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Dietary intake, eating behavior and health outcomes

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Dietary intake, eating behavior and health outcomes

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Editorial: Dietary intake, eating behavior and health outcomes

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Editorial on the Research Topic
Dietary intake, eating behavior and health outcomes

Dietary intake and eating behaviors are important determinants of non-communicable diseases (NCDs) and have been widely investigated (1). The association between dietary intakes (e.g., fruits, vegetables, processed meat, and trans-fat) and NCDs (e.g., obesity, cardiovascular diseases (CVD), diabetes, and cancer) have been described. The associations varied by socioeconomic and the burden differed by demographic conditions (2, 3). Nevertheless, due to the complexity of measuring exposures related to dietary intake, the evidence base is mainly observational and lacking experimental designs. Additionally, there is scant evidence on the effectiveness of health promotion strategies focusing dietary consumption and behavior, on health outcomes.

Bring in mind the complexity of studying dietary intake and its effects in health, the Frontiers in Nutrition dedicated a specific topic to *Dietary intake, eating behavior and health outcomes*. A total of 105 studies were submitted with 35 being selected for publication after peer-review.

In this editorial, we focus on those 35 studies addressing dietary and nutrition intake [e.g., fatty acids (Fan et al.; Tan and Shin), pro-inflammatory diet (Ruan et al.; Schütte et al.; Wang Q. et al.; Yan et al.; Zhao et al.), fruit and vegetables (Jin et al.; Wang R. et al.), meat (Chen et al.; Wu et al.), and others (AL-Mohaithef; Buso et al.; Jiang et al.; Li et al.; Lei et al.; Liu et al.; Sousa et al.; Yu et al.)], dietary patterns and behaviors (Bai et al.; Cui et al.; D'Esposito et al.; Di Maso et al.; Kim and Kim; Nguyen et al.; Palomar-Croset al.; Park; Zhang et al.) and dietary diversity (Hu et al.; Kim and Kim; Qu et al.; Zhou et al.). Also, two studies focused on socioeconomic disadvantage (Isaura et al.) and on the neighborhood effects on eating behaviors among elders (Liu and Yu).

In the study of Qu et al. the dietary diversity score was associated with a reduction in the risk of mortality. Hu et al. in their study with preschool children from poor ethnic minority areas found significant associations between dietary diversity, nutrient adequacy, and anthropometric status. Also Isaura et al. comprehensively discussed how childhood and early socioeconomic disadvantage is related to adult food security status and poor health in children. Focusing on older adults from China, the investigation of Liu and Yu about the effects of neighborhood diet quality on the eating behaviors of older adults living in the same community is sound. The neighborhood effects were manifested in increased consumption of vegetables and fruits, meat, eggs, and dairy products.

The studies from this Research Topic addressed several health outcomes, namely diabetes (Yu et al.; Zhao et al.), cancer (Fan et al.; Jiang et al.; Jin et al.; Li et al.; Palomar-Cros et al.; Park; Wang R. et al.; Wu et al.), obesity and weight status (AL-Mohaithef; Buso et al.;

Hu et al.; Kim and Kim; Lei et al.; Zhou et al.), CVD (Wang Q. et al.; Xie et al.; Zhang et al.), among others (Cui et al.; D'Esposito et al.; Di Maso et al.; Liu et al.; Ruan et al.; Schütte et al.; Tan and Shin; Yan et al.), including psychological issues (Berbegal et al.; Nguyen et al.; Sousa et al.).

In the Yu et al.'s study, intakes of branched-chain amino acids was associated with higher risk of type 2 diabetes, while Zhao et al., presented positive association between dietary acid load during early pregnancy and the risk of gestational diabetes mellitus.

In an updated systematic review and meta-analysis, Wang R. et al. found positive associations between dietary acid load and cancer risk and prognosis. Fan et al. found significant differences between dietary fatty acids intake in patients with oral cancer and controls. A positive association between the saturated fatty acids pattern and risk of oral cancer was observed, even after adjusting for potential confounders. Also beholding fatty acid intake, Tan and Shin discussed the preventative benefit of consuming oily fish and its fatty acid intake on non-alcoholic fatty liver disease, notably in South Korean women. In a large U.S. prospective cohort, Jiang et al. indicated that a moderate consumption of carrots was associated with a lower colorectal cancer incidence. In their Mendelian randomization study, Li et al., described direct associations between coffee and caffeine consumption and renal cell carcinoma, although suggesting the need to conduct further studies on the matter. Intake of dried fruit was considered protective on some site-specific cancers in the study of Jin et al.. The authors emphasized health education and an adjustment of dietary proportion for primary prevention of cancer. Regarding vegetable consumption, Park found inverse associations between their consumption and colon cancer. In the same study, the author reported positive associations between red meat and colon cancer and mortality. Also Wu et al. suggested that intake of processed meat was associated with an increased risk of lung cancer. In their study, Palomar-Cros et al. found significant associations between breakfast time and breast cancer.

Concerning weight status, Buso et al. suggested positive associations between consumption of both sugar-sweetened beverages and low/no-calorie beverages and weight-related outcomes. Dietary diversity was associated with body mass index in youth in the study of Zhou et al., while in the study of Hu et al. in preschool children, dietary diversity was associated with nutrient adequacy and other health outcomes. In addition, Kim and Kim suggested that psychosocial stress contributed to abdominal obesity by interacting with a low dietary variety score. Low carbohydrate diets and low fat diets had significant effects on metabolic risk factors and weight loss in the study of Lei et al. in adults with overweight and obesity, although the long-term effects of various sources of carbohydrates or fat in both diets need to be further studied. AL-Mohaithef addressed that vegan and vegetarian diets have increased in Saudi adults and those with a vegetarian diet showed a better lifestyle (e.g., higher physical activity level, higher consumption of fruits, vegetables, dairy products), low intake of fast-foods and fizzy beverages and was significantly associated with body mass index.

Concerning CVD, Zhang et al. reported prospective inverse associations between adherence to the 2015-2020 Dietary Guidelines for Americans and CVD risk. Also, Wang Q. et al., found inverse associations between urinary thiocyanate, a candidate biomarker of cruciferous vegetable intake, and CVD and total mortality among non-smoking adults. When studying people with diabetes, Xie et al. found that higher eating frequency was independently related to lower all-cause and CVDs-related mortality.

Anxiety was addressed in the study of Sousa et al. suggesting that the consumption of fermented dairy products had a positive effect on reducing anxiety in young university students. Also, Nguyen et al. found that during the pandemic, fear of COVID-19 and cigarette smoking had adverse impacts on medical students' psychological health. The results suggested that staying physically active and having healthy eating behaviors could potentially protect medical students from anxiety and depressive symptoms. Finally, Berbegal et al. provided further insights about the importance of adiposity in health and memory function, reporting that some measures of adiposity (e.g., weight, BMI, waist-hip ratio index) were inversely associated with memory function.

In their study, Schütte et al. found inverse associations between a pro-inflammatory dietary pattern and atopic outcome in children. In the same study, it was emphasized that this pattern reduced the buffering capacity of the individual against harmful environmental exposures or triggers. Also, D'Esposito et al. showed that age, body mass index, and lifestyles were associated with specific cytokines, potential markers for low-grade chronic inflammation.

In their study, Yan et al. confirmed the hypothesis that proinflammatory diets contribute to increase all-cause mortality in adults with chronic kidney disease. Concerning breastfeeding mothers, Di Maso et al., on behalf of MEDIDIET Working Group Members, found that a high adherence to the Mediterranean diet was associated with human milk composition, namely the milk's content of specific fatty acids.

In their study, Liu et al. confirmed a dose-response association between higher alcohol consumption and inflammatory bowel disease risk. Concerning other health outcomes, Chen et al. established associations between red meat, remarkably beef intake, and risk of rheumatoid arthritis. Ruan et al. when studying the dietary inflammatory potential, as estimated by the dietary inflammation index score, found that it is positively associated with erectile dysfunction among US males. Bai et al. focusing on some lifestyles determinants such as cigarette smoking, alcohol abuse, and decaffeinated coffee are associated with gallstone disease, whereas tea consumption can decrease the risk of gallstones due to the effect of caffeine metabolism on polyphenol intake. Interestingly, Cui et al. identified three dietary patterns in their study (i.e., "high protein pattern," "snack food pattern," and "vegetarian food pattern"), all of them showing a high linear association with high-altitude polycythemia.

Author contributions

RR formulated a draft. IF and TD revised the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Pro-inflammatory Diet Pictured in Children With Atopic Dermatitis or Food Allergy: Nutritional Data of the LiNA Cohort

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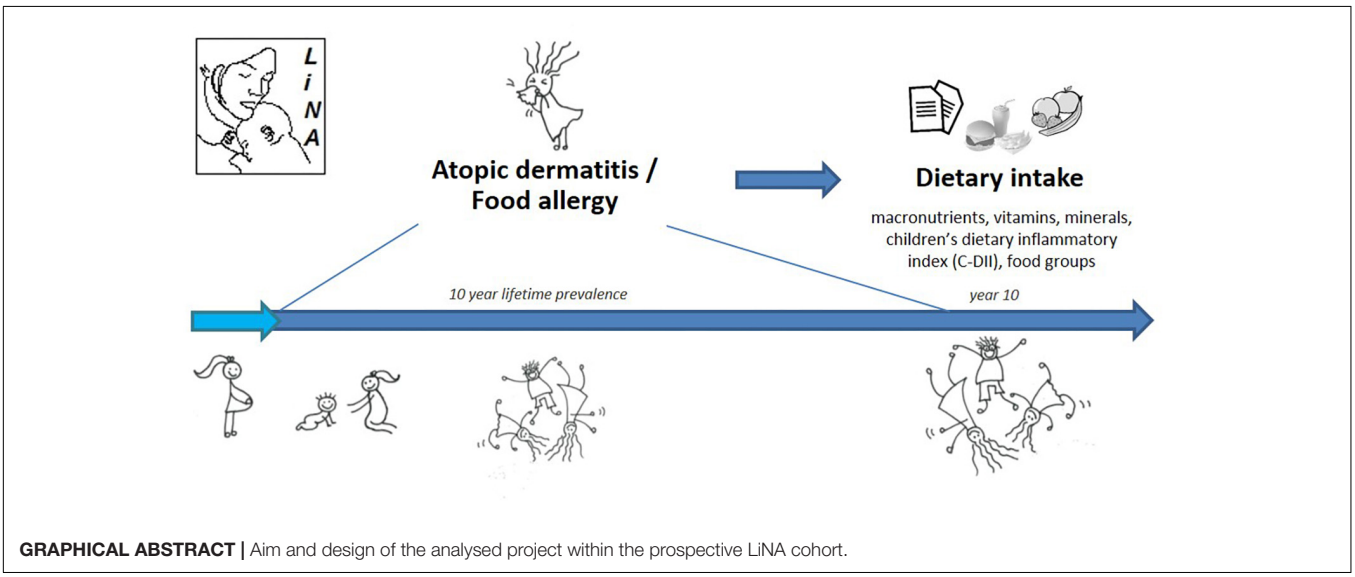
Background: Lifestyle and environmental factors are known to contribute to allergic disease development, especially very early in life. However, the link between diet composition and allergic outcomes remains unclear.

Methods: In the present population-based cohort study we evaluated the dietary intake of 10-year-old children and analyses were performed with particular focus on atopic dermatitis or food allergy, allergic diseases known to be affected by dietary allergens. Dietary intake was assessed *via* semi-quantitative food frequency questionnaires. Based on these data, individual nutrient intake as well as children's Dietary Inflammatory Index (C-DII^{TM}) scores were calculated. Information about atopic manifestations during the first 10 years of life and confounding factors were obtained from standardized questionnaires during pregnancy and annually thereafter.

Results: Analyses from confounder-adjusted logistic regression models (n = 211) revealed that having atopic outcomes was associated with having a pro-inflammatory pattern at the age of 10 years: OR = 2.22 (95% CI: 1.14–4.31) for children with atopic dermatitis and OR = 3.82 (95% CI: 1.47–9.93) for children with food allergy in the first 10 years of life.

Conclusion: A pro-inflammatory dietary pattern might worsen the atopic outcome and reduce the buffering capacity of the individual against harmful environmental exposures or triggers. For pediatricians it is recommended to test for the individual tolerance of allergenic foods and to increase the nutrient density of tolerable food items to avoid undesirable effects of eating a pro-inflammatory diet.

Keywords: atopic dermatitis (AD), food allergy (FA), food frequency questionnaires (FFQ), nutrients, food group consumption, C-DII, 10-year-old children



GRAPHICAL ABSTRACT | Aim and design of the analysed project within the prospective LiNA cohort.

INTRODUCTION

In Western countries the increasing prevalence of atopic diseases has become a major problem in human health. Because allergy onset and atopic march begin in infancy (1), early prevention is advisable. Worldwide, approximately 15%–30% of children live with dermatitis (2, 3) and 4–10% of children suffer from food allergy (4–6). Both atopic dermatitis and food allergy have the earliest onset within the atopic march, resulting in highest prevalence in children before school age (7). Many risk factors for the development of atopic diseases – acting independently or in multifactorial combination – have been identified (8, 9). These include genetic background, individual immune response, barrier dysfunctions, microbiome alterations, as well as lifestyle behaviors (10, 11) and environmental conditions (12).

Diet represents a source of components that could affect atopy in a number of ways. First, diet is a potential source of allergens. Diet also could provide substrate for components that interfere with the pathology of atopy. Finally, it is well-known that diet can modulate inflammatory and related immune responses that can ameliorate or exacerbate allergic or atopic reactions. However, the link between diet composition and the pathogenesis of allergies is complex and not well understood. Though the important role of breastfeeding and timing/manner of introducing solid food is well understood, there are few dietary factors consumed in early life that are described to alter the risk for allergic diseases [e.g., vitamin D, pro/prebiotics or omega(ω)-3 long-chain polyunsaturated fatty acids (13, 14)]. A position paper of the European Association of Asthma, Allergy and Clinical Immunology (EAACI) outlined that it is of high importance to understand how diet diversity modulates allergic outcomes (15). The task force also recommends to use indices in the future to better describe the allergic potential of foods or food patterns within the context of diet diversity. There are several dietary indices available that have been analyzed with respect to nutritional quality, adherence to dietary guidelines or recommendations as well as in association with specific outcomes, such as cardiovascular disease risk (16, 17). Because allergies are characterized by inflammatory processes (18, 19), it would be appropriate to apply an index representing the inflammatory potential of the individual diet in this context (20, 21).

In addition to the role of specific dietary factors contributing to the pathogenesis of allergic and atopic conditions, it also is important to consider that individuals suffering from food allergen-triggered symptoms develop a specific dietary pattern due to their mandatory avoidance of causative allergens (7, 22–25). Such dietary restrictions are, themselves, known to be associated with adverse health issues (22). Knowing the dietary intake of individuals affected by allergies, in particular of children, might offer possibilities to improve their immune response, reduce symptom severity or relapse frequency which

Abbreviations: AD atopic dermatitis, C-DII children's Dietary Inflammatory Index, FA food allergy, fx5 specific IgE for food allergen (mix), IL interleukin, sx1 specific IgE for respiratory allergen (mix), OR odds ratio.

is even more important in growing individuals. Therefore, the aim of the present study was to evaluate the nutritional pattern in a cohort of 10-year-old children with respect to their development of allergic diseases known to be directly affected by dietary allergens such as atopic dermatitis or food allergy. Because of the role of inflammation in these conditions, the children's Dietary Inflammatory Index (C-DII) was used to describe dietary exposure.

MATERIALS AND METHODS

Study Design

Within the population-based, prospective birth cohort study LiNA (Lifestyle and environmental factors and their Influence on Newborn's Allergy risk) 629 mother-child-pairs (622 mothers and 629 children; 7 twins) were recruited during regular appointments with their midwife during May 2006 and December 2008 in Leipzig, Germany. The aim of the study is to investigate how lifestyle and environmental factors in the pre and postnatal period influence the immune system of the newborn and the child later in life with consequences for future allergy risk. Mothers suffering from chronic immune or infectious diseases during pregnancy were excluded from the study, as well as mothers with non-German ancestry/non-Caucasian ethnicity. Further, only term ($\geq$ 37th week of pregnancy, $\geq$ 2,500 g birth weight) and healthy newborns (without postnatal infections that needed medical treatment) were included. General characteristics (such as sex of the child, mothers age at birth, birth mode, breastfeeding duration, presence of older siblings, parental school education, environmental tobacco smoke (ETS) exposure, pet keeping, family history of atopy, etc.) or outcome data were assessed during pregnancy and annually thereafter using questionnaires and in-person examinations. All questionnaires were self-administered by the parents (together with the children when they were old enough). Study participation was voluntary and written informed consent was obtained of all participants. The LiNA study was approved by the Institutional Review Board of the University of Leipzig and the Saxonian Board of Physicians (046-2006, 160-2008, 160b/2008, 144-10-31052010, 113-11-18042011, 206-12-02072012, 169/13-ff, 150/14-ff, EK-allg-28/14-1, 008/17-ek).

Dietary Assessment

Dietary intake was assessed at the age of 10 years using a semi-quantitative food frequency questionnaire (FFQ) asking for children's intake of foods in the past 12 months. The FFQ contained 106 food items from 14 different food/beverage groups (bread and rolls, spreads, cheese and cold sausage, cereals and cornflakes, milk (-products) and eggs, basic carbohydrates, meat, fish, vegetables, fruits, cake and desserts, (salty) sweets and nuts, fats and oils and beverages) with nine non-overlapping frequency categories (never to $\geq$4 times/d) as well as five relative portion size options (1/4, 1/2, 1, 2, 3) referring to an exemplified or pictured standard portion size (i.e., Equal to 1). Relevant data on the fat content and type of preparation were recorded (e.g., fat content of milk products/raw or cooked vegetables etc.). The data based on FFQ where then analyzed using DGExpert (version 1.9, based on codes by the German Food Code and Nutrient Database – BLS 3.02) which outlined the individual intake of 158 macro- and micronutrients for every child. A comparison to applicable reference values (D (Germany) –A (Austria) –CH (Switzerland) reference) is provided by DGExpert considering the children's personal data [age, sex, weight, height and Physical Activity Level (PAL)] to calculate individual energy- and nutrient requirements. Average PAL was considered to be 1.6 according to the German Society of Nutrition (DGE) and was further adopted according to children's activity on their way to school (how children went to school (walk/bike/car) and how long this took). In addition to the nutrient calculation, the consumption of specific food groups was analyzed according to the optimized mixed diet (OptimiX) recommendation (beverages [mL/d], bread and cereals [g/d], pasta, rice and potatoes [g/d], vegetables [g/d], fruits [g/d], milk and dairy products [g/d], meat and sausages [g/d], eggs [pieces/week], fish [g/week], fats [g/d] or tolerated food group [portions/d, sweets, snacks, soft drinks] (26).

Children's Dietary Inflammatory Index

Using data from the FFQ the inflammatory potential of the participant's diet was evaluated by calculating the children's Dietary Inflammatory Index (C-DII) for each child. The detailed C-DII methodology has been established and described earlier (27). Briefly, the Dietary Inflammatory Index (DII) classifies human dietary patterns on a continuous scale from anti-inflammatory (values <0) to pro-inflammatory (values >0) based on a broad literature database with respect to 45 foods or nutrients that were described to be associated with inflammatory markers such as interleukin (IL)-1b, IL-4, IL-6, IL-10, tumor necrosis factor (TNF)-a and C-reactive protein (CRP) (28). The DII was further adapted for children (C-DII) using 25 nutrients or food parameters (27). All parameters except selenium (missing software database information) were included for the LiNA C-DII; Anti-inflammatory parameters included: vitamin A, thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), vitamin B6, folic acid (vitamin B9), vitamin D, vitamin C, vitamin E, beta carotene, fiber, mono-unsaturated fatty acid (MUFA), poly-unsaturated fatty acid (PUFA), magnesium (Mg) and zinc (Zn); Pro-inflammatory parameters included: vitamin B12, energy, carbohydrates, total fat, saturated fat, cholesterol, protein, alcohol and iron (Fe). Next to data on the inflammatory potential of the diet, dietary intakes from a wide range of diverse populations from different countries representing six continents were used to construct a consumption database that was referred to as Z-Scores (27).

Atopic Outcomes

Atopic dermatitis and food allergy were used as atopic outcomes in the present analyses. Atopic dermatitis was recorded annually *via* parental report of a doctor-diagnosed atopic dermatitis or as the diagnosis of the study physician at the annual LiNA medical examination. For food allergy the annual parental report of a doctor-diagnosed food allergy was used. Outcome prevalence was defined as at least one positive indication within the first 10 years of life.

IgE Measurements

Total immunoglobulin E (IgE), as well as IgE specific for food allergens (fx5) or inhalative allergens (sx1) were determined at children's age of 10 years by Phadia ImmunoCAP system (Thermo Fisher Scientific, Freiburg, Germany) from serum samples. Total IgE concentration >34.6 kU/l was classified as "increased," as well as specific IgE (sx1 or fx5) >0.35 U/l (29). Values below the detection limit were included in the analyses using half of the defined detection limit.

Statistical Analyses

After testing for normal distribution with Shapiro-Wilk test, descriptive analyses were performed using non-parametric tests for parameters found not to be distributed normally. Data are presented as medians with 25–75th percentile (1st to 3rd quartile) or as frequencies (%). χ^2-tests were used to compare characteristics in the analyzed sub-cohort at age 10 years with the total cohort recruited during pregnancy (sex of the child, mothers age at birth, birth mode, breastfeeding duration, presence of older siblings, parental school education (highest level), environmental tobacco smoke (ETS) exposure during pregnancy, pet keeping during pregnancy, family history of atopy and body mass index). Further, these characteristics were compared within the cohort for analysis with respect to children's anti-/pro-inflammatory C-DII. Characteristics known to be associated with atopic outcomes that were also associated with the C-DII were included as confounders in the regression models.

The relationship between atopic outcomes and nutrients/C-DII/food groups as well as the association between C-DII and food groups was addressed using the Mann–Whitney *U*-test. To adjust for confounders, multiple logistic regression models were used to calculate the risk of having a pro-inflammatory C-DII at the age of 10 years (dependent variable) with respect of the atopic outcome development within the first 10 years of life (independent variable) while adjusting for potential confounding factors (sex of the child, breastfeeding duration, parental school education, pet keeping during pregnancy and body mass index age 10). Data are presented as odds ratios with 95% confidence interval. All p-values <0.05 were considered to be significant. Statistical analyses were performed with STATISTICA for Windows, Version 13 (Statsoft Inc.), R (version 3.6.1; R development Core Team) or GraphPad Prism (Version 8.1.2.).

RESULTS

Characteristics of the Analyzed LiNA Sub-Cohort

From the total cohort (n = 629), 268 participated in the 10-year campaign and 211 of whom were available with complete FFQ as well as confounding data (**Supplementary Figure 1**). Drop outs resulted from loss to follow-up over 10 years (average annual drop out 8.95%). Reasons for drop out – if available – were for example family moving or less available time when kids entered school. General characteristics (sex of the child, mothers age at birth, birth mode, breastfeeding duration, presence of older siblings, parental school education (highest level), environmental tobacco smoke (ETS) exposure during pregnancy, pet keeping during pregnancy, family history of atopy and body mass index) of the analyzed sub-cohort compared to the total LiNA cohort are presented in **Table 1** with no differences seen between the two groups.

Atopic Outcomes

Within the analyzed sub-cohort prevalence of atopic dermatitis and food allergy during the first 10 years of life was 37.4 and 11.8%, respectively (**Supplementary Table 1**). From the 79 children diagnosed with atopic dermatitis, 76.2% had increased total IgE levels measured at the age of 10 years compared to the allergy-diagnostic reference value of 34.6 kU/l for 10-year-old children. Further, 25.4% of these children had increased food-allergen-specific fx5 levels as well as 61.2% increased airway-allergen-specific sx1 levels. In addition, from the 25 children with food allergy 90.5% had increased total IgE levels, 33.3% increased fx5 levels as well as 71.4% increased sx1 levels at the age of 10 years. For 16 children both atopic dermatitis and food allergy was reported.

General Dietary Intake

Because it was the first time that nutrients were assessed *via* FFQ and DGExpert in the LiNA cohort, a comparison of the final LiNA nutrient data set was performed with data from a study with similar design/geographical region as were available from EsKiMo, a nutritional module from the Robert Koch institute's KiGGs study (30). All analyzed macronutrients (% of total energy intake for fat, carbohydrates and proteins, absolute amounts of fatty acids, cholesterol, sugar and fibers) or absolute amounts of consumed minerals or vitamins were in a very similar range and thus comparable between LiNA and EsKiMo (31) for 10-year-old boys and girls (**Supplementary Table 2**; overall median difference between LiNA and EsKiMo was 8%).

For the following investigations, a representative subset of 35 nutrients (macronutrients, minerals, and fat/water soluble vitamins) was analyzed. In general, the overall intake of macro- and micronutrients of the LiNA participants was displayed as % of total energy intake (carbohydrates, fat, and proteins) or as absolute values; both compared to the D-A-CH-reference values which is shown in **Supplementary Figure 2** for all children and in **Supplementary Figure 3** for boys/girls separately. For macronutrients in all children, data exceeded the recommendation for total fat intake (30% of energy) and total protein intake (0.9 g/kg body weight; in LiNA adequate to an overall 6.6% of the total energy intake) as pictured in **Supplementary Figure 2A**, with the girls being significantly lower in protein intake than the boys (**Supplementary Figure 3A**). Children's minerals intake exceeded the D-A-CH reference for sodium (Na), chloride (Cl), magnesium (Mg), zinc (Zn), copper (Cu), and manganese (Mn), while calcium (Ca), phosphorus (P), iron (Fe) and iodine (I) and fluoride (F), in particular, were below D-A-CH reference values (**Supplementary Figure 2B**). According to sex differences, girls had a significant lower Na, Cl, K, Ca, P, Mg, Fe, I, F, and Cu intake than the boys (**Supplementary Figure 3B**).

TABLE 1 | Study characteristics.

	Analyzed sub-cohort Age 10 $N = 211^a$ n (%)	Entire LiNA cohort Pregnancy $N = 629^a$ n (%)	*p*-value χ^2 test
Sex of child			0.80
Male	107 (50.7)	330 (52.5)	
Female	104 (49.3)	299 (47.5)	
Mothers age at birth			0.74
≤25 years	16 (7.58)	66 (10.5)	
>25 – 30 years	72 (34.1)	239 (38.0)	
>30 – 35 years	77 (36.5)	214 (34.0)	
>35	46 (21.8)	110 (17.5)	
Birth mode			0.72
Spontaneous	152 (72.0)	471 (74.9)	
Cesarean section	56 (26.5)	132 (21.0)	
Others	2 (1.00)	7 (1.10)	
Breastfeeding duration			0.56
No	11 (5.20)	26 (4.10)	
3 months	27 (12.8)	112 (17.8)	
6 months	60 (28.4)	190 (30.2)	
12 months	107 (50.7)	254 (40.4)	
Presence of older siblings			0.79
Yes	74 (35.1)	208 (33.1)	
No	136 (64.5)	414 (65.8)	
Parental school education[b]			0.64
Low	2 (1.00)	16 (2.50)	
Medium	43 (20.4)	142 (22.6)	
High	165 (78.2)	464 (73.8)	
ETS[c] exposure pregnancy			0.34
No	168 (81.6)	464 (76.1)	
Yes	38 (18.4)	146 (23.9)	
Pet keeping pregnancy			0.62
No	128 (61.2)	358 (57.8)	
Yes	81 (38.8)	261 (42.2)	
Family history of atopy			0.97
None	69 (32.7)	212 (33.7)	
One	103 (48.8)	296 (47.1)	
Both	39 (18.5)	121 (19.2)	
Body mass index age 10[d]			
Underweight	25 (12.0)	–	
Normal weight	166 (79.4)	–	
Overweight/obese	18 (8.6)	–	

[a] *n may differ from 211/629 due to missing data.*
[b] *Low = 8 years school education; medium = 10 years school education; high = at least 12 years school education.*
[c] *Environmental tobacco smoke.*
[d] *Underweight (body mass index equivalent to <18,5 kg/m² at 18 years), normal weight (body mass index equivalent to 18,5 −<25 kg/m² at 18 years), overweight/obese (body mass index equivalent to ≥25 kg/m² at 18 years).*
General characteristics of the analyzed sub-cohort compared to the total LiNA cohort.

Potassium (K) intake was according to the recommendations. With respect to fat-soluble vitamins shown in **Supplementary Figure 2C** for the total sub-cohort, children were above (for vitamin A and K) and below (for vitamin E and in particular for vitamin D) the recommendation, with no differences between girls/boys (**Supplementary Figure 3C**). Water soluble vitamins (**Supplementary Figure 2D**) were all clearly on or above the recommended intake (for vitamin C, B1 (thiamine), B2 (riboflavin), B3 (niacin), B6, B7 (biotin), B9 (folate) and B12), with girls being significant lower in B5 (pantothenic acid), B7 and B12 intake than the boys (**Supplementary Figure 3D**). According to our data only vitamin B5 was consumed in amounts below the current recommendations (**Supplementary Figure 2D**), in particular by girls (**Supplementary Figure 3D**).

Dietary Intake With Respect to Atopic Diseases

The dietary intake assessed at the age of 10 years was analyzed with respect to children's development of atopic dermatitis or food allergy within the first 10 years of life (**Table 2**). Children with atopic dermatitis/food allergy consumed significantly lower amounts of fiber (in % of the total energy intake) than children without atopic dermatitis/food allergy. Sugar intake was lower in children with atopic dermatitis; however, overall sugar intake was above the recommendation of 10% of the total energy intake in all children. The intake of minerals was not different in children with or without atopic dermatitis/food allergy. Children with atopic dermatitis had a significant lower intake of vitamins C, E, and B7 compared to children who did not develop an atopic dermatitis within the first 10 years of life. However, both groups had either higher (for vitamin C and B7) or lower levels (for vitamin E) compared to the D-A-CH reference. Vitamin intake of children with food allergy was not different from those without food allergy within the first 10 years of life.

Children's Dietary Inflammatory Index

Children's dietary inflammatory index scores were calculated to quantify the inflammatory potential of the diet of LiNA children. In general, values above 0 indicate a more pro-inflammatory pattern, whereas values below 0 indicate an anti-inflammatory pattern. Overall, the LiNA children had a median C-DII of −0.97 (interquartile range (IQR): −2.06 to 0.26; $n = 211$), with girls being lower than boys (i.e., −1.22 (IQR: −2.17 to −0.24; $n = 104$ compared to −0.53 (IQR: −1.93 to 0.77; $n = 107$), respectively. Furthermore, general characteristics were compared between children who had a more anti-inflammatory (C-DII <0) and those who had a pro-inflammatory (C-DII >0) dietary pattern (**Table 3**). Data revealed that sex, breastfeeding duration, parental school education and children's body mass index at the age of 10 years differed between children with pro-inflammatory and those with anti-inflammatory C-DII score. Next, the C-DII was analyzed in the context of atopic dermatitis and food allergy. As shown in **Table 4**, there were no significant differences in C-DII between children with and those without atopic outcomes, although C-DII levels tended to be lower (indicating a more anti-inflammatory diet) in children without atopic outcomes. When C-DII was grouped into anti-inflammatory (<0) and pro-inflammatory (>0), regression models revealed that having atopic outcomes was associated with having a pro-inflammatory

TABLE 2 | Single nutrients and atopic outcomes.

	Atopic dermatitis within the first 10 years							Food allergy within the first 10 years						
	Without (*n* = 132)			With (*n* = 79)				Without (*n* = 186)			With (*n* = 25)			
	Median	**Q 1st**	**Q 3rd**	**Median**	**Q 1st**	**Q 3rd**	***p*-value**	**Median**	**Q 1st**	**Q 3rd**	**Median**	**Q 1st**	**Q 3rd**	***p*-value #**
Macronutrients														
Energy (kcal)	**2109**	1780	2590	**2152**	1715	2719	0.91	**2097**	1764	2608	**2464**	1826	2859	0.19
Fat (%E)	**35.5**	31.2	39.4	**36.4**	32.9	40.0	0.10	**35.8**	31.8	39.4	**36.3**	32.9	40.0	0.36
SFA (%E)	**15.3**	13.7	17.9	**16.0**	14.7	18.0	0.07	**15.7**	14.0	17.9	**15.8**	14.1	17.8	0.71
PUFAs (%E)	**4.7**	4.1	5.7	**4.7**	4.3	5.4	0.58	**4.7**	4.1	5.5	**4.6**	4.3	5.3	0.87
MUFAs (%E)	**12.3**	10.9	13.8	**12.6**	11.3	14.0	0.17	**12.3**	10.9	13.9	**12.8**	11.7	14.5	0.15
Chol (mg)	**286**	227	360	**293**	228	389	0.46	**283**	225	364	**330**	255	394	0.08
Carbs (%E)	**49.0**	45.5	53.5	**47.3**	44.3	51.6	0.09	**48.3**	44.5	53.1	**47.7**	44.4	52.0	0.53
Sugar (%E)	**21.9**	17.7	26.3	**19.5**	15.8	25.2	0.05	**20.6**	17.0	26.0	**20.6**	17.5	23.2	0.71
Fiber (%E)	**2.0**	1.7	2.5	**1.9**	1.5	2.2	0.04	**2.0**	1.6	2.4	**1.7**	1.5	2.0	0.02
Protein (%E)	**14.4**	13.2	15.8	**14.9**	13.3	16.2	0.23	**14.6**	13.2	16.1	**14.7**	13.8	15.5	0.91
Minerals*														
Na	**210.0**	166.4	280.5	**209.1**	162.7	298.2	0.67	**210.0**	165.5	286.4	**212.7**	186.4	300.9	0.39
K	**101.0**	79.0	121.0	**93.4**	73.8	117.2	0.23	**97.1**	75.9	120.7	**103.4**	84.8	111.0	0.75
Ca	**64.5**	53.6	89.5	**71.8**	50.9	91.8	0.92	**65.9**	51.8	88.2	**70.9**	55.5	91.8	0.64
Mg	**120.0**	96.5	151.3	**115.2**	90.0	154.8	0.36	**119.0**	95.7	151.3	**123.0**	96.0	153.2	0.69
P	**90.8**	74.4	116.8	**92.8**	72.8	119.2	0.91	**91.6**	72.8	115.2	**91.2**	77.6	124.0	0.48
Fe	**71.7**	56.0	88.9	**72.0**	54.2	94.2	0.90	**68.8**	55.0	90.0	**78.7**	56.0	97.5	0.33
Zn	**123.8**	94.8	144.3	**112.4**	93.7	155.0	0.71	**118.5**	93.7	147.0	**129.7**	102.4	144.6	0.83
J	**48.3**	38.6	60.3	**46.7**	38.3	61.1	0.48	**48.3**	38.3	60.0	**47.8**	38.3	66.7	0.96
Cl	**215.9**	173.8	293.2	**222.4**	161.2	305.3	0.89	**215.9**	170.6	288.8	**222.4**	183.5	315.9	0.42

(Continued)

TABLE 2 | (Continued)

	Atopic dermatitis within the first 10 years							Food allergy within the first 10 years						
	Without (*n* = 132)			With (*n* = 79)				Without (*n* = 186)			With (*n* = 25)			
	Median	**Q 1st**	**Q 3rd**	**Median**	**Q 1st**	**Q 3rd**	***p*-value**	**Median**	**Q 1st**	**Q 3rd**	**Median**	**Q 1st**	**Q 3rd**	***p*-value #**
Fl	**33.8**	27.8	42.8	**32.5**	26.5	44.0	0.66	**33.0**	27.0	42.5	**35.0**	30.0	45.5	0.30
Cu	**135.5**	109.5	165.0	**120.0**	98.0	180.0	0.20	**131.5**	106.0	168.0	**137.0**	109.0	169.0	0.85
Mn	**214.8**	163.3	286.3	**187.0**	152.5	281.5	0.21	**205.0**	157.5	284.5	**194.5**	161.5	281.0	0.84
Vitamins*														
A	**125.8**	93.8	172.9	**124.7**	95.7	176.2	0.94	**125.1**	94.0	175.4	**125.0**	98.1	161.9	0.92
C	**241.1**	164.8	327.8	**180.8**	125.7	344.3	0.02	**223.1**	145.4	327.5	**201.2**	135.2	352.5	0.84
D	**9.0**	7.0	13.0	**8.5**	5.5	13.5	0.59	**8.5**	6.5	13.0	**9.0**	5.5	13.5	0.66
E	**83.7**	68.8	114.3	**77.7**	60.0	103.6	0.03	**81.2**	63.6	108.2	**80.9**	66.2	110.0	0.94
K	**303.0**	210.4	417.4	**252.5**	175.3	400.5	0.14	**285.6**	197.8	411.5	**239.8**	182.3	396.5	0.68
B1	**146.9**	116.2	177.9	**140.0**	105.8	179.9	0.40	**143.8**	108.0	175.0	**156.0**	125.0	188.0	0.27
B2	**139.5**	109.5	169.5	**130.9**	101.8	169.1	0.43	**136.2**	106.0	167.0	**149.1**	120.9	178.2	0.41
B3	**240.5**	194.5	292.7	**230.9**	192.3	300.0	0.82	**236.7**	193.1	300.0	**260.8**	209.2	293.1	0.39
B5	**92.9**	74.8	116.8	**85.8**	66.8	109.4	0.11	**151.0**	118.0	194.0	**162.0**	131.0	203.0	0.48
B6	**157.0**	121.5	194.5	**150.0**	114.0	196.0	0.53	**223.5**	181.0	286.5	**252.5**	171.5	277.5	0.91
B7	**236.3**	188.3	285.3	**201.0**	158.5	285.5	0.047	**97.9**	75.8	135.0	**116.7**	72.9	133.3	0.85
B9	**105.6**	80.0	132.3	**88.8**	72.1	135.0	0.12	**216.0**	169.5	303.5	**270.5**	204.0	322.5	0.12
B12	**216.0**	162.3	301.8	**237.0**	177.5	319.5	0.24	**89.4**	73.2	113.6	**96.2**	76.0	108.4	0.67

**% of D-A-CH reference.*

 # p-values from Mann–Whitney U-test, for medians with first/third quartile (Q 1st/Q 3rd).

%E - percentage of energy intake.

Median daily nutritional intake of 10-year old children with or without atopic dermatitis/food allergy within the first 10 years of life.

All Median values are printed in bold.

TABLE 3 | C-DII and study characteristics.

	C-DII class 1 Anti-inflammatory (n = 151[a])		C-DII class 2 Pro-inflammatory (n = 60[a])		p-value
	n	%	n	%	χ^2 test
Sex of the child					
Male	70	46.4	37	61.7	0.02
Female	81	53.6	23	38.3	
Mothers age at birth					
≤25 years	13	8.6	3	5.0	0.45
>25 – 30 years	48	31.8	24	40.0	
>30 – 35 years	58	38.4	19	31.7	
>35	32	21.2	14	23.3	
Birth mode					
Spontaneous	108	72.0	44	73.3	0.52
C. section	40	26.7	16	26.7	
Others	2	1.3	0	0	
Breastfeeding duration					
no	10	6.8	0	0	0.047
3 month	18	12.2	9	15.8	
6 month	41	27.7	19	33.3	
12 months	79	53.4	28	49.1	
Presence of older siblings					
Yes	52	34.7	22	36.7	0.77
No	98	65.3	38	63.3	
Parental school education[b]					
Low	2	1.3	0	0	0.049
Medium	25	16.7	18	30	
High	123	82.0	42	70	
ETS exposure pregnancy					
No	122	83.6	46	76.7	0.22
Yes	24	16.4	14	23.3	
Pet keeping during pregnancy					
No	97	65.1	31	51.7	0.06
Yes	52	34.9	29	48.3	
Family history of atopy					
None	47	31.1	22	36.7	0.19
One	72	47.7	31	51.7	
Both	32	21.2	7	11.7	
Body mass index age 10[c]					
Under weight	17	11.4	8	13.3	0.02
Normal weight	115	77.2	51	85	
Overweight/obese	17	11.4	1	1.7	

[a] *n may differ from 151/60 due to missing data.*
[b] *Low = 8 years school education; medium = 10 years school education; high = at least 12 years school education.*
[c] *Underweight (body mass index equivalent to 18,5 kg/m^2 at 18 years), normal weight (body mass index equivalent to 18,5 −<25 kg/m^2 at 18 years), overweight/obese (body mass index equivalent to ≥25 kg/m^2 at 18 years).*
ETS - environmental tobacco smoke.
General characteristics of the analyzed sub-cohort with respect of having an anti-inflammatory (C-DII <0) or pro-inflammatory (C-DII >0) children's dietary inflammatory index at the age of 10 years.

pattern at the age of 10 years (**Table 5**; OR = 2.22, 95% CI: 1.14–4.31) for children with atopic dermatitis, OR = 3.82 (95% CI: 1.47–9.93) for children with food allergy in the first 10 years of life). These associations were independent of confounders. This more pro-inflammatory pattern in children with atopic outcomes was supported by analyses of specific consumed food groups: children that developed atopic dermatitis within the first 10 years of life consumed significantly less fruits and nuts, children with food allergy consumed significantly more of the tolerated food group including sweets/snacks etc. (**Figure 1**). Children with a pro-inflammatory diet (C-DII >0) consumed fewer vegetables, fruits and nuts, but more meat/sausages and more sweets/snacks (**Figure 2**).

DISCUSSION

In this project we assessed the dietary intake of 10-year-old children for the first time within the prospective birth cohort, LiNA. According to participants' overall dietary intake, we were able to show that they had an adequate intake of the majority of nutrients, with some even exceeding the recommendations (e.g., for total fat, SFA, protein, sugar, Na and Cl). In contrast, for some nutrients children did not even reach half of the recommendation (e.g., vitamin D, F, I). When compared to other studies, LiNA results on the intake of specific nutrients, as well as the consumed food groups [according to the OptiMix recommendation (26)] were very similar to other studies; for example, compared to the nutritional assessment within Robert-Koch-Institute's EsKiMo module (31). The overall median nutrient difference between children from LiNA and EsKiMo was 8%, supported by similar data on food consumption with respect to the recommendation as shown for 6–11 year-old children from EsKiMo: lower consumption of vegetables, fruits and carbohydrates (bread, cereals, pasta, rice and potatoes) as well as higher consumption of meat (meat, sausages, etc.) and sweets and snacks (31). Further, our data indicated that girls' intakes of minerals and water soluble vitamins was higher than boys' intakes. Sex-specific differences in food groups that provide these nutrients such as vegetables were also described previously (32–34).

In addition to the overall dietary pattern of the LiNA children, analyses on the intake of single nutrients and atopic outcomes were performed. We were not able to show a clear allergy-specific dietary pattern, although there were some changes in vitamins (B7, C, and E), sugar and fiber. Still, when interpreted according to the D-A-CH-references, these nutrients were lower (vitamin E) or higher (vitamin B7 and C and sugar) than the recommendation values independent of children's allergy development. However, children with atopic dermatitis/food allergy within the last 10 years of life were more likely to show a less anti-inflammatory/more pro-inflammatory dietary pattern at the age of 10 years as assessed *via* the C-DII. In line with this, we were also able to show that children who developed atopic dermatitis within the first 10 years of life consumed significantly less fruits and nuts – food groups which, next to others, provide mostly nutrients that would drive the C-DII toward an anti-inflammatory pattern. This was supported by the significantly lower intake of vitamin C, E, and B7 in LiNA children with atopic dermatitis within the first 10 years of life. In addition, children with food allergy within the first

10 years of life consumed significantly more of the tolerated food group [which emphasizes sweets/snacks etc., according to Kersting et al. (26)]. This food group provides mostly nutrients that drive the C-DII toward a pro-inflammatory pattern such as sugar or saturated fat (with the consequence that these energy-dense foods result in higher overall energy intake and greater inflammation). This was confirmed by showing that children's pro-inflammatory diet was associated with a lower intake of vegetables, fruits and nuts and a higher intake of meat products and sweets/snacks. We hypothesize that children might have developed this less-anti-inflammatory/more pro-inflammatory diet due to an avoidance of possible anti-inflammatory – but allergy triggering – food items. It was described earlier that therapeutic strategies in atopic dermatitis and food allergy often involve dietary exclusions, which may be seen as mandatory in children sensitive to food allergens for whom accidental and potentially life threatening anaphylactic reactions can occur (6). It was also described that these exclusions may impact diet quality, nutrient intake and nutrient demands. It was even shown that an unsupervised elimination diet in childhood might lead to malnutrition, growth retardation, vitamin deficiencies and associated health issues (22, 35). In the context of this study, a pro-inflammatory diet consumed by the children might worsen the atopic outcome itself (20) and furthermore reduce

TABLE 4 | C-DII and atopic outcomes.

Atopic dermatitis within the first 10 years									
	Without disease outcome				With disease outcome				
	n	Median	1st quartile	3rd quartile	n	Median	1st quartile	3rd quartile	*p*-value[#]
All	132	**−1.19**	−2.13	−0.06	79	**−0.53**	−2.00	0.79	0.05
Boys	60	**−0.97**	−2.07	0.17	47	**−0.27**	−1.58	1.07	0.12
Girls	72	**−1.26**	−2.15	−0.32	32	**−1.19**	−2.18	0.40	0.57
Food allergy within the first 10 years									
	Without disease outcome				With disease outcome				
	n	Median	1st quartile	3rd quartile	n	Median	1st quartile	3rd quartile	*p*-value
All	186	**−1.07**	−2.13	0.07	25	**−0.25**	−1.79	0.73	0.08
Boys	92	**−0.62**	−1.98	0.75	15	**−0.25**	−1.79	1.07	0.42
Girls	94	**−1.31**	−2.18	−0.35	10	**−0.03**	−2.06	0.73	0.12

 # p-values from Mann-Whitney U-test, for medians with first/third quartile.
Descriptive data on the children's dietary inflammatory index (C-DII) assessed at the age of 10 with respect to their development of atopic dermatitis or food allergy within the first 10 years of life. Shown among all 211 children from the LiNA-cohort, as well as separately for boys and girls, p-value from Mann-Whitney-U-test.

TABLE 5 | Pro-inflammatory C-DII and atopic outcomes.

	Pro-inflammatory diet age 10 (C-DII >0)											
AD	Crude						Adjusted*					
	n total	n C-DII >0	OR	(95% CI)		*p*-value	n total	n C-DII >0	OR	(95% CI)		*p*-value
All	211	60	1.89	1.02	3.49	0.04	201	57	2.22	1.14	4.31	0.02
Boys	107	37	1.87	0.83	4.23	0.13	102	36	2.65	1.04	6.72	0.04
Girls	104	23	1.62	0.61	4.31	0.33	99	21	2.52	0.86	7.36	0.09
	Pro-inflammatory diet age 10 (C-DII >0)											
FA	Crude						Adjusted*					
	n total	n C-DII >0	OR	(95% CI)		*p*-value	n total	n C-DII >0	OR	(95% CI)		*p*-value
All	211	60	2.65	1.13	6.24	0.03	201	57	3.82	1.47	9.93	0.01
Boys	107	37	1.81	0.59	5.53	0.29	102	36	4.57	1.17	17.9	0.03
Girls	104	23	4.22	1.09	16.4	0.04	99	21	7.24	1.51	34.8	0.01

**Logistic regression model adjusted for sex, breastfeeding duration, parental school education, pet keeping pregnancy and body mass index age 10; 10 missing cases on specific confounders, OR - odds ratio, CI - confidence interval.*
Logistic regression models – raw or adjusted for confounders - showing the risk for children consuming a pro-inflammatory diet at the age of 10 years (by having a C-DII >0) with respect to having developed atopic dermatitis (AD) or food allergy (FA) within the first 10 years of life.

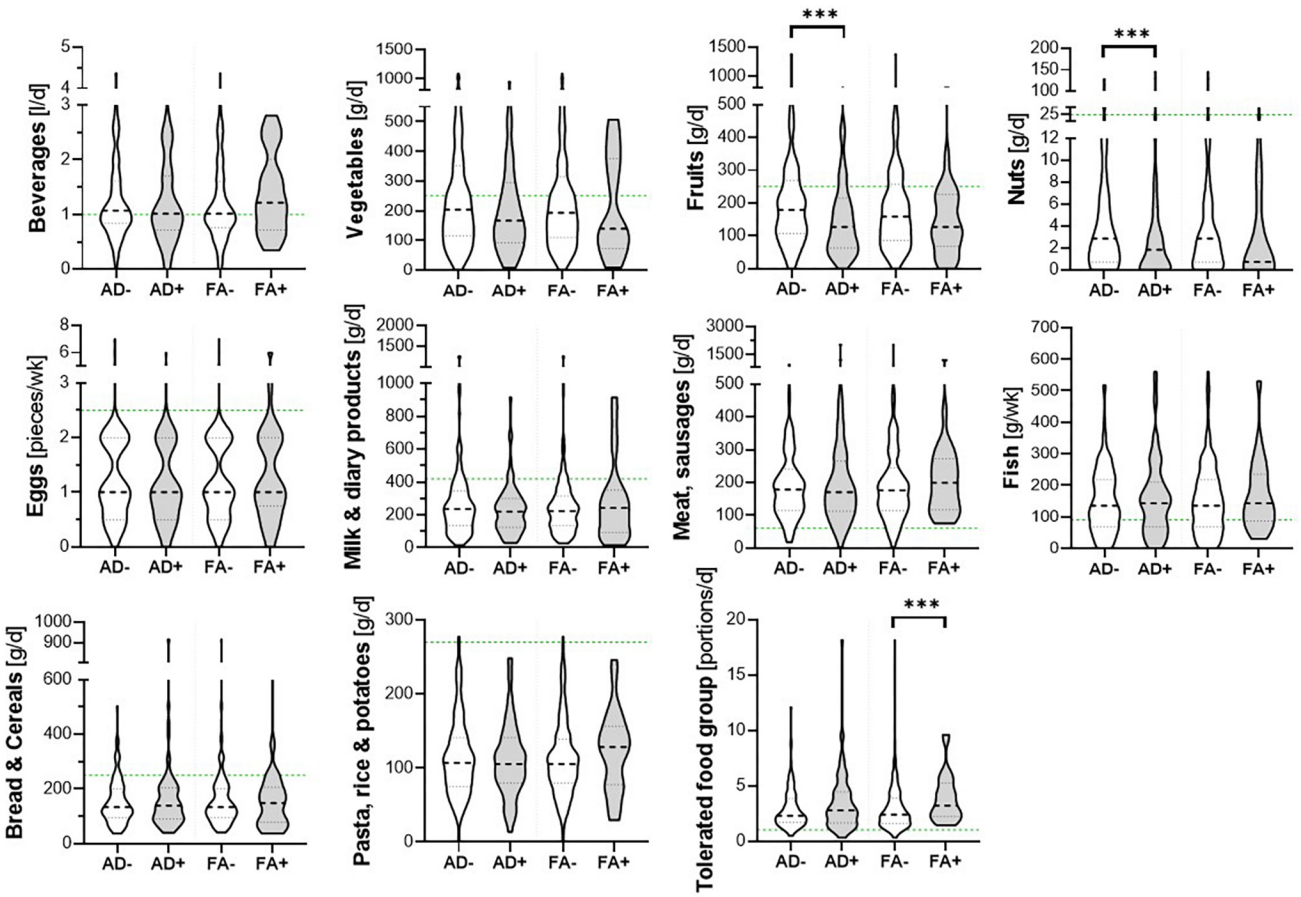


FIGURE 1 | Food groups and atopic outcomes. Children's consumed food groups at the age of 10 years according to having developed atopic dermatitis (AD +) or food allergy (FA +) within the first 10 years of life or not (AD- and FA-, respectively). Green line: OptiMix recommendations for 10–12 year old children. Data are presented as violin plots with median (bold dotted line) and 25 to 75th percentile (dotted line), $n = 211$. ***Significant difference ($p < 0.05$) between AD+/AD- or FA+/FA-, respectively (Mann–Whitney *U*-test).

their buffering capacity against harmful environmental exposures or triggers. An optimal nutritional status was described to be protective against both communicable and non-communicable diseases (36).

Because of the design of this study, reverse causality could not be ruled out. The diet as assessed at the age of 10 years could be a proxy for children's lifelong dietary pattern. The C-DII assessed in LiNA at the age of 10 was associated with markers of socio-economic status (SES) of their families assessed during pregnancy (such as parental school education). It has been shown previously that lower SES is associated with poorer nutrition (37). Studies also suggest that children begin to assimilate and mimic their parents' food choices at a very young age (38) and that this parent-child-transmission in dietary behaviors is dependent on SES (39). It was further shown that parental SES impacts childhood health issues (40) and that healthy lifestyle promotion alone might not substantially reduce the socioeconomic inequity in health (41). So, it also should be kept in mind that a pro-inflammatory diet consumed by the children on a daily basis throughout infancy also might have contributed to their allergy development. However, with the data available in our cohort, we are not able to examine the direction of temporal ordering of these effects in further detail.

To the best of our knowledge, this is the first use of the C-DII in association with atopic dermatitis and food allergy. Both atopic dermatitis and food allergy are characterized by inflammatory processes (18, 19), similar to other non-communicable diseases that are characterized by low-grade, chronic systemic inflammation. It was shown, for example, that in adults a pro-inflammatory diet (DII >0) was associated with an increased risk of certain cancers, cardiovascular disease, adverse mental health outcomes, and musculoskeletal disorders (21). It also is known that a pro-inflammatory diet is linked to greater all-cause mortality risk (42). The evidence for an association between DII and respiratory health, neurodevelopmental outcomes, metabolic syndrome, diabetes and obesity was described to be either conflicting or scarce (21). Furthermore, there are limited data in the context of DII and allergies, and the available data so far address mainly respiratory issues such as asthma or wheezing. For example, in children, a pro-inflammatory diet

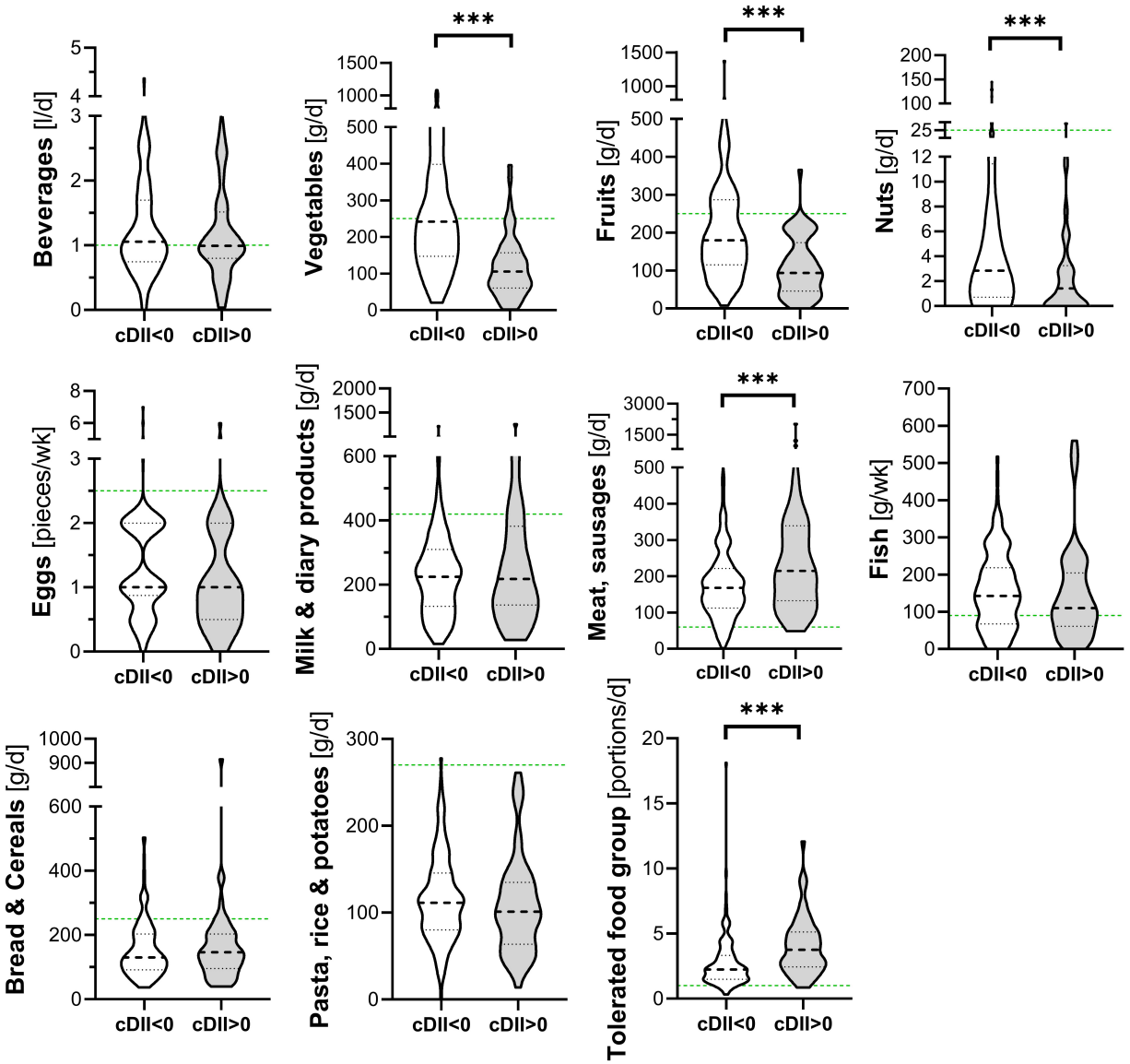


FIGURE 2 | Food groups and C-DII. Children's consumed food groups at the age of 10 years according to having a pro (C-DII >0) or anti-inflammatory (C-DII <0) diet at the age of 10 years. Green line: OptiMix recommendations for 10–12 year old children (26). Data are presented as violin plots with median (bold dotted line) and 25 to 75th percentile (dotted line), $n = 211$. ***Significant difference ($p < 0.05$) by Mann–Whitney *U*-test.

was not associated with current asthma or lung function, but in children with allergic airway inflammation, a higher DII score was associated with a 2.38 fold higher risk of wheezing (43). In addition, a pro-inflammatory diet was associated with asthma (20). Further, it was shown that higher inflammatory potential of the maternal diet was associated with increased odds of offspring asthma and/or wheeze by age 4 years, although results attenuated into non-significance after adjustment for confounders (44).

One strength of our study lies in the well-characterized participants regarding longitudinal atopic outcomes and exposure variable assessment, including diet. Therefore, a possible link between children's dietary intake of specific nutrients or specific indices such as the C-DII and allergy development could be investigated. The use of an index such as the C-DII offers an insight into the total dietary pattern compared to interpreting singular effects of specific nutrients. A limitation of the LiNA study in general is the potential bias by high rates of participating atopic parents (64.7%), limiting our ability to extrapolate findings to the general population (with approximately 30% prevalence of atopic outcomes). This fact is accumulating even more throughout the 10-year follow up, in detail 76% of the children positive for AD within our analyzed sub-cohort show a positive family history of atopy. This shift was also seen in the high rates of increased IgE levels in children negative for atopic dermatitis or food allergy. One further limitation of the study is the low number of cases in certain

outcomes, in particular when analyses are stratified for sex, which limited the power of the results. Furthermore, outcome data were obtained, in part, from parental questionnaire documented physician diagnosis of outcomes. This might reduce the strengths of the reported results. However, by including clinical allergy markers such as the IgE data we may overcome this limitation, at least in part. Further, the high rates of increased IgE levels at the age of 10 years in children positive of atopic dermatitis or food allergy at least once during their first 10 years outlines that these children have a persistent atopic phenotype also at the age of 10 years. Another major limitation is the missing questionnaire information on children's physical activity in general and in their leisure time in particular. Therefore, we probably have underestimated the energy expenditure in several children. However, our data are very similar to the study protocol from Eskimo (30) who also reported this limitation.

CONCLUSION

Children with atopic dermatitis/food allergy within their first 10 years of life were more likely to show a more pro-inflammatory dietary pattern assessed at the age of 10 years *via* the C-DII compared to children without allergic diseases. Because of their allergy history, these children may have developed a more pro-inflammatory dietary pattern due to avoidance of possible allergy triggers such as fruits or nuts for example. Overall, a pro-inflammatory dietary pattern might worsen the atopic outcome itself and reduce the buffering capacity of the individual against harmful environmental exposures or triggers. For pediatricians it is recommended to test affected children for their individual tolerance of allergenic foods to avoid a restrict elimination diet. Furthermore, an increased nutrient density of tolerable food items should be advised to omit undesirable effects of eating a pro-inflammatory diet.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because longitudinal LiNA datasets are not anonymized. Therefore, the raw cohort data cannot be provided as an open source file due to ethical declaration/data protection issues. Data can be requested in their analysed version from the corresponding author. Requests to access the datasets should be directed to KJ, kristin.junge@ufz.de.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of the University of Leipzig and the Saxonian Board of Physicians (046-2006, 160-2008, 160b/2008, 144-10-31052010, 113-11-18042011, 206-12-02072012, 169/13-ff, 150/14-ff, EK-allg-28/14-1, and 008/17-ek). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

KJ: conceptualization and project administration. OS, LB, NS, JH, and JF: methodology. OS, LB, and NS: software. OS, KJ, and SR: validation. OS and KJ: formal analysis, visualization, and writing – original draft preparation. AZ and GH: resources. MB, US, and WK: clinical resources. SR: data curation. GS, GH, JF, JH, AZ, OS, LB, SR, MB, US, NS, and WK: writing – review and editing. KJ and GS: supervision. GH: cohort PI. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.868872/full#supplementary-material

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Conflict of Interest: JH owns controlling interest in Connecting Health Innovations LLC (CHI), a company that has licensed the right to his invention of the dietary inflammatory index (DII®) from the University of South Carolina in order to develop computer and smart phone applications for patient counseling and dietary intervention in clinical settings. NS is an employee of CHI.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Association Between Dietary Fatty Acid Pattern and Risk of Oral Cancer

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Objective: To investigate the association between dietary fatty acid (FA) patterns and the risk of oral cancer.

Method: A case-control study which included 446 patients with oral cancer and 448 controls subjects was conducted in Southeast China. A structured food frequency questionnaire was used to assess the dietary FA consumption before cancer diagnosis. FA patterns were identified using the principal component analysis, and the relationship between the dietary FA patterns and oral cancer was analyzed by logistic regression.

Results: General differences in FA intake were observed between the patient and control groups. The intakes of saturated FAs (SFAs) C14:0, C16:0, C18:0, and monounsaturated FA C18:1 were higher in the patient group than the control group ($p < 0.001$). Four FA patterns were derived by principal component analysis. The "SFA" pattern, "Polyunsaturated FA" pattern, "Monounsaturated FA" pattern, and "Medium- and long-chain FA" pattern, which could explain 75.7% of the variance of the dietary FA intake, were submitted to logistic regression analysis. A positive association was observed between the "SFA" pattern and oral cancer risk. Compared with the lowest quartile score, the *OR* of the highest quartile score was 3.71 (95%*CI*: 2.31, 5.94, $P_{trend} < 0.001$) in the multivariate logistic regression model. No significant association was found among the other three patterns and oral cancer risk.

Conclusions: General differences in dietary FA intake were observed between patients with oral cancer and controls. A positive association between the "SFA" pattern and risk of oral cancer was observed after adjusting for potential confounders.

Keywords: fatty acid pattern, saturated fatty acids, oral cancer, principal component analysis, case-control study

INTRODUCTION

Oral cancer is one of the foremost cancers in head and neck cancers with nearly 40,000 new cases recognized in China in 2015 (1). According to GLOBOCAN 2018, the incidence and mortality of oral cancer in China were 2.0/100,000 and 0.97/100,000, respectively, in 2018 (2). The recognized etiologic factors of oral cancer consist of smoking, drinking, oral hygiene, human papillomavirus (HPV), and betel quid consumption (3–9). In addition to the above-mentioned traditional risk factors, diet is also involved in the etiology of oral cancer (10–12). Additionally, the potential role of fatty acids (FAs) in tumorigenesis has got increased interest.

Fatty acids, including saturated FA (SFA), n-3 and n-6 polyunsaturated FA (PUFA), and trans fatty acid (TFA), have been reported to be associated with the risk of varied types of cancer such as prostate cancer (13, 14), pancreatic cancer (15, 16), colorectal cancer (17, 18), and lung cancer (19). However, reports about the association between FA and head and neck tumors, especially oral cancer, are rare.

Most of the previous studies have taken individual FAs as separate exposures. However, individual FAs were consumed together and tended to be correlated with each other and to be interactive or synergistic, partially due to shared food sources and metabolic pathways (20, 21). Because of the complexity of diet and the highly interrelated nature of dietary exposures, FA pattern analysis could instead offer a more comprehensive view of separate FAs and shed light on the biological interactions between different FAs and their relation with disease risk (22–24).

Due to the limited evidence of the role of FA in oral cancer, we performed a case-control study to explore the potential FA intake patterns in oral cancer and their role in the development of oral cancer.

MATERIALS AND METHODS

Study Design and Population

In this case-control study from September, 2016, to July, 2020, 446 newly diagnosed patients with oral cancer and 448 control participants were recruited from the First Affiliated Hospital of Fujian Medical University in Fujian province, China. Cancers of the lip, oral cavities, and parotid corresponded to codes C00 to C07 according to the 10th revision of the International Classification of Diseases (ICD-10) (25) were referred to as oral cancer in this study. The inclusion criteria of the patients were as follows: (1) histologically confirmed primary oral cancer; (2) Chinese Han population and residence in Fujian Province; (3) age above 18 years old. Patients with second primary, recurrent, or metastasized cancer, and previous radiotherapy or chemotherapy were excluded. Control participants were recruited from the health examination center of the same hospital during the same period. Those with a history of cancer were excluded. Additionally, we excluded those with extreme daily caloric intake (>4,200 or <700 kcal/day for men; >3,500 or <500 kcal/day for women).

All participants provided signed informed consent. The study protocol was approved by the Institutional Review Board of Fujian Medical University (Approval number: 2011053; Approval date: March 10, 2011) and conducted following the ethical standards described in the Declaration of Helsinki.

TABLE 1 | Characteristics of the case ($n = 446$) and control ($n = 448$) group.

Variable	Case	Control	*P*
Age			**<0.001**
<49	94 (21.1%)	210 (46.9%)	
≥49	352 (78.9%)	238 (53.1%)	
Sex			**0.002**
Male	258 (57.8%)	213 (47.5%)	
Female	188 (42.2%)	235 (52.2%)	
Education			**<0.001**
Low	77 (17.3%)	204 (45.5%)	
High	369 (82.7%)	244 (54.5%)	
Marital status			0.699
Married	408 (91.5%)	413 (92.2%)	
Others	38 (8.5%)	35 (7.8%)	
BMI			**0.024**
<18.5	39 (8.7%)	19 (4.2%)	
18.5∼	284 (63.7%)	297 (66.3%)	
≥24	123 (27.6%)	132 (29.5%)	
Residence			**0.008**
Rural areas	258 (57.8%)	298 (66.5%)	
Urban areas	188 (42.2%)	150 (33.5%)	
Occupation			0.231
Farmer and worker	148 (33.2%)	132 (295%)	
Others	298 (66.8%)	316 (66.8%)	
Tobacco smoking			**0.001**
No	259 (58.1%)	307 (68.5%)	
Yes	187 (41.9%)	141 (31.5%)	
Alcohol drinking			**<0.001**
No	294 (65.9%)	349 (77.9%)	
Yes	152 (34.1%)	99 (22.1%)	
Family history of tumor			**<0.001**
No	374 (83.9%)	413 (92.2%)	
Yes	72 (16.1%)	35 (7.8%)	
Oral hygiene score			**<0.001**
0–2	79 (17.7%)	167 (37.3%)	
3–5	257 (57.6%)	248 (55.4%)	
6–8	110 (24.7%)	33 (7.4%)	

Data Collection

A structured questionnaire was used to collect information through face-to-face interviews conducted by well-trained interviewers. The questionnaire included socio-demographic characteristics (age, sex, education, marital status, residence, occupation, and family history of cancers) and lifestyle indicators (tobacco smoking, alcohol drinking, and oral hygiene). Subjects who had smoked at least 100 cigarettes during their lifetime were considered tobacco smokers. Alcohol drinker was defined as consuming at least one drink per week and lasting for more than 6 months continuously (26). A complete description

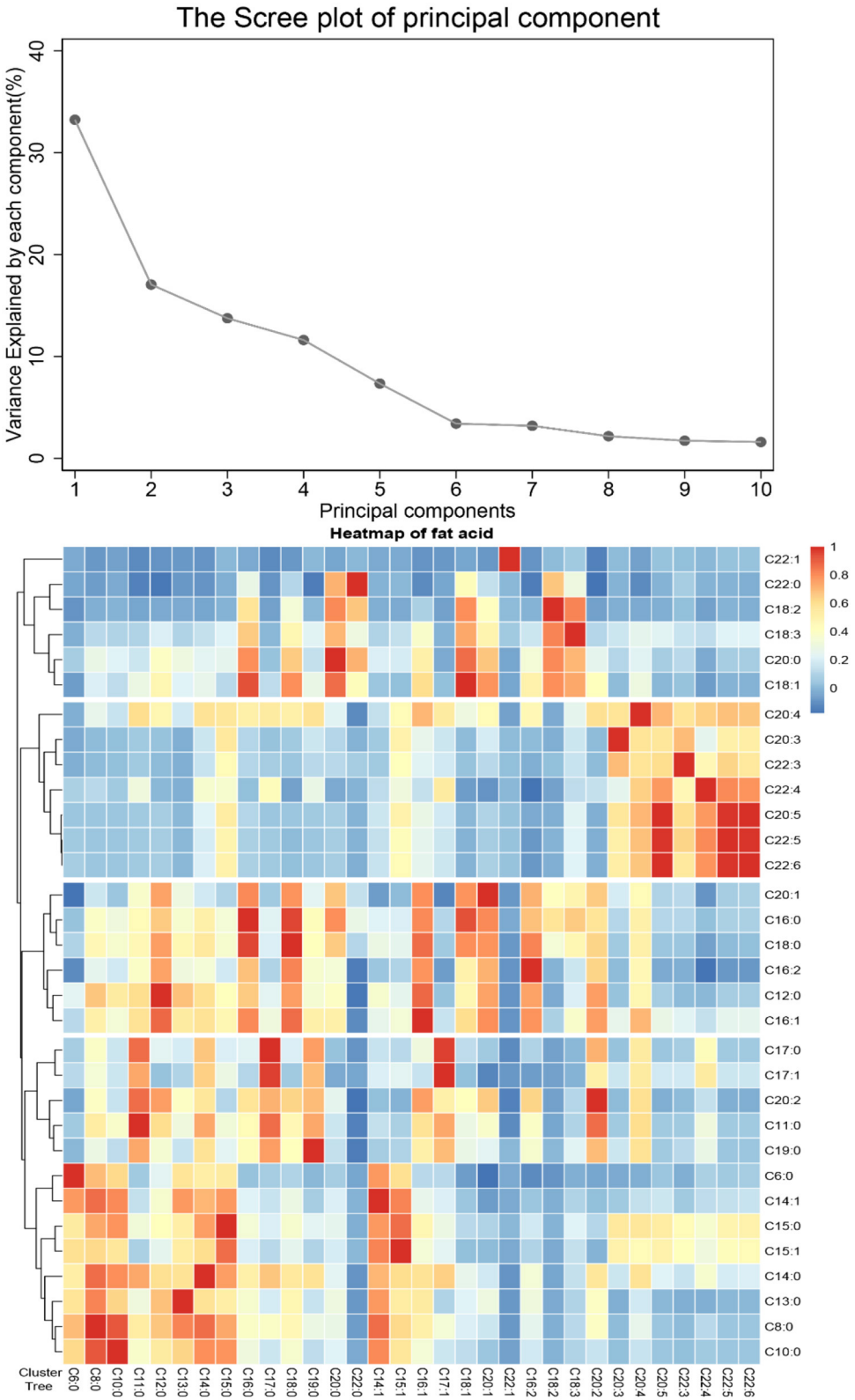

FIGURE 1 | Principal components and clusters of 32 fatty acids. **(A)** The proportion of total variance of 32 fatty acids explained by each principal component. **(B)** Association among 32 fatty acids, the hierarchical cluster tree on the left, and the heatmap of fatty acid on the right.

TABLE 2 | Factor-loading matrix for four fatty acid patterns.

Fatty acids	Name	Fatty acid patterns*			
		"SFA" pattern	"PUFA" pattern	"MUFA" pattern	"MLC-FA" pattern
Saturated fatty acids					
C6:0	Caproic			−0.538	
C8:0	Caprylic	**0.731**			
C10:0	Capric	**0.596**			
C11:0	Undecanoic	**0.702**			
C12:0	Lauric	**0.783**			
C13:0	Tridecanoic	**0.618**			
C14:0	Myristic	**0.848**			
C15:0	Pentadecanoic	**0.720**			
C16:0	Palmitic	**0.787**			
C17:0	Heptadecanoic				**−0.642**
C18:0	Stearic	**0.792**			
C19:0	Non-adecanoic	**0.586**			
C20:0	Arachidic	**0.559**			
C22:0	Behenic				**0.410**
Monounsaturated fatty acids					
C14:1	Myristoleic				**0.519**
C15:1	Pentadecanoic			−0.132	
C16:1	Palmitoleic	0.871			
C17:1	Heptadecenoic				**−0.549**
C18:1	Oleic			**0.355**	
C20:1	Eicosenoic			**0.337**	
C22:1	Erucic			**0.088**	
Polyunsaturated fatty acids					
C16:2	Hexadecatrienoic	0.577			
C18:2	Linoleic		−0.486		
C18:3	Octadecadienoic			0.477	
C20:2	Eicosadienoic	0.695			**−0.592**
C20:3	Eicosatrienoic		**0.433**		
C20:4	Arachidonic	0.769			
C20:5	Eicosapentaenoic		**0.658**		
C22:3	Docosatrienoic		**0.474**		
C22:4	Docosatetraenoic		**0.651**		
C22:5	Docosapentaenoic		**0.658**		
C22:6	Docosahexaenoic		**0.654**		

Four principal components explained 75.7% of the variation in all 32 fatty acids.

of the oral hygiene score is available in our previous study (3). Oral hygiene score = teeth brushing + the number of missing teeth + wearing dentures + regular dental visits + recurrent dental ulceration. The range of oral hygiene score was 0–8, and a higher score indicated worse oral hygiene. Detailed coding information of variables included in the analysis was as follows: age (<49 years/≥49 years, based on the median of controls), sex (male/female), marital status (married/others), residence (rural areas/urban areas), occupation (farmer and worker/others), tobacco smoking (no/yes), alcohol drinking (no/yes), oral hygiene (0–2/3–5/6–8), and family history of cancer (no/yes). Educational was defined as low (lower vocational training or primary school), or high (secondary school and above) level groups. Height and weight were measured by the nurse of the hospital. The body mass index (BMI) was calculated as weight (in kilograms) divided by the square of the height (in meters) and was classified into three categories (<18.5/18.5–23.9/≥24).

A validated food frequency questionnaire (FFQ) (27) was utilized to collect the habitual dietary intake from each participant. The dietary intake of the year before the interview was collected. The dietary items were grouped into 8 broad categories (grains; beans and soy products; vegetables; fruits; animal food; algal fungi and nuts; beverages and soup; fried foods and pickled foods) and 17 sub-categories (grains; beans and soy products; dark vegetables; light color vegetables; purple

TABLE 3 | Association between fatty acid patterns and oral cancer risk.

Model[#]	Quartiles of the fatty acid pattern score*				P_{trend}
	I	II	III	IV	
"SFA" pattern					
Case/control (*n*)	138/86	139/84	99/125	72/151	
Crude	1.0 (reference)	0.97 (0.66, 1.42)	2.06 (1.39, 2.95)	3.36 (2.28, 4.96)	<0.001
Model 1	1.0 (reference)	0.93 (0.60, 1.43)	2.24 (1.46, 3.44)	3.00 (1.93, 4.68)	<0.001
Model 2	1.0 (reference)	1.07 (0.68–1.68)	2.56 (1.62, 4.02)	3.71 (2.31, 5.94)	<0.001
"PUFA" pattern					
Case/control (*n*)	116/107	137/87	111/113	84/139	
Crude	1.0 (reference)	0.68 (0.47, 1.00)	1.10 (0.76, 1.59)	1.79 (1.23.2.62)	<0.001
Model 1	1.0 (reference)	0.58 (0.38, 0.89)	0.99 (0.65, 1.15)	1.59 (1.04, 2.44)	0.006
Model 2	1.0 (reference)	0.55 (0.35, 0.85)	0.92 (0.59, 1.42)	1.38 (0.88, 2.16)	0.038
"MUFA" pattern					
Case/control (*n*)	101/123	125/98	119/105	103/120	
Crude	1.0 (reference)	0.64 (0.44, 0.94)	0.73 (0.50, 1.05)	0.96 (0.66, 1.39)	0.980
Model 1	1.0 (reference)	0.67 (0.44, 1.03)	0.75 (0.49, 1.14)	1.03 (0.67, 1.56)	0.762
Model 2	1.0 (reference)	0.68 (0.44, 1.06)	0.78 (0.50, 1.20)	1.15 (0.74, 1.78)	0.441
"MLC-FA" pattern					
Case/control (*n*)	111/113	117/106	123/101	97/126	
Crude	1.0 (reference)	0.89 (0.61, 1.29)	0.81 (0.56, 1.17)	1.28 (0.88, 1.85)	0.290
Model 1	1.0 (reference)	0.69 (0.45, 1.05)	0.69 (0.45, 1.06)	0.99 (0.65, 1.52)	0.993
Model 2	1.0 (reference)	0.72 (0.46, 1.12)	0.69 (0.45, 1.08)	1.02 (0.66, 1.58)	0.928

[*]Four categories were obtained by quartiles of the fatty acid pattern scores. Each participant was assigned a fatty acid pattern score for each pattern.
[#]Mode l adjusted for demographic characteristics including sex, age, marital status, residence, BMI, family history of tumor, occupation, education.
Model 2 adjusted for demographic characteristics and tobacco smoking, drinking, oral hygiene score.

vegetables; fresh beans; fruits; livestock; poultry; fish; processed meat; red meat; eggs; dairy; algal fungi and nuts; fried foods; pickled foods). For each food item or food group, participants were asked how frequently (daily, weekly, monthly, yearly, or never) they consumed the food or food group, which was followed by a question on the amount consumed in lians per unit of time. Lian is a unit of weight in China (1 lian = 50 g). The Chinese Food Composition Tables (28) were used to estimate the intake levels of macronutrients and FAs for participants.

Statistical Analysis

The intakes of energy and nutrients were log transformed and then FA intakes were adjusted for total energy intake using the residuals method (29). The quantitative data were presented as median with inter-quartile range, while the qualitative variables were presented as frequency (numbers and percentages). The chi-square test was used to compare the main characteristics between patients and controls. The Wilcoxon rank-sum test was used to analyze the distribution of dietary FAs. The Pearson correlation coefficients were calculated, and the hierarchical cluster tree and heatmap were generated to visualize the correlation between FAs (30). Hierarchical cluster analysis was performed using the Ward's method on correlation coefficient using the pheatmap package in R software.

Fatty acid patterns were derived by principal component analysis (PCA) using the intake of 32 FAs and PCs identified were referred to as FA patterns. The correlation pattern matrix from PCA was then used to calculate the scores of each pattern which were then categorized into quartiles, and the lowest quartiles were used as reference. The FA pattern score was evaluated categorically in the logistic regression model, and the *OR*s and their 95% *CI*s were calculated. Associations between FA pattern and intakes of 17 food groups and macronutrients were assessed by the Spearman correlation analysis. In addition, the restricted cubic spline (RCS) was used to plot and investigate the possible non-linear association between FA pattern and oral cancer risk.

All analyses were performed using the R software (version 4.0.3), with 2-tailed p-values <0.05 considered statistically significant.

RESULTS

Characteristics of the Study Population

The distributions of the demographical characteristics and lifestyle factors are shown in **Table 1**. Compared with the patient group, the case group was characterized by a higher proportion of subjects with tobacco abuse, alcohol consumption, tumor history, and worse oral hygiene. In addition, the distribution of gender, education levels, BMI, and residence was significantly different between the patient and control groups ($p < 0.05$). General differences in FA intake were observed between the patient and control groups. The intake of saturated FAs C14:0, C16:0, C18:0, and monounsaturated FA C18:1 were higher in the patient

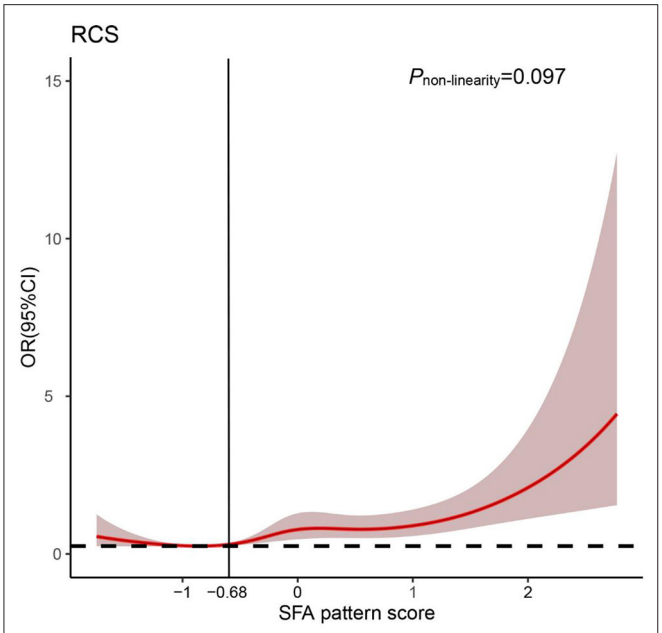

FIGURE 2 | Odds ratio (*OR*) based on saturated fatty acid "(SFA) pattern" score and restricted cubic spline ($P_{non-linearity} = 0.097$). OR was adjusted for the same variates as in model 2.

group than the control group ($p < 0.001$). The distribution of dietary FAs between the case and control groups are shown in **Supplementary Figure 1**.

Identification of FA Patterns

Four FA patterns were identified by applying PCA which could explain 75.7% of the variance of the dietary FA consumption, as the scree plot shown in **Figure 1**. Pattern 1 was characterized with saturated FA (the "SFA" pattern), which mainly included octanoic acid (C8:0), undecanoic acid (C11:0), lauric acid (C12:0), myristic acid (C14:0), and pentadecanoic acid (C15:0). Pattern 2 (the "PUFA" pattern) had high factor loading of eicosatrienoic acid (C20:3), eicosapentaenoic acid (C20:5), docosatrienoic acid (C22:3), docosatetraenoic acid (C22:4), docosapentaenoic acid (C22:5), and docosahexaenoic acid (C22:6). Pattern 3 (the "MUFA" pattern) was characterized with oleic acid (C18:1), eicosenoic acid (C20:1), and erucic acid (C22:1). Pattern 4 [the "medium- and long-chain FA (MLC-FA)" pattern] was dominated by heptadecanoic acid (C17:0), behenic acid (C20:0), myristoleic acid (C14:1), heptadecenoic acid (C17:1), and eicosadicnoic acid (C20:2). The factor loadings of individual FAs in the four FA patterns are shown in **Table 2**. Additionally, a correlation analysis among individual FAs was performed, and a heatmap was derived using correlation coefficients among individual FAs. A similar pattern was identified in the cluster analysis, as FAs adjacent in the tree had similar loading values (**Figure 1**).

Association Between FA Patterns and Oral Cancer Risk

Crude and multivariable-adjusted *OR* and 95% *CI* for oral cancer across quartile categories of dietary FA pattern scores are shown in **Table 3**. A positive association between the "SFA" pattern and the risk of oral cancer was observed. In the crude model, those in the highest quartile of the "SFA" pattern had an increased risk of oral cancer compared with the lowest quartile, with a statistically significant linear trend ($OR = 3.36$; 95% *CI*: 2.28–4.96; $P_{trend} < 0.001$). In model 1, after adjusting for sex, age, marital status, education levels, residence, BMI, occupation, and family history of tumor, the individuals in the highest quartile of the "SFA" pattern tended to have higher oral cancer risk ($OR = 3.00$; 95%*CI*: 1.93–4.68; $P_{trend} < 0.001$) compared with those in the lowest quartile. In model 2, the result remained statistically significant after further adjustment for lifestyle factors, including tobacco smoking, alcohol drinking, and oral hygiene score ($OR = 3.71$; 95% *CI*: 2.31–5.94; $P_{trend} < 0.001$). Compared with the lowest quartile, the *OR*s of the second quartile of the "PUFA" pattern were 0.58 (95% *CI*: 0.38–0.89) and 0.55 (95% *CI*: 0.35–0.85) in the crude model and model 2. Additionally, the *OR*s of the highest quartile of the "PUFA" pattern were 1.79 (95% *CI*: 1.23–2.62) and 1.59 (95% *CI*: 1.04–2.44) compared to the lowest quartile in the crude model and model 1. However, the result showed no statistical significance after further adjustment in model 3 ($OR = 1.38$; 95% *CI*: 0.88–2.16). Neither the "MUFA" nor the "MLC-FA" pattern was observed to be associated with oral cancer in all the three models ($P > 0.05$).

Additionally, we evaluated the correlations between the "SFA" pattern with intakes of macronutrients and food groups, the result of which is shown in **Supplement Table 1**. The "SFA" pattern was positively associated with the intake of protein, total fat ($r = 0.207$, 0.368, respectively, all $p < 0.001$), but negatively related to fiber ($r = -0.185$, $P < 0.001$). As for food groups, the "SFA" pattern was positively correlated with the intakes of fish, eggs, dairy, and red meat ($r = 0.372$, 0.320, 0.283, 0.282, respectively, all $p < 0.05$), but negatively correlated with grain and vegetables ($r = -0.403$, -0.100, respectively, all $p < 0.05$).

Furthermore, we visualized the association between the "SFA" pattern score and the risk of oral cancer using restricted cubic splines. Generally, the risk of oral cancer increased with the increase of the "SFA" pattern score and there is no evidence of non-linear association between the score and oral cancer risk ($P_{non-linearity} = 0.097$). However, the risk of oral cancer was relatively flat until around −0.68 of the "SFA" pattern scores and then started to increase rapidly afterward (**Figure 2**).

Association Between "SFA" Pattern and Oral Cancer Risk by Stratification Analysis

The association between the "SFA" pattern and oral cancer risk was stratified by the demographic characteristics and lifestyle factors, the result of which is shown in **Figure 3**. A positive association between oral cancer risk and the "SFA" pattern was observed in all subgroups except for the lower oral hygiene score group. No effect modification was observed by sex, tobacco

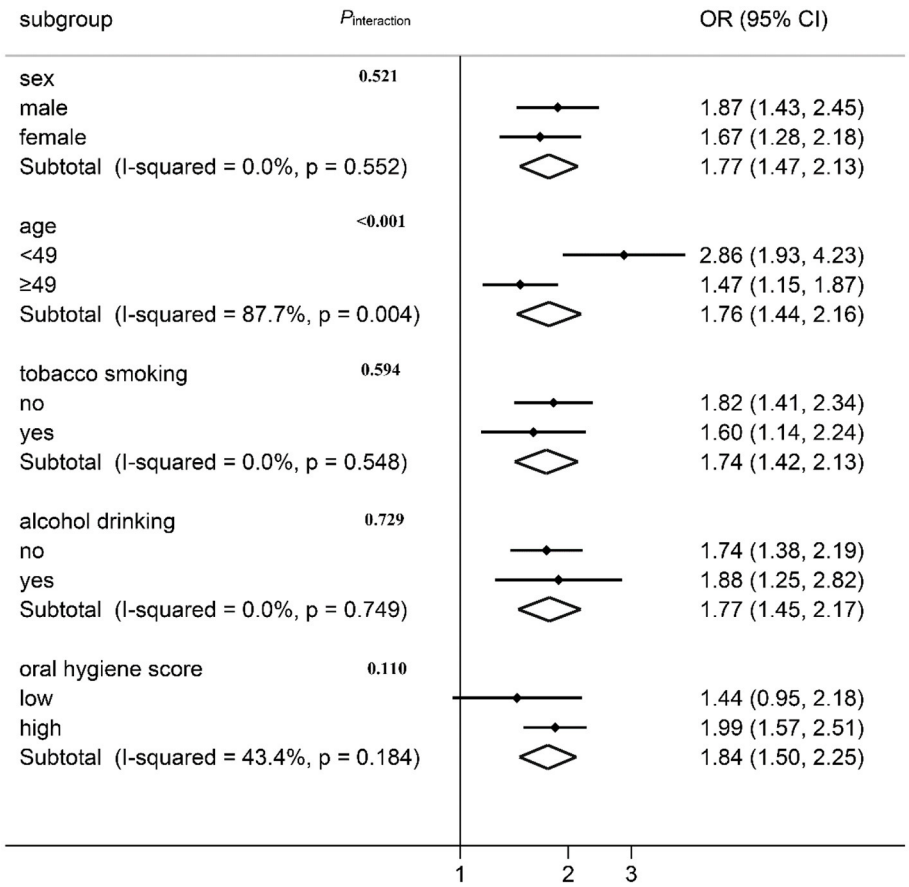

FIGURE 3 | Association between "SFA" pattern and oral cancer risk by stratification analysis.

smoking, alcohol drinking, or oral hygiene score ($P_{heterogeneity} > 0.05$). The association varied across different age groups (**Figure 3**; $I^2 = 87.8\%$, $P_{heterogeneity} = 0.004$). The interaction was further tested by multiplying the variates of "SFA" pattern score with age in the logistic regression model, and a multiplicative interaction was observed ($P_{interaction} < 0.001$).

DISCUSSION

In this case-control study conducted in Southeast China, we observed that the intake of FAs varied between patients with oral cancer and healthy controls. Four FA patterns, the "SFA" pattern, "PUFA" pattern, "MUFA" pattern, and "MLC-FA" pattern, were derived by PCA. The "SFA" pattern was found to be positively associated with oral cancer risk while no statistically significant association was found between the other three patterns and disease risk.

Dietary FAs, especially saturated FAs, have been hypothesized to increase cancer risk. Kim et al. performed a cross-sectional study, in which the results showed that the risk of colorectal cancer increased with higher SFA intake in Korean adults (31). Several epidemiological studies discovered that increased consumption of SFA correlated with increased odds of prostate cancer and may also be directly associated with the risk of biochemical recurrence and cancer progression (30, 32, 33). However, there is also evidence supporting that dietary SFA is not associated with cancer risk or even negatively associated with cancer risk. Cao et al. performed a meta-analysis of prospective cohort studies, in which the results showed that the highest vs. lowest levels of dietary SFA were not associated with the risk of breast cancer (34). A meta-analysis of prospective cohort research shows a null association between the SFA intake and colon cancer risk (35). No associations were observed in the Nurses' Health Study cohort of dietary SFAs and epithelial ovarian cancer risk (36). In the European Prospective Investigation into Cancer and Nutrition (EPIC), Aglago et al. (17) found an inverse association between dietary total SFA and colorectal cancer. To the best of our knowledge, reports of the association between dietary SFA and oral cancer are rare. A FA pattern characterized by SFA was identified in our study and was found to be positively associated with oral cancer risk. The inconsistent findings across studies may be partly due to differences in the type of cancer, study design and population, sample size, and varied measuring of dietary intake and confounding elimination.

The mechanism concerning dietary SFA and risk of cancer had also been widely discussed. It was reported that SFA intake influenced the risk of oral cancer through several mechanisms including chronic inflammation, insulin resistance, and fatty acylation, which were all related to carcinogenesis. Firstly, dietary SFA, particularly lauric acid and palmitic acid, were capable of stimulating inflammatory response through the toll-like receptors 4 (TLR4) (37), which could be exacerbated by the production of reactive oxygen species (ROS) *in vivo* (38). Inflammation was a key cause of the development and progression of many chronic diseases, including cancer (39). Moreover, inflammatory cytokines such as tumor necrosis factor (TNF)-a, induced by SFA, may influence insulin sensitivity (40), which favored the establishment of a pro-tumorigenic environment (41). Fatty acylation was another potential carcinogenic mechanism of SFA. It was shown that an SFA-rich diet could lead to an increase of myristoylated Src kinase and Src-mediated oncogenic signaling which accelerated tumor progression (42).

Dietary intake of SFAs consists of both animal and plant origins. The association between dietary FAs and cancer risk may depend on types and food sources of FAs (43, 44). The "SFA" pattern identified in this study was verified by performing a correlation analysis between the "SFA" pattern score and intakes of nutrients and food groups. It was found that the intake of red meat and dairy products was significantly higher in individuals with higher "SFA" pattern scores, which was consistent with previous studies about relation between varied food components and oral cancer. A study from Italy suggested that animal-derived foods such as dairy products and red meat could increase the risk of oral cancer (45). Epidemiological evidence from Greece also indicated that meat products were positively associated with the risk of oral cancer (46). However, we did not observe significant food components of plant origin that were related to SFA intake. So, it was unclear whether the association between the "SFA" pattern and oral cancer was partially attributed to the origin of SFA intake. This remains unclear for now and warrants investigation.

Additionally, in stratification analysis, we found that the association between the "SFA" pattern and oral cancer risk varied with age. The "SFA" pattern was positively associated with oral cancer risk in both age groups, but the association was more significant in the age group younger than 49 years. Compared with MUFA and PUFA, SFA is more likely to come from red meat, processed meat, and dairy products. Red meat is a primary source of total SFA, which has been identified as a dietary risk factor closely associated with various cancers (47, 48). In addition to red meat, excessive intake of dairy products could also contribute to cancer risks (49). Therefore, the origin of SFAs may modulate the effect of SFAs on oral cancer risk. Actually, in this study, we found that the "SFA" pattern was more strongly associated with dairy products in the younger-age group than the older-age group (**Supplement Table 2**). The results indicate that younger-age groups may consume more saturated FAs from dairy products, such as cakes, cheese, and ice cream bars, which may be positively associated with the risk of oral cancer.

There were several limitations in this study. Firstly, the selection of controls was not well-matched with the case, which resulted in distribution differences between the case and control groups in characteristics such as sex, age, and education. This could imply a selection bias, even when these variables were adjusted in the models. Secondly, recall bias and measurement error in dietary assessment using FFQ could be hardly avoided in a case-control study. Lastly, this was a single-center study and the sample size was limited. A prospective study with a large-scale sample size is needed to verify the current findings

CONCLUSION

In conclusion, the study provides support for a possible positive relationship between the "SFA" pattern and the risk of oral cancer. In addition, potential interactions were found between "SFA" pattern and age in oral cancer risk. Our findings support previous findings that there is suggestive evidence of a link between dietary patterns with head and neck cancer, but go beyond this by highlighting the role of specific FA patterns in oral cancer susceptibility.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board (IRB) of Fujian Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YF, JW, QC, and FL conceptualized the original idea for the study and have been involved in data collection, data analysis, and manuscript drafting. YQ, LL, LP, and BS were involved in data and blood samples collection. SW, YWa, YL, YWe, and JQ carried out the initial analysis. FC and BH assisted with revisions. All authors have made substantial contributions to the conception and design of the study, read and approved the final manuscript, and agreed to be accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.864098/full#supplementary-material

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The Dietary Branched-Chain Amino Acids Transition and Risk of Type 2 Diabetes Among Chinese Adults From 1997 to 2015: Based on Seven Cross-Sectional Studies and a Prospective Cohort Study

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Background: The situation is grim for the prevention and control of type 2 diabetes (T2D) and prediabetes in China. Serum and dietary branched-chain amino acids (BCAAs) were risk factors for T2D. However, there is a lack of information on trends in consumption of BCAAs and the risk of T2D associated with BCAAs intake, based on nationally representative data in China. Thus, we aimed to comprehensively describe the dietary BCAAs transition and risk of T2D, at a national level among Chinese adults from 1997 to 2015.

Methods: The data sources were the China Health and Nutrition Survey (CHNS) and China Nutrition and Health Survey (CNHS). Cross-sectional data on intake were obtained from CHNS (1997, $n = 9{,}404$), CHNS (2000, $n = 10{,}291$), CHNS (2004, $n = 9{,}682$), CHNS (2006, $n = 9{,}553$), CHNS (2009, $n = 9{,}811$), CHNS (2011, $n = 12{,}686$) and CNHS (2015, $n = 71{,}695$). Prospective cohort data were obtained CHNS (1997–2015, $n = 15{,}508$).

Results: From 1997 to 2015, there was a significant decreasing trend in the BCAAs intake of Chinese adults in all subgroups ($P < 0.0001$) except for Leu in 80 or older, and a decreasing trend in the consumption of BCAAs after 40 years old ($P < 0.05$). The mean intake of BCAAs in the population of cohort study was 11.83 ± 3.77g/day. The 95% CI was above the HR of 1.0, when the consumptions were higher than 14.01, 3.75, 6.07, 4.21 g/day in BCAAs, Ile, Leu and Val, based on RCS curves. According to the Cox proportional hazards models, Compared with individuals with BCAAs consumption of 10.65–12.37 g/day, the multivariable-adjusted HR for diabetes was 2.26 (95% CI 1.45 to 3.51) for individuals with consumption of BCAAs more than 18.52 g/day. A statistically significant positive association between BCAAs intake and risk of T2D was observed in males or participants aged 45 years and older, but not in females or participants younger than 45 years.

Conclusion: Our results reveal a trend toward decreased BCAAs intake in Chinese from 1997 to 2015. After 40 years of age, consumption of BCAAs declined with increasing age. Higher BCAAs intake was associated with higher risk of T2D. This relationship is more stable among men and middle-aged and elderly people.

Keywords: nutritional epidemiology, branched chain amino acids, transition, nutrient effects, type 2 diabetes, risk analysis

INTRODUCTION

Branched-chain amino acids (BCAAs), including leucine (Leu), isoleucine (Ile), and valine (Val), are essential amino acids for mammals (1) and are supplied considerably from diet. Previous studies have shown that the main food sources of BCAAs in the US population were meat (37%), milk (12%), and fish (8%), while in the Japanese population the main contributors were cereals, potatoes and starches (23–25%), fish and shellfish (21–23%) and meat (14–15%) (2). BCAAs were critical components of dietary protein. Elevations in branched-chain amino acids (BCAAs) associated with numerous systemic diseases, including cancer, type 2 diabetes (T2D), and heart failure (3). Reports since the 1960's have noted that elevations in circulating BCAAs tightly associate with insulin resistance (4).

The prevalence of diabetes in China has increased dramatically in the past two decades (5, 6). Elevated plasma branched chain amino acids (BCAAs) has been implicated in development of insulin resistance and T2D. However, whether consumption of BCAAs contribute to the disease is controversial. Some studies have shown that high intake of BCAAs is associated with an increased risk of T2D (2, 7, 8) and may have adverse effects on the development of IR (9). On the contrary, a study from a Japanese population reported that high intake of BCAAs may be associated with reduced diabetes risk (10). Research in this area has remained relatively limited. Thus, the association between dietary BCAAs and the risk of T2D in Chinese adults is unclear. Also, the quantity of BCAAs intake causing risk of T2D is not clearly defined. It could have significant clinical and public health implications that finding out exact BCAAs consumption threshold values of developing diabetes.

In the past few decades, dietary structure and food intakes of Chinese have undergone substantial changes (11). However, there is a lack of information on trends in BCAAs consumption and the risk of T2D associated with BCAAs intake, based on nationally representative data. Using data from 1997 to 2015 China Health and Nutrition Survey (CHNS) and China Nutrition and Health Survey (CNHS), the current study was aimed to systematically describe the changes in dietary BCAAs intake in Chinese adults from 1997 to 2015 and the risk of T2D caused by BCAAs intake.

METHODS

Study Population

All datasets used in this study were from two independent national project, CHNS and CNHS. CHNS was an international collaborative project cohosted by the Carolina Population Center at the University of North Carolina at Chapel Hill and the National Institute for Nutrition and Health (NINH) at Chinese Center for Disease Control and Prevention (CCDC), which aimed to examine the effects of the healthand nutrition. CNHS was a national survey conducted by the CCDC to survey the national health and nutrition status. The sampling method, dietary survey method, anthropometric measurement method, and quality control method of CNHS are almost identical to those of CHNS in terms of cross-section. The provincial staff for both projects are the same team. The core structure of the two surveys is the same in terms of cross-section. Both projects used stratified, multistage, random cluster sampling method, and further detailed information could be referred elsewhere (12, 13).

In the dietary BCAA transition trend analysis, seven cross-sectional data were obtained from CHNS (1997), CHNS (2000), CHNS (2004), CHNS (2006), CHNS (2009), CHNS (2011) and CNHS (2015). Data were included for analysis if dietary intake records were available and the age of the study object was 18 years or older at the time of survey. And data of 9,404, 10,291, 9,682, 9,553, 9,811, 12,686 and 71,695 participants in 1997, 2000, 2004, 2006, 2009, 2011, and 2015 were used for analysis, respectively.

In the BCAAs risk analysis, prospective cohort data were extracted from CHNS (1997–2015). Participants diagnosed with diabetes at baseline, those aged <18 years, and those without dietary records were excluded for analysis, and 15,508 participants with 9.9 ± 5.6 (mean ± SD) follow-up years were finally included for analysis.

Dietary BCAA Intake Assessment

BCAAs intake were calculated from 24-h dietary recall records and household condiment weighing records for three consecutive days (2 working days and 1 weekend). All field staff are professionally trained nutritionists who work in nutrition in their own county. BCAAs intakes was estimated by multiplying the consumed grams of each food by the amino acid contents of each food (referred from Chinese Food Composition Tables) (14–16) before BCAAs intake for all food items was summed by individual.

In the BCAA risk analysis, dietary exposure to BCAAs were calculated by using the average BCAAs intake values in each record before the onset of diabetes.

Identification of the New-Onset Diabetes

Since 1997, participants have been asked to report their previous diabetes history in the form of questionnaire interviews at each follow-up. Three questions were used to identify the new onset diabetes in the CHNS project: (1) Have the doctor told you that

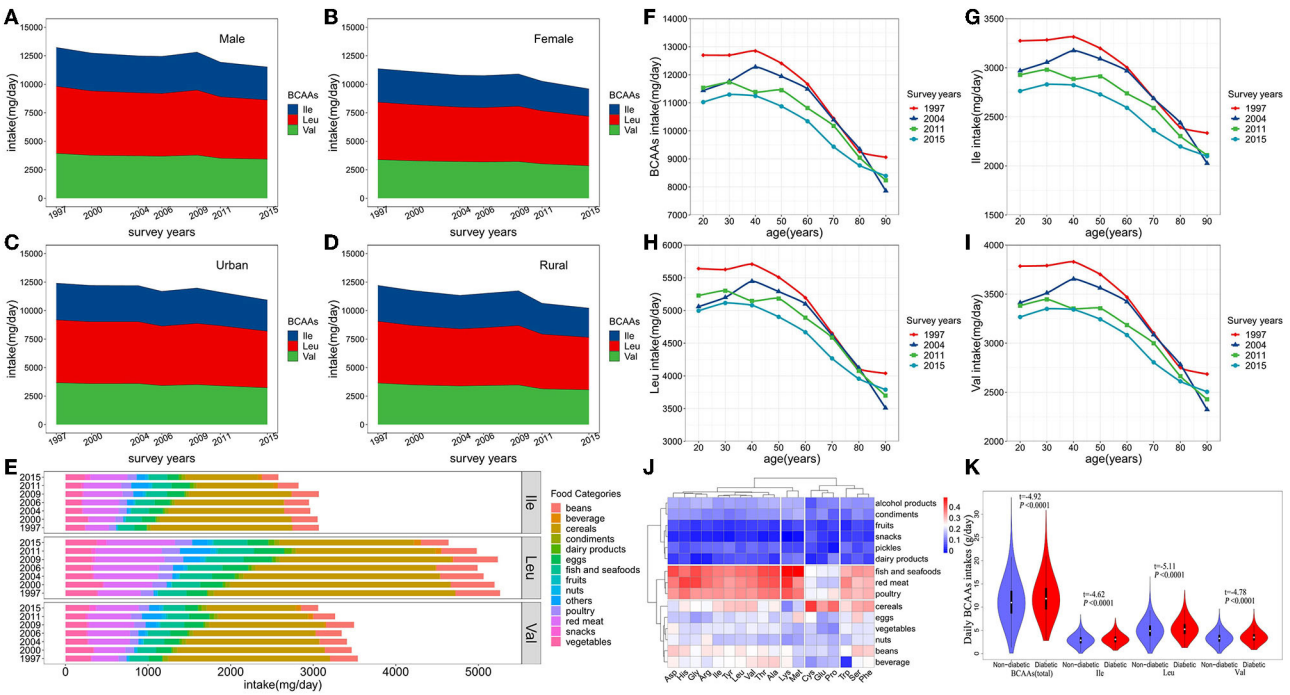

FIGURE 1 | (A–D) Trends in consumption of BCAAs and food sources from 1997 to 2015. *P* value for the trend, $P < 0.0001$ (Male, Female, Urban, Rural). **(E)** Food sources of dietary BCAAs. **(F–I)** Average daily BCAAs (Ile, Leu and Val) intakes in adults (aged 18–90 years) during 1997-2015. **(J)** Clustering and correlation heat map between dietary amino acids and food categories. **(K)** Comparison of BCAAs intakes between the diabetic onset group and non-onset group in the cohort.

you suffer from type 2 diabetes? (2) How old (age) were you when this happened? (3) Have you used the following treatment methods, such as special diet, weight control, oral medication, insulin injection, Chinese medicine, etc.? The diagnosis of T2D was based on patient-reported physicians' diagnoses and/or the presence of diabetes-specific medication.

Statistical Analysis

We provided the demographic characteristics of each survey year. We also calculated the mean (SD) of dietary BCAAs by sex, age group and urban/rural status. A generalized linear model was used to test trends for consumption of BCAAs from 1997 to 2015, adjusting for sex, age, BMI and region. Heatmaps were generated and clustered using hierarchical clustering. For the comparison between the two groups, *t*-test was applied in **Figure 1K**, and generalized linear model was used in **Table 4**.

Based on the Cox proportional hazard model, a restricted cubic spline (RCS) curve was used to assess the association between dietary BCAAs levels and T2D risk on a continuous scale. In the statistical analyses, we adjusted for age, sex, energy intake, BMI, region, smoking status (previous or present, never), alcohol consumption (yes, no), which were well known risk factors for diabetes. In the Cox proportional hazards models, participants with previously diagnosed diabetes, were excluded when first entry into the survey. To balance best fit and overfitting in the main splines for incident diabetes, the number of knots, between three and six, was chosen as the lowest value for the Akaike information criterion, but if within two of each other for different knots, the lowest number of knots was chosen (17). In the non-linearity test, $P < 0.1$ was considered statistically significant for data exploration and visualization. Otherwise, two-sided significance tests were used throughout, and a two-sided $P < 0.05$ was considered statistically significant. All analyses were conducted using SAS software, version 9.4 (SAS Institute, Cary, NC) and R software, version 4.1.2.

Patient and Public Involvement

Participants were not involved in setting the research question or the outcome measures, nor were they involved in the design or implementation of the study. No participants were asked to advise on interpretation or writing of the manuscript.

RESULTS

From 1997 to 2015, the number of participants in the survey increased from 9,404 to 71,695, and the proportion of the elderly and urban residents continued to increase, reflecting increasing trends of aging and urbanization in China (**Table 1**). From 1997 to 2015, there was a significant decreasing trend in the BCAAs intake of Chinese adults in all subgroups (including the type of BCAAs, age subgroups, sex and urbanization status) ($P < 0.0001$) except for Leu in 80 or older, and a decreasing trend in the consumption of BCAAs after 40 years old ($P < 0.05$) (**Table 2**; **Figure 1**). From 1997 to 2015, cereals

TABLE 1 | Sociodemographic distribution of participants in the 1997962015.

	1997	2000	2004	2006	2009	2011	2015
Total	9,404	10,291	9,682	9,553	9,811	12,686	71,695
Age group (years)							
18–34	3,339 (35.5)	3,019 (29.3)	2,142 (22.1)	1,780 (18.6)	1,692 (17.3)	2,090 (16.5)	8,695 (12.1)
35–49	3,010 (32.0)	3,636 (35.3)	3,237 (33.4)	3,156 (33.0)	3,122 (31.8)	3,967 (31.3)	18,782 (26.2)
50–64	1,949 (20.7)	2,319 (22.5)	2,782 (28.7)	2,989 (31.3)	3,208 (32.7)	4,278 (33.7)	27,697 (38.6)
65–79	956 (10.2)	1,157 (11.2)	1,333 (13.8)	1,412 (14.8)	1,518 (15.5)	2,004 (15.8)	14,739 (20.6)
80 or older	150 (1.6)	160 (1.6)	188 (1.9)	216 (2.3)	271 (2.8)	347 (2.7)	1,782 (2.5)
Sex (%)							
Male	4,562 (48.5)	4,980 (48.4)	4,614 (47.7)	4,538 (47.5)	4,676 (47.7)	5,933 (46.8)	34,140 (47.6)
Female	4,842 (51.5)	5,311 (51.6)	5,068 (52.3)	5,015 (52.5)	5,135 (52.3	6,753 (53.2)	37,555 (52.4)
Living area (%)							
Urban	2,971 (31.6)	3,256 (31.6)	3,007 (31.1)	2,984 (31.2)	3,082 (31.4)	5,281 (41.6)	29,145 (40.7)
Rural	6,433 (68.4)	7,035 (68.4)	6,675 (68.9)	6,569 (68.8)	6,729 (68.6)	7,405 (58.4)	42,550 (59.4)

continued to be the first primary source for dietary BCAA intake, but the proportion of its contribute decreased from 55.6% to 34.9%. Similarly, beans decreased from 10.1 to 7.2%. In contrast, the percent contribution of red meat increased from 9.5 to 17.5%. In addition, the contribution of fish and seafoods increased from 6.5 to 8.6%, and eggs increased from 4.7 to 5.4%.

As shown in **Figure 1J**, the types of food were clustered into three major groups. Fish and seafoods, red meat and poultry were clustered into one category. Cereals, eggs, vegetables, nuts, beans, and beverages were clustered into one category. Additionally, alcohol products, condiments, fruits, snacks, pickles and dairy products were clustered into one category. Dietary BCAAs (Leu, Ile, and Val) were clustered together with aspartate (Asp), histidine (His), glycine (Gly), arginine (Arg), threonine (Thr) and alanine (Ala). Furthermore, the top 4 types of food, exhibiting the strongest correlation with dietary BCAAs, were fish and seafoods, red meat, poultry and cereals.

At the endpoint of observation, mean BCAAs intake was higher in participants with new-onset diabetes onset than in non-diabetic participants ($t = -4.92$, $P < 0.0001$) (**Figure 1K**). The same phenomenon were also observed in Ile ($t = -4.62$, $P < 0.0001$), Leu ($t = -5.11$, $P < 0.0001$) and Val ($t = -4.78$, $P < 0.0001$).

The mean intake of BCAAs in the population of cohort study was 11.83 ± 3.77 g/day (**Table 3**). The impact of dietary BCAA intake on risk of T2D was shown in **Figure 2**. The consumption of BCAAs and risk of T2D was U-shape-associated and higher dietary BCAAs (≥14.01 g/day) increased the risk of T2D. When upon a closer look, higher intake of each BCAA also increased the risk of T2D (**Figure 2**). The 95% confidence interval (CI) was above the HR of 1.0, when the consumptions were higher than 14.01, 3.75, 6.07, 4.21 g/day in BCAAs, Ile, Leu and Val. Those with higher dietary BCAAs (Group B ≥ 14.01 vs. Group A < 14.01 g/day) also consumed more food in amounts (1616.96 ± 755.83 vs. 1244.92 ± 524.68 g/day, $P < 0.0001$) (**Table 4**). The average food intake of group A was 1244.92 (95% reference value 216.55 to 2273.29) g/day.

Compared with individuals with BCAAs consumption of 10.65–12.37 g/day, the multivariable-adjusted HR for diabetes was 2.26 (95% CI 1.45 to 3.51) for individuals with consumption of BCAAs more than 18.52 g/day (**Table 5**). The same trends were found in Ile and Leu, except for Val. The results were unaffected by multivariable adjustments in BCAAs, Ile and Leu.

Sensitivity Analyses

When fractional polynomials was applied, the U-shaped association between dietary BCAAs intake and T2D risk also exist, and the BCAAs consumption cut-off that increased the T2D risk was 18.52 g/day (**Table 5**). When further stratified by sex and age, the association between the two was unaltered in men or in participants aged 45 years and older. However, the association between BCAAs intake and risk of T2D diminished in females or in participants younger than 45 years (**Figures 2E–H**).

DISCUSSION

Using seven large-scale nationally representative survey data, a decreasing trend in dietary BCAAs intake was observed in the study population at all ages from 1997 to 2015. Consumption of BCAAs also declined as age increased for those aged 40 years older. In all food categories, the strongest correlations with BCAAs were with red meat, poultry, fish and seafoods. And the risk analysis showed that increased BCAAs intake was associated with an elevated risk of T2D. This association was more stable among men and among people with middle-age and elder. The people with risk of T2D accounted for about 23.86% of the total population due to BCAAs.

To the best of our knowledge, this is the largest study including the most recent national survey data to first address the dietary BCAA intake trend and its risk on T2D. The reliability of our result could be guaranteed by the strict quality control of the CHNS and CNHS, including standardized protocols, standardized data collection procedures and standardized training of the field working stuff. This study contributes to the

TABLE 2 | Trends in mean BCAAs intake among Chinese adults from 1997962015.

		1997	2000	2004	2006	2009	2011	2015	*P* for trend*	Δ
	BCAAs (mg/day)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**		
Total	Ile	3165.81 (1124.99)	3102.89 (1122.43)	3004.50 (1189.17)	3018.33 (1172.68)	3063.48 (1181.62)	2805.68 (1159.1)	2635.44 (3301.12)	<0.0001	530.37
	Leu	5449.48 (1995.36)	5277.97 (1947.35)	5140.51 (2076.7)	5115.99 (2036.53)	5261.45 (2065.60)	4995.45 (2110.14)	4746.61 (4820.84)	<0.0001	702.87
	Val	3657.37 (1282.00)	3520.52 (1242.34)	3456.76 (1344.52)	3427.45 (1305.43)	3489.38 (1313.68)	3248.66 (1314.82)	3128.64 (4268.14)	<0.0001	528.73
	BCAAs	12272.66 (4363.69)	11901.38 (4280.58)	11601.77 (4573.22)	11561.77 (4487.12)	11814.32 (4532.28)	11049.79 (4554.87)	10510.69 (12334.01)	<0.0001	1761.97
Age group (years)										
18-34	Ile	3277.85 (1127.07)	3174.12 (1128.90)	3015.09 (1157.40)	3054.45 (1149.93)	3206.70 (1228.80)	2965.23 (1196.04)	2812.25 (1441.48)	<0.0001	465.60
	Leu	5627.67 (2006.46)	5375.61 (1939.71)	5129.59 (2006.18)	5190.55 (1985.61)	5519.91 (2148.54)	5284.22 (2157.42)	5084.05 (2633.88)	<0.0001	543.62
	Val	3786.87 (1288.29)	3592.22 (1245.41)	3465.16 (1304.44)	3473.67 (1277.97)	3646.99 (1361.33)	3426.45 (1360.96)	3327.38 (1702.73)	<0.0001	459.49
	BCAAs	12692.39 (4379.5)	12141.95 (4283.15)	11609.85 (4430.17)	11718.68 (4383.86)	12373.61 (4712.2)	11675.9 (4684.62)	11223.68 (5755.53)	<0.0001	1468.71
35-49	Ile	3290.14 (1125.94)	3226.16 (1099.01)	3159.97 (1200.06)	3171.33 (1176.59)	3192.13 (1132.73)	2891.4 (1154.24)	2791.73 (1454.93)	<0.0001	498.41
	Leu	5659.13 (1990.15)	5489.12 (1906.01)	5415.63 (2096.72)	5366.09 (2035.04)	5470.76 (1985.76)	5149.94 (2105.13)	5026.23 (2643.82)	<0.0001	632.90
	Val	3800.32 (1280.56)	3663.71 (1214.83)	3637.73 (1353.41)	3593.17 (1310.39)	3630.29 (1258.27)	3348.39 (1306.01)	3306.51 (1714.99)	<0.0001	493.81
	BCAAs	12749.59 (4359.19)	12378.99 (4186.5)	12213.33 (4611.5)	12130.59 (4495.47)	12293.18 (4345.43)	11389.73 (4535.5)	11124.47 (5789.78)	<0.0001	1625.12
50-64	Ile	3081.67 (1089.61)	3070.33 (1119.17)	3026.88 (1197.89)	3066.46 (1156.92)	3102.08 (1188.87)	2811.08 (1140.81)	2651.93 (5023.03)	<0.0001	429.74
	Leu	5325.64 (1947.39)	5243.37 (1973.22)	5191.05 (2102.94)	5195.02 (2022.77)	5343.00 (2074.06)	5015.5 (2080.58)	4766.21 (7085.95)	<0.0001	559.43
	Val	3565.45 (1242.93)	3489.68 (1242.32)	3488.43 (1358.41)	3486.48 (1284.42)	3542.52 (1322.31)	3258.84 (1294.74)	3156.31 (6561.03)	<0.0001	409.14
	BCAAs	11972.77 (4237.81)	11803.38 (4299.01)	11706.36 (4620.93)	11747.96 (4433.15)	11987.6 (4554.36)	11085.43 (4485.39)	10574.45 (18615.04)	<0.0001	1398.32
65-79	Ile	2693.07 (1012.55)	2709.12 (1066.37)	2666.63 (1105.89)	2653.19 (1127.01)	2699.41 (1105.36)	2561.84 (1123.27)	2357.05 (1164.32)	<0.0001	336.02
	Leu	4656.38 (1810.65)	4617.87 (1857.61)	4558.38 (1935.65)	4506.80 (1974.96)	4621.23 (1936.37)	4536.59 (2048.59)	4254.54 (2153.32)	<0.0001	401.84
	Val	3103.42 (1139.38)	3074.82 (1183.03)	3056.87 (1248.90)	3014.44 (1260.65)	3073.02 (1229.80)	2965.56 (1271.8)	2798.81 (1353.3)	<0.0001	304.61
	BCAAs	10452.88 (3933.61)	10401.81 (4083.21)	10281.88 (4256.11)	10174.43 (4338.58)	10393.67 (4245.9)	10064 (4416.96)	9410.4 (4642)	<0.0001	1042.48
80 or older	Ile	2282.67 (900.67)	2276.84 (964.06)	2271.67 (1057.54)	2206.28 (988.86)	2269.76 (1035.85)	2206.43 (1006.53)	2171.86 (1166.13)	0.0001	110.81
	Leu	3939.90 (1536.34)	3912.45 (1682.44)	3907.61 (1833.79)	3735.89 (1733.01)	3857.34 (1755.69)	3892.75 (1822.17)	3918.36 (2169.47)	0.0979	21.54
	Val	2631.25 (987.66)	2583.53 (1058.60)	2611.61 (1189.12)	2508.16 (1097.33)	2585.18 (1146.85)	2546.96 (1138.18)	2582.16 (1358.49)	0.0304	49.09
	BCAAs	8853.82 (3397.75)	8772.81 (3688.53)	8790.9 (4055.09)	8450.34 (3805)	8712.28 (3920.74)	8646.13 (3944.96)	8672.37 (4668.85)	0.0174	181.45
Sex										
Male	Ile	3409.84 (1168.50)	3321.68 (1145.03)	3233.38 (1210.77)	3249.92 (1213.03)	3327.02 (1210.04)	3033.45 (1189.13)	2891.54 (4583.11)	<0.0001	518.30
	Leu	5880.03 (2082.90)	5648.08 (1986.64)	5534.46 (2133.91)	5505.95 (2113.17)	5706.89 (2121.96)	5393.28 (2159.4)	5195.51 (6539.97)	<0.0001	684.52
	Val	3943.19 (1332.62)	3768.90 (1267.69)	3721.41 (1374.79)	3690.08 (1350.08)	3785.64 (1342.90)	3509.78 (1345.81)	3431.11 (5964.2)	<0.0001	512.08
	BCAAs	13233.07 (4541.18)	12738.66 (4366.18)	12489.25 (4677.37)	12445.96 (4646.04)	12819.56 (4642.75)	11936.51 (4662.03)	11518.16 (17038.23)	<0.0001	1714.91
Female	Ile	2935.88 (1030.95)	2897.74 (1060.69)	2796.13 (1129.64)	2808.77 (1093.56)	2823.50 (1101.69)	2605.57 (1093.67)	2402.64 (1263.12)	<0.0001	533.24
	Leu	5043.83 (1818.40)	4930.93 (1843.63)	4781.85 (1955.57)	4763.11 (1896.92)	4855.83 (1925.45)	4645.92 (2001.83)	4338.53 (2266.43)	<0.0001	705.30
	Val	3388.08 (1170.32)	3287.61 (1171.29)	3215.81 (1269.37)	3189.80 (1215.85)	3219.60 (1225.85)	3019.24 (1242.58)	2853.68 (1510.68)	<0.0001	534.40
	BCAAs	11367.79 (3983.52)	11116.29 (4044.61)	10793.8 (4321.02)	10761.68 (4180.55)	10898.93 (4226.6)	10270.74 (4310.95)	9594.84 (4976.1)	<0.0001	1772.95
Region										
Urban	Ile	3218.94 (1116.88)	3184.62 (1104.34)	3158.1 (1227.17)	3046.42 (1137.22)	3103.34 (1164.94)	2938.09 (1193.25)	2738.25 (1387.03)	<0.0001	480.69
	Leu	5510.39 (1956.58)	5428.92 (1904.09)	5424.62 (2118.00)	5217.25 (1992.88)	5366.65 (2051.88)	5271.77 (2163.02)	4957.73 (2399.85)	<0.0001	552.66
	Val	3676.81 (1260.35)	3595.33 (1218.96)	3605.08 (1382.04)	3427.72 (1260.11)	3511.99 (1285.59)	3406.9 (1361.22)	3235.19 (1698.24)	<0.0001	441.62
	BCAAs	12406.13 (4306.65)	12208.87 (4205.73)	12187.8 (4699.57)	11691.39 (4365.89)	11981.98 (4477.58)	11616.77 (4693.54)	10931.17 (5348.8)	<0.0001	1474.96
Rural	Ile	3141.27 (1127.96)	3065.06 (1128.79)	2935.31 (1165.14)	3005.58 (1188.31)	3045.23 (1188.82)	2711.25 (1124.71)	2565.02 (4126.97)	<0.0001	576.25
	Leu	5421.35 (2012.54)	5208.11 (1963.25)	5012.52 (2045.13)	5069.99 (2054.55)	5213.27 (2070.22)	4798.38 (2049.12)	4602 (5929.87)	<0.0001	819.35
	Val	3648.4 (1291.88)	3485.90 (1251.59)	3389.94 (1321.95)	3427.32 (1325.60)	3479.02 (1326.32)	3135.8 (1268.79)	3055.66 (5357.86)	<0.0001	592.74
	BCAAs	12211.02 (4388.75)	11759.07 (4307.65)	11337.77 (4490.58)	11502.89 (4540.22)	11737.53 (4555.39)	10645.43 (4409.33)	10222.68 (15379.57)	<0.0001	1988.34

SD, standard deviation. Linear trends in the mean BCAAs intake from 1997 to 2015 were tested using generalized linear model adjusted for sex, age, BMI and region. Δ1997–2015.

TABLE 3 | Baseline characteristics of 15,508 individuals in the CHNS Study.

	Dietary BCAAs Centile (g/day)							
	1st-5th	**6th-20th**	**21st-40th**	**41st-60th**	**61st-80th**	**81st-95th**	**96th-100th**	**All**
No. of individuals	778 (5.0)	2,330 (15.0)	3,091 (19.9)	3,113 (20.1)	3,096 (20.0)	2,324 (15.0)	776 (5.0)	15,508
Women	547 (70.3)	1,587 (68.1)	1,949 (63.1)	1,624 (52.2)	1,346 (43.5)	836 (36.0)	268 (34.5)	8,157 (52.6)
Age	50.6 (17.3)	48.3 (16.4)	44 (15)	42.6 (14)	41.7 (13.6)	41 (13.2)	41.1 (14)	43.6 (14.9)
Smoker	174 (22.4)	543 (23.3)	803 (26.0)	960 (30.8)	1,115 (36.0)	942 (40.5)	327 (42.1)	4,864 (31.4)
Drinker	177 (22.8)	557 (23.9)	847 (27.4)	1,072 (34.4)	1,299 (42.0)	1,071 (46.1)	397 (51.2)	5,420 (34.9)
Height	157.5 (8.3)	158.3 (8.4)	159.6 (8.2)	161.1 (8.2)	162.4 (8)	163.9 (8.2)	165.7 (7.8)	161.1 (8.5)
Weight	57.3 (11.5)	57.3 (11.1)	58.4 (10.7)	59.3 (10.7)	60.5 (10.3)	62.6 (11.2)	64.7 (11.6)	59.7 (11.1)
BMI	23 (3.9)	22.8 (3.5)	22.9 (3.4)	22.8 (3.2)	22.9 (3.2)	23.3 (3.5)	23.5 (3.5)	22.9 (3.4)
Systolic blood pressure (mm Hg)	125.4 (20.3)	121.9 (19.4)	119.4 (18.1)	118.9 (16.9)	118.4 (15.4)	119.4 (15.7)	120.9 (15.3)	119.9 (17.3)
Diastolic blood pressure (mm Hg)	79.1 (11.6)	77.8 (11)	77.4 (11.1)	77.3 (10.7)	77.3 (10)	77.8 (10.3)	78.6 (10.9)	77.6 (10.7)
Triceps skin fold (mm)	16.5 (8.1)	15.5 (8)	15 (8.1)	14.5 (7.9)	14.5 (8)	15.3 (8.4)	16.6 (8.6)	15.1 (8.1)
Hip Circumference (cm)	92.7 (9)	92.5 (8.5)	92.8 (8.4)	92.7 (8.3)	93.1 (8)	93.9 (8.2)	94.8 (9.5)	93 (8.4)
Waist Circumference (cm)	80.6 (10.6)	79.5 (10.2)	79.3 (10.6)	79.3 (9.9)	79.6 (9.9)	81 (10.4)	82.6 (11)	79.9 (10.3)
Upper Arm Circumference (cm)	26.3 (4.5)	26.0 (4.3)	26.0 (3.9)	26.2 (4)	26.4 (4)	26.9 (4)	27.9 (5.9)	26.4 (4.2)
BCAAs intake(g/day)	5.14 (1.18)	7.81 (0.66)	9.75 (0.52)	11.49 (0.49)	13.37 (0.61)	16.08 (1.08)	21.44 (2.72)	11.83 (3.77)

Values are means (standard deviation) or number (%). BCAAs, Branched-Chain Amino Acids.

discovery of the relationship between dietary BCAAs and chronic diseases in the Chinese population.

The declines in BCAAs intake may well have contributed to the declining T2D morbidity. According to a recent study, the incidence of diabetes decreased from 2007 to 2017 in both men and women in China (18). And, the trend in consumption of BCAAs paralleled with the decreased trend of T2D incidence, which reduced by 14.36% from 1997 to 2015 in the adult population (**Table 2**). The declined BCAAs intake reflected changes of society and behavioral lifestyle in China. Accompanying with the decreasing BCAAs consumption, it was also observed that energy and protein intake decreased substantially from 1992 to 2012 among Chinese adults (11). One possible reason for these declines could be decreased physical activity. In China, although leisure-time physical activity have generally increased since 2000 (19), total physical activity have dropped sharply from 1991 to 2009 (20), and classical literatures showed a J-shaped relationship between physical activity and energy intake (21, 22). However, physical activity was also inversely related to incident diabetes (23). Still, the age-standardized incidence rates of diabetes subsequently decreased from 2007 to 2017 (18).

In dietary BCAA risk analysis of the cohort, increased dietary BCAAs intake was associated with an elevated risk of T2D. Men and older people were more sensitive to the risk of diabetes caused by BCAAs. The conclusions reached in this study were similar to previous studies in the US and northeastern China (2, 7, 8). In the prospective cohort study of United States, HR of diabetes for the highest quintile of BCAAs intake compared with the lowest quintile were 1.13 (95%CI, 1.07–1.19, $P < 0.001$) in leucine, 1.13 (95%CI, 1.07–1.19, $P < 0.001$) in isoleucine and 1.11 (95%CI, 1.05–1.17, $P < 0.001$) in valine (2). In Harbin, China and the American population, it has been observed that higher dietary BCAA intake will promote the risk of T2D. The Harbin population study showed that the OR and 95% CI across quartiles of total BCAA intakes for T2D within the 4th quartile were 1.0, 1.337 (0.940–1.903); 1.579 (1.065–2.343); 2.412 (1.474–3.947) (8). In a meta-analysis study, higher total intake of BCAAs causes increased T2DM risk with an OR and 95% CI of 1.32 (1.14, 1.53) (24). However, the results may seem in contrast to the study from Japan (10). The Japanese study showed that increased intake of BCAAs may be associated with a reduced risk of diabetes. The HR between the highest tertile and the lowest tertile was 0.70 (95% CI: 0.48–1.02; P for trend = 0.06). In that study, total BCAA, leucine and valine intakes were inversely associated with T2D risk in women, and no associations were found in men. Studies have shown that dietary BCAAs affect human metabolism and the risk of chronic diseases (25). A study of young people in northern China showed that a higher dietary BCAA ratio was negatively correlated with postprandial blood glucose (26). Reducing the intake of dietary BCAAs can improve glucose tolerance and body composition (27, 28). Although studies have shown that dietary BCAAs were closely related to multiple chronic diseases, this paper bridges a gap in large cohort studies of representative populations of Chinese.

Of serum BCAAs levels, 80% were determined by protein or BCAAs from diet or supplements, and the remaining 20% are related to their catabolites (29, 30). Studies have shown that oral BCAAs supplementation can affect the leucine content in blood circulation. The relationship between serum BCAAs levels and the occurrence and development of chronic diseases were well established. Studies have found that elevated levels of serum BCAAs are closely related to weight gain, insulin resistance, and abnormal glucose metabolism in adults (31, 32). Animal experiments have shown that in non-obesity, insulin resistance, and fructose-fed rat models, elevated serum BCAA levels were associated with insulin resistance (33). Previous studies also showed higher plasma levels of BCAAs were associated with

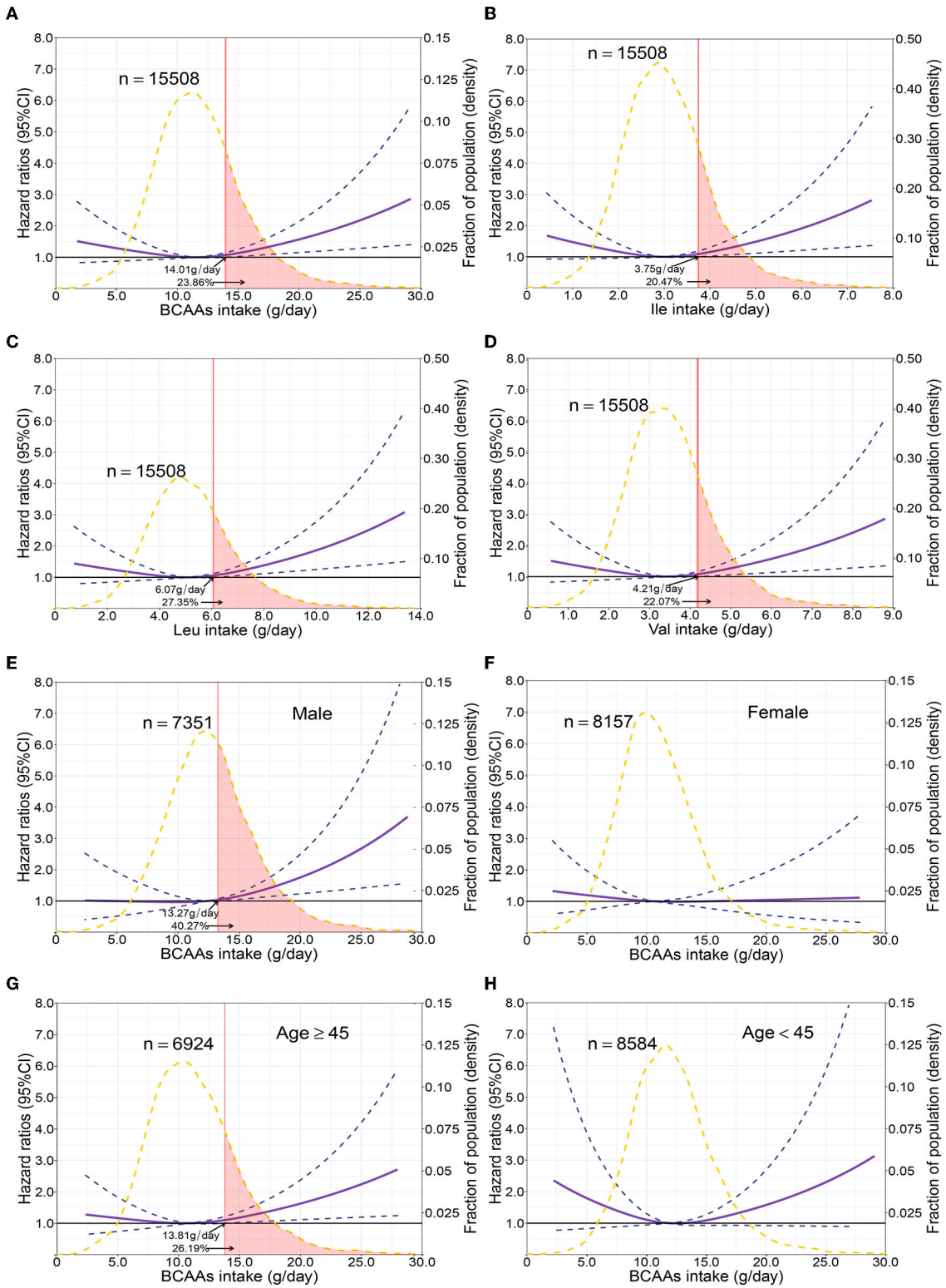

FIGURE 2 | Multivariable adjusted hazard ratios of incident type 2 diabetes according to levels of BCAAs consumption on a continuous scale in the overall population. Solid blue lines are multivariable adjusted hazard ratios, with dashed blues lines showing 95% confidence intervals derived from restricted cubic spline regressions with three knots. Reference lines for no association are indicated by solid bold lines at a hazard ratio of 1.0. Dashed yellow curves show fraction of population with different levels of BCAAs intake. Arrows indicate the lowest consumption of BCAAs and fraction of population with risk of T2D. Analyses were adjusted for age, sex,

(Continued)

FIGURE 2 | smoking status, alcohol consumption, BMI, physical activity levels and energy intake at baseline. Based on individuals from the CHNS followed for a mean 9.9 years. **(A–D)** Representation of restricted cubic spline cox regression models for dietary BCAAs, Ile, Leu, Val and risk of type 2 diabetes. **(E–H)** Representation of restricted cubic spline cox regression models for dietary BCAAs and risk of type 2 diabetes in different age and gender subgroups.

TABLE 4 | Differences in diet with upper and lower thresholds of BCAAs.

Variables	Group A BCAAs < 14.01 g/day (*n* = 11,808)	Group B BCAAs ≥ 14.01 g/day (*n* = 3,700)	χ^2/F	*P-value*
	n (%)/Mean (SD)	*n* (%)/Mean (SD)		
Demographic characteristics				
Female	6,800 (57.59%)	1,357 (36.68%)	494.1568	<0.0001
Age	44.43 (15.16)	41.07 (13.48)	12.85	<0.0001
≥60 years	2,161 (18.30%)	386 (10.43%)	127.0792	<0.0001
BMI	22.85 (3.37)	23.25 (3.45)	−6.03	<0.0001
Smoking history	3,371 (28.55%)	1,493 (40.35%)	182.3113	<0.0001
Alcohol consumption history	3,685 (31.21%)	1,735 (46.89%)	304.8290	<0.0001
Energy intake	2,042.12 (707.20)	2593.34 (1215.11)	−26.23	<0.0001
Food categories (g/day)				
Cereals	451.00 (194.16)	526.54 (262.78)	206.97	<0.0001
Beans	53.51 (64.61)	84.63 (90.13)	329.59	<0.0001
Vegetables	393.83 (196.80)	453.22 (253.42)	166.45	<0.0001
Pickles	3.39 (10.05)	3.77 (10.34)	7.47	0.0063
Fruits	59.74 (104.03)	75.91 (125.65)	27.23	<0.0001
Nuts	4.24 (14.08)	8.69 (23.29)	149.07	<0.0001
Red meat	74.94 (68.94)	121.36 (106.93)	568.14	<0.0001
Poultry	13.75 (29.13)	30.87 (52.40)	445.93	<0.0001
Dairy products	17.19 (58.41)	37.23 (91.20)	169.43	<0.0001
Eggs	31.09 (34.04)	45.86 (49.85)	247.45	<0.0001
Fish and seafoods	29.49 (45.91)	66.49 (81.14)	982.46	<0.0001
Snacks	11.24 (37.46)	15.43 (51.95)	8.52	0.0035
Sugar and starch	2.91 (10.40)	3.87 (13.70)	6.75	0.0094
Sauce	0.52 (3.12)	0.43 (2.65)	2.27	0.1321
Alcohol products	10.93 (55.62)	27.27 (104.20)	58.74	<0.0001
Fast food	10.32 (37.51)	17.08 (62.32)	17.47	<0.0001
Beverage	3.26 (60.59)	9.84 (58.11)	21.2	<0.0001
Vegetable oil and condiments	56.17 (32.71)	64.25 (35.98)	72.53	<0.0001
Others	17.43 (38.21)	24.21 (64.06)	26.63	0.0004
Total food intake	1244.92 (524.68)	1616.96 (755.83)	671.59	<0.0001

SD, standard deviation. Demographic characteristics were tested using Chi-square test and student's t-test. Group differences of food consumption were calculated using the generalized linear models after adjusting for age, sex, energy intake, BMI, region, physical activity levels, smoking status (previous or present, never), alcohol consumption (yes, no).

an increased risk of T2D (34, 35). Prospective population studies have proved that serum BCAA levels can predict the future risk of diabetes (36). In patients with overweight and metabolic syndrome, there was also a correlation between plasma BCAA levels and red meat or animal protein (37). Therefore, control of serum BCAAs can start from dietary BCAAs intake. Our results link dietary BCAAs with population health, especially the risk of diabetes. In this study, group B (BCAAs ≥ 14.01 g/day) was significantly higher than group A (BCAAs < 14.01 g/day) in total food intake and most food categories ($P < 0.0001$), (**Table 4**). From this point of view, high consumption of BCAAs is accompanied by high consumption of food. Our results are in accordance with a recent study. When the quantity of food intake exceeded certain thresholds, the risks of new-onset diabetes increased or reached a plateau (38).

In all food categories, the strongest correlations with BCAAs were with red meat, poultry, fish and seafoods. Our research found that although BCAA intake is decreasing, sources have changed over time. Now animal sources are main sources and previously cereals. Meanwhile, there was also a correlation between plasma BCAA levels and red meat or animal protein (37). A similar phenomenon was also found in the Brazilian population that the main food sources of BCAA were unprocessed red meat, unprocessed poultry, bread and toast, beans and rice (39). Epidemiological studies have shown that high consumption of animal protein, especially red meat with high levels of methionine and BCAAs, have promoted the progression

TABLE 5 | Hazard ratios for incident type 2 diabetes according to categories of levels of BCAAs (Ile, Leu, Val) intake, sex and age adjusted, and multivariable adjusted.

Centile	Consumption (g/day)	Individuals	Events	Event rate per 1,000 person years	Age and sex adjusted hazard ratio (95% CI)	Hazard ratio (95% CI)	Multivariable adjusted hazard ratio (95% CI)	Hazard ratio (95% CI)
BCAAs								
1st-5th	<6.44	778	21	4.98		1.25 (0.77–2.03)		1.72 (1.03–2.88)
6th-20th	6.44–8.82	2,330	61	2.97		0.86 (0.62–1.20)		1.12 (0.78–1.6)
21st-40th	8.82–10.65	3,091	108	3.32		1.20 (0.91–1.59)		1.43 (1.05–1.95)
41st-60th	10.65–12.37	3,113	90	2.54		1.0		1.0
61st-80th	12.37–14.53	3,096	106	3.05		1.27 (0.96–1.68)		1.35 (0.99–1.84)
81st-95th	14.53–18.52	2,324	79	3.38		1.47 (1.08–1.99)		1.27 (0.9–1.78)
96th-100th	>18.52	776	31	5.56	0.4 1 1.6 2.2 2.8 3.4	2.46 (1.63–3.72)	0.4 1 1.6 2.2 2.8 3.4	2.26 (1.45–3.51)
Ile								
1st-5th	<1.64	769	22	5.41		1.43(0.88–2.30)		1.84 (1.11–3.05)
6th-20th	1.64–2.27	2,332	68	3.38		1.03 (0.74–1.42)		1.27 (0.89–1.79)
21st-40th	2.27–2.75	3,069	103	3.20		1.16 (0.88–1.55)		1.29 (0.94–1.76)
41st-60th	2.75–3.20	3,124	89	2.50		1.0		1.0
61st-80th	3.20–3.77	3,131	106	3.01		1.27 (0.96–1.68)		1.28 (0.94–1.75)
81st-95th	3.77–4.77	2,303	77	3.26		1.45 (1.06–1.97)		1.24 (0.88–1.74)
96th-100th	>4.77	780	31	5.43	0.4 1 1.6 2.2 2.8 3.4	2.44 (1.62–3.69)	0.4 1 1.6 2.2 2.8 3.4	2.14 (1.38–3.32)
Leu								
1st-5th	<2.83	783	19	4.34		1.07 (0.65–1.78)		1.37 (0.80–2.35)
6th-20th	2.83–3.88	2,305	62	3.02		0.88 (0.63–1.23)		1.28 (0.90–1.84)
21st-40th	3.88–4.72	3,126	107	3.22		1.18 (0.89–1.57)		1.52 (1.11–2.07)
41st-60th	4.72–5.51	3,110	89	2.51		1.0		1.0
61st-80th	5.51–6.49	3,081	107	3.13		1.32 (1.00–1.76)		1.46 (1.07–2.00)
81st-95th	6.49–8.34	2,330	78	3.37		1.46 (1.07–1.98)		1.32 (0.93–1.86)
96th-100th	>8.34	773	34	6.16	0.4 1 1.6 2.2 2.8 3.4	2.72 (1.83–4.06)	0.4 1 1.6 2.2 2.8 3.4	2.67 (1.74–4.11)
Val								
1st-5th	<1.93	785	21	4.96		1.20 (0.74–1.95)		1.60 (0.96–2.66)
6th-20th	1.93–2.62	2,323	67	3.33		0.93 (0.67-1.28)		1.19 (0.84–1.68)
21st-40th	2.62–3.16	3,117	101	3.08		1.05 (0.79–1.39)		1.21 (0.89–1.65)
41st-60th	3.16–3.66	3,062	93	2.67		1.0		1.0
61st-80th	3.66–4.30	3,131	108	3.06		1.21 (0.92-1.60)		1.25 (0.92–1.70)
81st-95th	4.30–5.44	2,314	77	3.28		1.37 (1.01–1.86)		1.32 (0.95–1.85)
96th-100th	>5.44	776	29	5.13	0.4 1 1.6 2.2 2.8 3.4	2.17 (1.43–3.31)	0.4 1 1.6 2.2 2.8 3.4	1.45 (0.90–2.33)

Multivariable adjusted analyses were adjusted for age, sex, smoking status, alcohol consumption, BMI, physical activity levels and energy intake at baseline. Based on individuals from the CHNS followed for a mean 9.9 years. Interaction with consumption of red meat (P-value for interaction > 0.05), fish and sea foods (P-value for interaction > 0.05), poultry (P-value for interaction > 0.05).

of age-related diseases (40). And, reducing BCAAs consumption in the Western diet improved glucose tolerance and relieved insulin resistance. Previous research has indicated that reducing dietary BCAAs may represent a highly translatable option for the treatment of obesity and insulin resistance in animals (41). According to the results of this study, we propose dietary recommendations for the population's diet to prevent diabetes. The dietary intake should not exceed 2,273 g/day, and the intake of red meat, poultry, fish and seafoods should be controlled at the same time.

Our study also has several limitations. First, these surveys are not carried out annually, which could have allowed more details in trends. Second, dietary consumption data from the CHNS survey 2015 was not available. We used the dietary information from CNHS survey 2015 for make-up. Statistical processing was used to ensure the quality of the results and the comparability between the CNHS and CHNS. Third, our dietary intake estimates are mainly based on 3-day 24-h meal recall, so measurement errors are inevitable. In order to reduce selection biases and measurement errors, we averaged three 24-h dietary recalls for different age groups or urban/rural areas. The average long-term intakes were used to represent the dietary exposure level of the participants. Finally, when the CHNS survey was planned and implemented, the State Statistical Office of China would not share their sample frame with the CHNS team. Furthermore, the data sets for public distribution would not be released if the CHNS team had worked with them. However, the design used extant census data as best as we could for a multi-level random sample.

In conclusion, a trend toward decreased BCAAs intake was observed in Chinese of all subgroups (including age and sex) from 1997 to 2015. After 40 years of age, consumption of BCAAs declined with increasing age. In all food categories, the strongest correlations with BCAAs were with red meat, poultry, fish and seafoods. Higher BCAAs intake was associated with higher risk of T2D. This relationship is more stable among men and middle-aged and elderly people. The people with risk of T2D accounted for about 23.86% of the total population due to BCAAs. Based on the results of this study, in order to prevent diabetes, we recommend that dietary intake should be restricted, while controlling the intake of red meat, poultry, fish and seafood.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the copyright of the dataset is currently owned by the Chinese Center for Disease Control and Prevention and has not been fully disclosed yet. Requests to access the datasets should be directed to https://www.cpc.unc.edu/projects/china.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JZ is guarantor, designed the study, principal investigator, and attests that all the listed authors meet the authorship criteria and that no others meeting the criteria have been omitted. LY conducted the data analysis and drafted the manuscript. PS, QZ, YL, SJ, SZ, and ZW critically revised the manuscript for important intellectual content. All authors contributed to the article and approved the final version of the manuscript.

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Dietary Acid Load Is Positively Associated With Risk of Gestational Diabetes Mellitus in a Prospective Cohort of Chinese Pregnant Women

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Background: Growing evidence suggests that dietary acid load plays an important role in the development of type 2 diabetes. However, prospective studies on the relationship between dietary acid load and gestational diabetes mellitus (GDM) are limited in the pregnant population. This study aimed to investigate the effect of dietary acid load during early pregnancy on the risk of GDM in Chinese pregnant women.

Methods: A total of 1,327 pregnant women were enrolled from an ongoing prospective study of the Tongji Birth cohort (TJBC) in Wuhan, China. Dietary intake was assessed before 20 weeks using a 74-item semiquantitative food frequency questionnaire (FFQ). The dietary acid load was estimated using potential renal acid load (PRAL), net endogenous acid production (NEAP), and animal protein to potassium ratio (A:P ratio). A 75g 2-h oral glucose tolerance test (OGTT) was performed at 24-28 gestational weeks to diagnose GDM.

Results: The mean (standard deviation) values for PRAL score, NEAP score, and A:P ratio were 0.8 ± 11.3 mEq/day, 45.3 ± 16.5 mEq/day, and 9.8 ± 6.0, respectively. There was a significant positive correlation of dietary acid load with the intake of red meat, poultry, fish, and eggs, and a negative correlation with the intake of vegetables, fruits, nuts, and legumes (all $P < 0.05$). Compared to the lowest tertile, the highest tertile of dietary acid load, including PRAL score (odds ratio [OR]: 2.26, 95% confidence interval [CI] = 1.38–3.71, P-trend = 0.002), NEAP score (OR: 2.02, 95% CI = 1.25–3.27, P-trend = 0.009), and A:P ratio (2.08, 95% CI = 1.30–3.31, P-trend = 0.005), significantly increased the risk of GDM. In addition, the dietary acid load was also significantly associated with an increase in 1-h and 2-h post-load blood glucose concentrations (all P-trend < 0.05).

Conclusion: We found a significant positive association between dietary acid load during early pregnancy and the risk of GDM in a Chinese population, suggesting that the reduction of food sources of dietary acid load may be an effective strategy for preventing the risk of GDM.

Keywords: dietary acid load, gestational diabetes mellitus (GDM), potential renal acid load (PRAL), net endogenous acid production (NEAP), animal protein to potassium ratio (A:P ratio), cohort

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INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as glucose intolerance that is new onset or first recognized during pregnancy (1). As one of the most common complications of pregnancy, GDM affects approximately 5.8-20.7% of pregnant women worldwide (2, 3). A systematic review and meta-analysis showed that the prevalence of GDM in Chinese pregnant women was 14.8% (95% confidence interval [CI]: 12.8-16.7%) (4). In the short term, pregnant women with GDM are at higher risk of preterm birth, macrosomia, and cesarean section (5, 6). In addition, it can also have long-term effects, leading to overweight (7) and neurodevelopmental disorders (8) in the offspring and a higher risk of type 2 diabetes in the mothers (9). Several risk factors for the development of GDM have been identified in previous studies, such as maternal age, family history of diabetes, pre-pregnancy body mass index (pre-pregnancy BMI), gestational weight gain, and multiple births (10, 11). The identification of modifiable risk factors that contribute to the prevention of GDM is of great importance in promoting the health of mothers and offspring.

In recent years, the role of dietary acid load in the etiology of insulin resistance and type 2 diabetes has attracted increasing attention (12–17). It has been suggested that acid-base disturbance may contribute to the development of insulin resistance (18, 19). Randomized controlled trials have demonstrated that a short-term vegan dietary intervention is effective in reducing dietary acid load and raising 24-h urine pH in healthy individuals (20, 21). Similarly, observational studies have shown that Western dietary patterns (high intake of acidogenic foods including animal products, and low intake of alkalizing foods including fruits and vegetables) might lead to excessive production of endogenous acids and dietary acid-base imbalances, which in turn might contribute to the development of type 2 diabetes (16, 22). Currently, there are three main indicators for evaluating dietary acid load produced by overall diet, including potential renal acid load (PRAL), net endogenous acid production (NEAP), and animal protein to potassium ratio (A:P ratio). A recent meta-analysis of observational studies showed that higher dietary acid load levels, particularly PRAL scores, were associated with an increased risk of type 2 diabetes (17). The results of a longitudinal study suggested that higher diet-dependent acid load, both PRAL and NEAP scores, is positively associated with the development of insulin resistance (12). However, prospective evidence for the effect of dietary acid load on GDM risk is limited. Only one case-control study in Iran has examined the association between dietary acid load and GDM risk, showing that higher dietary acid load was associated with greater odds of GDM (23). Given the wide variation in dietary habits across regions, it is valuable to provide additional data from the Chinese Population to improve the generalizability of the findings.

Therefore, this study aimed to prospectively evaluate the relationship between dietary acid load in early pregnancy and the risk of GDM in Chinese pregnant women using the PRAL score, NEAP score, and A:P ratio.

MATERIALS AND METHODS

Study Population

Data was used from the prospective cohort study of Tongji Birth Cohort (TJBC) in Wuhan, China. The TJBC study was established in 2018 to assess the role of nutritional status and environmental exposures in maternal and child health. Pregnant women with a single pregnancy, gestational age < 20 weeks, planning to deliver at a participating hospital, and agreeing to complete a face-to-face questionnaire were included in the study ($n = 2261$). For the present analyses, we excluded participants with pre-pregnancy diabetes ($n = 7$), no dietary data on early pregnancy ($n = 857$), extreme energy intake (< 500 kcal/day or > 3500 kcal/day) ($n = 8$), and lack of GDM diagnosis ($n = 62$), with a total of 1,327 participants finally being included (**Figure 1**). Ethical approval was obtained from the Ethics Committee of Tongji Medical College of Huazhong University of Science and Technology, and all participants provided written informed consent before enrollment.

Dietary Assessment

Dietary intake was assessed through face-to-face interviews using a 74-item semiquantitative food frequency questionnaire (FFQ), which has been proven in the previous study to be a reasonable tool for assessing nutrient and food intakes of pregnant women in China (24). The description of FFQ has been described in detail in the previous study (25). In brief, pregnant women were asked about the frequency and amount of the 74 food items consumed over the past four weeks. The frequency of food intake ranged from "less than once a month" to "more than three times a day" among the 13 frequency options. Trained dietitians used a color food photography atlas containing different portion sizes of all foods and food models representing the standard portions to make the estimation more accurate. The daily intake of energy and nutrients was calculated by FFQ based on the Chinese Food Composition Tables (26). Food and nutrient intakes were adjusted according to the energy residual method (27).

Dietary Acid Load

In this study, we calculated the dietary acid load through three different measures: potential renal acid load (PRAL) (28), net endogenous acid production (NEAP) (29), and animal protein-to-potassium ratio (A:P ratio) (30).

The equations are as follows:

(1) PRAL (mEq/day) = (0.4888 × protein (g/day)) + (0.0366 × phosphorus (mg/day)) − (0.0205 × potassium (mg/day)) − (0.0263 × magnesium (mg/day))−(0.0125 × calcium (mg/day));
(2) NEAP (mEq/day) = 54.5 × protein intake (g/day)/potassium intake (mEq/day) − 10.2;
(3) A:P ratio = animal protein (g/day)/potassium (g/day).

Outcome Definitions

A 75 g 2-h oral glucose tolerance test (OGTT) was performed for all pregnant women at 24-28 gestational weeks after at least 8-h of fasting. Fasting blood glucose (FBG), 1-h post-load

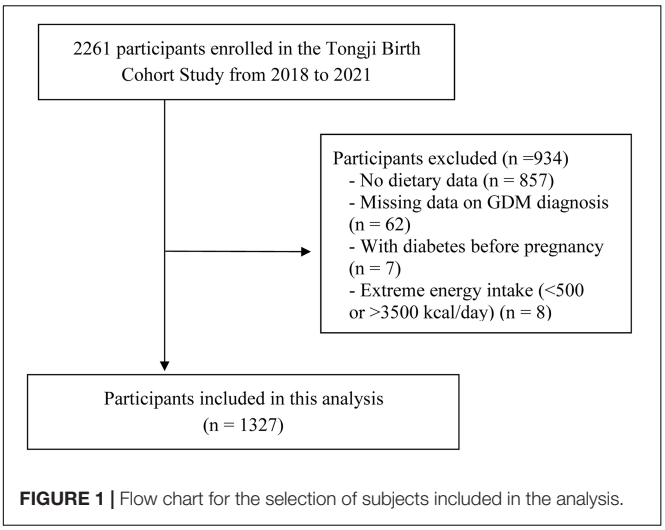

FIGURE 1 | Flow chart for the selection of subjects included in the analysis.

blood glucose (PBG), and 2-h PBG levels were collected from medical records. According to the criteria established by the International Association of the Diabetes and Pregnancy Study Groups, subjects were diagnosed with GDM if they met any of the following criteria: FBG $\geq$ 5.1 mmol/L; 1-h PBG $\geq$ 10.0 mmol/L; or 2-h PBG $\geq$ 8.5 mmol/L (31).

Other Variables

Information on covariates was obtained through a structured questionnaire completed at enrolment, including maternal age, education level, gravidity, parity, personal and family history of diabetes, and lifestyle habits before pregnancy such as smoking status, alcohol intake, and physical activity. Alcohol consumers (or smokers) were defined as drinking (or smoking) more than one time a week before pregnancy. Participants were considered to have regular physical activity if they reported physical activity at least once a week before pregnancy. We also collected data on anthropometric measurements, including maternal height and pre-pregnancy weight. Pre-pregnancy BMI was calculated using self-reported pre-pregnancy weight (kg) divided by height^2 (m^2).

Statistical Analyses

Data are presented as mean (standard deviation [SD]) for continuous variables and n (%) for categorical variables. One-way analysis of variance and the Chi-squared test were used to compare continuous and categorical variables, respectively. The PRAL score, NEAP score, and A:P ratio were categorized in tertiles, with the lowest tertile as the reference group. Multivariable logistic regression analyses were used to assess the associations between dietary acid load levels in early pregnancy and risk of GDM, with the results expressed as odds ratios (ORs) and 95% CIs. In order to test the significance of linear trends across tertiles, the median value of each tertile of dietary acid load measures was considered to be a continuous variable. Generalized linear models were conducted to examine the association of dietary acid load levels with FBG, 1-h PBG, and 2-h PBG, and the results were presented as coefficients (β) with 95% CIs. All potential confounders in the multivariable models were chosen based on both biological and statistical considerations (changed main effect estimates > 10%). Multivariate models were as followed: (1) model 1 was the crude model; (2) model 2 adjusted for maternal age (continuous), pre-pregnancy BMI (continuous), education years ($\leq$ 12, 13–15, $\geq$ 16 years), primiparity (yes/no), smoking status before pregnancy (yes/no), alcohol intake before pregnancy (yes/no), regular physical activity before pregnancy (yes/no), and family history of diabetes (yes/no); (3) model 3 further adjusted for energy-adjusted nutrient intake (i.e., carbohydrate, dietary fiber, cholesterol, vitamin A, vitamin C, vitamin E, saturated fatty acids (SFAs), and Monounsaturated fatty acids (MUFAs)).

To evaluate the potential modification effect, stratified analyses were conducted according to the median value of maternal age (< 29.2 or $\geq$ 29.2 years), pre-pregnancy BMI (< 20.5 or $\geq$ 20.5 kg/m^2), primiparity (yes or no), gravidity (yes or no), regular physical activity (yes or no), and family history of diabetes (yes or no). The likelihood ratio tests were used to assess the interactions between stratified variables and freshwater fish intake. In addition, we performed different sensitivity analyses to assess the stability of the study results. First, we excluded participants who were over 30 years old at the time of pregnancy. Second, we excluded participants with abnormal pre-pregnancy BMI (< 18.5 or $\geq$ 24 kg/m^2). Third, we separately excluded participants with smoking or alcohol consumption habits before pregnancy. All analyses were performed using statistical packages R (The R Foundation; v. 3.4.3)[1] and Empower(R) (X&Y Solutions Inc.)[2]. We considered $P < 0.05$ in the two-sided test as significant.

RESULTS

Characteristics of Participants

A total of 1,327 subjects were included in the present study (**Figure 1**). The mean (SD) values for PRAL score, NEAP score, and A:P ratio in the study population were 0.8 $\pm$ 11.3 mEq/day, 45.3 $\pm$ 16.5 mEq/day, and 9.8 $\pm$ 6.0, respectively. **Table 1** shows the characteristics of study participants by tertiles of the PRAL score distribution. Compared to those with the lowest tertile of PRAL scores (< −3.2 mEq/day), individuals with the highest tertile of PRAL scores ($\geq$ 5.3 mEq/day) were more likely to be multiparous and to have a family history of diabetes. For specific food groups, participants with higher PRAL scores consumed more grains and animal products (red meat, poultry, fish, eggs) and fewer vegetables, fruit, and legumes than participants with lower PRAL scores. In addition, they also had higher intakes of protein, cholesterol, SFAs, and MUFAs, and lower intakes of carbohydrates, dietary fiber, vitamin A, vitamin C, vitamin E, potassium, calcium, and magnesium.

Correlation Between Dietary Acid Load and Food Intake

Table 2 shows the Pearson correlation coefficients between dietary acid load scores and food intake. There were statistically

[1]http://www.r-project.org

[2]http://www.empowerstats.com

TABLE 1 | Characteristics of study participants according to tertiles of the PRAL score[a].

Variables	Overall (n = 1327)	Tertiles of PRAL score (mEq/day)			P value[b]
		T1 (n = 442)	T2 (n = 442)	T3 (n = 443)	
Maternal Characteristics					
Maternal age (years)	29.5 ± 3.3	29.4 ± 3.6	29.5 ± 3.1	29.7 ± 3.4	0.365
Pre-pregnancy BMI (kg/m^2)	21.1 ± 3.1	20.9 ± 2.8	21.0 ± 3.2	21.3 ± 3.2	0.170
Education (years), n (%)					0.919
≤ 12	304 (22.9%)	105 (23.8%)	97 (21.9%)	102 (23.0%)	
13–15	427 (32.2%)	146 (33.0%)	139 (31.4%)	142 (32.1%)	
≥ 16	590 (44.5%)	189 (42.8%)	203 (45.9%)	198 (44.7%)	
Income (CNY/month), n (%)					0.193
≤ 4999	189 (14.2%)	60 (13.6%)	70 (15.8%)	59 (13.3%)	
5000–9999	734 (55.3%)	240 (54.3%)	242 (54.8%)	252 (56.9%)	
≥ 10000	376 (28.3%)	128 (29.0%)	127 (28.7%)	121 (27.3%)	
Gravidity (times), n (%)					0.451
1	789 (59.5%)	266 (60.2%)	270 (61.1%)	253 (57.1%)	
≥ 2	538 (40.5%)	176 (39.8%)	172 (38.9%)	190 (42.9%)	
Primiparity (yes), n (%)	1069 (80.6%)	362 (81.9%)	366 (82.8%)	341 (77.0%)	0.062
Alcohol intake (yes), n (%)	29 (2.2%)	8 (1.8%)	8 (1.8%)	13 (2.9%)	0.418
Smoking status (yes), n (%)	36 (2.7%)	11 (2.5%)	12 (2.7%)	13 (2.9%)	0.920
Regular physical activity (yes), n (%)	503 (37.9%)	177 (40.0%)	167 (37.8%)	159 (35.9%)	0.444
Family history of diabetes (yes), n (%)	150 (11.3%)	31 (7.0%)	56 (12.7%)	63 (14.2%)	0.010
GDM, n (%)	217 (16.4%)	53 (12.0%)	82 (18.6%)	82 (18.5%)	0.010
Food intake[c]					
Grains (g/day)	245.2 ± 58.9	223.8 ± 58.7	245.3 ± 54.9	266.2 ± 55.3	< 0.001
Vegetables (g/day)	309.8 ± 139.0	376.0 ± 155.7	309.6 ± 120.2	244.0 ± 103.2	< 0.001
Fruits (g/day)	485.7 ± 219.3	649.0 ± 229.4	453.0 ± 152.5	355.3 ± 154.0	< 0.001
Red meats (g/day)	31.3 ± 34.6	18.6 ± 18.1	27.8 ± 21.2	47.4 ± 48.9	< 0.001
Poultry (g/day)	7.4 ± 11.7	5.3 ± 8.6	7.4 ± 10.8	9.5 ± 14.7	< 0.001
Fish (g/day)	27.7 ± 27.5	22.8 ± 21.9	26.3 ± 24.7	33.9 ± 33.5	< 0.001
Eggs (g/day)	31.7 ± 23.9	28.1 ± 24.8	30.6 ± 21.5	36.4 ± 24.6	< 0.001
Dairy products (ml/day)	165.2 ± 136.0	167.2 ± 144.0	170.2 ± 131.8	158.2 ± 131.8	0.394
Nuts (g/day)	13.3 ± 13.2	14.4 ± 14.5	13.0 ± 12.7	12.6 ± 12.2	0.117
Legumes (g/day)	7.9 ± 8.2	8.1 ± 6.8	8.1 ± 9.0	7.5 ± 8.6	0.473
Nutrient intake[c]					
Energy (kcal/day)	1899.9 ± 492.8	1915.5 ± 516.0	1923.1 ± 451.6	1861.1 ± 507.1	0.125
Protein (g/day)	57.7 ± 14.4	51.1 ± 10.0	55.9 ± 8.4	66.1 ± 18.2	< 0.001
Animal protein (g/day)	21.8 ± 12.9	16.5 ± 8.1	20.4 ± 8.9	28.4 ± 16.7	< 0.001
Plant protein (g/day)	35.9 ± 7.3	34.4 ± 7.0	35.3 ± 5.8	38.1 ± 8.4	< 0.001
Fat (g/day)	68.8 ± 15.5	67.3 ± 15.1	70.1 ± 15.9	69.2 ± 15.6	0.022
Carbohydrates (g/day)	289.9 ± 35.8	296.8 ± 34.2	287.3 ± 36.4	285.5 ± 35.8	< 0.001
Dietary fiber (g/day)	14.6 ± 3.5	16.8 ± 3.6	14.3 ± 2.8	12.6 ± 2.6	< 0.001
Cholesterol (mg/day)	296.0 ± 164.9	260.9 ± 165.6	286.4 ± 149.0	340.7 ± 169.6	< 0.001
Vitamin A (ugRAE/day)	781.3 ± 331.9	922.7 ± 355.3	764.7 ± 302.0	656.7 ± 278.6	< 0.001
Vitamin C (mg/day)	186.7 ± 68.5	238.9 ± 70.8	179.9 ± 47.4	141.6 ± 45.0	< 0.001
Vitamin E (mg/day)	39.6 ± 12.7	42.3 ± 13.0	39.9 ± 13.1	36.8 ± 11.4	< 0.001
Dietary SFAs (g/day)	14.8 ± 4.2	13.9 ± 3.9	15.1 ± 4.0	15.5 ± 4.5	< 0.001
Dietary MUFAs (g/day)	22.7 ± 8.1	21.7 ± 8.0	23.0 ± 8.1	23.4 ± 8.1	0.005
Dietary PUFAs (g/day)	23.1 ± 8.7	23.3 ± 9.0	23.5 ± 9.0	22.5 ± 8.1	0.156
Sodium (mg/day)	385.4 ± 155.8	391.6 ± 136.6	381.5 ± 145.9	383.2 ± 181.3	0.589
Potassium (g/day)	2.3 ± 0.4	2.6 ± 0.4	2.2 ± 0.3	2.0 ± 0.3	< 0.001
Calcium (mg/day)	536.3 ± 166.8	576.1 ± 170.3	535.2 ± 161.6	497.7 ± 159.5	< 0.001
Magnesium (mg/day)	314.7 ± 46.2	341.0 ± 45.7	310.0 ± 40.4	293.0 ± 38.6	< 0.001
Phosphorus (mg/day)	937.3 ± 119.7	936.6 ± 116.8	931.1 ± 120.6	944.1 ± 121.7	0.266
PRAL score (mEq/day)	0.8 ± 11.3	−11.0 ± 7.1	1.1 ± 2.4	12.3 ± 7.7	< 0.001
NEAP score (mEq/day)	45.3 ± 16.5	31.2 ± 6.9	43.4 ± 4.0	61.1 ± 17.3	< 0.001
A:P ratio	9.8 ± 6.0	6.2 ± 2.7	9.0 ± 3.2	14.1 ± 7.7	< 0.001

[a] *Values are expressed as mean ± standard deviation or n (%).*

[b] *P value was obtained using the chi-square test for categorical variables and ANOVA tests for continuous variables.*

[c] *Energy-adjusted using the residual method.*

A:P ratio, animal protein to potassium ratio; pre-pregnancy BMI, pre-pregnancy body mass index; Eq, equivalent; GDM, gestational diabetes mellitus; MUFAs, Monounsaturated fatty acids; NEAP, net endogenous acid production; PRAL, potential renal acid load; PUFAs, Polyunsaturated fatty acids; SFAs, saturated fatty acids; T, tertile.

TABLE 2 | Pearson correlations between food group intake and three dietary acid load measures[a].

Food group[b]	Dietary acid load					
	PRAL score	*P* value	NEAP score	*P* value	A:P ratio	*P* value
Grains (g/day)	0.177	< 0.001	0.278	< 0.001	−0.100	< 0.001
Vegetables (g/day)	−0.223	< 0.001	−0.305	< 0.001	−0.197	< 0.001
Fruits (g/day)	−0.261	< 0.001	−0.452	< 0.001	−0.263	< 0.001
Nuts (g/day)	−0.059	0.132	−0.090	0.002	−0.047	0.104
Legumes (g/day)	−0.071	0.065	−0.059	0.040	−0.102	< 0.001
Total meats g/day)	0.140	< 0.001	0.246	< 0.001	0.608	< 0.001
Red meats (g/day)	0.280	< 0.001	0.453	< 0.001	0.571	< 0.001
Poultry (g/day)	0.135	0.002	0.214	< 0.001	0.355	< 0.001
Fish (g/day)	0.086	0.024	0.121	< 0.001	0.360	< 0.001
Eggs (g/day)	0.055	0.144	0.078	0.007	0.253	< 0.001
Dairy products (ml/day)	−0.024	0.533	−0.017	0.546	0.281	< 0.001

[a]*Food group intakes and three dietary acid load measures were log10-transformed to improve normality.*
[b]*Energy-adjusted using the residual method.*
A:P ratio, animal protein to potassium ratio; NEAP, net endogenous acid production; PRAL, potential renal acid load.

significant positive correlations between intake of most animal foods (red meats, poultry, fish, and eggs) and dietary acid load scores (all $P < 0.05$), except for dairy products. Regarding plant foods, we observed significant negative correlations of vegetables, fruits, nuts, and legumes intake with dietary acid load (all $P < 0.05$). However, grains intake was positively correlated with PRAL and NEAP scores, while it was negatively correlated with the A:P ratio.

Association Between Maternal Dietary Acid Load and GDM Risk

The associations between indices of dietary acid load and GDM risk were shown in **Table 3**. In the multivariable models, PRAL score, NEAP score, and A:P ratio were all associated with an increased risk of GDM after adjusting for covariates of maternal age, pre-pregnancy BMI, education, primiparity, smoking status, alcohol intake, regular physical activity, family history of diabetes, and other dietary factors. The multivariable-adjusted ORs (95% CIs) of GDM for the lowest to the highest tertiles of PRAL score were 1.00 (reference), 2.06 (1.35, 3.15), and 2.26 (1.38, 3.71) (*P*-trend = 0.002). Similar findings were found for the NEAP score (OR for T3 vs. T1: 2.02, 95% CI: 1.25–3.27; T2 vs. T1: 2.05, 95% CI: 1.36–3.10; *P*-trend = 0.009). In addition, those in the highest tertile of the A:P ratio had a 108% higher risk of GDM than those in the lowest tertile after controlling for potential covariates (OR: 2.08, 95% CI: 1.30–3.31, *P*-trend = 0.005).

Association Between Maternal Dietary Acid Load and Blood Glucose Concentrations

In the crude model, the highest tertile of dietary acid load (PRAL score, NEAP score, and A:P ratio) in early pregnancy was associated with an increase in FBG, 1-h PBG, and 2-h PBG compared to the lowest tertile. After controlling for potential covariates, we found that women in the highest tertile of the PRAL score significantly increased FBG by 0.09 mmol/L (95% CI: 0.02, 0.17, *P*-trend = 0.017), 1-h PBG by 0.50 mmol/L (95% CI: 0.19, 0.81, *P*-trend = 0.002) and 2-h PBG by 0.54 mmol/L (95% CI: 0.28, 0.80, *P*-trend < 0.001), respectively, compared to women in the lowest tertile. Similarly, we identified the significant positive relationships of NEAP score and A:P ratio with 1-h PBG ($\beta = 0.47$, 95% CI: 0.17, 0.77, *P*-trend = 0.003 for NEAP; $\beta = 0.31$, 95% CI: 0.01, 0.61, *P*-trend = 0.044 for A:P ratio) and 2-h PBG ($\beta = 0.43$, 95% CI: 0.18, 0.69, *P*-trend = 0.001 for NEAP; $\beta = 0.28$, 95% CI: 0.03, 0.53, *P*-trend = 0.041 for A:P ratio) when the highest tertile compared to the lowest tertile (**Table 4**).

Subgroup and Sensitivity Analyses

To assess whether other confounding factors modified the association between dietary acid load and risk of GDM, we performed stratified analyses by maternal age (< 29.2 or ≥ 29.2 years), pre-pregnancy BMI (< 20.5 or ≥ 20.5 kg/m^2), primiparity (yes or no), gravidity (< 1 or ≥ 2), regular physical activity (yes or no), and family history of diabetes (yes or no). No significant modifications were observed between dietary acid load and GDM risk (all $P_{interaction} > 0.05$) (**Supplementary Table 1**). In sensitivity analyses that excluded participants with age >30 years, abnormal pregnancy BMI, smoking, and alcohol consumption, the results remained stable and the significantly positive associations were still observed (**Supplementary Table 2**).

DISCUSSION

In this study of pregnant Chinese women, we found that higher dietary acid load (as reflected by three different dietary acid load indices) in early pregnancy was associated with an increased risk of GDM, even after adjustment for characteristics, lifestyle, and other dietary factors (carbohydrate, dietary fiber, cholesterol, vitamin A, vitamin C, vitamin E, SFAs, and MUFAs). In addition, the positive associations tended to be stronger in women with pre-pregnancy BMI ≥ 20.5 kg/m^2, primiparity, gravidity ≥ 2,

TABLE 3 | Associations between maternal dietary acid load and risk of GDM.

Variables			OR (95% CI)		
	Median (IQR)	Cases/N	Crude model	Multivariate model I[a]	Multivariate model II[b]
PRAL score					
T1	−8.91 (−13.79–6.00)	53/442	1.00	1.00	1.00
T2	1.22 (−0.79-3.23)	82/442	1.67 (1.15, 2.43)	1.69 (1.14, 2.50)	2.06 (1.35, 3.15)
T3	10.15 (7.30-14.48)	82/443	1.67 (1.15, 2.42)	1.64 (1.11, 2.43)	2.26 (1.38, 3.71)
P-trend[c]			0.007	0.015	0.002
NEAP score					
T1	32.74 (27.73-35.92)	52/442	1.00	1.00	1.00
T2	43.47 (40.84-46.31)	86/442	1.81 (1.25, 2.63)	1.78 (1.21, 2.63)	2.05 (1.36, 3.10)
T3	55.62 (51.54-64.79)	79/443	1.63 (1.12, 2.38)	1.63 (1.10, 2.42)	2.02 (1.25, 3.27)
P-trend[c]			0.019	0.025	0.009
A:P ratio					
T1	5.32 (3.80-6.29)	50/442	1.00	1.00	1.00
T2	8.85 (8.11-9.62)	81/442	1.76 (1.20, 2.57)	1.65 (1.11, 2.46)	1.85 (1.23, 2.79)
T3	13.75 (12.02-16.34)	86/443	1.89 (1.30, 2.75)	1.77 (1.19, 2.62)	2.08 (1.30, 3.31)
P-trend[c]			0.002	0.008	0.005

[a] *Multivariate model I was adjusted for maternal age, pre-pregnancy BMI, education, primiparity, smoking status, alcohol intake, regular physical activity, and family history of diabetes.*

[b] *Multivariate model II was further adjusted for intake of carbohydrate, dietary fiber, cholesterol, vitamin A, vitamin C, vitamin E, SFAs, and MUFAs.*

[c] *Tests for linear trend were conducted by using the median value for each tertile and treating it as a continuous variable in the logistic regression.*

A:P ratio, animal protein to potassium ratio; GDM, gestational diabetes mellitus; IQR, interquartile range; NEAP, net endogenous acid production; OR, odds ratio; PRAL, potential renal acid load, T, tertile.

lack of regular physical activity, and having a family history of diabetes. After controlling for potential covariates, FBG, 1-h PBG, and 2-h PBG were all significantly increased in the highest tertile of PRAL scores compared to the lowest tertile. Also, higher NEAP scores and A:P ratio increased 1-h PBG and 2-h PBG, but not FBG levels.

In recent years, the interest of research has focused on the relationship between diet-induced acid load and type 2 diabetes and insulin resistance (12, 13, 17, 32). A prospective cohort study of 66,485 French women and the pooled results from three prospective cohort studies in the United States both showed that dietary acid load was positively associated with the risk of type 2 diabetes (13, 32). Similarly, the results of a meta-analysis that included 14 studies showed that participants in the highest categories of PRAL and NEAP scores had a 19 and 22% increased risk of developing diabetes, respectively, compared to the lowest categories (17). Another prospective study in a Korean middle-aged and elderly population found that a higher diet-dependent acid load was associated with an increased risk of insulin resistance in the future (12). To our knowledge, however, research investigating the relationship between dietary acid load and the risk of GDM has been limited to date. In line with our findings, a case-control study conducted in Iran reported a positive association between dietary acid load and risk of GDM measured by PRAL score and A:P ratio (23). Furthermore, apart from finding a positive relationship between PRAL scores and FBG as in the previous study (23), we also identified significant relationships of dietary acid load with 1-h PBG and 2-h PBG.

Our study used three different methods to calculate dietary acid load: PRAL, NEAP, and A:P ratio. These methods are calculated based on the intake of protein, phosphorus, potassium, magnesium, and calcium. All these nutrients are acid-base precursors and may be in relation to pH homeostasis in the body (28, 30, 33). Studies have suggested that foods from animals, such as cheese, fish, and meat, have more acid precursors, while fruits and vegetables are net alkalinizing in nature (20, 21, 34). In the current study, the results showed that the PRAL score, NEAP score, and A:P ratio were all strongly positively correlated with the intake of red meat, poultry, fish, and eggs, while there were significant negative correlations with the intake of vegetables, fruits, legumes, and nuts. The findings are consistent with those of previous studies in other populations (32, 35). Notably, previous studies have only confirmed that consumption of single acidic foods (e.g., meat, milk) or alkaline foods (e.g., fruit, vegetables) was associated with the risk of GDM (36–39); however, taking an integrated approach considering the balance of acidic and alkaline foods may be more important than assessing single acidic and alkaline foods.

The underlying mechanisms linking dietary acid load to glucose homeostasis and GDM risk remain to be elucidated. In the current study, we found that individuals in the highest tertile of dietary acid load had higher protein intakes and lower potassium, calcium, and magnesium intakes. Studies have shown that meat and dairy products, as the main sources of animal protein, were significantly associated with a higher risk of GDM (36, 37). Moreover, animal protein and cereal grains have higher contents of sulfur-containing amino acids (methionine, homocysteine, and cysteine), which produce sulfates with acidifying effects during their metabolism and constitute the

TABLE 4 | Associations between maternal dietary acid load and blood glucose levels.

Variables	β (95% CI), mmol/L		
	Crude model	Multivariate model I[a]	Multivariate model II[b]
PRAL score			
FBG			
T1	0.00	0.00	0.00
T2	0.08 (0.02, 0.14)	0.06 (0.00, 0.12)	0.07 (0.01, 0.14)
T3	0.10 (0.04, 0.16)	0.08 (0.02, 0.13)	0.09 (0.02, 0.17)
P-trend[c]	0.001	0.009	0.017
1-h PBG			
T1	0.00	0.00	0.00
T2	0.21 (−0.04, 0.46)	0.09 (−0.15, 0.33)	0.26 (−0.01, 0.53)
T3	0.37 (0.12, 0.61)	0.23 (0.00, 0.47)	0.50 (0.19, 0.81)
P-trend[c]	0.003	0.051	0.002
2-h PBG			
T1	0.00	0.00	0.00
T2	0.29 (0.09, 0.50)	0.18 (−0.02, 0.38)	0.40 (0.18, 0.63)
T3	0.33 (0.13, 0.53)	0.23 (0.03, 0.42)	0.54 (0.28, 0.80)
P-trend[c]	0.001	0.022	< 0.001
NEAP score			
FBG			
T1	0.00	0.00	0.00
T2	0.05 (−0.01, 0.12)	0.03 (−0.03, 0.09)	0.04 (−0.02, 0.11)
T3	0.08 (0.02, 0.14)	0.06 (−0.00, 0.11)	0.06 (−0.01, 0.14)
P-trend[c]	0.010	0.053	0.113
1-h PBG			
T1	0.00	0.00	0.00
T2	0.27 (0.02, 0.52)	0.15 (−0.09, 0.39)	0.31 (0.04, 0.57)
T3	0.36 (0.11, 0.60)	0.25 (0.02, 0.49)	0.47 (0.17, 0.77)
P-trend[c]	0.004	0.034	0.003
2-h PBG			
T1	0.00	0.00	0.00
T2	0.36 (0.15, 0.56)	0.25 (0.05, 0.45)	0.43 (0.20, 0.65)
T3	0.29 (0.08, 0.49)	0.21 (0.02, 0.41)	0.43 (0.18, 0.69)
P-trend[c]	0.008	0.039	0.001
A:P ratio			
FBG			
T1	0.00	0.00	0.00
T2	0.08 (0.02, 0.14)	0.05 (−0.01, 0.11)	0.06 (−0.01, 0.12)
T3	0.09 (0.03, 0.15)	0.06 (0.00, 0.12)	0.05 (−0.03, 0.12)
P-trend[c]	0.006	0.043	0.309
1-h PBG			
T1	0.00	0.00	0.00
T2	0.13 (−0.12, 0.38)	0.01 (−0.23, 0.25)	0.15 (−0.12, 0.41)
T3	0.27 (0.03, 0.52)	0.13 (−0.10, 0.37)	0.31 (0.01, 0.61)
P-trend[c]	0.031	0.245	0.044
2-h PBG			
T1	0.00	0.00	0.00
T2	0.18 (−0.03, 0.39)	0.09 (−0.11, 0.29)	0.23 (0.01, 0.45)
T3	0.20 (−0.01, 0.40)	0.09 (−0.11, 0.29)	0.28 (0.03, 0.53)
P-trend[c]	0.075	0.411	0.041

[a]*Multivariate model I was adjusted for maternal age, pre-pregnancy BMI, education, primiparity, smoking status, alcohol intake, regular physical activity, and family history of diabetes.*

[b]*Multivariate model II was further adjusted for intake of carbohydrate, dietary fiber, cholesterol, vitamin A, vitamin C, vitamin E, SFAs, and MUFAs.*

[c]*Tests for linear trend were conducted by using the median value for each tertile and treating it as a continuous variable in the logistic regression.*

A:P ratio, animal protein to potassium ratio; FBG, fasting blood glucose; NEAP, net endogenous acid production; PBG, post-load blood glucose; PRAL, potential renal acid load, T, tertile.

main contributor to the daily acid load (33, 40). The main food sources of potassium are vegetables and fruits, which also provide other basic cations (e.g., magnesium). A low-potassium diet can lead to the development of impaired glucose tolerance, through impairments in insulin secretion from pancreatic β-cells (41). Also, potassium can involves in acid-base homeostasis by exchanging hydrogen ions across the cell membrane to assist in electroneutrality (42). Low blood pH could reduce the uptake of glucose by muscle tissue, disrupt the binding of insulin to its receptors (43), and further inhibit insulin signaling pathways, which could lead to the development of insulin resistance and diabetes (44). In addition, the high acidity of the diet may stimulate cortisol secretion from the adrenal cortex, and chronically elevated cortisol levels may induce insulin resistance (45, 46). Furthermore, a higher dietary acid load may stimulate the expression of induced NO synthase and increase levels of inflammatory factors, which may in turn be triggers for GDM (47, 48).

The strengths of this study include detailed information on potential confounders and a prospective design, which greatly reduces the chance of reverse causality and provides strong evidence for examining the associations between dietary acid load and GDM risk. Secondly, we used three indicators, PRAL score, NEAP score, and A:P ratio, which could provide a more comprehensive assessment of dietary acid load during pregnancy from different perspectives. In addition, as dietary habits vary considerably between populations, this study provides evidence from the population of Chinese pregnant women, filling a data gap in the relationship between dietary acid load and GDM in this population.

The current study also has some limitations that need to be considered. Firstly, we used the validated FFQ for dietary assessment, which may still be subject to measurement error and inaccuracy. To partially control for reporting bias, we excluded all participants with extreme values of total energy intake (< 500 kcal/day or > 3500 kcal/day) from the analysis and also adjusted food and nutrient intakes according to the energy residual method. Secondly, we only assessed dietary acid load in early pregnancy, whereas subsequent dietary changes in mid and late pregnancy may have some influence on the results. However, diet before the onset of GDM may more accurately reflect the true causal relationship between exposure and outcome, which could exclude the effect of changes to diet after the occurrence of GDM. Finally, we cannot completely exclude the impact of unmeasured residual factors that may influence the association between dietary acid load and GDM risk. However, it is worth noting that our analysis has adjusted for several confounding factors identified in the previous studies including maternal age, education level, pre-pregnancy BMI, primiparity, smoking status, alcohol intake, regular physical activity, and family history of diabetes.

CONCLUSION

To our knowledge, this is the first prospective cohort study using a combination of three indicators to assess the association

between dietary acid load and the risk of GDM. Collectively, we found that dietary acid load scores in early pregnancy were positively related to GDM risk in Chinese pregnant women. Decreasing dietary acid load may be a preventive strategy to reduce the occurrence of GDM. Underlying biological mechanisms involved in these associations should be identified and further explored in future studies. Besides, further large-scale studies are needed to confirm our findings in other populations.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Tongji Medical College of Huazhong University of Science and Technology. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LH, GX, and XY contributed to the conceptualization of the study. RZ and YL performed the analysis. SW, LZ, and GL conducted data collection. RZ wrote the manuscript. LH and GX critically revised the manuscript. All authors read and approved the submitted manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.892698/full#supplementary-material

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Adherence to Mediterranean Diet of Breastfeeding Mothers and Fatty Acids Composition of Their Human Milk: Results From the Italian MEDIDIET Study

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Background and Aims: The content of fatty acids (FA) in human milk may be influenced by maternal nutrition. We evaluated the role of a Mediterranean diet in influencing the content of FA in human milk among 282 breastfeeding mothers participating in the MEDIDIET study.

Materials and Methods: Mediterranean Diet Score (MDS), a composite index, was used to evaluate adherence to the Mediterranean diet. It includes 9 components (i.e., vegetables, fruit, cereals, legumes, monounsaturated to saturated FA ratio – MUFA/SFA, fish, dairy products, meat, and alcohol) and therefore potentially ranges from 0 (no adherence) to 9 (complete adherence) points. None of the mothers obtained the highest score due to the low alcohol consumption in the study population. Mothers were categorized in approximate tertiles of adherence: 0–3 (34.4%), 4 (26.2%), and 5–8 points (39.4%). The mean content of FA across categories of MDS was compared using ANOVA and test for trend.

Results: A high adherence of breastfeeding mothers to the Mediterranean diet was associated with lower content of SFA in human milk (42.58 ± 4.36 for MDS = 0–3, 42.58 ± 4.89 for MDS = 4, and 40.92 ± 5.22% of fats for MDS = 5–8 points; p ANOVA and p for trend = 0.02). Conversely, a high adherence was associated with higher content of MUFA in human milk (43.27 ± 4.27 for MDS = 0–3, 43.27 ± 4.47

for MDS = 4, and 45.24 ± 5.22% of fats for MDS = 5–8 points; *p* ANOVA and *p* for trend < 0.01), ω-3 FA (1.07 ± 0.25 for MDS = 0–3, 1.22 ± 0.49 for MDS = 4, and 1.31 ± 0.51% of fats for MDS = 5–8 points; *p* ANOVA and *p* for trend < 0.01), and the major types of ω-3 FA (i.e., α-linolenic acid – ALA, eicosapentaenoic acid – EPA, docosahexaenoic acid – DHA, docosapentaenoic acid – DPA). These associations were mainly driven by the adherence to the vegetables, MUFA/SFA, fish, and dairy products components of the Mediterranean diet.

Conclusion: A high adherence to the Mediterranean diet was associated with human milk showing a lower content of SFA and higher content of MUFA and ω-3 FA, including DHA. The Mediterranean diet may contribute in human milk production with higher content of specific FA which is directly involved in infant's neural and visual development, as reported by previous studies.

Keywords: maternal nutrition, Mediterranean diet, breastfeeding, lipids, fatty acids profile

INTRODUCTION

Human milk is a mixture of nutritive and bioactive compounds (e.g., nutrients, hormones, antibodies, immune and stem cells, digestive enzymes, and macrophages) that contribute to the growth and development of the infant (1). Maternal dietary habits may influence the composition of human milk, especially the fatty acids (FA) content (2). In particular, it has been shown that maternal dietary habits modify the content of polyunsaturated FA (PUFA) in human milk and, to a lesser extent, the content of saturated FA (SFA) and monounsaturated FA (MUFA) (3).

Many studies investigated the relationship between maternal nutrition and the composition of human milk according to the intake of single foods or nutrients (4), while a few studies evaluated the role of *a posteriori* dietary patterns (5–8). In particular, only one study from China (7) and one of our previous analyses (5) investigated the adherence of breastfeeding mothers to *a posteriori* dietary patterns concerning the content of FA in human milk. Two additional studies evaluated the relationship between the adherence of breastfeeding mothers to *a posteriori* dietary patterns and the composition of their human milk focusing on macronutrients, without taking into account FA (6, 8).

The traditional Mediterranean diet is a plant-oriented dietary pattern characterized by a high intake of minimally processed foods, fresh fruits, bread and other cereals (generally minimally refined), potatoes, legumes, and nuts; a moderate intake of dairy products (mostly cheese and yogurt); a moderate to low intake of fish and poultry; a low intake of red meat; a high intake of olive oil (especially virgin and extra-virgin olive oils) used as the main source of fat; and a moderate intake of wine, consumed with meals (9). Adherence to the Mediterranean diet has been associated with a range of health benefits, including a reduced risk of cardiovascular diseases, diabetes, metabolic syndrome, overweight and obesity, several cancer types, as well as a lower disease-specific and overall mortality (10–13). However, no study has investigated so far the association between maternal adherence to the Mediterranean diet, measured by one of the proposed scores, and the content of FA in human milk.

The Italian MEDIDIET study aims to evaluate the relationship between the habitual diet of breastfeeding mothers and the nutritional composition, as well as oxidative/anti-oxidative properties of their milk (14). The role of the Mediterranean diet in influencing human milk composition is at the core of the MEDIDIET study. Thus, we present here the FA human milk profile of breastfeeding mothers participating in the MEDIDIET study according to their adherence to the traditional Mediterranean diet.

MATERIALS AND METHODS

Study Design and Participants

Between 2012 and 2014, the MEDIDIET study was carried out in five Italian maternity wards (i.e., Turin, Florence, Rome, San Giovanni Rotondo – SGR, and Palermo) enrolling 300 healthy breastfeeding mothers. Information on study design, inclusion criteria, maternal diet assessment, human milk collection, and analysis of human milk samples has been provided in detail elsewhere (14). Briefly, mothers were 25–41 years old and gave birth to healthy term infants. In particular, inclusion criteria for mothers were

- not chronically diseased (i.e., free of diabetes, autoimmune diseases, cardiovascular disease, renal disease, and hypertension);
- seronegative for hepatitis B, hepatitis C, and human immunodeficiency viruses;
- non-smokers during pregnancy and lactation;
- non-abusers of drugs or alcohol;
- non-severely obese (i.e., BMI < 35 kg/m2);
- not on a restricted diet.

Inclusion criteria for infants were:

- born at ≥37 gestational weeks;
- weight range of 2,500–4,500 g;
- body length range of 46–56 cm;

- exclusively breastfed from birth to the day of human milk collection (i.e., 6 ± 1 week postpartum).

All mothers signed an informed consent form to participate in the study. The Ethics Committee of participating hospitals approved the study (protocol number: 31060 MD) as per the ethical standards of the Declaration of Helsinki.

Human Milk Collection and Analysis

On the day of human milk collection (i.e., 6 ± 1 weeks postpartum), mothers provided a sample of their foremilk (30–50 ml) expressed in the morning after breakfast and before lunch, using a breast pump. The time elapsed since the end of the previous breastfeeding session ranged from 1 to 3 h. The freshly expressed human milk was stirred and divided in sterile 10 ml tubes, overlaid with nitrogen gas to avoid oxidation, and then stored at −70°. Human milk analyses included macronutrient composition (i.e., protein, lactose, fat, and energy density), oxidative parameters, and complete FA profile as reported in Moro et al. (14). Briefly, FAs with a chain length between 4 and 24 carbon atoms of total lipids of human milk samples were analyzed as methyl esters by capillary gas chromatography with flame ionization detection ("GC-FID") according to the method proposed by Beermann et al. (15). In addition, the lipid content was analyzed using the method outlined by Lucas et al. (16) and Jones et al. (17). In particular, the FA analyzed included SFA, MUFA, trans-FA (TFA), branched-chain FA (BFA), and PUFA. Peak identification of SFA, MUFA, TFA, and both ω-3 and ω-6 FA methyl esters were verified and calibrated by comparison with authentic standards (NuChek Prep; Elysian, MN, United States: GLC-463, GLC-473, GLC-642, GLC-643, GLC-674, and Sigma-Aldrich Ltd., St. Louis, MO, United States: Supelco PUFA3). The standard mix of BFA methyl esters was originally analyzed by Danone Nutricia Research, Utrecht, the Netherlands. Lastly, FA concentrations were recorded as% of fats.

Assessment of Maternal Diet and Adherence to the Mediterranean Diet

A validated and reproducible food frequency questionnaire (FFQ) (18–20) was administered by trained interviewers to investigate the habitual diet of mothers (from partum to the day of human milk collection). The FFQ included 7 sections to collect information on the weekly intake of 78 food items, recipes, and beverages as follows: (1) milk, hot beverages, and sweeteners; (2) typical Italian first courses (e.g., pasta, risotto, gnocchi, lasagne); bread, and cereals; (3) second courses (including meat, fish, and cheese); (4) side dishes (e.g., vegetables); (5) fruits; (6) sweets, desserts, and soft drinks; and (7) alcoholic beverages. The serving size was defined in "natural" units (e.g., 1 cup of milk, 1 coffee spoon of sugar, 1 egg, 1 apple) or as smaller, on average, or larger than a standard Italian serving (e.g., 80 g of pasta, 100 g of mixed salad, 175 g of potatoes, and 150 g of beef). Occasional intakes (i.e., less than once a week, but greater than once per month) were coded as 0.5 servings per week. Dietary data collected using the FFQ were used to estimate maternal intake of energy and nutrients using an Italian food composition database (21). In this computation, we weighted the fat composition of each food or recipe according to information on the type of fat used for cooking or as dressing.

To evaluate the adherence of the maternal diet to the Mediterranean one, we used an *a priori* score (i.e., Mediterranean Diet Score – MDS) developed by Trichopoulou et al. (22, 23). According to the proposed methodology, the MDS includes 9 dietary components: (1) vegetables; (2) fruit; (3) cereals (including bread and potatoes); (4) legumes; (5) MUFA to SFA ratio (MUFA/SFA) as a proxy of olive oil consumption (23); (6) fish; (7) dairy products (including milk); (8) meat (including poultry, red and processed meat); (9) and alcohol. Each of the 9 components is assigned a score of 0 or 1 using fixed or median intakes as cutoffs according to the component considered. In particular, for components more frequently consumed in the Mediterranean diet (i.e., vegetables, fruit, cereals, legumes, fish, and MUFA/SFA), a score of 1 is given if the individual intake is greater or equal to the median, and 0 if the individual intake is lower than the median; for components less frequently consumed (i.e., dairy products and meat), a score of 1 is given if the individual intake is lower than the median, and 0 if the individual intake is greater or equal to the median. For alcohol component, a score of 1 is given for an individual consumption ranging from 5 to less than 25 g of ethanol/day, and 0 if the individual consumption is outside this range. The MDS is obtained by adding up the 9 component scores, and therefore, it potentially ranges from 0 (no adherence) to 9 (complete adherence) points.

Statistical Analyses

The FA content was not available for 18 human milk samples due to insufficient milk volume, thus leaving 282 samples for the present analysis. We categorized the MDS in approximate tertiles: 0–3 points (n = 97; 34.4%) for a low adherence, 4 points (n = 74; 26.4%) for a medium adherence, and 5–8 points (n = 111; 39.4%) for a high adherence to the Mediterranean diet. None of the mothers obtained the theoretical highest score (i.e., MDS = 9) mainly due to the common Italian behavior of excluding or substantially reducing alcohol from the diet during the lactation period (24). We compared the mean content of FA in human milk according to categories of MDS using ANOVA adjusting for the ratio between the maternal energy intake and maternal pre-pregnancy weight (kcal/day/kg). In addition, we used the test for trend to evaluate the linear relationship of the mean contents of FA in human milk across categories of MDS. All analyses were conducted using R version 4.0.5.

RESULTS

Description of the Study Population and Dietary Intake of Breastfeeding Mothers According to the Mediterranean Diet Score Components

Table 1 reports the demographic characteristics, pre-pregnancy BMI, and energy intake of the 282 breastfeeding mothers. Mothers had a mean age of 33 ± 4 years, a mean pre-pregnancy

TABLE 1 | Distribution of 282 Italian breastfeeding mothers according to center, age, pre-pregnancy weight and BMI, and energy intake.

Variable	Descriptive statistic[a]
Center	
Turin	96 (34.0)
Florence	21 (7.5)
Rome	46 (16.3)
SGR	99 (35.1)
Palermo	20 (7.1)
Age (years)	28–38; 33 ± 4
Pre-pregnancy weight (kg)	50–71; 60 ± 9
Pre-pregnancy BMI (kg/m^2)	18.8–26.2; 22.3 ± 3.2
Energy intake (kcal/day)	1485–2456; 1947 ± 443

[a]*Categorical variables are expressed as absolute frequency and relative frequency in parenthesis; continuous variables are expressed as 10th and 90th percentile range, and as mean ± standard deviation.*
SGR, San Giovanni Rotondo; BMI, body mass index. MEDIDIET study, 2012–2014.

BMI of 22.3 ± 3.2 kg/m^2, and a mean energy intake of 1947 ± 443 kcal/day.

The distributions of MDS components (10th, 25th, 50th, 75th, and 90th percentile and mean ± standard deviation) are reported in **Table 2**. Overall, the median intakes (servings/week) of components more frequently consumed in the Mediterranean diet were 11.0 for vegetables, 15.0 for fruit, 22.0 for cereals, 1.0 for legumes, and 2.0 for fish; the median intake of the MUFA/SFA component was 1.24. The median intakes (servings/week) of components less frequently consumed in the Mediterranean diet were 11.0 for dairy products, and 8.0 for meat; the median intake for the alcohol component was 0.0 g ethanol/day.

Content of Fatty Acids in Human Milk According to the Adherence of Breastfeeding Mothers to the Mediterranean Diet

High adherence to the Mediterranean diet was associated with a significantly lower content of SFA in human milk (p ANOVA = 0.02 and p for trend = 0.02; **Table 3**). In particular, the mean contents of SFA were 42.58 ± 4.36% of fats for mothers with low adherence to the Mediterranean diet (MDS = 0–3 points), 42.58 ± 4.89% of fats for mothers with medium adherence (MDS = 4 points), and 40.92 ± 5.22% of fats for mothers with high adherence (MDS = 5–8 points). Considering the major types of SFA, a high adherence of breastfeeding mothers to the Mediterranean diet was associated with a significantly lower content of palmitic acid (PA) and stearic acid (SA).

Conversely, high adherence to the Mediterranean diet was associated with a significantly higher content of MUFA in human milk (43.27 ± 4.27% of fats for MDS = 0–3 points; 43.27 ± 4.47% of fats for MDS = 4 points; 45.24 ± 5.22% of fats for MDS = 5–8 points; p ANOVA and p for trend < 0.01). Both the oleic acid (OA) and the erucic acid (EA), the major types of MUFA, significantly increased according to the adherence to the Mediterranean diet.

The content of PUFA in human milk was not associated with the adherence to the Mediterranean diet (p ANOVA = 0.62 and p for trend = 0.35). However, considering the major types of PUFA, a significantly lower content of arachidonic acid (AA) in human milk was observed among mothers with high adherence to the Mediterranean diet (0.48 ± 0.09% of fats for MDS = 0–3 points; 0.47 ± 0.09% of fats for MDS = 4 points; 0.46 ± 0.07% of fats for MDS = 5–8 points; p for trend = 0.01). Conversely, a significantly higher content of ω-3 FA in human milk was observed among mothers highly adherent to the Mediterranean diet (1.07 ± 0.25% of fats for MDS = 0–3 points, 1.22 ± 0.49% of fats for MDS = 4 points, and 1.31 ± 0.51% of fats for MDS = 5–8 points; p ANOVA and p for trend < 0.01). Likewise, significantly higher content of the major types of ω-3 FA in human milk (i.e., α-linolenic acid – ALA, eicosapentaenoic acid – EPA, docosahexaenoic acid – DHA, docosapentaenoic acid – DPA) was observed among mothers with high adherence to the Mediterranean diet. In addition, high adherence to the Mediterranean diet was associated with a significantly lower ratio between ω-6 and ω-3 FA (p ANOVA and p for trend < 0.01) and the ratio between LA and ALA (p ANOVA and p for trend < 0.01) in human milk. Conversely, high adherence to the Mediterranean diet was associated with a higher DHA/AA (p ANOVA = 0.01; p for trend < 0.01) and (EPA + DHA)/AA (p ANOVA and p for trend < 0.01) in human milk. No clear association was observed for the others FA considered.

Content of Fatty Acids in Human Milk According to the Adherence of Breastfeeding Mothers to Each Component of Mediterranean Diet Score

Table 4 reports the content of SFA in human milk according to the adherence of breastfeeding mothers to each component of MDS. For components more frequently consumed in the Mediterranean diet, one point (i.e., adherence) corresponded to an individual intake greater or equal to the median intake of vegetables, fruit, cereals, legumes, MUFA/SFA, and fish, respectively; for components less frequently consumed in the Mediterranean diet, one point corresponded to an individual intake less than the median intake of dairy products and meat, respectively; for alcohol component, one point corresponded to an individual alcohol intake ranging from 5 to less than 25 g of ethanol/day. Mothers who adhered to the vegetable component showed human milk with a significantly lower content of SFA (41.16 ± 5.29% of fats) than the human milk of non-adherent mothers (42.64 ± 4.41% of fats; p = 0.01). Likewise, mothers who were adherent to MUFA/SFA (41.07 ± 5.15 vs. 42.74 ± 4.52% of fats; p < 0.01) and dairy products (41.24 ± 4.71 vs. 42.58 ± 5.01; p = 0.02) components showed lower content of SFA in human milk. A similar pattern was observed for PA and SA.

Table 5 reports the content of MUFA in human milk according to the adherence of breastfeeding mothers to each component of MDS. A significantly higher content of MUFA was observed for mothers who adhered to the vegetables (44.73 ± 5.34 vs. 43.41 ± 4.14% of fats; p = 0.02), MUFA/SFA (44.90 ± 5.03 vs. 43.23 ± 4.42% of fats; p < 0.01), and dairy products

TABLE 2 | Distribution of components of the Mediterranean Diet Score.

MDS component	Percentile					Mean ± SD
	10th	25th	50th	75th	90th	
Vegetables (servings/week)	4.5	7.0	11.0	16.0	21.5	12.0 ± 7.0
Fruit (servings/week)	7.0	11.0	15.0	20.0	27.0	16.0 ± 8.5
Cereals (servings/week)	13.0	17.0	22.0	28.0	35.5	23.5 ± 10.0
Legumes (servings/week)	0.0	0.0	1.0	2.0	3.0	1.5 ± 1.5
MUFA/SFA	1.00	1.12	1.24	1.40	1.57	1.27 ± 0.23
Fish (servings/week)	0.5	1.0	2.0	2.0	3.0	1.5 ± 1.0
Dairy products (servings/week)	3.5	8.0	11.0	14.0	18.5	11.0 ± 5.5
Meat (servings/week)	5.0	6.5	8.0	10.0	12.5	8.5 ± 3.0
Alcohol (grams/day)	0.0	0.0	0.0	1.1	3.6	1.0 ± 2.5

MDS, Mediterranean Diet Score; MUFA, monounsaturated fatty acids; SD, standard deviation; SFA, saturated fatty acids. MEDIDIET study, 2012–2014.

TABLE 3 | Mean and standard deviation contents of selected fatty acids (expressed as% of fats) in human milk according to the adherence of breastfeeding mothers to the Mediterranean Diet Score (expressed approximately in tertiles).

FA in human milk	Total		MDS			*p*-value (ANOVA)[a]	*p*-value (trend)[a]
			0–3 points *n* = 97 (34.4)	4 points *n* = 74 (26.2)	5–8 points *n* = 111 (39.4)		
	Min-max	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
SFA (% of fats)	27.89–56.11	41.92 ± 4.90	42.58 ± 4.36	42.58 ± 4.89	40.92 ± 5.22	$p = 0.02$	$p = 0.02$
CPA (% of fats)	0.08–0.51	0.24 ± 0.07	0.23 ± 0.08	0.25 ± 0.07	0.25 ± 0.07	$p = 0.14$	$p = 0.13$
CA (% of fats)	0.01–3.04	1.50 ± 0.42	1.43 ± 0.44	1.55 ± 0.42	1.53 ± 0.40	$p = 0.11$	$p = 0.08$
LAU (% of fats)	1.90–12.11	5.18 ± 1.92	4.99 ± 1.81	5.54 ± 2.14	5.11 ± 1.85	$p = 0.15$	$p = 0.62$
MA (% of fats)	2.11–11.89	5.45 ± 1.65	5.40 ± 1.58	5.69 ± 1.79	5.33 ± 1.62	$p = 0.33$	$p = 0.80$
PA (% of fats)	15.65–28.61	22.45 ± 2.40	23.18 ± 1.98	22.56 ± 2.43	21.75 ± 2.53	$p < 0.01$	$p < 0.01$
SA (% of fats)	3.26–9.27	5.81 ± 1.04	6.05 ± 1.00	5.71 ± 1.00	5.66 ± 1.06	$p = 0.01$	$p < 0.01$
MUFA (% of fats)	31.12–60.82	44.05 ± 4.79	43.27 ± 4.27	43.27 ± 4.47	45.24 ± 5.22	$p < 0.01$	$p < 0.01$
OA (% of fats)	26.23–56.63	39.29 ± 4.56	38.49 ± 4.00	38.61 ± 4.19	40.45 ± 5.03	$p < 0.01$	$p < 0.01$
EA (% of fats)	0.04–0.20	0.08 ± 0.02	0.07 ± 0.02	0.07 ± 0.02	0.08 ± 0.08	$p = 0.01$	$p < 0.01$
PUFA (% of fats)	9.03–24.32	13.59 ± 2.52	13.71 ± 2.52	13.71 ± 2.42	13.41 ± 2.61	$p = 0.62$	$p = 0.35$
ω-6 (% of fats)	8.14–23.10	12.39 ± 2.51	12.64 ± 2.51	12.49 ± 2.38	12.10 ± 2.58	$p = 0.27$	$p = 0.11$
LA (% of fats)	6.41–21.42	10.89 ± 2.43	11.13 ± 2.47	10.97 ± 2.27	10.63 ± 2.51	$p = 0.33$	$p = 0.14$
AA (% of fats)	0.27–0.73	0.47 ± 0.08	0.48 ± 0.09	0.47 ± 0.09	0.46 ± 0.07	$p = 0.07$	$p = 0.01$
ω-3 (% of fats)	0.68–4.40	1.21 ± 0.45	1.07 ± 0.25	1.22 ± 0.49	1.31 ± 0.51	$p < 0.01$	$p < 0.01$
ALA (% of fats)	0.27–1.15	0.54 ± 0.17	0.49 ± 0.14	0.54 ± 0.17	0.57 ± 0.18	$p < 0.01$	$p < 0.01$
EPA (% of fats)	0.01–0.38	0.06 ± 0.04	0.04 ± 0.02	0.06 ± 0.05	0.06 ± 0.05	$p < 0.01$	$p < 0.01$
DHA (% of fats)	0.09–2.30	0.29 ± 0.22	0.25 ± 0.12	0.30 ± 0.24	0.33 ± 0.26	$p = 0.03$	$p = 0.01$
DPA (% of fats)	0.03–0.59	0.12 ± 0.06	0.11 ± 0.03	0.13 ± 0.06	0.13 ± 0.07	$p = 0.02$	$p = 0.02$
ω-6/ω-3	2.50–28.28	11.17 ± 3.70	12.40 ± 3.79	11.13 ± 3.57	10.13 ± 3.39	$p < 0.01$	$p < 0.01$
LA/ALA	5.95–54.09	21.87 ± 7.40	24.05 ± 7.21	21.90 ± 7.63	19.93 ± 6.92	$p < 0.01$	$p < 0.01$
AA/LA	0.02–0.09	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	$p = 0.71$	$p = 0.67$
DHA/ALA	0.12–4.56	0.57 ± 0.38	0.54 ± 0.30	0.56 ± 0.31	0.59 ± 0.46	$p = 0.63$	$p = 0.35$
DHA/AA	0.23–5.25	0.64 ± 0.48	0.52 ± 0.23	0.66 ± 0.61	0.72 ± 0.53	$p = 0.01$	$p < 0.01$
(EPA + DHA)/ALA	0.14–5.00	0.67 ± 0.43	0.64 ± 0.35	0.67 ± 0.37	0.70 ± 0.52	$p = 0.56$	$p = 0.29$
(EPA + DHA)/AA	0.29–6.21	0.76 ± 0.57	0.61 ± 0.27	0.79 ± 0.74	0.86 ± 0.62	$p < 0.01$	$p < 0.01$

[a]*Adjusted for maternal energy intake/maternal pre-pregnancy weight (kcal/day/kg).*

ω-3, omega-3; ω-6, omega-6; AA, arachidonic acid; ALA, α-linolenic acid; DHA, decosahexaenoic acid; DPA, docosapentaenoic acid; CA, capric acid; CPA, caprylic acid; EA, euricic acid; EPA, eicosapentaenoic acid; FA, fatty acid; LA, linoleic acid; LAU, lauric acid; MA, myristic acid; MDS, Mediterranean Diet Score; MUFA, monounsaturated fatty acid; OA, oleic acid; PA, palmitic acid; PUFA, polyunsaturated fatty acid; SA, stearic acid; SD, standard deviation; SFA, saturated fatty acid. MEDIDIET study, 2012–2017.

(44.87 ± 4.47 vs. 43.26 ± 4.97% of fats; $p < 0.01$) components. The adherence of such components resulted in human milk with higher contents of OA and EA.

Table 6 reports the content of PUFA in human milk according to the adherence of breastfeeding mothers to each component of MDS. A significantly higher content

TABLE 4 | Mean and standard deviation contents of major saturated fatty acids in human milk (expressed as% of fats) according to the adherence of breastfeeding mothers to single components of the Mediterranean Diet Score.

MDS component	FA in human milk (% of fats)						
	SFA Mean ± SD	CPA Mean ± SD	CA Mean ± SD	LAU Mean ± SD	MA Mean ± SD	PA Mean ± SD	SA Mean ± SD
Vegetables[a]							
0 point	42.64 ± 4.41	0.24 ± 0.07	1.47 ± 0.43	5.20 ± 1.88	5.50 ± 1.55	23.02 ± 2.29	5.92 ± 1.13
1 point	41.16 ± 5.29	0.25 ± 0.08	1.53 ± 0.41	5.16 ± 1.97	5.39 ± 1.77	21.84 ± 2.38	5.68 ± 0.91
p-value (*t*-test)[b]	$p = 0.01$	$p = 0.19$	$p = 0.22$	$p = 0.85$	$p = 0.60$	$p < 0.01$	$p = 0.06$
Fruit[a]							
0 point	41.41 ± 4.92	0.24 ± 0.07	1.44 ± 0.43	4.99 ± 1.91	5.26 ± 1.62	22.32 ± 2.38	5.85 ± 1.00
1 point	42.37 ± 4.86	0.25 ± 0.07	1.54 ± 0.41	5.34 ± 1.93	5.61 ± 1.67	22.56 ± 2.43	5.76 ± 1.06
p-value (*t*-test)[b]	$p = 0.10$	$p = 0.20$	$p = 0.04$	$p = 0.13$	$p = 0.08$	$p = 0.40$	$p = 0.47$
Cereals[a]							
0 point	41.89 ± 5.22	0.25 ± 0.08	1.49 ± 0.44	5.19 ± 2.07	5.45 ± 1.75	22.45 ± 2.48	5.76 ± 0.96
1 point	41.96 ± 4.58	0.24 ± 0.07	1.50 ± 0.40	5.17 ± 1.77	5.44 ± 1.55	22.46 ± 2.33	5.85 ± 1.11
p-value (*t*-test)[b]	$p = 0.91$	$p = 0.60$	$p = 0.90$	$p = 0.92$	$p = 0.95$	$p = 0.98$	$p = 0.46$
Legumes[a]							
0 point	41.90 ± 4.85	0.24 ± 0.07	1.46 ± 0.44	5.08 ± 1.85	5.37 ± 1.64	22.64 ± 2.29	5.81 ± 1.00
1 point	41.95 ± 4.97	0.25 ± 0.07	1.53 ± 0.41	5.28 ± 2.00	5.52 ± 1.67	22.26 ± 2.50	5.80 ± 1.08
p-value (*t*-test)[b]	$p = 0.93$	$p = 0.07$	$p = 0.18$	$p = 0.40$	$p = 0.46$	$p = 0.19$	$p = 0.89$
MUFA/SFA[a]							
0 point	42.74 ± 4.52	0.24 ± 0.08	1.49 ± 0.45	5.22 ± 1.94	5.58 ± 1.66	22.97 ± 2.14	5.94 ± 1.01
1 point	41.07 ± 5.15	0.25 ± 0.07	1.51 ± 0.39	5.14 ± 1.91	5.32 ± 1.65	21.91 ± 2.54	5.67 ± 1.05
p-value (*t*-test)[b]	$p < 0.01$	$p = 0.61$	$p = 0.71$	$p = 0.73$	$p = 0.19$	$p < 0.01$	$p = 0.03$
Fish[a]							
0 point	42.38 ± 4.34	0.24 ± 0.07	1.49 ± 0.38	5.23 ± 1.83	5.49 ± 1.51	22.74 ± 2.24	5.87 ± 1.02
1 point	41.52 ± 5.35	0.25 ± 0.08	1.50 ± 0.45	5.13 ± 2.01	5.41 ± 1.78	21.19 ± 2.52	5.74 ± 1.05
p-value (*t*-test)[b]	$p = 0.14$	$p = 0.58$	$p = 0.86$	$p = 0.67$	$p = 0.66$	$p = 0.06$	$p = 0.29$
Dairy products[c]							
0 point	42.58 ± 5.01	0.25 ± 0.08	1.53 ± 0.45	5.25 ± 1.94	5.63 ± 1.73	22.70 ± 2.35	5.88 ± 1.03
1 point	41.24 ± 4.71	0.24 ± 0.07	1.47 ± 0.39	5.10 ± 1.91	5.26 ± 1.55	22.19 ± 2.43	5.72 ± 1.04
p-value (*t*-test)[b]	$p = 0.02$	$p = 0.16$	$p = 0.26$	$p = 0.53$	$p = 0.06$	$p = 0.07$	$p = 0.19$
Meat[c]							
0 point	41.96 ± 4.77	0.24 ± 0.07	1.48 ± 0.45	5.12 ± 2.07	5.37 ± 1.73	22.57 ± 2.28	5.89 ± 1.05
1 point	41.89 ± 5.05	0.25 ± 0.07	1.51 ± 0.39	5.24 ± 1.77	5.53 ± 1.58	22.33 ± 2.52	5.72 ± 1.02
p-value (*t*-test)[b]	$p = 0.90$	$p = 0.61$	$p = 0.58$	$p = 0.57$	$p = 0.43$	$p = 0.40$	$p = 0.16$
Alcohol[d]							
0 point	41.96 ± 4.93	0.24 ± 0.07	1.50 ± 0.42	5.19 ± 1.92	5.46 ± 1.66	22.46 ± 2.44	5.81 ± 1.04
1 point	41.26 ± 4.50	0.23 ± 0.08	1.47 ± 0.47	5.05 ± 1.97	5.15 ± 1.52	22.36 ± 1.61	5.77 ± 0.97
p-value (*t*-test)[b]	$p = 0.60$	$p = 0.55$	$p = 0.80$	$p = 0.79$	$p = 0.48$	$p = 0.89$	$p = 0.89$

*[a] 1 point is assigned to an individual intake greater or equal to the median intake reported in the **Table 1**; [b] Adjusted for maternal energy intake/maternal pre-pregnancy weight (kcal/day/kg); [c] 1 point is assigned to an individual intake less than the median intake reported in the **Table 1**; [d] 1 point is assigned to an individual intake ranging from 5 to less than 25 g of ethanol/day.*

CA, capric acid; CPA, caprylic acid; FA, fatty acid; LAU, lauric acid; MA, myristic acid; MDS, Mediterranean Diet Score; PA, palmitic acid; SA, stearic acid; SD, standard deviation; SFA, saturated fatty acid. MEDIDIET study, 2012–2017.

of ω-3 FA was observed for mothers who adhered to the vegetables (1.30 ± 0.48 vs. 1.12 ± 0.39% of fats $p < 0.01$), MUFA/SFA (1.30 ± 0.56 vs 1.12 ± 0.27; $p < 0.01$), and fish (1.29 ± 0.50 vs. 1.12 ± 0.36; $p < 0.01$). Similarly, the adherence of breastfeeding mothers to vegetables, MUFA/SFA, and fish components was significantly associated with human milk with higher contents of ALA, EPA, DHA, and DPA.

DISCUSSION

We assessed the association between the adherence of breastfeeding mothers to the Mediterranean diet and the FA content of their human milk using data from the Italian MEDIDIET study. High adherence to the Mediterranean diet was associated with lower content of SFA (including PA and SA), ω-6/ω-3, and LA/ALA, and higher content of MUFA

TABLE 5 | Mean and standard deviation contents of major monounsaturated fatty acids in human milk (expressed as% of fats) according to the adherence of breastfeeding mothers to single components of the Mediterranean Diet Score.

MDS component	FA in human milk (% of fats)		
	MUFA Mean ± SD	OA Mean ± SD	EA Mean ± SD
Vegetables[a]			
0 point	43.41 ± 4.14	38.65 ± 3.84	0.07 ± 0.02
1 point	44.73 ± 5.34	39.98 ± 5.15	0.08 ± 0.02
p-value (*t*-test)[b]	$p = 0.02$	$p = 0.01$	$p < 0.01$
Fruit[a]			
0 point	44.49 ± 4.86	39.76 ± 4.69	0.07 ± 0.02
1 point	43.67 ± 4.72	38.89 ± 4.42	0.08 ± 0.02
p-value (*t*-test)[b]	$p = 0.15$	$p = 0.11$	$p = 0.80$
Cereals[a]			
0 point	43.90 ± 5.02	39.14 ± 4.75	0.08 ± 0.02
1 point	44.19 ± 4.57	39.44 ± 4.37	0.07 ± 0.02
p-value (*t*-test)[b]	$p = 0.62$	$p = 0.58$	$p = 0.08$
Legumes[a]			
0 point	44.06 ± 4.81	39.29 ± 4.54	0.08 ± 0.02
1 point	44.03 ± 4.79	39.29 ± 4.59	0.08 ± 0.02
p-value (*t*-test)[b]	$p = 0.95$	$p = 0.99$	$p = 0.88$
MUFA/SFA[a]			
0 point	43.23 ± 4.42	38.47 ± 4.11	0.07 ± 0.02
1 point	44.90 ± 5.03	40.14 ± 4.85	0.08 ± 0.02
p-value (*t*-test)[b]	$p < 0.01$	$p < 0.01$	$p < 0.01$
Fish[a]			
0 point	43.73 ± 4.15	38.95 ± 3.95	0.07 ± 0.02
1 point	44.33 ± 5.31	39.59 ± 5.05	0.08 ± 0.02
p-value (*t*-test)[b]	$p = 0.29$	$p = 0.24$	$p = 0.34$
Dairy products[c]			
0 point	43.26 ± 4.97	38.55 ± 4.67	0.07 ± 0.02
1 point	44.87 ± 4.47	40.07 ± 4.32	0.08 ± 0.02
p-value (*t*-test)[b]	$p < 0.01$	$p < 0.01$	$p = 0.05$
Meat[c]			
0 point	43.97 ± 4.59	39.18 ± 4.42	0.08 ± 0.02
1 point	44.13 ± 5.01	39.40 ± 4.71	0.07 ± 0.02
p-value (*t*-test)[b]	$p = 0.78$	$p = 0.69$	$p = 0.76$
Alcohol[d]			
0 point	44.00 ± 4.80	39.24 ± 4.58	0.08 ± 0.02
1 point	44.97 ± 4.72	40.24 ± 4.23	0.08 ± 0.01
p-value (*t*-test)[b]	$p = 0.46$	$p = 0.42$	$p = 0.74$

*[a] 1 point is assigned to an individual intake greater or equal to the median intake reported in the **Table 1**; [b] Adjusted for maternal energy intake/maternal pre-pregnancy weight (kcal/day/kg); [c] 1 point is assigned to an individual intake less than the median intake reported in the **Table 1**; [d] 1 point is assigned to an individual intake ranging from 5 to less than 25 g of ethanol/day.*

EA, euricic acid; FA, fatty acid; MDS, Mediterranean Diet Score; MUFA, monounsaturated fatty acid; OA, oleic acid; SD, standard deviation; SFA, saturated fatty acid. MEDIDIET study, 2012–2017.

(including OA and EA), ω-3 FA (including ALA, EPA, DHA, and DPA), and DHA/AA and (EPA + DHA)/AA) in human milk. These associations were mainly driven by the adherence to the vegetables, MUFA/SFA, fish, and dairy products components of the Mediterranean diet.

Previous studies investigated the relationship between maternal intakes of single foods or nutrients and the FA composition in human milk. A longitudinal Finnish study (25) reported a weak positive correlation ($r = 0.21$) between maternal intake of high-fat dairy products and the content of SFA in human milk, collected at 3 months of lactation. Likewise, we observed a lower content of SFA in human milk for mothers with high adherence to the Mediterranean diet, which is characterized by a moderate to low consumption of dairy products. Accordingly, two studies (26, 27) found positive correlations between maternal intake of SFA and the content of SFA in human milk ($r = 0.60$ and $r = 0.215$, respectively). The lower content of SFA in human milk observed among mothers

TABLE 6 | Mean and standard deviation contents of major polyunsaturated fatty acids in human milk (expressed as% of fats) according to the adherence of breastfeeding mothers to single components of the Mediterranean Diet Score.

MDS component	FA in human milk (% of fats)								
	PUFA Mean ± SD	ω-6 Mean ± SD	LA Mean ± SD	AA Mean ± SD	ω-3 Mean ± SD	ALA Mean ± SD	EPA Mean ± SD	DHA Mean ± SD	DPA Mean ± SD
Vegetables[a]									
0 point	13.52 ± 2.40	12.40 ± 2.34	10.90 ± 2.27	0.47 ± 0.08	1.12 ± 0.39	0.50 ± 0.15	0.05 ± 0.04	0.27 ± 0.18	0.11 ± 0.05
1 point	13.67 ± 2.66	12.37 ± 2.69	10.88 ± 2.61	0.46 ± 0.08	1.30 ± 0.48	0.57 ± 0.17	0.06 ± 0.05	0.32 ± 0.25	0.13 ± 0.06
p-value (*t*-test)[b]	$p = 0.61$	$p = 0.93$	$p = 0.94$	$p = 0.26$	$p < 0.01$	$p < 0.01$	$p = 0.06$	$p = 0.04$	$p < 0.01$
Fruit[a]									
0 point	13.66 ± 2.43	12.48 ± 2.41	10.99 ± 2.34	0.47 ± 0.09	1.18 ± 0.41	0.53 ± 0.15	0.05 ± 0.04	0.29 ± 0.21	0.12 ± 0.05
1 point	13.53 ± 2.61	12.30 ± 2.59	10.81 ± 2.52	0.47 ± 0.08	1.23 ± 0.47	0.54 ± 0.18	0.06 ± 0.05	0.30 ± 0.23	0.12 ± 0.06
p-value (*t*-test)[b]	$p = 0.65$	$p = 0.55$	$p = 0.53$	$p = 0.43$	$p = 0.39$	$p = 0.50$	$p = 0.49$	$p = 0.62$	$p = 0.49$
Cereals[a]									
0 point	13.76 ± 2.32	12.51 ± 2.29	11.02 ± 2.23	0.47 ± 0.08	1.26 ± 0.51	0.55 ± 0.19	0.06 ± 0.05	0.32 ± 0.27	0.12 ± 0.06
1 point	13.42 ± 2.70	12.27 ± 2.70	10.77 ± 2.62	0.47 ± 0.09	1.15 ± 0.36	0.52 ± 0.14	0.05 ± 0.04	0.27 ± 0.15	0.12 ± 0.05
p-value (*t*-test)[b]	$p = 0.25$	$p = 0.43$	$p = 0.39$	$p = 0.74$	$p = 0.05$	$p = 0.09$	$p = 0.19$	$p = 0.06$	$p = 0.23$
Legumes[a]									
0 point	13.61 ± 2.51	12.42 ± 2.48	10.93 ± 2.42	0.47 ± 0.09	1.19 ± 0.44	0.53 ± 0.16	0.06 ± 0.05	0.29 ± 0.22	0.12 ± 0.05
1 point	13.57 ± 2.55	12.35 ± 2.55	10.85 ± 2.45	0.47 ± 0.08	1.23 ± 0.45	0.54 ± 0.17	0.06 ± 0.04	0.30 ± 0.22	0.12 ± 0.06
p-value (*t*-test)[b]	$p = 0.90$	$p = 0.80$	$p = 0.79$	$p = 0.45$	$p = 0.44$	$p = 0.48$	$p = 0.93$	$p = 0.86$	$p = 0.83$
MUFA/SFA[a]									
0 point	13.59 ± 2.65	12.47 ± 2.65	10.97 ± 2.58	0.48 ± 0.09	1.12 ± 0.27	0.51 ± 0.15	0.05 ± 0.03	0.26 ± 0.12	0.11 ± 0.04
1 point	13.59 ± 2.40	12.30 ± 2.36	10.81 ± 2.28	0.46 ± 0.08	1.30 ± 0.56	0.57 ± 0.18	0.06 ± 0.06	0.33 ± 0.29	0.13 ± 0.07
p-value (*t*-test)[b]	$p = 0.98$	$p = 0.57$	$p = 0.60$	$p = 0.24$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p = 0.01$	$p = 0.01$
Fish[a]									
0 point	13.45 ± 2.40	12.34 ± 2.41	10.83 ± 2.34	0.47 ± 0.09	1.12 ± 0.36	0.51 ± 0.16	0.05 ± 0.04	0.25 ± 0.15	0.11 ± 0.05
1 point	13.72 ± 2.63	12.43 ± 2.60	10.94 ± 2.52	0.47 ± 0.08	1.29 ± 0.50	0.55 ± 0.17	0.06 ± 0.05	0.33 ± 0.26	0.13 ± 0.06
p-value (*t*-test)[b]	$p = 0.39$	$p = 0.75$	$p = 0.71$	$p = 0.98$	$p < 0.01$	$p = 0.04$	$p < 0.01$	$p < 0.01$	$p = 0.03$
Dairy products[c]									
0 point	13.71 ± 2.76	12.50 ± 2.77	10.99 ± 2.70	0.47 ± 0.09	1.20 ± 0.42	0.52 ± 0.16	0.06 ± 0.04	0.30 ± 0.21	0.12 ± 0.06
1 point	13.47 ± 2.25	12.26 ± 2.19	10.79 ± 2.12	0.46 ± 0.08	1.21 ± 0.47	0.55 ± 0.17	0.05 ± 0.05	0.29 ± 0.23	0.12 ± 0.05
p-value (*t*-test)[b]	$p = 0.44$	$p = 0.42$	$p = 0.49$	$p = 0.39$	$p = 0.87$	$p = 0.16$	$p = 0.61$	$p = 0.83$	$p = 0.32$
Meat[c]									
0 point	13.64 ± 2.36	12.42 ± 2.37	10.89 ± 2.30	0.48 ± 0.09	1.22 ± 0.48	0.54 ± 0.17	0.06 ± 0.05	0.29 ± 0.24	0.12 ± 0.06
1 point	13.54 ± 2.68	12.35 ± 2.64	10.89 ± 2.57	0.45 ± 0.08	1.19 ± 0.41	0.53 ± 0.16	0.05 ± 0.04	0.29 ± 0.20	0.12 ± 0.05
p-value (*t*-test)[b]	$p = 0.76$	$p = 0.82$	$p = 0.99$	$p < 0.01$	$p = 0.65$	$p = 0.58$	$p = 0.73$	$p = 0.99$	$p = 0.32$
Alcohol[d]									
0 point	13.60 ± 2.55	12.39 ± 2.53	10.90 ± 2.46	0.47 ± 0.08	1.21 ± 0.45	0.54 ± 0.17	0.06 ± 0.05	0.29 ± 0.23	0.12 ± 0.06
1 point	13.38 ± 1.96	12.31 ± 2.01	10.68 ± 1.89	0.51 ± 0.10	1.07 ± 0.16	0.48 ± 0.10	0.05 ± 0.01	0.27 ± 0.08	0.11 ± 0.02
p-value (*t*-test)[b]	$p = 0.75$	$p = 0.91$	$p = 0.74$	$p = 0.07$	$p = 0.25$	$p = 0.17$	$p = 0.41$	$p = 0.63$	$p = 0.49$

*[a] 1 point is assigned to an individual intake greater or equal to the median intake reported in the **Table 1**; [b] Adjusted for maternal energy intake/maternal pre-pregnancy weight (kcal/day/kg); [c] 1 point is assigned to an individual intake less than the median intake reported in the **Table 1**; [d] 1 point is assigned to an individual intake ranging from 5 to less than 25 g of ethanol/day.*

ω-3, omega-3; ω-6, omega-6; AA, arachidonic acid; ALA, α-linolenic acid; DHA, decosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MDS, Mediterranean Diet Score; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SD, standard deviation; SFA, saturated fatty acid. MEDIDIET study, 2012–2017.

with high adherence to the Mediterranean diet could not provide optimal food for the infant's needs (2, 28–31). However, the mean content of SFA in human milk among mothers who highly adhered to the Mediterranean diet (40.92 ± 5.22% of fats) was in agreement with the typical human milk composition of FA, which contains approximately 35–45% of SFA, 45–50% of MUFA, and 15% of PUFA (32, 33).

The Italian cross-sectional study by Scopesi et al. (26) reported a positive correlation of 0.63 between maternal intake of MUFA and the content of MUFA in human milk (collected during the 1st month of lactation) which is in agreement with the higher content of MUFA in human milk observed in this study for mothers who were adherent to the Mediterranean diet. The higher content of MUFA in human milk for these mothers likely derives from the consumption of olive oil, the major fat source of the Mediterranean diet, which is rich in MUFA, especially in the form of OA (34).

A cross-sectional study conducted in Denmark (35) reported a higher content of ω-3 FA (including EPA, DHA, and DPA) in human milk (collected at 4 months postpartum) for mothers who had a high intake of fish, as compared to mothers who did not consume it. A longitudinal Greek study (36) reported positive correlations between maternal PUFA intake and the contents of ω-3 FA ($r = 0.26$) and DHA ($r = 0.27$) in human milk, collected in the 1st month of lactation. Likewise, the cross-sectional study of Kim et al. (27) reported a weak positive correlation ($r = 0.211$) between maternal intake of PUFA and the content of ω-3 FA in human milk (collected from 1 to 11 months postpartum) for South Korean mothers. The cross-sectional study by Olafsdottir et al. (37) reported a positive correlation of 0.43 between maternal PUFA intake and the content of ALA in human milk (collected from 2 to 4 months postpartum) for Icelandic mothers. We observed a higher content of ω-3 FA and the major types of ω-3 FA in human milk for mothers with high adherence to the Mediterranean diet. The moderate consumption of fish jointly with the high consumption of vegetables (various of them are relatively rich in ω-3 FA) results in an appreciable intake of ω-3 FA for populations belonging to the Mediterranean area (34).

Three Chinese studies assessed the association between *a posteriori* dietary patterns of breastfeeding mothers and the composition of human milk (6–8). Dietary patterns were derived through principal component analysis based on food group intakes of mothers. Among the dietary patterns identified in the cross-sectional study by Hu et al. (6), the "high-in-plant-foods" dietary pattern was negatively associated with total fats content in human milk, collected within 2 months postpartum. We observed a lower content of SFA, a higher content of MUFA, and a stationary content of PUFA in human milk for high adherence to the Mediterranean diet. In the cross-sectional study by Tian et al. (7), the dietary pattern characterized by "dairy and soybean products, and nuts" showed higher content of SFA in human milk; the dietary pattern characterized by "meats, mushrooms and algae, and marine products" and the dietary pattern characterized by "vegetables and fruit" showed higher content of PUFA and ω-6 FA in human milk (collected from 22 days to 6 months postpartum). In our study, neither the adherence to the Mediterranean diet nor the adherence to vegetables, fruit, and fish components was associated with the contents of PUFA and ω-6 FA in human milk. No dietary pattern identified in the cross-sectional study of Huang et al. was associated with the FA profile in human milk, collected in the postpartum period from 7 days to 6 months and beyond (8). In our previous analysis of the same data, we identified five *a posteriori* dietary patterns based on nutrient intakes of breastfeeding mothers (5). The nutritional profile of three out of five dietary patterns, named "Proteins and FA with legs," "FA with fins," and "FA with leaves," was consistent with the Mediterranean diet and therefore they showed similar associations with the FA content in human milk. In particular, the "Proteins and FA with legs" dietary pattern, characterized by high maternal intake of animal proteins (including dairy products), was weakly positively correlated ($r = 0.12$) with the content of SFA and weakly negatively correlated ($r = -0.20$) with the content of MUFA in human milk. The "FA with fins" dietary pattern, characterized by high maternal intake of fish, was weakly positively correlated ($r = 0.23$) with the content of ω-3 FA (including ALA, EPA, DHA, and DPA) in human milk. The "FA with leaves," dietary pattern characterized by a high intake of vegetables, was weakly positively correlated ($r = 0.17$) with the content of MUFA and weakly negatively correlated ($r = -0.19$) with the content of SFA in human milk.

The FA in human milk derives from the endogenous synthesis in the mammary gland and uptake from maternal plasma. In both cases, the content of FA in human milk may be influenced by maternal nutrition (2, 38, 39). Furthermore, the role of FA in the infant diet is gaining interest because FA is involved in several growth processes, especially in visual and neural functions (28, 40). In particular, DHA is selectively enriched in a few specific membrane lipids, which include the glycerophospholipids of the retina and brain gray matter (29). High concentrations of DHA are present in phosphatidylserine and the ethanolamine phosphoacylglycerols of gray matter and the outer segments of rod and cone photoreceptors in the retina (41). Considerable evidence showed that poor maternal DHA status increased the risk of inadequate transferring of DHA in human milk and consequently a delayed or reduced neural and visual system development of the infant (30, 42–44).

Strengths and Limitations

We observed some maternal dietary behaviors diverging from the typical Mediterranean diet such as an overall low intake of legumes, an overall quite high intake of dairy products and meat, and a low intake of vegetables for mothers from Southern Italy (**Supplementary Table 1**). This could be partially explained by the results of a recent internet survey describing the nutritional behavior of the Italian population and their compliance with the Mediterranean pyramid recommendations. The authors reported "a rather low adherence" of participants to some Mediterranean pyramid recommendations, especially by females and in the South (45). In addition, we observed that the human milk of mothers in the South had a lower content of ω-6 and ALA (**Supplementary Table 2**), likely due to these changing dietary habits. Nevertheless, we generally did not find other modified associations between the adherence to the Mediterranean diet and the content of FA in human milk across the strata of geographical area (**Supplementary Table 2**).

Some studies showed differences in the FA profile of human milk according to maternal pre-pregnancy weight and/or BMI (46, 47). However, no difference emerged in the distributions of maternal pre-pregnancy weight and BMI according to the adherence to the MDS, excluding possible confounding effects of these variables (**Supplementary Table 3**). Nevertheless, mothers highly adherent to the Mediterranean diet tended to be older than those with low or medium adherence, not excluding a possible residual confounding due to age (**Supplementary Table 3**). In addition, the cross-sectional design of this study did not allow to establish a causal relationship between the Mediterranean diet and the content of FA in human milk. Nevertheless, it is unlikely that differences in the contents of FA in human milk could have modified the maternal adherence to the Mediterranean diet. However, it could not be ruled out that the existence of external

factors (e.g., socioeconomic status or healthy lifestyles) trigger the adherence to the Mediterranean diet and the composition of human milk in parallel and causatively influence the content of FA. Dietary information of breastfeeding mothers was self-reported and possibly biased from incomplete recall. However, it was collected by trained interviewers using structured FFQ which has been validated (18) and tested for the reproducibility (19, 20) in the Italian adult population, minimizing possible recall bias. Although we used a validated and reproducible FFQ, the maternal nutrient intakes could be underestimated. Nevertheless, we previously compared maternal nutrient intakes of mothers participating in the MEDIDIET study with those of mothers from other developed countries in a reviewing framework. The energy intake of mothers participating in the MEDIDIET study was lower than the average energy requirement (i.e., 2,300 kcal/day) recommended during the lactation period, but it was in agreement with those of the majority of studies included in the review (48).

Possible systematic and random errors in the assessment of the content of FA in human milk were reduced by a standard protocol for the collection and analysis of human milk samples. In addition, we collected all human milk samples in a short window of time (i.e., 6 ± 1 weeks postpartum) and in the morning for all mothers participating in the MEDIDIET study, avoiding the variability due to different lactation stages or different times of the day. However, we did not collect human milk samples repeatedly over time making it difficult to make conclusions on the impact of maternal diet on the composition of human milk in a comprehensive way.

Another limitation of this study is the lack of information on follow-up of the infant growth. Thus, we can establish the relationship between maternal nutrition and human milk composition and only infer the relationship between human milk properties and infant outcomes using results from external studies.

The multicentric design of the MEDIDIET study allowed us to include mothers from different Italian regions strengthening the findings of this study. The inclusion of healthy breastfeeding mothers with healthy infants allowed transferring of these results to the general population. In addition, another strength includes the detailed set of FA in human milk provided in this analysis. Lastly, to the best of our knowledge, this is the first study aimed to evaluate the relationship between the Mediterranean diet and the content of FA in human milk using a formal score of adherence.

CONCLUSION

High adherence to the Mediterranean diet was associated with human milk with lower content of SFA and higher contents of MUFA, ω-3 FA, and the major types of ω-3 FA, including DHA. The Mediterranean diet may contribute to human milk production with higher content of specific FA which is directly involved in the growth and development of neural and visual functions of the infant, as reported by previous studies. If the results of this study were confirmed by further analyses, possibly including infant outcomes, this would help in the development of dietary guidelines for breastfeeding mothers to promote good dietary practices – such as the Mediterranean diet – and their positive implications for infants.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of participating hospitals. The patients/participants provided their written informed consent to participate in this study.

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AUTHOR CONTRIBUTIONS

GM, MF, and AD designed the research idea. FB and MD performed the statistical analyses and wrote the manuscript. MF, FB, MD, CA, SE, EB, GM, and BS interpreted the results. GM directed data acquisition of the MEDIDIET study. PT, PQ, GS, CP, and IK managed data acquisition of the MEDIDIET study. All authors have read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.891376/full#supplementary-material

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Association of Dietary Carrot/Carotene Intakes With Colorectal Cancer Incidence and Mortality in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial

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Background: The evidence of dietary carrot/carotene intake's effect on the association with colorectal cancer (CRC) risk is conflicted. We sought to examine the association of carrot/carotene intake with CRC incidence and mortality in the Prostate, Lung, Colorectal, and Ovarian Cancer (PLCO) Screening cohort.

Methods: In all, 101,680 participants were enrolled between November 1993 and July 2001 from the PLCO cohort. We employed the multivariable Cox regression analyses to estimate the hazard ratios and 95% confidence interval. Subgroup analyses and interaction tests were performed to examine the potential effect modifiers. We further applied the generalized additive model to explore the non-linear trend of the exposure to cancer-related outcomes.

Results: A total of 1,100 CRC cases and 443 cancer-related deaths were documented. We noted that the 4th quintile of dietary carrot intakes was associated with a 21% lower risk of CRC incidence, compared with the lowest quintile group (full-adjusted $HR_{quintile4vs.quintile1} = 0.79$, 95%CI = 0.65–0.97, p for trend = 0.05), while the adjusted-HR was 0.95 (95%CI = 0.89–1.02) with per SD increment of carrot intakes, and no statistically significant associations were detected between dietary α-, and β-carotene intake and CRC incidence. There were no statistically significant associations observed between carrot/carotene intakes and CRC mortality. Furthermore, there were no non-linear dose-response relationships between dietary carrot, α-, and β-carotene intake and CRC incidence and mortality (all $p_{nonlinearity} > 0.05$). Of note, smoking status as a modifier on the association of dietary carrot intakes with CRC incidence but not mortality was observed.

Conclusions: In summary, this large U.S. prospective cohort study indicated that a moderate consumption of carrots was associated with a lower CRC incidence, which suggested that a certain dose-range of carrots consumed might contribute to a potential cancer-prevention effect, not the more the better.

Keywords: carrot, carotene, colorectal cancer, cohort, PLCO

INTRODUCTION

Colorectal cancer (CRC) is the third most common cause of cancer-related death in the United States, with nearly 147,950 incident cases and 53,200 cancer deaths in 2020 (1). Apart from well-established risk factors (i.e., environmental and genetic factors) that play a crucial role in the pathogenesis of CRC (2, 3), more than half of patients can be attributed to other risk factors including smoking, diet, drinking, obesity, and thus may be potentially preventable (4). Although emerging evidence implies that cancer prevention dietary nutrients or food, including calcium (5, 6), fiber (7), dairy products (8), and whole grain (9) have been associated with a lower risk of colorectal cancer, it remains controversial.

Carrots are rich in high amounts of carotenoid antioxidants (α- and -β-carotene) that might have a potential role in cancer prevention (10, 11). Several meta-analyses on carrot consumption have indicated that carrot intake was inversely associated with the risk of several cancers, including gastric (12), lung (13), prostate (14), breast (15), and urothelial cancer (16). However, epidemiological studies depicted an inverse (17–20) or a null association (21–23) of dietary α-/β-carotene intakes with the risk of CRC. Recently, a prospective cohort study of 57,053 Danes has shown that the consumption of raw carrots was associated with a 17% decrease in the risk of CRC (24). Given the differences in geography and eating habits, it remains unclear whether the results would be stable for U.S. adults. Meanwhile, there is no evidence of the effect of dietary carrot intake on mortality of CRC.

Therefore, to provide the most reliable prospective evidence on the association of dietary carrot intake with the risk of CRC, we, respectively, analyze the association of dietary carrot, α- and -β-carotene intakes with the risk of CRC incidence and mortality using a multicenter randomized controlled trial data from the prostate, lung, colorectal, and ovarian (PLCO) screening trial.

METHODS

Data Source and Study Population

The PLCO Cancer Screening Trial is a randomized, controlled trial conducted to investigate whether certain screening examinations would reduce the mortality from PLCO cancers. The specific study design and methods were previously illustrated elsewhere (25). Approximately 155,000 participants were recruited from 1993 to 2001 via 10 screening centers across the United States, through a detailed recruitment plan. Then, they were randomly assigned to two groups (the control group receiving usual care, whereas the intervention arm receiving screening tests). The PLCO study was approved by the Institutional Review Boards of the US National Cancer Institute and each study center, and written informed consents were obtained from all eligible participants.

We established inclusion criteria to identify eligible cases in our final cohort. They would be further excluded as follows: (1) no baseline questionnaire returned (BQ) (n = 4,918) and any history of colorectal cancer before BQ (n = 34); (2) did not complete Diet History Questionnaire (DHQ) (n = 33,230), and invalid DHQ for missing the date of DHQ completion, the date of DHQ completion before the date of death, the presence of ≥8 missing frequency responses or extreme values of calorie intake [i.e., top 1% or bottom 1%]) (n = 5,221); (3) a history of any cancer before DHQ entry (n = 9,682), and no follow-up time after the DHQ (n = 122). At last, 101,680 subjects were included in our cohort.

Data Collection and Dietary Assessment

The self-reported information of sex, race, trial arm, body mass index (BMI), educational level, marital status, aspirin use, cigarette smoking, family history of colorectal cancer, and diabetes history, were collected from the BQ. Dietary data, including age at DHQ, alcohol drinking, dietary carrot/carotene intake, energy intake from diet, and supplemental nutrients (Beta-Carotene, Calcium, Vitamin A, Vitamin C, Vitamin D, and Vitamin E), were collected from the DHQ (version 1.0, National Cancer Institute 2007), which was a self-administered food frequency questionnaire (FFQ) designed to assess the portion size and consumption frequency of 124 food items and supplement use over the past year, and has been validated with better performance in estimating dietary intake than two widely used FFQs (26) at the time of PLCO study carried out. The 1994–96 Continuing Survey of Food Intakes by Individuals, available from the USDA Food Surveys Research Group, and the Nutrition Data Systems for Research (NDS-R) from the University of Minnesota was applied to calculate the daily intake of all nutrients in the database (27). Three exposure variables (dietary carrot, α-, and dietary β-carotene intakes) were acquired in this study.

Ascertainment of Colorectal Cancer Incidence and Mortality

The time metric was followed up from the date of DHQ completion to the date of events that firstly occurred, including colorectal cancer diagnosis, dropout, colorectal cancer death, or the end of follow-up (incidence through December 31, 2009; and mortality through December 31, 2015). CRC diagnosis was ascertained via an annually updated medical record. Deaths were mainly identified by (1) annually mailed questionnaires, (2)

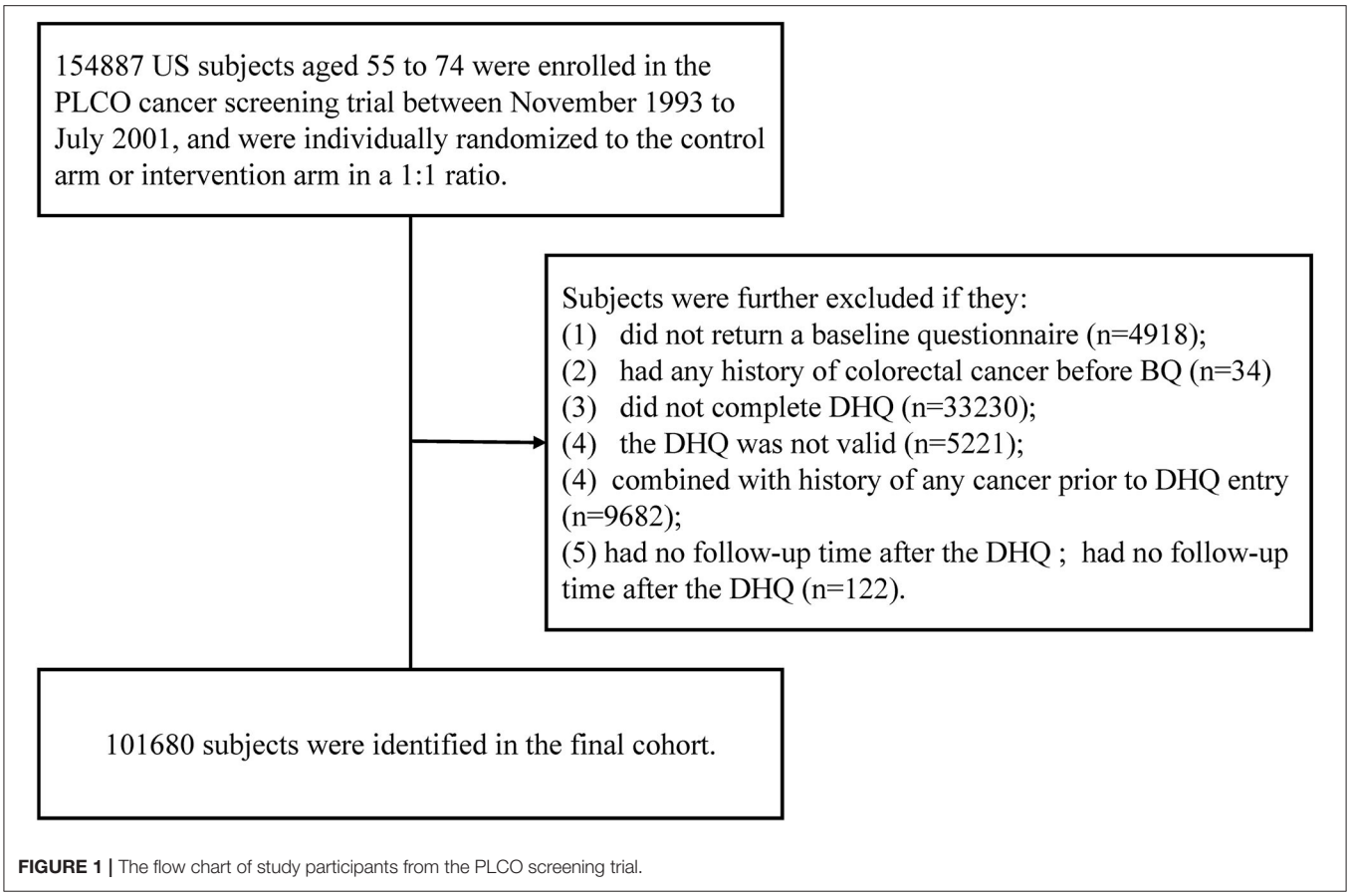

FIGURE 1 | The flow chart of study participants from the PLCO screening trial.

reports from relatives, friends, or physicians, and (3) periodic linkage to the National Death Index. Our interested endpoints were the incidence and mortality of colorectal cancer.

Statistical Analysis

Dietary carrot, α-, and β-carotene intakes were adjusted for energy intake with the residual method (28). The distributions of them were transformed with Z-score as continuous variables, and then were divided into quintiles as categorical variables, and the lowest quintile was as the referent. Continuous variables are expressed as median (IQR, interquartile range), and categorical variables are presented as numbers (frequency). We applied the Kruskal–Wallis H test and Pearson's chi-squared test to compare the baseline differences, if appropriate. Multivariable Cox regression analyses were employed to estimate the hazard ratios (HR) and 95% confidence interval (CI). The proportional hazard assumption of baseline covariates for the Cox model was verified using the Schoenfeld residuals (29) (all $p > 0.05$). Potential confounders were selected according to the change-in-estimate strategy (30) (more than 10% change in effect estimates) and literature-known risk factors. Missing values of covariates were treated as dummy variables in the multivariable Cox regression analyses. Specifically, the full-adjusted model included age, sex, race, trial arm, marital status, BMI, educational level, aspirin use, cigarette smoking, alcohol drinking, diabetes, family history of colorectal cancer, and energy intake from the diet, and supplemental use of Beta-Carotene, Calcium, Vitamin A, Vitamin C, Vitamin D, and Vitamin E. We also analyzed the linear trend of each quintile of energy-adjusted dietary carrot, α-, and β-carotene intakes, by entering the median value as a continuous variable in the model.

Subgroup analyses were performed, including age, sex, race, trial arm, marital status, BMI, educational level, aspirin use, cigarette smoking, alcohol drinking, diabetes, and family history of colorectal cancer. The interaction effect on each stratum was compared using likelihood-ratio tests. To address the dose-response trend between dietary carrot/carotene intakes and colorectal cancer incidence and mortality, the smooth curve fitting was conducted with a multivariable Cox regression model using the generalized additive model (GAM, Restricted Cubic Spline Functions). Here, we excluded subjects with <1st or >90th percentile values of energy-adjusted dietary carrot/carotene intakes (i.e., to reduce the potential impacts on the association of dose-response analyses).

The following sensitivity analyses were conducted: (1) excluding cases diagnosed or died within the first 5 years of follow-up; (2) excluding the extreme values of energy intake from the diet (<800/>4,000 kcal/day for men and <500/>3,500 kcal/day for women); (3) additional adjusted for other factors, including fruit (continuous), dietary Magnesium (continuous), dietary Sodium (continuous), dietary Potassium (continuous),

TABLE 1 | Baseline characteristics of study population according to quintiles of energy-adjusted dietary carrot intake in 101680 US participants.

		Quintiles of energy-adjusted dietary carrot intake, g/day				
Variables[a]	**Overall**	**Q1 (<1.95)**	**Q2 (1.95–3.88)**	**Q3 (3.88–7.60)**	**Q4 (7.60–15.30)**	**Q5 (>15.30)**
Number of participants	101,680	20,336	20,336	20,336	20,336	20,336
Age at DHQ (years)	65.0 (61.0, 70.0)	64.0 (60.0, 69.0)	65.0 (61.0, 70.0)	66.0 (61.0, 70.0)	66.0 (61.0, 70.0)	65.0 (61.0, 70.0)
Sex						
Male	49,441 (48.6)	14,802 (72.8)	10,366 (51)	8,618 (42.4)	8,299 (40.8)	7,356 (36.2)
Female	52,239 (51.4)	5,534 (27.2)	9,970 (49)	11,718 (57.6)	12,037 (59.2)	12,980 (63.8)
Trial arm						
Intervention	51,767 (50.9)	10,432 (51.3)	10,362 (51)	10,308 (50.7)	10,384 (51.1)	10,281 (50.6)
Control	49,913 (49.1)	9,904 (48.7)	9,974 (49)	10,028 (49.3)	9,952 (48.9)	10,055 (49.4)
Race						
White, Non-Hispanic	92,465 (90.9)	17,726 (87.2)	17,982 (88.4)	18,563 (91.3)	19,034 (93.6)	19,160 (94.2)
Black, Non-Hispanic	3,352 (3.3)	1,269 (6.2)	947 (4.7)	521 (2.6)	322 (1.6)	293 (1.4)
Hispanic	1,493 (1.5)	418 (2.1)	342 (1.7)	272 (1.3)	215 (1.1)	246 (1.2)
Others[b]	4,333 (4.3)	912 (4.5)	1,055 (5.2)	974 (4.8)	759 (3.7)	633 (3.1)
Missing	37 (0.0)	11 (0.1)	10 (0)	6 (0)	6 (0)	4 (0)
Marital status						
Married	79,578 (78.3)	15,647 (76.9)	15,742 (77.4)	15,851 (77.9)	16,351 (80.4)	15,987 (78.6)
Unmarried	21,916 (21.6)	4,648 (22.9)	4,553 (22.4)	4,445 (21.9)	3,951 (19.4)	4,319 (21.2)
Missing	186 (0.2)	41 (0.2)	41 (0.2)	40 (0.2)	34 (0.2)	30 (0.1)
Education level						
College below	64,704 (63.6)	13,517 (66.5)	13,500 (66.4)	13,408 (65.9)	12,499 (61.5)	11,780 (57.9)
College graduate	17,838 (17.5)	3,466 (17)	3,372 (16.6)	3,385 (16.6)	3,724 (18.3)	3,891 (19.1)
Postgraduate	18,941 (18.6)	3,314 (16.3)	3,418 (16.8)	3,500 (17.2)	4,080 (20.1)	4,629 (22.8)
Missing	197 (0.2)	39 (0.2)	46 (0.2)	43 (0.2)	33 (0.2)	36 (0.2)
Aspirin use						
No	53,472 (52.6)	10,082 (49.6)	10,763 (52.9)	10,849 (53.3)	10,789 (53.1)	10,989 (54)
Yes	47,775 (47.0)	10,148 (49.9)	9,475 (46.6)	9,401 (46.2)	9,467 (46.6)	9,284 (45.7)
Missing	433 (0.4)	106 (0.5)	98 (0.5)	86 (0.4)	80 (0.4)	63 (0.3)
Diabetes						
No	94,353 (92.8)	18,702 (92)	18,695 (91.9)	18,867 (92.8)	19,002 (93.4)	19,087 (93.9)
Yes	6,801 (6.7)	1,536 (7.6)	1,525 (7.5)	1,353 (6.7)	1,241 (6.1)	1,146 (5.6)
Missing	526 (0.5)	98 (0.5)	116 (0.6)	116 (0.6)	93 (0.5)	103 (0.5)
Cigarette smoking						
Never	48,532 (47.7)	7,396 (36.4)	9,063 (44.6)	10,071 (49.5)	10,674 (52.5)	11,328 (55.7)
Current	9,393 (9.2)	3,132 (15.4)	2,151 (10.6)	1,768 (8.7)	1,322 (6.5)	1,020 (5)
Former	43,742 (43.0)	9,805 (48.2)	9,120 (44.8)	8,493 (41.8)	8,337 (41)	7,987 (39.3)
Missing	13 (0.0)	3 (0)	2 (0)	4 (0)	3 (0)	1 (0)
BMI, kg/m^2						
<25	34,426 (33.9)	5,729 (28.2)	6,550 (32.2)	7,066 (34.7)	7,269 (35.7)	7,812 (38.4)
≤25	65,915 (64.8)	14,318 (70.4)	13,507 (66.4)	12,988 (63.9)	12,823 (63.1)	12,279 (60.4)
Missing	1,339 (1.3)	289 (1.4)	279 (1.4)	282 (1.4)	244 (1.2)	245 (1.2)
Family history of colorectal cancer						
No	88,113 (86.7)	17,659 (86.8)	17,603 (86.6)	17,649 (86.8)	17,668 (86.9)	17,534 (86.2)
Yes	10,300 (10.1)	1,894 (9.3)	2,014 (9.9)	2,039 (10)	2,127 (10.5)	2,226 (10.9)
Possibly	2,493 (2.5)	625 (3.1)	554 (2.7)	495 (2.4)	408 (2)	411 (2)
Missing	774 (0.8)	158 (0.8)	165 (0.8)	153 (0.8)	133 (0.7)	165 (0.8)

(Continued)

TABLE 1 | Continued

Variables[a]	Overall	Quintiles of energy-adjusted dietary carrot intake, g/day				
		Q1 (<1.95)	Q2 (1.95–3.88)	Q3 (3.88–7.60)	Q4 (7.60–15.30)	Q5 (>15.30)
Energy intake from diet, kcal/day	1607.0 (1222.0, 2101.0)	2132.0 (1727.0, 2668.0)	1372.0 (1083.0, 1721.0)	1300.0 (991.6, 1868.0)	1610.0 (1299.0, 1985.0)	1597.0 (1236.0, 2118.0)
Alcohol drinking						
Never	10,110 (9.9)	1,370 (6.7)	1,941 (9.5)	2,153 (10.6)	2,188 (10.8)	2,458 (12.1)
Former	14,746 (14.5)	3,304 (16.2)	3,088 (15.2)	2,991 (14.7)	2,656 (13.1)	2,707 (13.3)
Current	73,944 (72.7)	15,125 (74.4)	14,707 (72.3)	14,569 (71.6)	14,943 (73.5)	14,600 (71.8)
Missing	2,880 (2.8)	537 (2.6)	600 (3)	623 (3.1)	549 (2.7)	571 (2.8)
Supplemental Beta-Carotene, mcg/day	142.9 (0.0, 200.0)	57.1 (0.0, 200.0)	142.9 (0.0, 200.0)	142.9 (0.0, 200.0)	200.0 (0.0, 200.0)	200.0 (0.0, 200.0)
Supplemental Vitamin A, i.u./day	3571.0 (0.0, 5000.0)	1429.0 (0.0, 5000.0)	3571.0 (0.0, 5000.0)	3571.0 (0.0, 5000.0)	5000.0 (0.0, 5000.0)	5000.0 (0.0, 5000.0)
Supplemental Vitamin E, mg/day	20.1 (0.0, 288.1)	20.1 (0.0, 268.0)	20.1 (0.0, 268.0)	20.1 (0.0, 288.1)	50.2 (5.7, 288.1)	115.8 (14.4, 288.1)
Supplemental Vitamin C, mg/day	60.0 (0.0, 400.0)	60.0 (0.0, 310.0)	60.0 (0.0, 310.0)	60.0 (0.0, 400.0)	60.0 (1.0, 500.0)	60.0 (17.1, 500.0)
Supplemental Calcium, mg/day	4.1 (0.0, 500.0)	0.0 (0.0, 171.4)	0.0 (0.0, 500.0)	16.4 (0.0, 500.0)	33.2 (0.0, 600.0)	142.9 (0.0, 600.0)

[a] *Data are presented as median (IQR) or number (percentage).* [b] *"Others" refers to Asian, Pacific Islander, or American Indian. DHQ, dietary history of questionnaire; BMI, body mass index.*

whole grain (continuous), vegetables (continuous), added sugars (continuous), fiber (continuous), and saturated fatty acids (continuous). All analyses were performed using R Statistical Software (http://www.R-project.org, The R Foundation) and the Free Statistics analysis platform (31). Tests were two-tailed, and the significance level was set at 0.05.

RESULTS

Participant Characteristics

Accordingly, 101,680 subjects were selected for the final data analysis (**Figure 1**). The baseline characteristics are presented in **Table 1**. The median value of dietary carrot intake (without adjustment for energy intake) was 4.3 g/day, ranging from 0 to 205.3 g/day. Compared to participants with the highest quintile (Q5) of dietary carrot consumption, participants with the lowest quintile (Q1) showed a higher proportion of males (72.8% vs. 36.2%), Black race, aspirin usage, higher BMI (70.4% vs. 60.4%), and higher energy intake (2132.0 kcal/day vs. 1597.0 kcal/day), and former/current cigarette smoking (48.2% vs. 39.3%;15.4% vs. 5.0%).

Energy-Adjusted Dietary Carrot/Carotene Intakes and Colorectal Cancer Incidence

A total of 1,100 participants were diagnosed with CRC after a median follow-up of 9.4 years (896,327.8 person-years), and the incidence rate was 12.27 per 10,000 person-years. As shown in **Table 2**, we observed an inverse effect on the association between carrot intakes and CRC incidence at the quintile 4 level, which showed the multivariable-adjusted HR was 0.79 (95%CI = 0.65–0.97, $p_{\text{trend}} = 0.05$), compared with the referent group; and corresponding adjusted HR of cancer risk was 0.95 (95%CI = 0.89–1.02) with per SD increment of carrot intakes. However, no statistical association was detected between dietary α- and β-carotene intakes and CRC incidence after adjusting for covariates.

The results of subgroup analyses indicated that the associations between dietary carrot intakes and the risk of CRC were stable in almost all subgroups (**Supplementary Table 1**). An exception of significant effect modifier was represented by smoking status, which showed a stronger association for the participants who never smoked than those with a history of smoking (p for interaction = 0.027). Sensitivity analyses revealed that the results of the correlation between carrot/carotene intake and CRC risk were substantially robust to the findings in **Table 2** and **Supplementary Table 3**. The smooth curve fitting plots of carrot/carotene intakes with CRC risk revealed no evidence of a non-linear trend (all p for non-linearity > 0.05, **Figures 2A–C**).

Energy-Adjusted Dietary Carrot/Carotene Intakes and Colorectal Cancer Mortality

A total of 443 cases died from CRC after a median follow-up of 14.5 years (1,353,326.28 person-years), and the mortality rate was 3.27 per 10,000 person-years. As shown in **Table 3**, in the fully-adjusted Cox model, only a suggestive but no significant association with cancer mortality was noted for carrot intakes ($HR_{\text{quintile5vs.quintile1}} = 0.87$, 95%CI = 0.64–1.18, $p_{\text{trend}} = 0.297$), and corresponding adjusted HR was 0.94 (95%CI = 0.84–1.05), with per SD increment of carrot intakes. Similar results on the association of dietary α-carotene intake with colorectal mortality were obtained (model 3: $HR_{\text{quintile5vs.quintile1}} = 0.91$, 95%CI = 0.62–1.35, $p_{\text{trend}} = 0.417$; and HR with per SD increment = 0.94, 95%CI = 0.8–1.11); and for dietary β-carotene intake (model

TABLE 2 | Association between energy-adjusted dietary carrot/carotene intakes and colorectal cancer incidence risk in the PLCO cancer screening trial.

					HR (95%CI), *p*-value			
Variable	**Cohort (*n*)**	**Cases (*n*)**	**Person-years**	**Incidence rate per 10,000 person-years**	**Unadjusted**	**Model 1**	**Model 2**	**Model 3**
Dietary carrot intakes, g/day								
Q1 (<1.95)	20,336	239	175,553.87	13.61	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
Q2 (1.95–3.88)	20,336	240	177,803.05	13.50	0.99 (0.83–1.19), $p = 0.928$	0.99 (0.82–1.18), $p = 0.874$	1 (0.82–1.21), $p = 0.997$	1 (0.82–1.21), $p = 0.995$
Q3 (3.88–7.60)	20,336	235	179,671.94	13.08	0.96 (0.8–1.15), $p = 0.668$	0.96 (0.8–1.16), $p = 0.701$	0.99 (0.82–1.2), $p = 0.922$	1 (0.82–1.21), $p = 0.968$
Q4 (7.60–15.30)	20,336	185	181,363.20	10.20	0.75 (0.62–0.91), $p = 0.003$	0.75 (0.62–0.91), $p = 0.004$	0.79 (0.64–0.96), $p = 0.019$	0.79 (0.65–0.97), $p = 0.025$
Q5 (>15.30)	20,336	201	181,935.76	11.05	0.81 (0.67–0.98), $p = 0.03$	0.83 (0.69–1.01), $p = 0.062$	0.88 (0.73–1.08), $p = 0.225$	0.9 (0.74–1.1), $p = 0.306$
*T*rend					0.001	0.004	0.029	0.05
Per SD increment	101,680	1,100	896,327.81	12.27	0.91 (0.85–0.98), $p = 0.011$	0.93 (0.87–0.99), $p = 0.034$	0.95 (0.88–1.01), $p = 0.117$	0.95 (0.89–1.02), $p = 0.169$
Dietary α-carotene intakes, mcg/day								
Q1 (<317.9)	20,336	236	176,237.15	13.39	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
Q2 (317.9–475.86)	20,336	238	178,171.74	13.36	1 (0.83–1.19), $p = 0.981$	1 (0.83–1.19), $p = 0.961$	1.02 (0.84–1.24), $p = 0.84$	1.02 (0.84–1.24), $p = 0.82$
Q3 (475.86–697.34)	20,336	233	179,532.58	12.98	0.97 (0.81–1.16), $p = 0.74$	0.97 (0.8–1.16), $p = 0.717$	1.01 (0.83–1.23), $p = 0.928$	1.01 (0.83–1.23), $p = 0.892$
Q4 (697.35–1135.29)	20,336	188	180,743.42	10.40	0.78 (0.64–0.94), $p = 0.01$	0.77 (0.63–0.94), $p = 0.009$	0.82 (0.67–1.02), $p = 0.076$	0.83 (0.67–1.03), $p = 0.087$
Q5 (>1135.37)	20,336	205	181,642.92	11.29	0.84 (0.7–1.02), $p = 0.075$	0.85 (0.7–1.03), $p = 0.107$	0.96 (0.75–1.24), $p = 0.773$	0.97 (0.76–1.25), $p = 0.832$
*T*rend					0.006	0.01	0.217	0.249
Per SD increment	101680	1,100	896327.81	12.27	0.93 (0.87–0.99), $p = 0.027$	0.94 (0.88–1), $p = 0.059$	0.99 (0.89–1.1), $p = 0.814$	0.99 (0.89–1.1), $p = 0.833$
Dietary β-carotene intakes, mcg/day								
Q1 (<1707.28)	20,336	238	176,127.95	13.51	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
Q2 (1707.28–2389.73)	20,336	250	177,981.12	14.05	1.04 (0.87–1.24), $p = 0.668$	1.04 (0.87–1.25), $p = 0.651$	1.06 (0.88–1.28), $p = 0.513$	1.07 (0.89–1.29), $p = 0.477$
Q3 (2389.74–3239.34)	20,336	199	180,083.18	11.05	0.82 (0.68–0.99), $p = 0.037$	0.83 (0.69–1.01), $p = 0.062$	0.86 (0.7–1.05), $p = 0.147$	0.87 (0.71–1.07), $p = 0.177$
Q4 (3239.4–4920.97)	20,336	227	180,603.16	12.57	0.93 (0.78–1.12), $p = 0.44$	0.95 (0.79–1.15), $p = 0.59$	1 (0.81–1.23), $p = 0.991$	1.01 (0.83–1.24), $p = 0.893$
Q5 (>4921.39)	20,336	186	181,532.41	10.25	0.76 (0.63–0.92), $p = 0.005$	0.78 (0.64–0.95), $p = 0.015$	0.82 (0.63–1.07), $p = 0.151$	0.84 (0.64–1.1), $p = 0.197$
*T*rend					0.002	0.009	0.181	0.243
Per SD increment	101,680	1,100	896327.8137	12.27	0.92 (0.86–0.98), $p = 0.013$	0.93 (0.87–1), $p = 0.036$	0.96 (0.86–1.07), $p = 0.44$	0.97 (0.87–1.07), $p = 0.519$

PLCO, prostate, lung, colorectal, and ovarian; HR, hazard ratio; CI, confidence interval; SD, standard deviation.

Unadjusted was the crude model.

Model 1 adjusted for age (continuous) and sex (male vs. female).

Model 2 adjusted for model 1 plus trial arm (intervention vs. control), race (white, non-Hispanic vs. black, non-Hispanic vs. Hispanic vs. others), marital status (married vs. unmarried), education level (college below vs. college graduate vs. postgraduate), aspirin use (yes vs. no), diabetes (yes vs. no), cigarette smoking (never vs. current vs. former), BMI (<25 vs. ≤25 kg/m^2),family history of colorectal cancer (yes vs. no vs. possibly), energy intake from diet (continuous), alcohol drinking (never vs. former vs. current), supplemental Beta-Carotene (continuous).

Model 3 adjusted for model 2 plus supplemental vitamin A (continuous), supplemental vitamin E (continuous), supplemental vitamin C (continuous), supplemental calcium (continuous).

For the association of dietary α-carotene intake with colorectal cancer incidence, model 2 was further adjusted for energy-adjusted dietary β-carotene intake (mcg/day). For the association of dietary β-carotene intake with colorectal cancer incidence, model 2 was further adjusted for energy-adjusted dietary α-carotene intake (mcg/day).

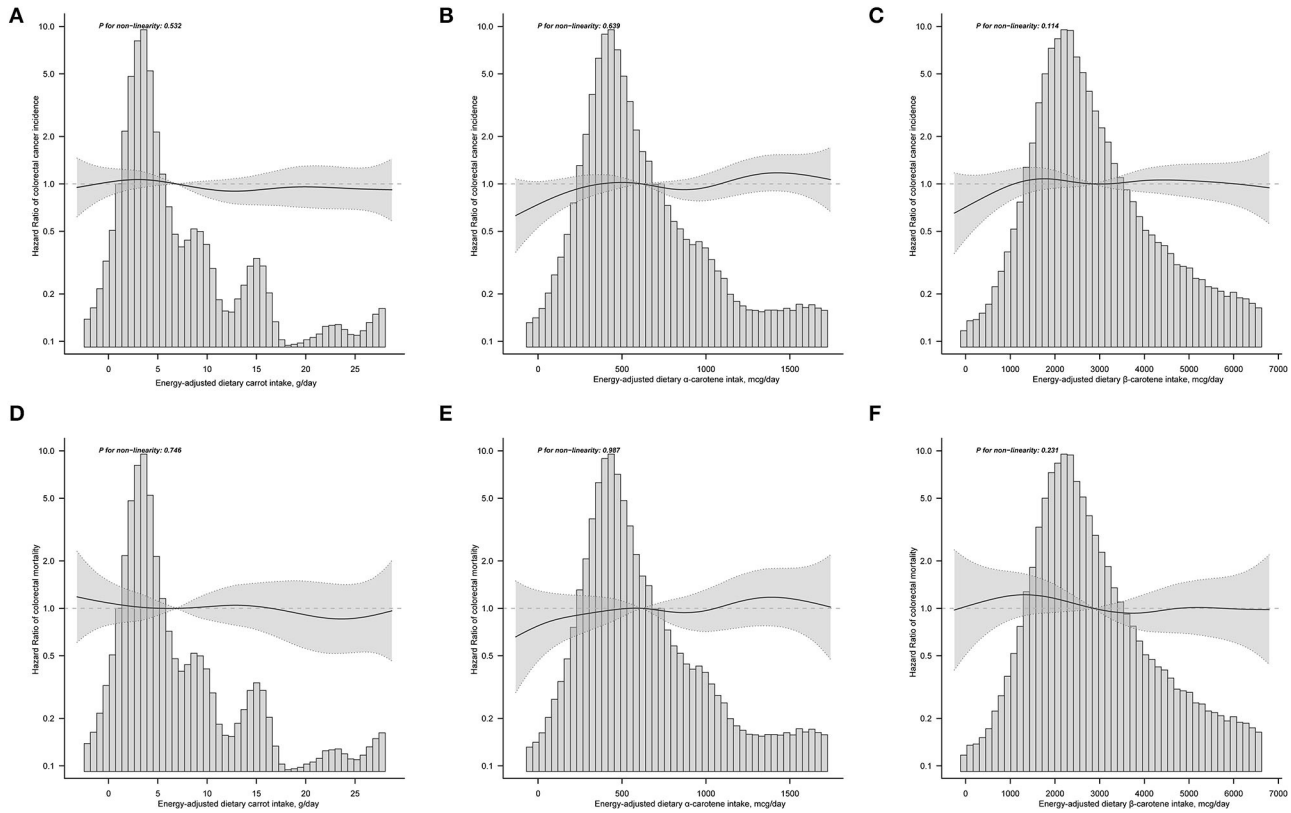

FIGURE 2 | Does-response analyses for the associations between energy-adjusted dietary carrot intake **(A)**, dietary α-carotene intake **(B)**, dietary β-carotene intake **(C)** and colorectal cancer incidence; and the associations between energy-adjusted dietary carrot intake **(D)**, dietary α-carotene intake **(E)**, dietary β-carotene intake **(F)** and colorectal cancer mortality were performed with smooth curve fitting using the generalized additive model. Hazard ratios and 95% confidence interval were calculated by the fully-adjusted multivariable Cox regression model, including age, sex, race, trial arm, marital status, BMI, educational level, aspirin use, cigarette smoking, alcohol drinking, diabetes, family history of colorectal cancer, energy intake from diet, supplemental Beta-Carotene, supplemental Calcium, supplemental Vitamin A, supplemental Vitamin C, supplemental Vitamin D, and Supplemental Vitamin E. Solid lines represent point estimates and dashed lines represent corresponding 95% confidence intervals. The histograms show the percentage of participants belonging to each level of specific energy-adjusted dietary carrot/carotene intake.

3: HR quintile 5 *vs.* quintile 1 = 0.91, 95%CI = 0.61–1.36, p trend = 0.344; and HR with per SD increment = 1.00, 95%CI = 0.86–1.17). Results of subgroup analyses showed no significant effect modifies in the prespecified groups (all p for interaction > 0.05, **Supplementary Table 2**). In the sensitivity analyses, the null associations of dietary carrot/carotene intakes were robust to colorectal cancer mortality (**Supplementary Table 4**). Dose-response analyses suggested no non-linear relationship between carrot/carotene intakes and colorectal cancer mortality (all p for non-linearity > 0.05, **Figures 2D–F**).

DISCUSSION

In this prospective cohort of 101,680 U.S. adults, we found that only the quintile 4 group of dietary carrot intakes but not dietary α-, and β-carotene intakes, had a lower risk of colorectal cancer, compared with the referent group. Meanwhile, the null associations were also detected between dietary carrot, α-, and β-carotene intakes and colorectal cancer mortality. Similar results were supported in several sensitivity analyses. Of note, the association of dietary carrot intakes was modified by smoking status with colorectal cancer incidence but not mortality.

Although the effect of carrot consumption on multiple types of cancer risk has been widely investigated, the findings were mixed. In our analysis, except for the quintile 4 group of carrot intakes, however, there were no significant associations shown in other quintile groups. The observed inverse association of our study was close to a previous prospective cohort from Danes (17), in which they illustrated that a higher intake of raw carrots (>32 g/day) was related to a 17% lower risk of CRC, compared with no intake of raw carrots (HR = 0.83, 95%CI = 0.71–0.98), but as for the raw carrot intakes <32 g/day, no significant association was observed with decreased risk of CRC (HR = 0.93, 95%CI = 0.82–1.06). Of note, there were also some differences. Our results, based on a large prospective cohort of 101,680 US adults, found that moderate consumption of carrots (Q4, >7.60–15.30 g/day) but not the highest consumption of carrots (Q5, >15.30 g/day) was associated with a lower risk of CRC incidence, compared with the lowest consumption of carrot (Q1, <1.95 g/day), while the Danes cohort showed that a higher raw carrots

TABLE 3 | Association between energy-adjusted dietary carrot/carotene intakes and colorectal cancer mortality risk in the PLCO cancer screening trial.

					HR (95%CI), *P*-value			
Variable	**Cohort (*n*)**	**Cases (*n*)**	**Person-years**	**Mortality rate per 10,000 person-years**	**Unadjusted**	**Model 1**	**Model 2**	**Model 3**
Dietary carrot intakes, g/day								
Q1 (<1.95)	20,336	103	264,449.34	3.90	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
Q2 (1.95–3.88)	20,336	92	267,822.85	3.44	0.88 (0.66–1.17), $p = 0.374$	0.88 (0.66–1.17), $p = 0.377$	0.94 (0.69–1.28), $p = 0.695$	0.94 (0.7–1.28), $p = 0.71$
Q3 (3.88–7.60)	20,336	87	270,611.78	3.22	0.82 (0.62–1.09), $p = 0.178$	0.83 (0.62–1.12), $p = 0.223$	0.9 (0.67–1.23), $p = 0.522$	0.91 (0.67–1.24), $p = 0.569$
Q4 (7.60–15.30)	20,336	82	274,063.17	2.99	0.76 (0.57–1.02), $p = 0.067$	0.77 (0.57–1.03), $p = 0.082$	0.85 (0.63–1.15), $p = 0.296$	0.87 (0.64–1.18), $p = 0.359$
Q5 (>15.30)	20,336	79	276,379.15	2.86	0.73 (0.54–0.97), $p = 0.033$	0.76 (0.56–1.02), $p = 0.07$	0.84 (0.62–1.14), $p = 0.27$	0.87 (0.64–1.18), $p = 0.376$
*T*rend					0.019	0.044	0.199	0.297
Per SD increment	101,680	443	1,353,326.28	3.27	0.89 (0.79–0.99), $p = 0.034$	0.9 (0.81–1.01), $p = 0.081$	0.93 (0.83–1.03), $p = 0.173$	0.94 (0.84–1.05), $p = 0.248$
Dietary α-carotene intakes, mcg/day								
Q1 (<317.9)	20,336	102	265,741.25	3.84	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
Q2 (317.9–475.86)	20,336	91	268,878.31	3.38	0.88 (0.66–1.17), $p = 0.375$	0.88 (0.66–1.18), $p = 0.402$	0.95 (0.71–1.29), $p = 0.755$	0.95 (0.7–1.28), $p = 0.742$
Q3 (475.86–697.34)	20,336	90	269,982.27	3.33	0.87 (0.65–1.15), $p = 0.319$	0.87 (0.65–1.17), $p = 0.358$	0.96 (0.71–1.31), $p = 0.811$	0.96 (0.7–1.3), $p = 0.772$
Q4 (697.35–1135.29)	20,336	78	272,597.94	2.86	0.74 (0.55–1), $p = 0.046$	0.74 (0.55–1), $p = 0.053$	0.85 (0.6–1.19), $p = 0.347$	0.83 (0.6–1.16), $p = 0.277$
Q5 (>1135.37)	20,336	82	276,126.51	2.97	0.77 (0.57–1.03), $p = 0.073$	0.79 (0.58–1.06), $p = 0.115$	0.98 (0.6–1.61), $p = 0.947$	0.91 (0.62–1.35), $p = 0.649$
*T*rend					0.035	0.057	0.531	0.417
Per SD increment	101,680	443	1,353,326.28	3.27	0.9 (0.81–1), $p = 0.06$	0.92 (0.82–1.02), $p = 0.113$	0.94 (0.8–1.11), $p = 0.475$	0.94 (0.8–1.11), $p = 0.49$
Dietary β-carotene intakes, mcg/day								
Q1 (<1707.28)	20,336	103	264,087.73	3.90	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
Q2 (1707.28–2389.73)	20,336	101	267,252.54	3.778	0.97 (0.73–1.27), $p = 0.806$	0.98 (0.74–1.29), $p = 0.866$	1.05 (0.78–1.4), $p = 0.759$	1.06 (0.79–1.42), $p = 0.705$
Q3 (2389.74–3239.34)	20,336	76	271,162.62	2.80	0.71 (0.53–0.96), $p = 0.026$	0.74 (0.54–1), $p = 0.048$	0.81 (0.59–1.11), $p = 0.184$	0.82 (0.6–1.13), $p = 0.225$
Q4 (3239.4–4920.97)	20,336	82	273,732.67	3.00	0.76 (0.57–1.02), $p = 0.064$	0.79 (0.58–1.06), $p = 0.116$	0.88 (0.63–1.21), $p = 0.422$	0.9 (0.65–1.25), $p = 0.521$
Q5 (>4921.39)	20,336	81	277,090.73	2.92	0.74 (0.55–0.99), $p = 0.042$	0.77 (0.57–1.04), $p = 0.093$	0.88 (0.58–1.31), $p = 0.52$	0.91 (0.61–1.36), $p = 0.64$
*T*rend					0.01	0.033	0.253	0.344
Per SD increment	101,680	443	1,353,326.28	3.27	0.92 (0.83–1.02), $p = 0.096$	0.93 (0.84–1.04), $p = 0.192$	0.99 (0.85–1.16), $p = 0.915$	1.00 (0.86–1.17), $p = 0.959$

PLCO, prostate, lung, colorectal, and ovarian; HR, hazard ratio; CI, confidence interval; SD, standard deviation.

Unadjusted was the crude model.

Model 1 adjusted for age (continuous) and sex (male vs. female).

Model 2 adjusted for model 1 plus trial arm (intervention vs. control), race (white, non-Hispanic vs. black, non-Hispanic vs. Hispanic vs. others), marital status (married vs. unmarried), education level (college below vs. college graduate vs. postgraduate), aspirin use (yes vs. no), diabetes (yes vs. no), cigarette smoking (never vs. current vs. former), BMI (<2 vs. ≥25 kg/m^2), family history of colorectal cancer (yes vs. no vs. possibly), energy intake from diet (continuous), alcohol drinking (never vs. former vs. current), supplemental Beta-Carotene (continuous).

Model 3 adjusted for model 2 plus supplemental vitamin A (continuous), supplemental vitamin E (continuous), supplemental vitamin C (continuous), supplemental calcium (continuous).

For the association of dietary α-carotene intake with colorectal cancer mortality, model 2 was further adjusted for energy-adjusted dietary β-carotene intake (mcg/day). For the association of dietary β-carotene intake with colorectal cancer mortality, model 2 was further adjusted for energy-adjusted dietary α-carotene intake (mcg/day).

intake was associated with a lower risk of CRC incidence. We further categorized the dietary carrot intake into three groups (0, 0–32 g/day, and ≥32 g/day), and the highest carrot intake was also null associated with CRC incidence (HR ≥32 g/day vs. none = 0.75, 95%CI = 0.46–1.24) after fully-adjustment. The small size of events might reduce the statistical power, in which only 56 (0.8%) incident cases and 26 (0.4%) CRC deaths were in the group of carrot intake ≥32 g/day, and lower the association with CRC risk. Some residual confounders, such as dietary habits, and assessment of exposure may account for the difference between the dose-response analyses of carrot consumption and CRC incidence in a different population and thus needed to interpret cautiously. In addition, we explored the association of carrot intake with CRC mortality, but no significant correlation was detected in the multivariable Cox models and other analyses.

The cancer-prevention effect of carrot intake might be explained that carrots are rich in carotenoid antioxidants such as α- and β-carotene, which showed a potential prevention effect on cancer development. Hence, we further examined the association between dietary α-, and β-carotene intakes and CRC incidence, and mortality, but no statistically significant inverse associations were observed, respectively. That was in line with the results of a meta-analysis of nine randomized controlled trials (32), in which no significant association was found between dietary intakes of α- and β-carotene and colorectal cancer incidence. However, another two case-control studies in the Chinese population (18, 19) noted an inverse association of dietary α- and β-carotene intake with the risk of colorectal cancer. The differences in retrospective study design, number of samples, exposure assessment, potential recall bias, and the unadjusted confounders may contribute to the conflict findings. Another biological interpretation might be that carrots are also a major source of falcarinol (FaOH), falcarindiol (FaDOH), which have been demonstrated to inhibit neoplastic transformations in the rat models with a prevention effect on the development of colorectal cancer (33, 34). Thus, our findings supported that the potential cancer-prevention effects on CRC might derive from other specific components, rather than α-, and β-carotene.

Interestingly, we found that smoking status was an effect modifier on the relationship between carrot intake and colorectal cancer incidence (p for interaction = 0.027). In the strata of never smokers, the HR of the risk of CRC was 0.99 (95%CI = 0.99–1, $p < 0.01$), even though the association was weak. The result was constant with the previous research that smoking as a well-established risk factor could attenuate or reversed the observed protective effect of dietary carotenoids on CRC occurrence (17).

There have several inevitable limitations in the current study. First, the nature of observational studies resulted in residual confounding that could not be fully ruled out. Second, the generalizability of conclusions may limit by the geographical location and population differences. Third, we could not take into account the dynamic change data because of the absence of repeated measurements of dietary nutrients. Forth, we lack the data on raw, cooked carrots, and carrot juice due to the limited raw data, thus we were impossible to further distinguish and analyze their effect on colorectal cancer risk. In addition, some epidemiological studies published inconsistent results about the association of serum α-, and β-carotene concentration with CRC incidence (18, 35). We were interested in this, but for the limited raw data, thus we were unable to further analyze the association between serum carrot/carotene and the risk of CRC.

In summary, this U.S. prospective cohort indicated that moderate consumption of dietary carrots was associated with a lower risk of CRC incidence, while no statistically significant associations were observed between dietary α-, and β-carotene intakes and CRC incidence. Dietary carrot, α-, and β-carotene intakes were null associated with CRC mortality. Furthermore, we found smoking status modified the association of dietary carrot intakes with CRC incidence but not mortality. The results added evidence for the potential cancer-prevention effect of dietary carrot intakes on CRC incidence but should be cautiously interpreted. More large, prospective, well-designed cohort studies are warranted to verify the findings in other populations.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The PLCO study was approved by the Institutional Review Boards of the US National Cancer Institute and each study center and written informed consents were obtained from all eligible participants. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ZJ: conception, design, and acquisition of data. WW, CF, and FL: administrative support. ZJ and HC: data analysis and interpretation. ZJ, CF, and ML: manuscript revising. All authors: manuscript writing, final approval, and accountable for all aspects of work ensuring integrity and accuracy.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.888898/full#supplementary-material

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Dose-Response and Substitution Analyzes of Sweet Beverage Consumption and Body Weight in Dutch Adults: The Lifelines Cohort Study

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Background/Methods: Prospective studies investigating sweet beverages and body weight associations show inconsistent results. Within the SWEET project, we examined prospective dose-response associations of sugar-sweetened beverages (SSB), low/no-calorie beverages (LNCB), and fruit juice with body weight-related outcomes among 78,286 Dutch adults followed for ∼4 years. Baseline intakes were assessed using a validated food-frequency questionnaire (FFQ) with 150 ml representing a standard serving. Outcome variables were body weight change, waist circumference change, overweight/obesity, and abdominal obesity. Associations were investigated by using linear and non-linear dose-response analysis, as well as substitution models while adjusting for multiple socio-demographic, lifestyle, health, and dietary variables.

Results: Participants were 46 ± 13 (mean ± SD) years old and 60% were women. Adjusted dose-response analyzes indicated an association between SSB and LNCB, and both body weight (+0.02 kg/year; SE 0.01 and +0.06 kg/year; SE 0.01) and waist circumference changes (+0.04 cm/year; SE: 0.01 and +0.11 cm/year; SE: 0.01). Associations for overweight/obesity and abdominal obesity incidence were +3% (95%CI: 1.00–1.06) and +2% (95%CI: 0.99–1.06) for SSB and +8% (95%CI: 1.06–1.11) and +5% (95%CI: 1.03–1.07) for LNCB, respectively. Substitution of SSB with LNCB was associated with higher weight change (+0.04 kg/year), waist circumference change (+0.09 cm/year), overweight/obesity incidence (+6%), but not abdominal obesity incidence. For fruit juice, we observed beneficial associations for intake levels below ∼1 serving/day with weight, waist circumference change, and overweight/obesity incidence, and no association with abdominal obesity. Subsequent substitution analyzes indicated a small beneficial association for the replacement of SSB with fruit juice on weight (−0.04 kg/year) and waist circumference (−0.04 cm/year), but not with other outcomes.

Conclusions: Overall, our results suggest that habitual consumption of both SSB and LNCB may adversely affect weight-related outcomes. In contrast, fruit juice consumption <150 ml may be beneficial with respect to weight and waist circumference.

Keywords: waist circumference, overweight, abdominal obesity, population study, non-calorie sweeteners

INTRODUCTION

From 1975 to 2016, the prevalence of obesity among adults increased from 100 million to 671 million worldwide (1). As obesity has been associated with major health consequences, such as type 2 diabetes and cardiovascular diseases (2), effective interventions to decrease prevalence of overweight and obesity are urgently needed. Weight gain has been partly attributed to higher caloric intakes, including the consumption of added sugar in the form of high-calorie sugar-sweetened beverages (SSB) (3–5). Consequently, major efforts have been made to replace sugars with low/no-calorie sweeteners such as aspartame, acesulfame-K, saccharin, and sucralose (6), and to develop low/no-calorie beverages (LNCB) (7, 8).

Despite the large body of research on alternatives for SSB, their impact on body weight remains a topic of sustained debate due to inconsistent findings. Available randomized control trials (RCTs) and meta-analyzes of RCTs on weight loss generally indicate a beneficial impact of consuming LNCB instead of SSB (9–13). In contrast, meta-analyzes of observational studies observed either no association (9) or a modest positive association between LNCB consumption and body mass index (BMI) (12, 13). Similar inconsistencies have also been observed for fruit juice (14, 15). Thus, although limited SSB consumption is recommended, there is insufficient evidence on whether or not LNCB and fruit juice could serve as healthier alternatives.

The conflicting findings in current literature may be explained by several methodological aspects. Although RCTs have better internal validity and are often considered superior over observational studies, the majority of the RCTs had small sample sizes and were short-term (≤6 months) (9, 13). Here, observational studies offer the benefit to explore long-term associations between SSB, LNCB, fruit juice, and body weight. Nevertheless, so far only few observational studies explored both linear and non-linear dose-response associations of SSB and LNCB consumption with weight-related outcomes (16, 17). Exploration of non-linear dose-response associations for fruit juice and weight-related outcomes is even lacking while such associations have been reported for fruit juices, cardiovascular diseases (CVD), and type 2 diabetes (18, 19). Moreover, existing large-scale prospective studies on body weight mostly used self-reported measures, and few investigated outcomes beyond body weight such as waist circumference. Finally, only a limited number of studies have conducted substitution analyzes to investigate the replacement of SSB with other beverages on body weight (20–22).

Therefore, we examined prospective dose-response associations of habitual SSB, LNCB, and fruit juice consumption with measured changes in body weight and waist circumference, and incidence of overweight/obesity and abdominal obesity, and evaluated the theoretical substitution of SSB with LNCB and fruit juices while using data of 78,286 Dutch adults followed for ∼4 years.

MATERIALS AND METHODS

The SWEET Project

The SWEET project is a Horizon 2020 funded project that aims to develop and review evidence on long-term benefits and potential risks involved with replacing sugars with low or non-calorie sweeteners and sweetness enhancers in the context of public health and safety, obesity, and sustainability (https://sweetproject.eu/). The current study using data from the Lifelines Cohort Study was conducted as part of a work package that aims to investigate long-term associations between sweeteners and health outcomes in population studies.

Study Population and Design

The Lifelines Cohort Study is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design, the health and health-related behaviors of 167,729 persons living in the North of The Netherlands, including children (0–18 years old), adults (18–65 years old) and older adults (>65 years old) (23). It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioral, physical, and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics. Participants were recruited between 2006 and 2013 and will be followed for over 30 years. Potential participants with severe psychiatric or physical illness, limited life expectancy (<5 years), or insufficient knowledge of the Dutch language were not eligible for participation. Every 1.5 years, participants are invited to complete a follow-up questionnaire, and on average every 5 years, several physical measurements are performed and additional questionnaires are administrated. At the time of the current analysis, baseline data of 152,728 adults were available. After excluding those with unreliable dietary data, i.e., total energy intakes <500 and >3,500 kcal/day for women and <800 and >4,000 kcal/day for men (24, 25), 128,612 adults were included of which 84,545 had data on body weight and waist circumference change. A total of 78,286 adults met the inclusion criteria for the prospective analysis after the exclusion of missing data for covariates (**Supplementary Figure 1**). These 78,286 participants had their first follow-up exam between 1 and 9 years after baseline, with a median of 4 years after baseline. The Lifelines Cohort Study was conducted in accordance with the principles of the Declaration of Helsinki and the research

code University Medical Center Groningen (UMCG). The Lifelines Cohort Study has been approved by The Medical Ethical Review Committee of the University Medical Center in Groningen. All participants provided written informed consent before participation.

Anthropometry

Measurements of body weight, waist circumference, and BMI were carried out at baseline and follow-up by trained professionals. Body weight was measured to the nearest 0.1 kg with a digital scale (SECA 761) after participants were asked to wear light clothing and remove shoes. Height and waist circumference were measured to the nearest 0.1 cm, with a stadiometer (SECA 222) and measuring tape (SECA 200), respectively. BMI was obtained by dividing the weight of participants by height squared (kg/m^2). Weight change (kg/year) and waist circumference change (cm/year) were calculated by subtracting the baseline measure to the follow-up measurements and dividing by the follow-up time, (i.e., weight follow-up – weight baseline)/years of follow-up). Incidence of overweight/obesity were defined by a BMI $\geq$25 kg/m^2 at follow-up and abdominal obesity with a waist circumference $>$94 cm for men and $>$80 cm for women, based on the World Health Organization (WHO) cuts offs points (26). Additionally, participants were asked whether they wanted to lose weight, which we interpreted as "desire to lose weight (yes/no)."

Dietary Assessment

In the Lifelines Cohort Study, dietary intake was assessed with a semi-quantitative 110-item food-frequency questionnaire (FFQ). A detailed description of the FFQ can be found elsewhere (23, 27). In short, average energy and nutrient intakes were calculated by multiplying the frequency of consumption by portion size and nutrient content per gram using the 2011 Dutch food composition table (28). For the current analyzes, SSB was defined as soda sugar drinks or lemonade (both carbonated and non-carbonated). Fruit juice corresponded to 100% fruit juice and other fruit drinks. LNCB was defined as all items covering "diet soda or light soda." Baseline measurement of the diet was performed between 2006 and 2013. For this analysis, a standardized serving of 150 g ($\sim$150 ml) was calculated in all studies based on the smallest standard packaging for soft drinks.

Covariates

Covariates, including age (years), sex (men/women), educational level (low, medium, or high), smoking status (never, former or current), and medical history (yes/no), were assessed with either self- or interview-administered questionnaires (23). Educational level was categorized into less than secondary school qualification (low), secondary school diploma up to university classes but no Bachelor's degree (medium), and Bachelor, Master or PhD degree (high). Participant history of diseases (type 2 diabetes, CVD, hypertension, and hypercholesterolemia) were assessed by self-report or medical staff at recruitment and subsequent visits. Physical activity was assessed using the Short Questionnaire to Assess Health (SQUASH) (29) and the Activity Questionnaire for Adults and Adolescents (AQuAA) (24) and physical activity is thus reported as MET-min/week for light, moderate and intense exercise and in min/week for sedentary behavior (i.e. watching TV).

Statistical Analyzes

Baseline characteristics are presented by mean (SD), median (25th, 75th percentile), or *n* (%) where appropriate. To evaluate the nature of the dose-response relationships between beverages and weight related-outcomes, restricted cubic spline analysis (three knots) was performed (30). The fit of the spline model was tested against a linear model with a likelihood-ratio test. To evaluate the association between beverage consumption and weight and waist circumference changes, multiple linear regression was used. To evaluate the associations between beverage consumption and incidence of overweight/obesity and abdominal obesity, Cox proportional hazards regression with robust variance estimation and a constant follow-up time was used to obtain unbiased incidence proportion ratios (IPR) (31, 32). To investigate the association with weight-related outcomes when replacing each serving of SSB with a serving of either LNCB or fruit juice, theoretical substitution analyzes were conducted by means of a leave-one-out model (33). This model included the sum of all beverages as one variable followed by the beverages defined as replacement, as well as all other covariates as modeled in the analyzes. In a sub-sample of Lifelines where water consumption was available (N = 22,859), we additionally studied the replacement of SSB and LNCB with water for comparaison purposes. In all models, potential confounders were identified based on a priori knowledge. Models were adjusted for sex and age (model 1) + height and baseline weight (or baseline BMI for overweight/obesity incidence models) or baseline waist circumference (for models with waist circumference or abdominal obesity as outcome; model 2), + education (low, medium, and high), physical activity (light, moderate and intense in METs-min/week), sedentary behavior (min/week), alcohol intake (ethanol categories: non-consumers, $\leq$10 g, $>$10–20 g, and $>$20 g/day), smoking (never, former or current), dietary variables (g/day), namely meat, dairy, legumes, vegetables, nuts, fruits, potatoes, fats, grains, tea, coffee, sugary food intakes and other beverages (servings/day, i.e., SSB adjusted for fruit juice and LNCB and vice-versa) and history of diseases (self-reported diabetes and history of CVD, hypertension, and hypercholesterolemia; model 3). As total energy may mediate and thus attenuate the associations under investigation, particularly in the case of SSB and fruit juice, the final models were tested with and without adjustment for total energy intake (model 4). Additional analyzes were performed adjusting for desire to lose weight (yes/no) and sensitivity analyzes were conducted by excluding participants with any self-reported health conditions at baseline (i.e., diabetes type 2, CVD, hypercholesterolemia, or hypertension). We also tested the interaction for BMI ($<$25 and $\geq$25 kg/m^2), sex and age ($<$46 years old and $\geq$46 years old) and studied the stratified data accordingly. All analyzes were performed using R 3.6.1 and RStudio 1.0.

TABLE 1 | General characteristics of the Lifelines Cohort Study.

Characteristics[a]	Overall	BMI < 25 kg/m^2	BMI ≥ 25 kg/m^2[b]
N	78,286	35,202	43,084
Women, *n* (%)	46,663 (59.6)	23,617 (67.1)	22,046 (53.5)
Age, years	45.9 (12.7)	43.1 (12.9)	48.1 (12.1)
Education, *n* (%)			
Low	3,077 (3.9)	1,073 (3.0)	2,004 (4.7)
Intermediate	50,690 (64.7)	20,931 (59.5)	29,759 (69.1)
High	24,519 (31.3)	13,198 (37.5)	11,321 (26.3)
Height (cm)	174.7 (9.3)	174.6 (9.0)	174.8 (9.5)
Body weight, kg	79.5 (15.0)	69.1 (9.1)	88.1 (13.3)
Waist circumference, cm	90.1 (12.2)	81.3 (7.7)	97.3 (10.3)
BMI, kg/m²	26.0 (4.2)	22.6 (1.7)	28.8 (3.5)
Desire to lose weight[c]	44,411 (56.8)	10,331 (29.4)	34,080 (79.2)
Physical activity (METs min/week)			
Intense	0 [0,630]	0 [0, 840]	0.0 [0, 420]
Moderate	1,665 [806, 2,948]	1,605 [788, 2,847]	1,702 [818, 3,045]
Sedentary (min/week)	840 [630,1,260]	840 [630, 1,260]	1,050 [840, 1,470]
Smoking, *n* (%)			
Never	36,461 (46.6)	18,020 (51.2)	18,441 (43.8)
Former	27,376 (35.0)	10,337 (29.4)	17,039 (39.5)
Current	14,449 (18.5)	6,845 (19.4)	7,604 (17.6)
Alcohol (ethanol) intake, *n* (%)			
No alcohol	1,919 (2.5)	702 (2.0)	1,217 (2.8)
Medium (0–≤10g)	55,888 (71.4)	25,925 (73.6)	29,963 (69.5)
High (10–≤20g)	15,032 (19.2)	6,593 (18.7)	8,439 (19.6)
Very high (>20 g)	5,447 (7.0)	1,982 (5.6)	3,465 (8.0)
Total energy, g/day	1,977 [1,640, 2,380]	1,997 [1,665, 2,387]	1,959 [1,619, 2,373]
SSB servings/day	0.11 [0.0, 0.62]	0.14 [0.00, 0.63]	0.09 [0.00, 0.60]
LNCB servings/day	0.07 [0.0, 0.61]	0.04 [0.00, 0.36]	0.12 [0.0, 0.71]
Fruit Juice servings/day	0.18 [0.04, 0.64]	0.18 [0.04, 0.71]	0.18 [0.00, 0.64]
Type 2 diabetes, *n* (%)	1,853 (2.4)	297 (0.8)	1,556 (3.6)
CVD, *n* (%)	1,805 (2.3)	541 (1.5)	1,264 (2.9)
Hypertension, *n* (%)	17,499 (22.4)	4,841 (13.8)	12,647 (29.4)
Hypercholesterolemia, *n* (%)	11,070 (14.1)	3,226 (9.2)	7,834 (18.2)
Body weight change (kg/year)	0.02 (1.58)	0.21 (1.20)	−0.13 (1.82)
Waist circumference change (cm/year)	0.01 (2.04)	0.10 (1.88)	−0.07 (2.15)
Overweight/obesity incidence	–	4,884/35,202 (13.9)	–
Abdominal obesity incidence	–	6,896/31,292 (22.0)	–

[a] *Mean (SD), median [25th-75th percentile] or n (%).*
[b] *All P-values for the difference between BMI categories were <0.01.*
[c] *Data was missing for 134 participants.*
BMI, body mass index; SSB, sugar-sweetened beverages; LNCB, low/no-calorie beverages; CVD, cardiovascular diseases.

RESULTS

Participants ($n = 78,286$) had a mean age of 45.9 (SD 12.7) years and 60% were women (**Table 1**). Baseline mean BMI was 26.0 (SD 4.2) kg/m^2 and 45% of the participants had a normal BMI < 25 kg/m^2. Mean body weight change during follow-up was +0.02 (SD 1.58) kg/year and mean waist circumference change was +0.01 (SD 2.04) cm/year. On average, participants with normal BMI gained weight and waist circumference [+0.21 (SD 1.20) kg/year and + 0.10 (SD 1.88) cm/year], while participants with higher BMI lost weight and waist circumference [−0.13 (SD 1.82) kg/year; and −0.07 (SD 2.15) cm/year]. Participants with overweight/obesity also reported a higher desire to lose weight at baseline (79 vs. 29% in participants with normal BMI). Of the 35,202 participants with normal BMI at baseline, 4,884 (14%) developed overweight or obesity and out of the 31,292 participants with normal waist circumference at baseline, 6,896 (22%) developed abdominal obesity (**Table 1**).

Overall, 62% of participants consumed SSB, 57% LNCB, and 77% fruit juice. Median [25th−75th percentile] baseline intakes

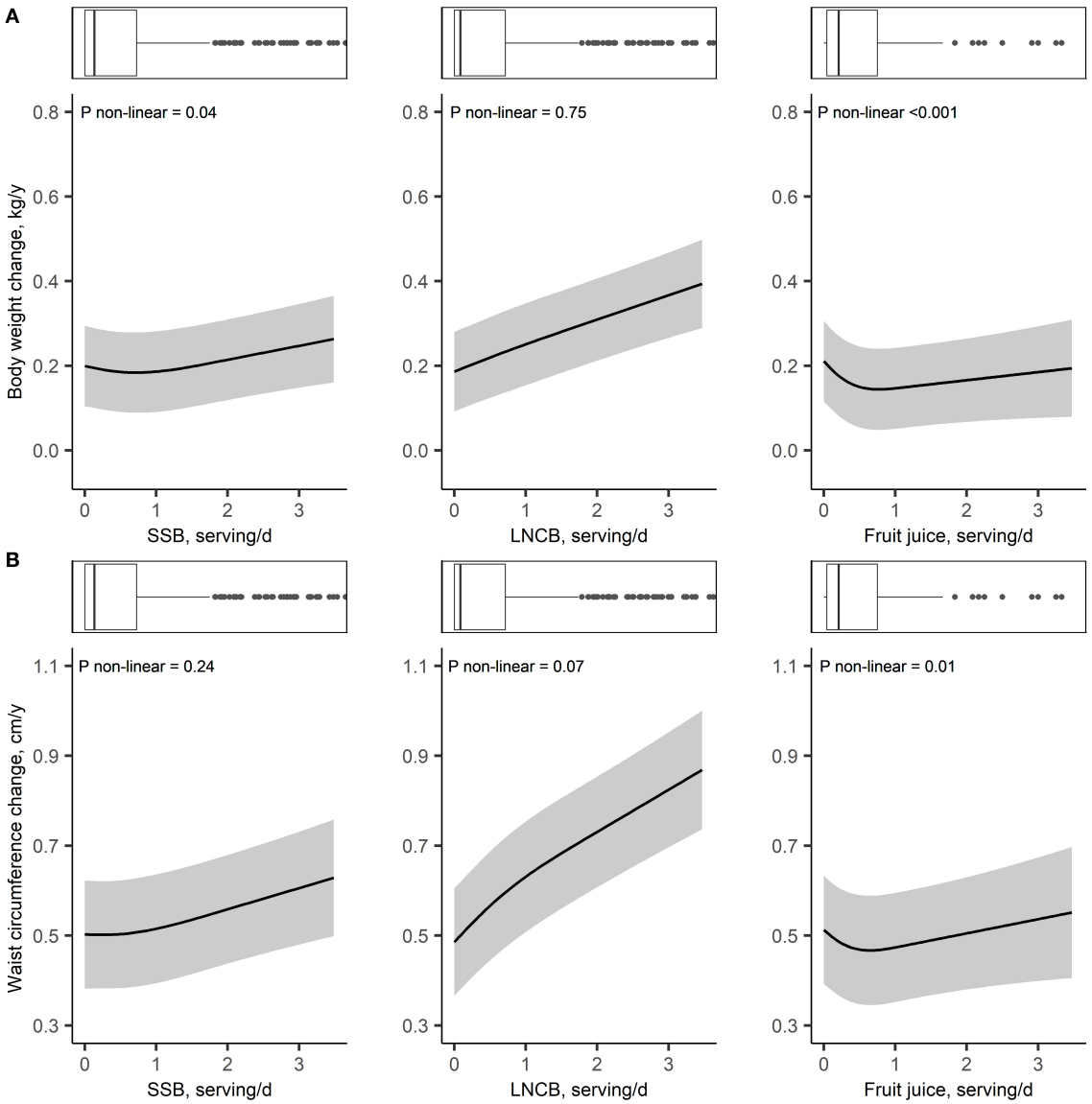

FIGURE 1 | Adjusted dose-response associations of SSB, LNCB, and fruit juice consumption with body weight change (kg/year) **(A)** and waist circumference change (cm/year) **(B)** in the Lifelines Cohorts Study; Models were adjusted for age, sex, height, baseline weight or baseline waist circumference (for models with waist circumference as outcome), education, alcohol intake, smoking, physical activity, all dietary factors, total energy intake and history of diseases. SSB, sugar-sweetened beverages; LNCB, low/no-calorie beverages.

were 0.1 [0.0; 0.6] servings/day for SSB and LNCB, and 0.2 [0.0; 0.6] servings/day for fruit juices. In general, those consuming the highest levels of SSB and fruit juice were more likely to be men, were younger, had less often a chronic disease history, and had a slightly lower BMI and higher energy intake (all P-trend < 0.001; **Supplementary Table 1**). Concerning LNCB, those consuming the highest levels were more likely to be women, to be younger, and to have a higher BMI (all P-trend <0.001). SSB and fruit juice consumption were both positively correlated with energy intake ($r = 0.34$ and $r = 0.21$ respectively; $P < 0.001$), while no correlation was observed between LNCB use and energy intake ($r = 0.005$).

Dose-response analyzes suggested a weak non-linear association between SSB and body weight (P non-linear $= 0.04$), but not with waist circumference (P non-linear $= 0.24$), overweight/obesity incidence (P non-linear $= 0.27$) or abdominal obesity incidence (P non-linear $= 0.70$; **Figures 1**, **2**). Each increase in SSB serving/day was associated with a +0.03 (SE 0.01) kg/year increase in weight change and a +0.05 (SE 0.01) cm/year increase in waist circumference change after adjusting for height and baseline weight or waist circumference (model 2). Further adjustment for lifestyle variables and total energy intake slightly attenuated this association, +0.02 (SE 0.01) kg/year and 0.04 (SE 0.01) cm/year (**Table 2**). Similarly, each

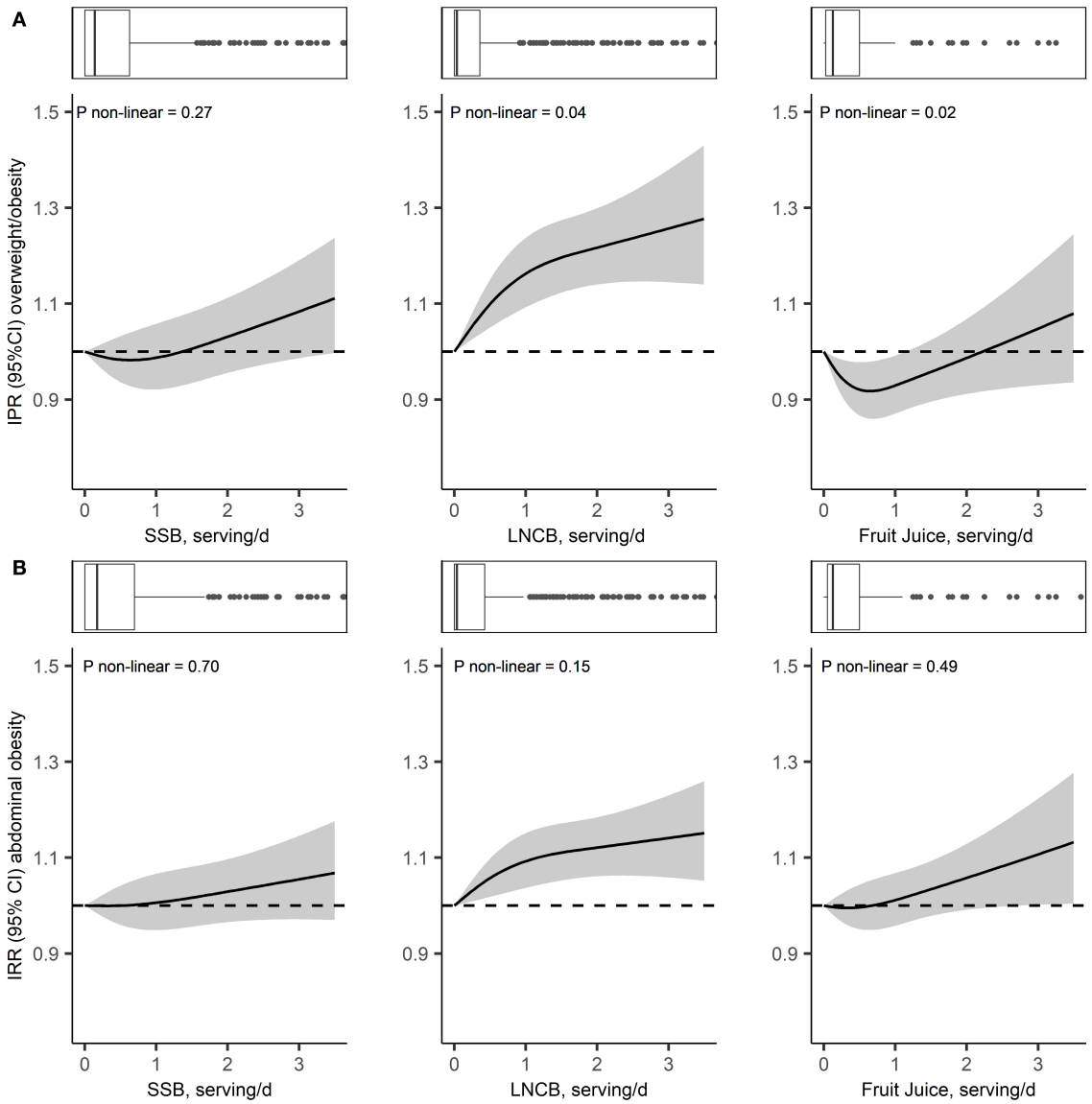

FIGURE 2 | Adjusted dose-response associations of SSB, LNCB, and fruit juice consumption with overweight/obesity incidence **(A)** and abdominal obesity incidence **(B)** in participants with normal values at baseline (i.e., <25 kg/m^2 for overweight/obesity and ≤94 cm in men and ≤80cm in women for abdominal obesity) in the Lifelines Cohort Study; Models were adjusted for age, sex, baseline BMI or baseline waist circumference and height (for models with abdominal obesity as outcome), education, alcohol intake, smoking, physical activity, all dietary factors, total energy intake and history of diseases; SSB, sugar-sweetened beverages; LNCB, low/no-calorie beverage; IPR, incidence proportion ratio.

SSB serving/day increase was associated with a 5% increase in incidence of overweight/obesity (IPR 1.05, 95%CI: 1.03–1.08) and a 5% increase in abdominal obesity incidence (IPR: 1.05, 95%CI: 1.02–1.07; model 2). After adjustment for dietary and lifestyle variables, these associations were attenuated to 3% (IPR: 1.03, 95%CI: 1.00–1.06) and 2% (IPR: 1.02, 95%CI: 0.99–1.05), respectively (**Table 2**).

Dose-response analyzes showed linear associations between LNCB consumption and weight and waist circumference (P = 0.75 and P = 0.07; **Figure 1**), and a non-linear association between LNCB and overweight/obesity incidence (P non-linear = 0.04; **Figure 2**). LNCB consumption was associated with neither changes in body weight nor waist circumference after adjusting for age and sex (model 1; **Table 2**). However, in fully adjusted models, each increase of one serving/day LNCB was associated with a +0.06 (SE 0.01) kg/year body weight change and a +0.11 (SE 0.01) cm/year waist circumference change. Moreover, after adjustment for age and sex, each LNCB serving/day increase was associated with a 20% increase in incidence of overweight/obesity (IPR: 1.20, 95%CI: 1.17–1.22) and a 12% higher incidence of abdominal obesity (IPR: 1.12, 95%CI: 1.04–1.09; model 1; **Table 2**), which attenuated to an 8% (IPR: 1.08, 95%CI: 1.06–1.11) and a 5% (IPR: 1.05, 95%CI: 1.02–1.07) increase after full adjustment

TABLE 2 | Linear associations between sugar-sweetened beverages, low/no-calorie beverages, fruit juice consumption, and weight-related outcomes in the Lifelines Cohort Study.

Outcomes[a]	Total *N*/cases *N*	SSB (serving/day)	LNCB (serving/day)	Fruit Juice (serving/day)
Body weight change (kg/year)	78,286			
Model 1		0.03 (0.01)	0.01 (0.01)	−0.01 (0.01)
Model 2		0.03 (0.01)	0.07 (0.01)	−0.02 (0.01)
Model 3		0.02 (0.01)	0.06 (0.01)	−0.02 (0.01)
Model 4		0.02 (0.01)	0.06 (0.01)	−0.02 (0.01)
Waist circumference change (cm/year)	78,286			
Model 1		0.03 (0.01)	0.02 (0.01)	−0.00 (0.01)
Model 2		0.05 (0.01)	0.13 (0.01)	−0.00 (0.01)
Model 3		0.03 (0.01)	0.11 (0.01)	−0.01 (0.01)
Model 4		0.04 (0.01)	0.11 (0.01)	−0.00 (0.01)
Overweight/obesity incidence (IPR, 95%CI)[b]	35,202/4,884			
Model 1		1.02 (0.99–1.05)	1.20 (1.17–1.22)	0.98 (0.94–1.03)
Model 2		1.05 (1.03–1.08)	1.10 (1.07–1.13)	1.00 (0.96–1.04)
Model 3		1.02 (0.99–1.05)	1.08 (1.05–1.11)	0.99 (0.95–1.03)
Model 4		1.03 (1.00–1.06)	1.08 (1.06–1.11)	1.00 (0.96–1.04)
Abdominal obesity incidence (IPR, 95%CI)[c]	31,292/6,896			
Model 1		1.04 (1.01–1.07)	1.12 (1.10–1.14)	1.03 (1.00–1.06)
Model 2		1.05 (1.02–1.07)	1.06 (1.04–1.09)	1.03 (0.99–1.06)
Model 3		1.01 (0.99–1.04)	1.05 (1.02–1.07)	1.03 (0.99–1.06)
Model 4		1.02 (0.99–1.05)	1.05 (1.02–1.07)	1.03 (1.00–1.07)

[a]Results given are β (SE) for body weight and waist circumference changes or as IPR (95%CI) for overweight/obesity and abdominal obesity incidences.

[b]In participants with normal BMI (<25 kg/m^2) at baseline.

[c]In participants with normal waist circumference (≤94 cm for men and ≤80 cm for women) at baseline.

Model 1: adjusted for age and sex, Model 2: model 1 + height and baseline weight (or baseline BMI for overweight/obesity incidence models) or baseline waist circumference (for models with waist circumference or abdominal obesity as outcome). Model 3: model 2 + education (categorical), physical activity (continuous), sedentary behavior (continuous), smoking (categorical), alcohol intake (categorical) + intakes of fruits, vegetables, legumes, nuts, meat, dairy, sugary foods, potatoes, fats, grains, coffee and tea (g/day) + LNCB and Fruit juice (if model SSB and vice versa) + history of diseases. Model 4: model 3 + total energy intake (kcal/day).

BMI, body mass index; SSB, sugar-sweetened beverages; LNCB, low/no-calorie beverages; CVD, cardiovascular diseases; IPR, incidence proportion ratio.

(model 4). The non-linear association between LNCB and overweight/obesity incidence showed a steeper increase in risk <1 serving/day intake and a more gradual increase at higher levels (**Figure 2** and **Table 3**).

Dose-response analyzes also indicated non-linearity for the associations between fruit juice with weight and waist circumference ($P < 0.001$ and $P = 0.01$, respectively; **Figure 1**). Below an intake of ∼1 serving fruit juice/day, body weight and waist circumference decreased, while no associations were observed above this threshold (**Table 3**). Accordingly, a J-shaped association was present for the association between fruit juice intake and overweight/obesity incidence (P non-linear = 0.02; **Figure 2**). Compared to non-users, participants consuming ≤1 serving/day had an 11% reduced overweight/obesity risk (IPR: 0.89, 95%CI: 0.83–0.95) while no association was observed at higher intake levels (**Table 3**). A weak linear association was observed for fruit juice and abdominal obesity incidence (IPR: 1.03, 95%CI 1.00–1.06; P non-linear = 0.49; **Figure 2** and **Table 3**).

Adjusting for or excluding participants with "desire to lose weight" or participants with self-reported health conditions at baseline (i.e. type 2 diabetes, CVD, hypertension, and hypercholesterolemia) did not substantially alter the associations for any beverage (**Supplementary Tables 2**, **3**).

Substitution analyzes are shown in **Figures 3**, **4**. Replacing one serving SSB with an equal amount of water or fruit juice was associated with decreased weight (−0.02 kg/year, SE 0.01 and −0.04 kg/year, SE 0.01, respectively) and waist circumference (−0.04 cm/year, SE 0.02; for both beverages) (**Figure 3**). Substituting LNCB with water also showed an inverse association with weight (−0.05 kg/year, SE 0.01) and waist circumference (−0.08 cm/year, SE 0.01). In contrast, replacing SSB with LNCB was positively associated with increased weight (+0.04 kg/year, SE 0.01) and waist circumference (+0.08 cm/year, SE 0.01). Only the substitution of one serving SSB with an equal amount of LNCB was associated with higher overweight/obesity incidence (IPR: 1.06; 95%CI: 1.02–1.10) while the substitution of one serving LNCB with one serving water was associated with reduced overweight/obesity incidence (IPR: 0.91; 95%CI: 0.86–0.97) (**Figure 4**). None of the other substitution analyzes related to overweight or abdominal obesity incidences showed any association.

Stratified analyzes are included as **Supplementary Tables 4**–**6**. Analyzes stratified by BMI category showed an interaction for SSB consumption and body weight change (P interaction < 0.001; **Supplementary Table 4**). In participants with normal BMI (< 25 kg/m^2), each increase in SSB serving/day was associated with a +0.04 (SE 0.01) kg/year change in body

TABLE 3 | Adjusted associations between sugar-sweetened beverages, low/no-calorie beverages, fruit juice consumption, and weight-related outcomes categorized by intake levels.

Outcomes[a]		SSB				LNCB				Fruit Juice			
		None	≤1 serving/day	1–2 servings/day	>2 servings/day	None	≤1 serving/day	1–2 servings/day	>2 servings/day	None	≤1 serving/day	1–2 servings/day	>2 servings/day
	***N* total/cases**	29,637	36,967	8,134	3,548	33,938	33,497	7,697	3,154	18,220	51,831	6,945	1,290
Body weight change (kg/year)	78,286	ref	−0.04 (0.01)	−0.03 (0.02)	0.04 (0.03)	ref	0.03 (0.01)	0.09 (0.02)	0.20 (0.03)	ref	−0.07 (0.01)	−0.07 (0.01)	−0.09 (0.05)
Waist circumference change (cm/year)	78,286	ref	−0.03 (0.02)	−0.01 (0.03)	0.09 (0.04)	ref	0.08 (0.02)	0.20 (0.03)	0.40 (0.04)	ref	−0.06 (0.02)	−0.03 (0.03)	−0.02 (0.03)
Overweight/ obesity[b]	35,202/4,884	ref	0.94 (0.88–1.00)	0.92 (0.83–1.01)	1.16 (1.04–1.28)	ref	1.06 (1.01–1.11)	1.18 (1.10–1.26)	1.26 (1.13–1.38)	ref	0.89 (0.83–0.95)	0.94 (0.84–1.03)	1.00 (0.82–1.18)
Abdominal obesity [c]	31,292/6,896	ref	0.99 (0.95–1.04)	1.01 (0.93–1.08)	1.06 (0.95–1.16)	ref	1.06 (1.02–1.10)	1.12 (1.05–1.19)	1.13 (1.02–1.25)	ref	0.99 (0.94–1.04)	1.05 (0.97–1.13)	1.04 (0.90–1.21)

[a] *Results given are β (SE) for body weight and waist circumference changes or as IPR (95%CI) for overweight/obesity and abdominal obesity incidences.*

[b] *For overweight/obesity incidence in each intake category n = 11,858; n = 177,740; n = 3,861 and n = 1,743 for SSB; n = 16,935; n = 14,745; n = 2,658 and n = 864 for LNCB, and n = 7,124; n = 24,230; n = 3,255 and n = 593 for Fruit Juice.*

[c] *For abdominal obesity incidence in each intake category n = 9,631, n = 16,187; n = 3,731 and n = 1,743 for SSB; n = 14,927, n = 13,042, n = 2,436 and n = 887 for LNCB and n = 6,128; n = 24,446; n = 3,113; and n = 605 for Fruit Juice.*

All models were adjusted by age, sex, height, and baseline weight (or baseline BMI for overweight/obesity) or baseline waist circumference (for models with waist circumference and abdominal obesity models as outcome), education (categorical), physical activity (continuous), sedentary behavior (continuous), smoking (categorical), alcohol intake (categorical), intakes of fruits, vegetables, legumes, nuts, meat, dairy, sugary foods, potatoes, fats, grains, coffee and tea (g/day), LNCB and Fruit juice (if model SSB and vice versa), history of diseases (diabetes, CVD, hypertension, and hypercholesterolemia) and total energy intake (kcal/day) (model 4).

BMI, body mass index; SSB, sugar-sweetened beverages; LNCB, low/no-calorie beverage; CVD, cardiovascular diseases; IPR, incidence proportion ratio.

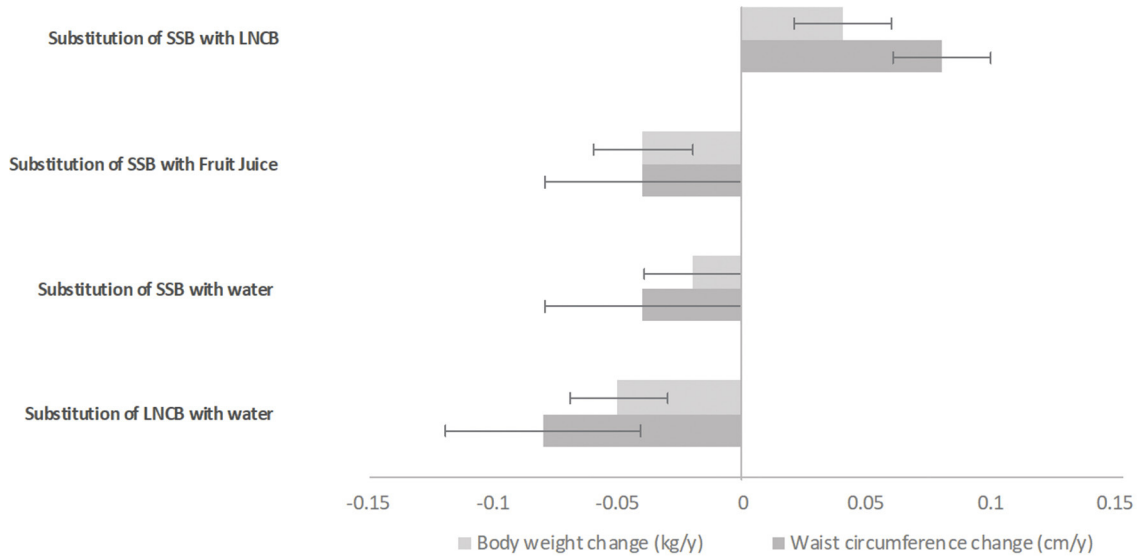

FIGURE 3 | Adjusted substitution associations of one serving of beverage with another with body weight change (kg/year) and waist circumference change (cm/year) in the Lifelines Cohorts Study; Models were adjusted for age, sex, height, baseline weight or baseline waist circumference (for models with waist circumference as outcome), education, alcohol intake, smoking, physical activity, all dietary factors and history of diseases. Error bars represent the 95% CIs. SSB, sugar-sweetened beverages; LNCB, low/no-calorie beverages.

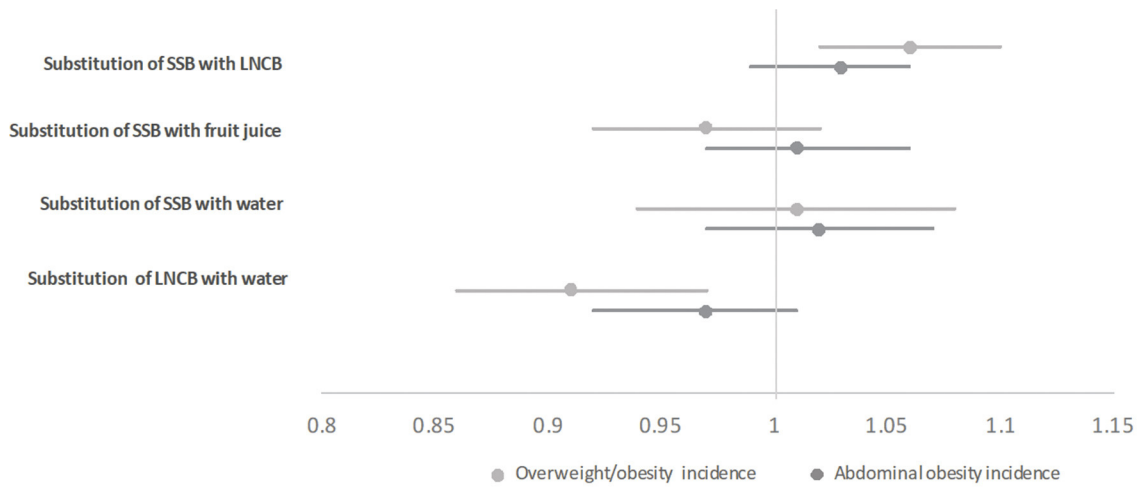

FIGURE 4 | Adjusted substitution associations of one serving of beverage with another with overweight/obesity incidence (IPR, 95%CI) and abdominal obesity incidence (IPR, 95%CI) in participants with normal values at baseline (i.e., <25 kg/m^2 for overweight/obesity and ≤94 cm in men and ≤80 cm in women for abdominal obesity) in the Lifelines Cohort Study; Models were adjusted for age, sex, baseline BMI or baseline waist circumference and height (for models with abdominal obesity as outcome), education, alcohol intake, smoking, physical activity, all dietary factors, and history of diseases; SSB, sugar-sweetened beverages; LNCB, low/no-calorie beverage; IPR, incidence proportion ratio.

weight while in participants with overweight/obesity no association was observed (0.00 kg/year, SE 0.01). Moreover, the impact of substituting one serving SSB with an equal amount of LNCB was more pronounced in overweight/obese participants (+0.06 kg/year, SE 0.01) compared to participants with normal BMI (+0.02 kg/year; SE 0.01, *P* interaction < 0.001; **Supplementary Table 6**). Stratification by BMI also showed an interaction for fruit juice and body weight (*P* interaction < 0.01) with a stronger inverse association among participants with overweight/obesity (**Supplementary Tables 4**, **5**). Additional stratification by sex indicated that the association of LNCB with abdominal obesity was slightly more pronounced in men (IPR: 1.06, 95%CI: 1.03–1.10) than in women (IPR: 1.03, 95%CI: 1.00–1.06, *P*-interaction = 0.04; **Supplementary Table 4**) with a similar observation for the substitution analysis (**Supplementary Table 6**). In contrast, the beneficial association observed between fruit juice and body weight change at moderate doses was stronger in women than in men (*P* interaction = 0.03).

We did not observe any other substantial evidence of effect modification with BMI, sex, or age.

DISCUSSION

In our study among 78,286 Dutch adults, habitual intakes of SSB and LNCB were linearly associated with most weight outcomes. A J-shaped association was observed for fruit juice showing a beneficial consumption below ∼1 serving/day for all outcomes except abdominal obesity incidence. In addition, the theoretical substitution of SSB with LNCB was associated with an increase in weight and waist circumference. Replacing one serving SSB with an equal amount of fruit juice was associated with decreases in weight and weight circumference.

The positive associations observed between SSB and weight outcomes in our study are generally in line with earlier studies (4, 16, 17). A meta-analysis including 174,252 adults, mostly from the US, showed that each daily serving (= 250 ml) increase in SSB was associated with a 0.22 kg (95%CI: 0.50–1.20) higher weight gain over 1 year (4). Furthermore, the positive associations between SSB consumption and overweight/obesity incidence align with recent meta-analyzes by Schlesinger et al. (16) and Qin et al. (17), which reported an increased overweight/obesity and obesity risk with each additional SSB serving, i.e., 5% (RR: 1.05, 95%CI:1.00–1.11) (16) and 12% (RR: 1.12, 95%CI:1.05–1.19), respectively (17). However, it needs to be emphasized that our results are rather modest compared to these – predominantly US - studies, which may be explained by lower SSB intake levels in our population (4, 16, 17) and a relatively short follow-up time of ∼4 years in our study. More consistent results may be observed once the follow-up time exceeds 5 years (34).

We also observed positive associations between LNCB consumption and body weight outcomes; stratifying the data for those with normal vs. overweight showed similar results. Moreover, substitution analyzes, showed that the replacement of SSB by LNCB was adversely associated with weight and waist circumference changes. Previous meta-analyzes of prospective studies on Low/No calorie sweeteners and weight outcomes generally report either no association or positive associations (9, 12, 13, 17, 35). To illustrate, a recent meta-analysis of prospective studies reported a 21% (RR: 1.21) increased risk of obesity for each 250 ml LNCB increment (17). The latest WHO report also acknowledges observed positive prospective associations of LNCB consumption with incidence obesity and BMI, but not with other adiposity measures (36). However, meta-analyzes of RCTs do not support these observational findings and generally report a beneficial impact of LNCB on body weight measures (9–11, 13, 36, 37). Conflicting findings between observational studies and RCTs might be due to differences in design and follow-up time where potential reverse causation or residual confounding may explain adverse findings in observational studies. It may be that overweight participants consume LNCB instead of SSB to manage their weight, while their overall weight management strategy is not sufficiently effective. This phenomenon of reverse causality may explain why the adverse association of replacing SSB with LNCB in our study was slightly stronger in the higher BMI category and why replacing LNCB – but not water – for SSB showed an adverse association with incidence overweight/obesity. To date, only a few other prospective studies have investigated the theoretical substitution of different beverages (20–22). In our study, substituting both SSB and LNCB with water was associated with less weight and waist circumference gain. Other studies have found similar beneficial results for the replacement of SSB with water and associations with weight change (20) or incidence obesity (21). However, substituting LNCB with water was not associated with any adiposity measures in other prospective analyzes (21, 22).

Interestingly, we found J-shaped associations between fruit juice and weight, waist circumference, and incidence of overweight/obesity during follow-up. Non-linear continuous dose-response associations between fruit juice consumption and weight-related outcomes have not been reported before. However, our results are in line with previous findings for CVD risk (18, 19). Khan et al. (19) observed a non-linear J-shaped curve with a beneficial association between 100% fruit juice and CVD incidence at moderate doses (∼150 ml) but no association at higher doses. D'Elia et al. (18) reported similar results in prospective studies of 100% fruit juice with CVD incidence. However, the borderline linear association observed with abdominal obesity incidence suggests that the consumption of fruit juice, even at moderate intake, might still be recommended against. Thus, further research on the potential beneficial effect of fruit juice is warranted.

Mechanically, the adverse association between SSB and increased body weight can be supported by several biological mechanisms (28, 29). The high-calorie content of SSBs and the lack of energy compensation can lead to a disturbed energy balance and thus weight gain. SSBs also contain rapidly absorbable carbohydrates that affect insulin secretion and blood glucose and possibly later insulin resistance (30). In contrast, biological mechanisms for the association between LNCB and weight are unclear. Factors other than energy intake may explain the adverse associations found with habitual LNCB and future weight gain. LNCB has been suggested to indirectly affect intestinal glucose absorption, appetite, and hormone dysregulation through activation of sweet taste receptors (38, 39). Other potential mechanisms include altered gut microbiota leading to glucose intolerance and insulin resistance (38, 39). Nevertheless, evidence for these mechanisms in human RCTs is limited and was not demonstrated by other studies when compared to water or unsweetened products (9, 40–42) or when used as a control in RCTs of SSB (43, 44). In contrast to SSB or LNCB, Fruit juices contain health-promoting nutrients such as antioxidants (i.e., polyphenols) and other bioactive substances (i.e., vitamins and minerals) (45). These nutrients could explain the benefits observed with moderate consumption of fruit juice on certain health outcomes as they may play a role in lowering oxidative stress, inflammation, and improving glucose metabolism (46, 47). After a certain level, these benefits may be counterbalanced by the sugar and calorie content of fruit juice leading to a detrimental effect on body weight measures through similar mechanisms as SSB. However, such a phenomenon remains a hypothesis (46).

An important strength of our study is the large sample size allowing for well-powered stratification and adjustment for multiple covariates. Our study is one of the largest

studies on the association and replacement of sweet beverages conducted in European adults so far. In addition, we used prospective measures of body weight and waist circumference, rather than self-reported anthropometrics. Furthermore, we included a variety of outcome measures, i.e., continuous waist circumference change, obesity, and abdominal obesity incidence. And last but not least, we evaluated both measures of continuous and dichotomous weight and waist-related outcomes together with the exploration of non-linear dose-response and substitution associations. A limitation of this study is that habitual dietary intake is only assessed at baseline. Dietary assessment at multiple time points might have provided more insight into whether the adverse associations observed in observational studies are caused by the beverage itself or other associated behaviors. Second, we were not able to distinguish between different types of fruit juice and different types of LNCB, and, therefore, we were not able to investigate potential differential effects of consumed sweeteners on weight gain. For example, a recent study demonstrated increased weight gain and hunger with saccharin intake but no change in energy intake, indicating that mechanisms other than energy intake might be implicated for this specific sweetener (48). With the use of different blends of sweeteners on the market, it is particularly relevant to further investigate specific effects in the future. Third, our questionnaire included 100% fruit juice along with other fruit drinks, which limits comparison with other studies that used specifically 100% fruit juice. However, according to the last consumption survey in the Netherlands, pasteurized orange, apple, and mixed juices composed 90% of the total fruit juice consumption in the Netherlands in the same time period as our study (49). Thus, we assume that the fruit juices consumed in this study were mostly 100% fruit juice.

To conclude, our study indicates that habitual consumption of both SSB and LNCB may adversely affect weight-related outcomes. In contrast, consumption of moderate amounts of fruit juice (<150 ml) may be beneficial with respect to body weight and waist circumference.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Data described in the manuscript, codebook, and analytic code will be made available upon request pending application, payment, and SWEET consortium agreement. Requests to access these datasets should be directed at: onderzoek@lifelines.nl.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Medical Ethical Review Committee of the University Medical Center in Groningen. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JHar, JHal, and AR are coordinators of the SWEET project and together with EF initiated the research question. MB and NN prepared the data for analyzes. MB, EB-B, and EF analyzed the data and drafted the manuscript. All authors critically revised the manuscript for important intellectual content and approved of the final version to be published.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.889042/full#supplementary-material

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Lifestyle and Dietary Habits Affect Plasma Levels of Specific Cytokines in Healthy Subjects

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Low-grade chronic inflammation (LGCI) is a common feature of non-communicable diseases. Cytokines play a crucial role in LGCI. This study aimed to assess how LGCI risk factors [e.g., age, body mass index (BMI), smoke, physical activity, and diet] may impact on specific cytokine levels in a healthy population. In total, 150 healthy volunteers were recruited and subjected to questionnaires about the last 7-day lifestyle, including smoking habit, physical activity, and food frequency. A panel of circulating cytokines, chemokines, and growth factors was analyzed by multiplex ELISA. BMI showed the heaviest impact on the correlation between LGCI-related risk factors and cytokines and was significantly associated with CRP levels. Aging was characterized by an increase in IL-1b, eotaxin, MCP-1, and MIP-1α. Smoking was related to higher levels of IL-1b and CCL5/RANTES, while physical activity was related to MIP-1α. Within the different eating habits, CRP levels were modulated by eggs, red meat, shelled fruits, and greens consumption; however, these associations were not confirmed in a multivariate model after adjusting for BMI. Nevertheless, red meat consumption was associated with an inflammatory pattern, characterized by an increase in IL-6 and IL-8. IL-8 levels were also increased with the frequent intake of sweets, while a higher intake of shelled fruits correlated with lower levels of IL-6. Moreover, IL-6 and IL-8 formed a cluster that also included IL-1b and TNF-α. In conclusion, age, BMI, smoke, physical activity, and dietary habits are associated with specific cytokines that may represent potential markers for LGCI.

Keywords: cytokines, low-grade chronic inflammation, biomarkers, body mass index, inflammaging

INTRODUCTION

Low-grade chronic inflammation (LGCI) is a common characteristic of many non-communicable diseases, such as obesity, type 2 diabetes, cardiovascular disease, chronic respiratory disease, and cancer. These frequent disorders are chronic conditions and are responsible for ∼71% of all global deaths (1, 2).

The LGCI has been recognized as a distinct type of inflammation. It is not accompanied by inflammatory classical signs and is not usually driven by pathological stimuli. LGCI is triggered by sentinel cells (i.e., macrophages and dendritic cells) that monitor for tissue stress and malfunction and, together with cells and molecules of the innate immune response, orchestrate the restoration of the normal/optimal homeostatic state (3). However, LGCI often does not resolve in a timely and controlled way and becomes chronic and smoldering (4).

Multiple cytokines preside over LGCI evolution. The cytokine network is a highly complex system that includes not only interleukins, chemokines, interferons, and tumor necrosis factors but also cellular and soluble receptors and serum mediators. Cytokines are small, non-structural proteins with pleiotropic effects. They have pro-inflammatory and/or anti-inflammatory functions, and some of them are able to control their own production (4–6).

Obesity, aging, tobacco use, physical inactivity, and unhealthy diets are considered the main risk factors for LGCI. These physiologic, environmental, and/or behavioral stimuli modify the cellular homeostasis, leading to cell stress and to the production of cytokines (3, 4, 7).

The association between obesity and LGCI is the most largely documented. Obesity drives the pathological expansion of the adipose tissue, which harbors enlarged hypertrophic adipocytes, impaired vasculogenesis, and enhanced fibrosis and hypoxia. Dysfunctional adipose tissue secretes pro-inflammatory cytokines that promote local and systemic inflammation, contributing to the onset of obesity-related diseases (8–10). The loosening of the cytokine balance between the pro-inflammatory and anti-inflammatory control is a characteristic feature also of aging. It has been defined as "inflamm-aging" and takes part in all aging-related diseases (4). In addition, smoking was found independently involved in the progression of LGCI, while physical activity has been recognized as an instrument to modulate LGCI (11, 12), although intense physical exercise is often paralleled by enhancement of inflammatory factors (13). Finally, it is now clear that dietary intake regulates inflammation through the complex interactions between foods and nutrients with bioactive properties. In this regard, several dietary indexes have attempted to assign inflammatory scores to specific foods/nutrients (14–16). Some nutrients have been classified as anti-inflammatory, and other nutrients have been defined as pro-inflammatory, according to their ability to promote the release of specific mediators (1, 7, 15).

Thus, cytokine levels may vary upon a plethora of stimuli. To date, critical levels of cytokines as biomarkers have not been defined, both in classical inflammatory diseases and in LGCI. Furthermore, only few studies have been performed to investigate cytokine levels in healthy subjects, and a limited number of LGCI-related risk factors have been explored when considering healthy subjects' cytokine profiles (5, 6, 17).

In this study, we have analyzed the cytokine profile in a cohort of healthy volunteers. We have assessed how gender, age, BMI, smoking, physical activity, and diet may modify specific cytokine levels potentially contributing to LGCI.

MATERIALS AND METHODS

Population Enrollment and Serum Collection

In total, 150 blood donors were recruited at the Transfusion Medicine Unit, Azienda Sanitaria Locale, Caserta, Italy, from January to July 2019. Exclusion criterion was ineligibility to donate blood, as indicated in D.M. 2/11/2015 (i.e., documented infectious diseases or other proliferative, degenerative, and autoimmune diseases; altered blood count and blood pressure; and drug assumption). The sample size was representative of the population of eligible donors, calculated on annual base. Since this is a pilot study with an exploratory nature, the power analysis has not been performed. All volunteers enrolled underwent detailed clinical phenotyping, including measurement of height, weight, and waist. Body mass index (BMI) was calculated as ratio of body weight (kg)/height (m^2). Moreover, a detailed questionnaire about anamnestic and anthropometric data, smoking habit, physical activity, and weekly food frequency was administrated to each subject (as described below). A serum sample from all donors was obtained and stored at −20°C. Transfusion Medicine Unit performed cytometric blood counts and biochemical analyses (AST, ALT, cholesterol, triglycerides, iron, total proteins, creatinine). Investigations were carried out following the rules of the Declaration of Helsinki of 1975, revised in 2013. Informed consent was obtained from every volunteer before the procedure. The protocol was approved by the ethical committee of the University of Naples (protocol no. 349/18).

Lifestyle Questionnaire

Lifestyle questionnaire was face-to-face administered by an expert nutritionist and included information about the last 7 days of physical activity and food frequency. The physical activity of enrolled volunteers was recorded and analyzed with the short form of the International Physical Activity Questionnaire (IPAQ) (18). The questionnaire reports the activity of four intensity levels: (1) vigorous-intensity activity, such as aerobics; (2) moderate-intensity activity, such as leisure cycling; (3) walking; and (4) sitting. For each activity, the *Metabolic EquivalenT (MET)*, a unit used to express energy spent and oxygen burned, was automatically calculated. MET value is given by the following equation (19):

$$MET = \frac{3.5\ ml\ (O_2)}{bodyweight\ \left[Kg\right]\ \times\ time\ [h]}$$

Based on the MET data analysis, as suggested by Craig et al. (18) and by the Guidelines for Data Processing and Analysis of the IPAQ (www.ipaq.ki.se), and considering MET value distribution in the enrolled population, volunteers have been classified into three groups, namely, volunteers with low or null physical activity (MET $<$ 1,000), volunteers with an average physical activity (1,000 $<$ MET $\leq$ 3,000), and volunteers with an intense physical activity (MET $>$ 3,000).

Dietary information was collected with a weekly food-frequency questionnaire (FFQ) conceived on that used in the framework of the Italian EPIC study (20, 21). Accordingly, a list

TABLE 1 | Clinical phenotyping of the population enrolled ($N = 150$).

Parameters [unit]	Total population ($N = 150$)	Female population ($N = 63$; 42%)	Male population ($N = 87$; 58%)	*p*-value
Age [years]	40.9 ± 11.2	40.33 ± 11.84	39.62 ± 10.88	0.707[a]
Weight [kg]	75 [50; 120]	65 [50; 94]	82 [64; 120]	<0.0001 [a]
Height [m]	170 [145; 186]	165 [145; 177]	175 [160; 186]	<0.0001 [a]
BMI [kg/m^2]	25.92 [19.16; 37.87]	23.44 [19.16; 36.51]	26.42 [20.98; 37.87]	0.0004 [a]
Smoker; yes (%)	43 (28.66%)	22 (34.92%)	21 (24.14%)	0.2 [b]
Physical activity [MET]	1,026 [0; 12,000]	1,026 [0; 11,784]	1,026 [0; 12,000]	0.828[a]

Age is expressed as mean ± SD. Smokers are indicated as number and percentage. Other data are expressed as median and range [min; max]. MET, Metabolic EquivalenT, an indicative unit of physical activity (refer to the "MATERIALS AND METHODS" section).

[a]*Welch's test.*

[b]*Chi-square test.*

of foods was developed in line with the local food availability, culturally specific and dietary habits (Mediterranean diet). Food frequency type with portion size was estimated by means of pictures. The participants were asked to report the frequency of consumption of food items listed in **Supplementary Table S1**. Pictures showing different portion sizes – arranged by increasing amount – followed the question on frequency and corresponded to a specific portion in grams. Additional questions addressed issues such as habitual cooking practices and types of cooking fats. Questionnaires were finally digitalized, and data relative to the weekly total consumption of each food item, expressed in grams, were gathered and analyzed.

Determination of Cytokines, Chemokines, and Growth Factors

Serum samples were screened for the concentration of interleukin (IL)-1ra, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, basic fibroblast growth factor (FGF), eotaxin, granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), interferon-γ inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 (MIP-1) α, MIP-1β, C-C motif chemokine ligand 5 (CCL5)/RANTES, TNF-α, platelet-derived growth factor (PDGF-BB), and vascular endothelial growth factor (VEGF) using the Bio-Plex Multiplex Human Cytokine, Chemokine, and Growth Factor Kit (cat. n. M500KCAF0Y, Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol, as previously described (17, 22). The magnetic bead-based assay was performed on a Bio-Plex 200 System (Bio-Rad, Hercules, CA, USA). All the values obtained were included within the detection limits indicated by the manufacturer (bio-rad.com/Bio-Plex/AnalyteGuide). High sensitivity C-reactive protein (CRP) assay (cat. n. L2KCR2, Siemens, USA) was performed using the IMMULITE® 2000 Analyzer (DPC, Los Angeles, CA, USA), according to the manufacturer's protocol.

Statistical Analysis

Statistical analyses were performed using the R statistical platform (https://www.R-project.org/) and the GraphPad 7.0 software (GraphPad Software Inc., La Jolla, Ca). D'Agostino-Pearson normality test was used to evaluate whether the continuous data were normally distributed, and according to the results, a Welch's two-tailed *t*-test for independent samples (for normally distributed data) or a Mann–Whitney *U*-test (for non-normally distributed data) was used. Multiple comparisons among more than two groups were made using the ANOVA test with Tukey's correction or the Kruskal Wallis test. The non-parametric Jonckheere-Terpstra test was used to analyze the trend between an ordinal independent variable. Categorical values were described by the number of occurrences and percentages and were compared using the chi-square test. Outliers have been detected and removed according to the ROUT method with *Q* coefficient 1%. To assess a correlation between cytokine levels and risk factors, a canonical correlation analysis (CCA) was performed (23). To investigate the collinearity effects among risk factors and food groups, data were analyzed with multivariate linear regression analysis. Generated models were analyzed with an ANOVA test to evaluate the goodness of fit. Regression coefficients were reported as an estimate and 95% confidence interval. Cytokine correlation matrix was obtained with Pearson's correlation test. Box plots denote median and 25th to 75th percentiles (boxes) and Tukey whiskers. *p*-value of <0.05 was considered statistically significant.

RESULTS

Anthropometric and Clinical Characteristics of the Study Population

The enrolled population was represented by 150 blood donors, with 63 female participants and 87 male participants. Overall, the mean age was 40.9 years, while the BMI median was 25.92; 43 (28.66%) individuals were smokers (**Table 1**). Female and male populations did not display statistically significant differences for age, smoker percentage, physical activity (**Table 1**), cytometric blood counts, and biochemical analyses (**Supplementary Table S2**). However, the male population showed statistically significant higher weight, height, and BMI compared with the female population (**Table 1**).

Serum samples of blood donors were then screened for the concentration of a panel of cytokines, chemokines, and

growth factors and of C-reactive protein (hsCRP). Overall, all factors were detectable in serum specimens, with a defined cytokine/chemokine profile for every individual (**Figure 1**; **Table 2**); 22% of total individuals had hsCRP concentrations >3 mg/L. However, these subjects did not display any significant difference in cytokine concentration, compared to those with

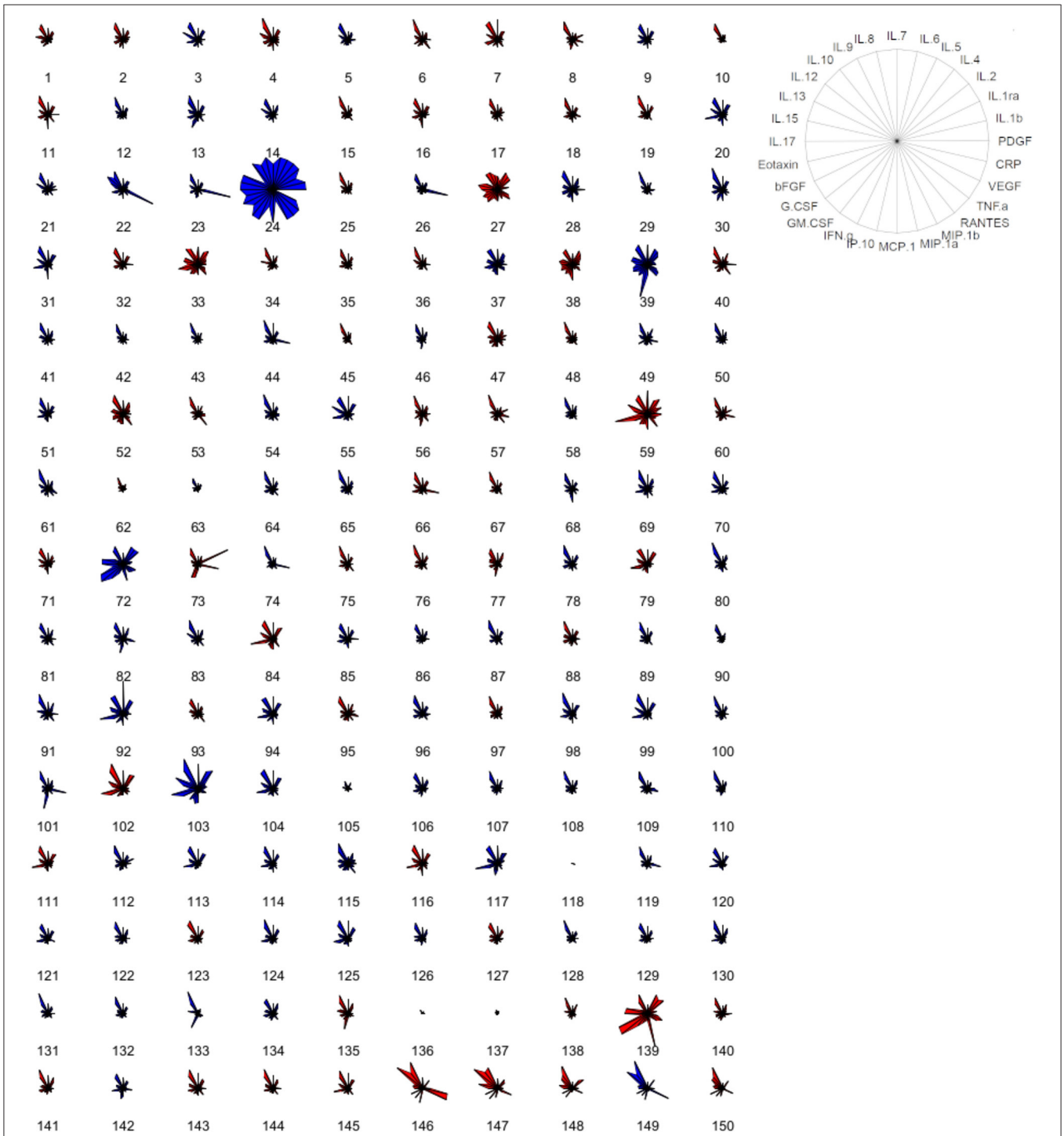

FIGURE 1 | Cytokine-based pattern of volunteers. Star plot obtained by multivariate data analysis of the whole cytokinome of every subject, consisting of a sequence of equi-angular spokes (radii), with each spoke representing one cytokine, as indicated in the figure on the right. Data length of a spoke is proportional to the magnitude of the variable for the data point relative to the maximum magnitude of the variable across all data points. A line is drawn connecting the data values for each spoke. Blue stars represent male subjects, and red stars represent female subjects.

TABLE 2 | Serum concentration of cytokines, chemokines, and growth factors.

Cytokine	Concentration (total)	Concentration (female)	Concentration (male)	*p*-value
IL-1b	1.89 [1.81; 2.22]	1.89 [1.81; 2.22]	1.89 [1.81; 2.22]	0.74
IL-1ra	352.5 [288.1; 463.6]	381.8 [284.4; 546.7]	341.7 [289.3; 400]	0.16
IL-2	15.46 [14.84; 17.20]	16.07 [14.52; 17.58]	15.46 [14.84; 16.68]	0.97
IL-4	5.93 [5.21; 7.22]	6.1 [5.27; 7.4]	5.81 [5.21; 7.04]	0.39
IL-5	55.51 [50.69; 61.5]	55.51 [49.42; 61.5]	55.51 [50.69; 61.5]	0.17
IL-6	7.85 [7.18; 8.91]	7.85 [7.29; 9.17]	7.85 [7.01; 8.91]	0.09
IL-7	43.69 [40.38; 46.93]	43.69 [40.38; 46.93]	43.69 [40.79; 48.52]	0.88
IL-8	18.82 [16.34; 23.02]	19.86 [16.19; 25.51]	18.56 [16.34; 20.98]	0.17
IL-9	252.1 [233.6; 264.4]	252.4 [234.6; 263.7]	251.3 [232.6; 266.2]	0.87
IL-10	24.85 [22.65; 26.9]	24.85 [22.65; 27.56]	24.85 [22.65; 26.73]	0.48
IL-12	11.26 [10.84; 12.88]	12.08 [11.26; 14.05]	11.26 [10.41; 12.08]	0.27
IL-13	5.41 [4.72; 6.08]	5.41 [4.72; 6.08]	5.41 [4.81; 6.16]	0.78
IL-15	354.9 [338.4; 379.4]	360.1 [344.1; 390.6]	349.6 [338.4; 372.4]	0.34
IL-17	26.28 [23.78; 30.3]	26.28 [23.56; 31.67]	26.69 [23.78; 29.51]	0.92
EOTAXIN	47.79 [33.82; 63.48]	47.62 [29.51; 59.58]	47.88 [36.96; 67.44]	0.4
b-FGF	78.43 [73.34; 85.86]	78.43 [73.34; 87.07]	78.43 [73.34; 83.41]	0.25
G-CSF	293.3 [260.3; 353.7]	283.8 [249; 360.1]	293.3 [263.1; 352.4]	0.75
GM-CSF	12.89 [12.45; 13.47]	12.97 [12.45; 13.96]	12.75 [12.3; 13.18]	0.3
IFN-γ	12.44 [11.43; 13.47]	12.78 [11.56; 14.36]	12.24 [11.36; 13.84]	0.13
IP-10	250.5 [203.5; 306.5]	260.8 [202.8; 333.6]	242.5 [203.8; 281.1]	0.09
MCP-1	33.63 [26.58; 44.53]	33.74 [25.94; 43.64]	33.32 [27.65; 44.77]	0.72
MIP-1α	2.6 [2.39; 2.91]	2.66 [2.39; 3]	2.53 [2.39; 2.82]	0.36
MIP-1β	81.52 [75.51; 90.01]	81.72 [74.91; 87.94]	81.37 [76.52; 91.43]	0.5
PDGF	1,538 [1,180; 1,994]	1,497 [1,180; 2,114]	1,553 [1,151; 1,956]	0.92
RANTES/ CCL5	6,573 [4,951; 7,858]	6,843 [4,825; 8,706]	6,251 [5,150; 7,557]	0.32
TNF-α	48.87 [44.63; 55.39]	48.49 [44.63; 56.91]	49.26 [45.4; 55.39]	0.27
VEGF	416.9 [398.7; 446.9]	421.4 [402.2; 455]	412.5 [397.5; 436.5]	0.29
CRP	1.22 [0.57; 2.59]	1.1 [0.47; 2.61]	1.29 [0.76; 2.47]	0.99

Cytokine, chemokine, and growth factor concentrations are expressed as pg/ml. hsCRP values are expressed as mg/L. Results are indicated as median and IQR [25th percentile; 75th percentile].

hsCRP <3 mg/L (**Supplementary Table S3**). No statistically significant differences between the male and female participants were observed for all circulating factors (**Figure 1**; **Table 2**).

Cytokines and LGCI Risk Factors

Next, we investigated the correlation between LGCI risk factors (age, BMI, smoke, physical activity) and the inflammatory cytokines IL-1b, IL-6, IL-8, IL-9, eotaxin, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, RANTES, TNF-α, and CRP. The CCA revealed that LGCI risk factors and inflammatory cytokines displayed a correlation coefficient of 0.521 (95% CI: 0.382–0.66), with a goodness of fit equal to 0.501 (**Figure 2A**). The standardized weights for the single risk factors indicated a stronger relevance of BMI, which concurred for 78.27% of the correlation; smoke contributed to 13.28%, while age and physical activity contributed to 8.44% and <0.002%, respectively (**Figure 2B**). The standardized weights for the single inflammatory cytokines showed that IL-1b, CRP, IFN-γ, and IL-6 were the principal components of the correlation, concurring for 47.83, 23, 11.23, and 7.88%, respectively (**Figure 2C**).

Next, the association between specific cytokines and LGCI-risk factors was investigated. Interestingly, IL-1b, eotaxin, MCP-1, and MIP-1α displayed a significant overall difference among four age-related groups (i.e., 20–29, 30–39, 40–49, and 50–59 years). Eotaxin and MCP-1 showed also an increasing trend associated with age (20–29 ≤ 30–39 ≤ 40–49 ≤ 50–59 years; **Figure 3**). In smokers, a significant increase in IL-1b and RANTES/CCL5 was detected (**Figure 3**). Overweight individuals (BMI: 25–30) showed significantly higher levels of CRP, compared with normal weight individuals (BMI: 20–24.9). Finally, physical activity was associated with higher levels of MIP-1α (**Figure 3**).

Cytokines and Diet

Dietary information was collected with a weekly food-frequency questionnaire, as described in the "Materials and Methods" section and in **Supplementary Table S1**. Data relative to the weekly total consumption of each food item (in g) were collected, included in 11 food groups, and analyzed. The main components of the dietary intake of the volunteers were "Grains," "Greens,"

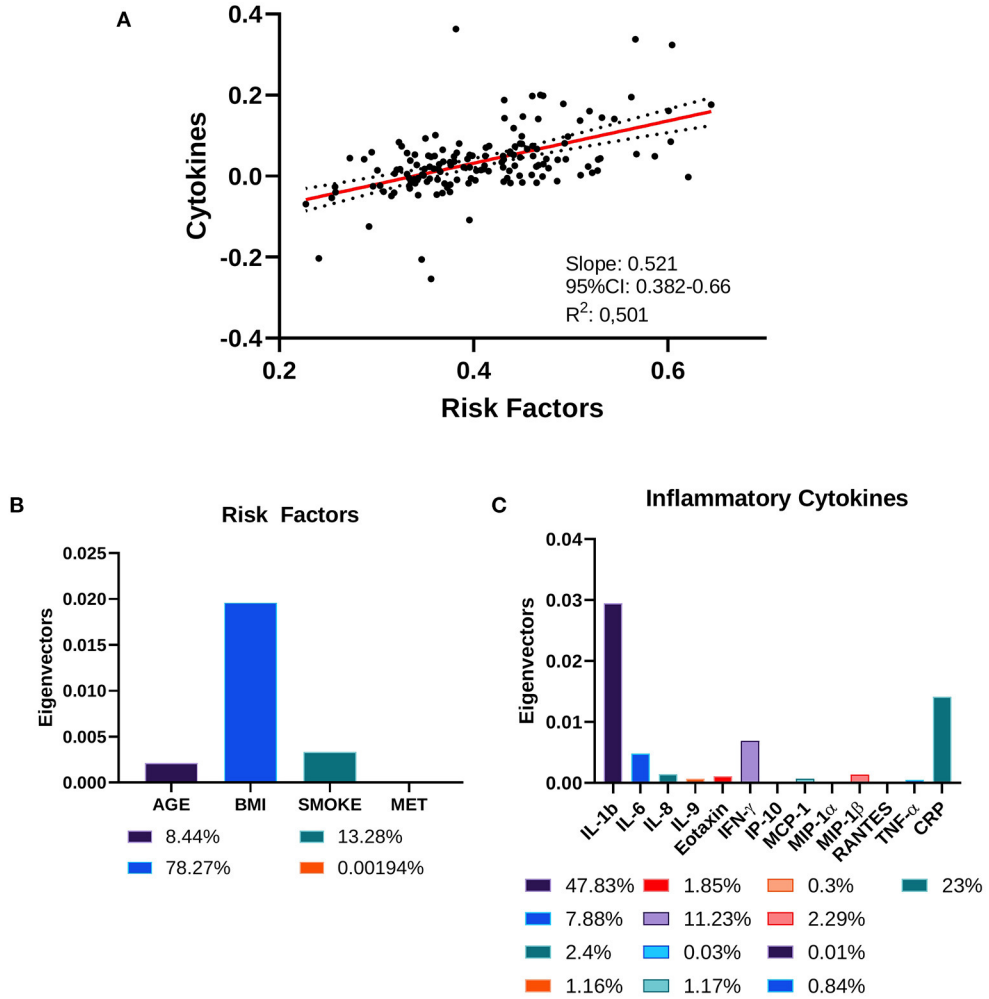

FIGURE 2 | Canonical correlation analysis of cytokines and risk factors. **(A)** Two-way CCA setting; each subject is described by two canonical variates per mode, represented on a scatter plot. The two variate groups are maximally correlated, and their linear regression slope corresponds to the canonical correlation coefficient. **(B)** Risk factors' standardized weights and **(C)** cytokines' standardized weights are represented as absolute values and as percentage in the color legends.

"Fresh Fruits," and "Milk and Dairy," which accounted for 29.79, 18.07, 17.17, and 11.01% of the total food consumption, respectively (**Figure 4**).

Food groups and inflammatory cytokines displayed a correlation coefficient of 0.462 (95% CI: 0.319–0.607), with a goodness of fit equal to 0.482 (**Figure 5A**). The standardized weights for the single food items indicated a stronger relevance of Red Meats and Shelled Fruits, which concurred for the 28.7 and 28.15% of the correlation, respectively (**Figure 5B**). Among the inflammatory cytokines, IFN-γ, IL-1b, and IL-6 were the principal components of the correlation, contributing to 36.62, 29.5, and 20.5%, respectively (**Figure 5C**).

At univariate analysis, individuals with a weekly grain intake higher than the 75th percentile displayed significantly higher levels of circulating IFN-γ, MCP-1, and TNF-α (**Figure 6**). The intake of red meat was associated with a significant increase in IL-6, IL-8, and CRP (**Figure 6**). Moreover, subjects who ate more fruits showed higher levels of IL-8, IFN-γ, and IP-10 (**Figure 6**). Increased levels of IL-8 were detected also in subjects consuming more sweets (**Figure 6**). A significant reduction in CRP was observed in individuals who ate more eggs, greens, or shelled fruits. In these latter subjects, a significant reduction of IL-1b and IL-6 was also found (**Figure 6**).

Multivariate Analysis and Cytokine Correlations in Healthy Subjects

In multivariate analysis, all significant associations among cytokines and lifestyle factors or food groups found at univariate analysis (**Figures 3**, **6**) were still retained upon adjusting for BMI (**Supplementary Table S4**).

Among all cytokines, MCP-1, IL-1b, and CRP levels were significantly associated both with risk factors and with specific food groups (**Figures 3**, **6**). As shown in **Table 3**, the association between MCP-1 and the intake of grains was not significant after adjusting for age; similarly, the association between IL-1b and shelled fruits was not

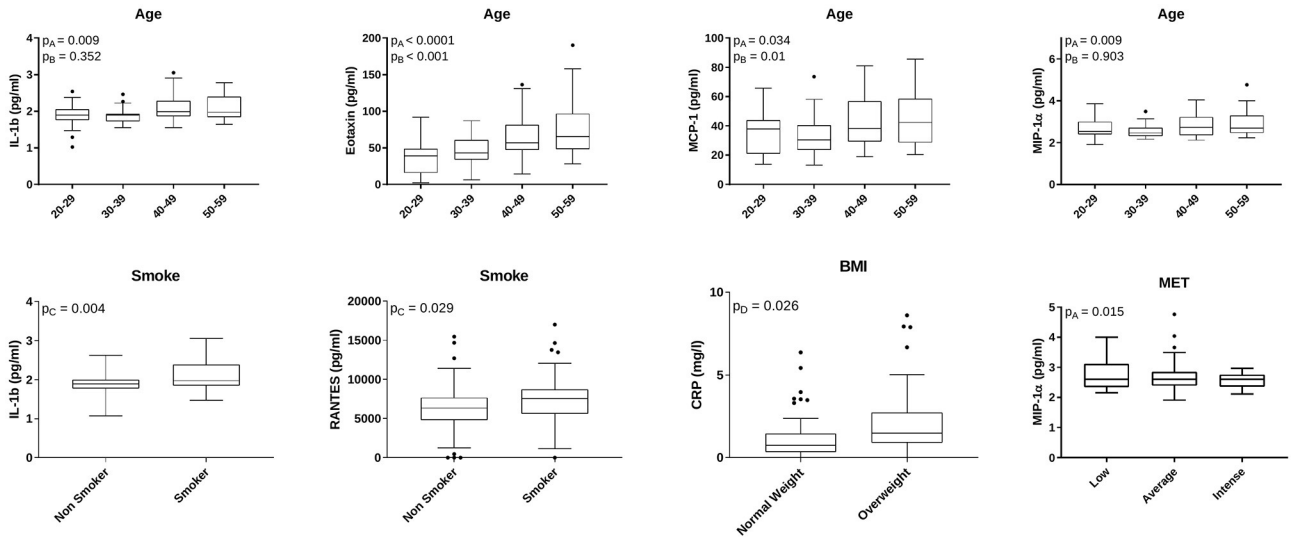

FIGURE 3 | Age, smoke, BMI, and physical activity-related cytokines. Box plots indicate cytokine concentrations in subjects classified for age ($N = 29$: 20–29 years; $N = 53$: 30–39 years; $N = 33$: 40–49 years; $N = 29$: 50–59 years), smoke habit ($N = 107$ non-smokers; $N = 43$ smokers), BMI ($N = 65$ normal weight; $N = 60$ overweight), and physical activity ($N = 74$ low physical activity; $N = 53$ average physical activity; $N = 23$ intense physical activity). IL-1b, eotaxin, MCP-1, MIP-1α, and CCL5/RANTES concentrations are expressed as pg/ml. CRP concentration is expressed as mg/L. The figure reports only factors with statistically significant different values. *p*A = overall ANOVA test; *p*B = Jonckheere-Terpstra trend test; *p*C = Welch's *t*-test for paired distributions; *p*D = Mann–Whitney test for non-parametric distributions.

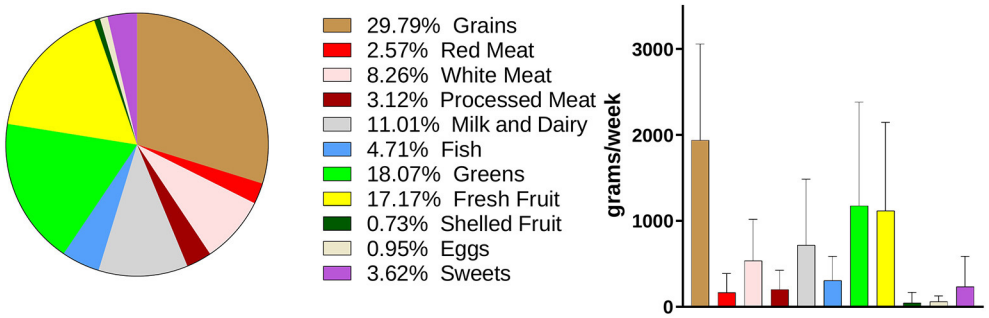

FIGURE 4 | Dietary habits of the enrolled volunteers. Weekly consumption of foods is expressed in grams. Data are showed in a pie-chart as percentage compared with the whole weekly food intake and in a histogram as mean ± SD for the single food groups.

significant when adjusted for age and smoke. Interestingly, CRP levels were not associated with the consumption of red meat, shelled fruits, eggs, and greens after adjusting for BMI (**Table 3**).

Finally, a correlation matrix based on cytokines significantly associated with at least one LGCI-related risk factor (age, BMI, smoke, physical inactivity, diet) indicated positive and significant correlations among all selected inflammatory cytokines, except for CRP, for which almost no correlations with cytokines were observed (**Figure 7**). Interestingly, IL-1b, TNF-α, IL-6, and IL-8 established a cluster of cytokines with r-values ranging from 0.88 (CI: 0.84–0.91) to 0.95 (CI: 0.93–0.96), thus representing a potential LGCI-related cytokine pattern biomarker (**Figure 7**, **Supplementary Table S5**).

DISCUSSION

Cytokines are key mediators in inflammatory, viral, and autoimmune diseases, as well as in LGCI (4–6, 10, 17, 24). However, it is still difficult to use cytokines as diagnostic or prognostic tools due to the problem of establishing "normal" vs. "abnormal" cytokine levels. Few studies investigated cytokine concentrations in healthy subjects, and often a limited number of variables have been examined.

In this study, we have analyzed the cytokine profile of a cohort of 150 healthy blood donors. We have addressed how age, BMI, smoke, physical activity, and dietary habits impact on specific cytokines, modulating their concentrations. We have shown that each individual has a peculiar cytokine pattern, influenced at a different degree, by physiologic and behavioral factors. No

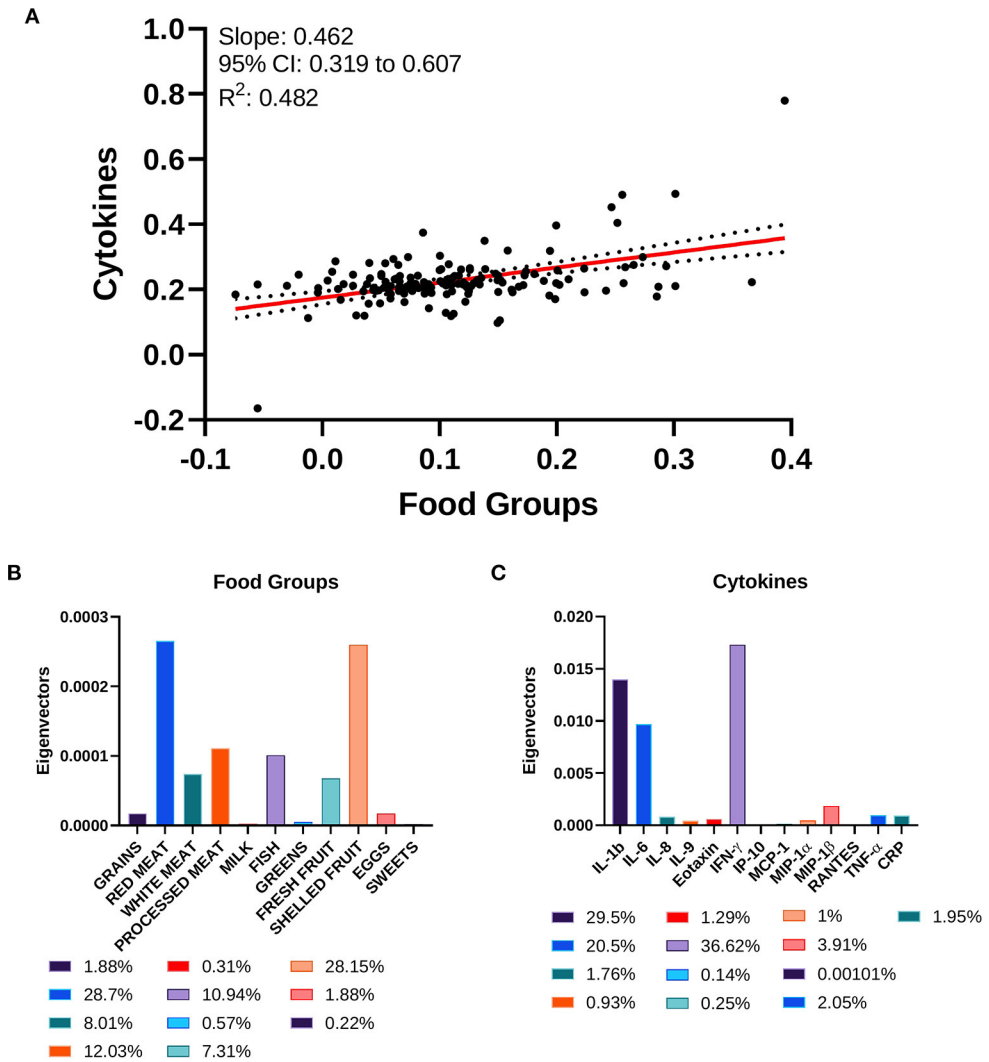

FIGURE 5 | Canonical correlation analysis of cytokines and food groups. **(A)** Two-way CCA setting; each subject is described by two canonical variates per mode, represented on a scatter plot. The two variate groups are maximally correlated, and their linear regression slope corresponds to the canonical correlation coefficient. **(B)** Food groups' standardized weights and **(C)** cytokines' standardized weights are represented as absolute values and as percentage in the color legends.

differences between male and female participants were detected, despite the different BMI. These results are in agreement with other studies performed with different populations (25, 26) and suggest that other factors, such as hormones, may balance the control elicited by BMI.

The BMI has the heaviest impact on the correlation between LGCI-related risk factors and cytokines. Indeed, many studies have characterized the cytokine profile in subjects with obesity and/or severe obesity detecting differences compared with normal weight individuals (10, 27, 28). In this study, we have found that, despite the removal from the analysis of subjects with obesity, BMI was still significantly associated with CRP levels. Interestingly, we have observed that CRP levels correlated also with the consumption of specific foods, i.e., eggs, red meat, shelled fruits, and greens; however, when corrected for BMI, all these associations were lost, thus highlighting the impact of the body mass.

The second most involved factor in the control of cytokine profile is tobacco use. Smokers displayed higher levels of IL-1b and CCL5/RANTES. These findings are in line with *in vivo* studies showing that cigarette smoke induces murine emphysema with lung inflammation and DNA injury/apoptosis *via* IL-1b and CCL5-CCR5 (29, 30). However, CCL5 exerts also regenerative functions, mainly through CCR1 (31). Thus, its increase may also suggest the activation of tissue repair mechanisms.

Age contributes 8.4% to the correlation between LGCI risk factors and inflammatory cytokines. Inflamm-aging is a dysregulation of the cytokine network well described at cellular levels, where cell senescence is associated with a defined secretory phenotype (SASP) characterized by specific cytokines, such as

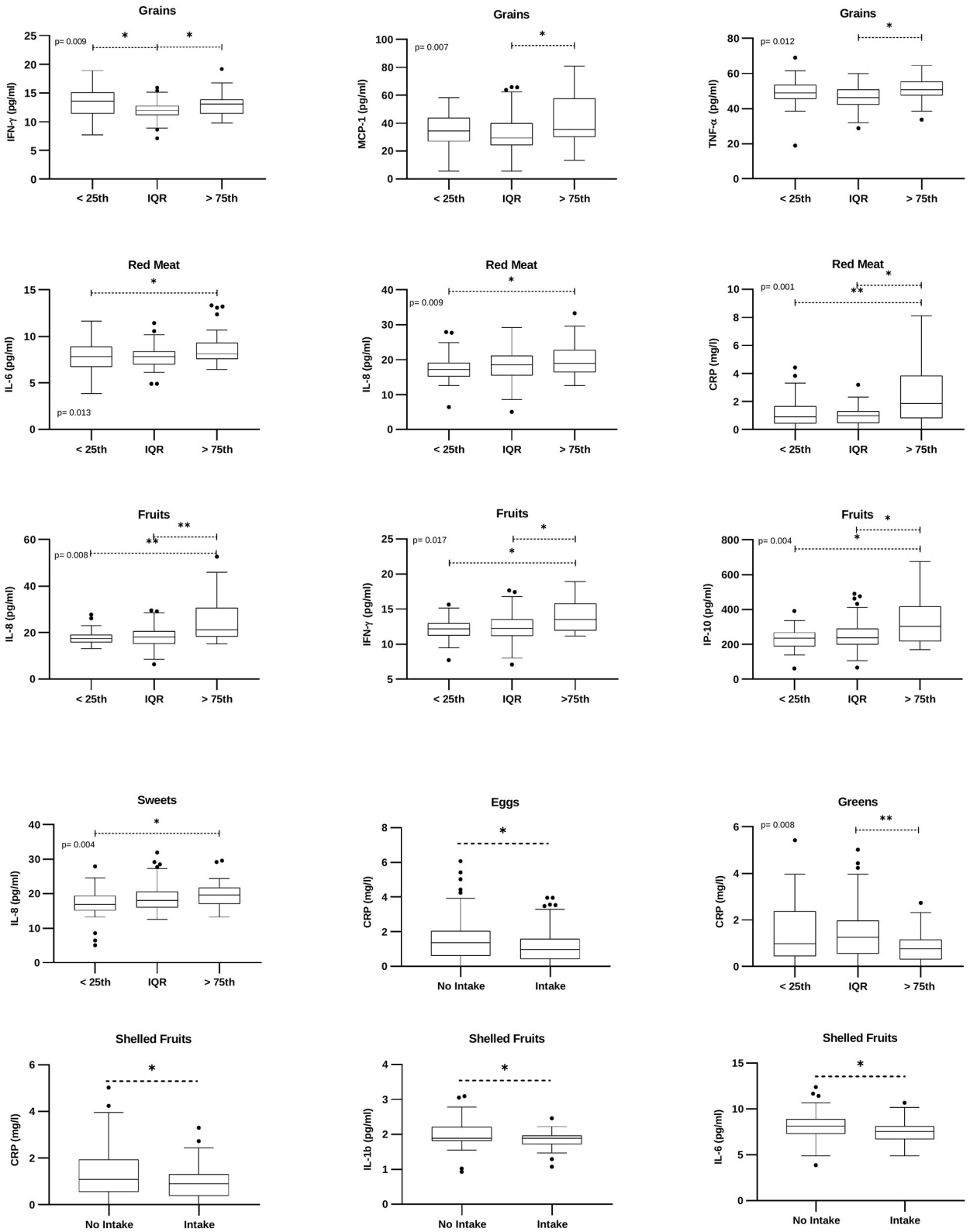

FIGURE 6 | Food groups-related cytokines. Box plots denote cytokine concentrations in subjects with low ("<25th" – subjects with a weekly intake lower than the 25th percentile), medium ("IQR" – subjects with a weekly intake between the 25th and the 75th percentile), or higher (">75th" – subjects with a weekly intake higher than the 75th percentile) food intake; or intake/no intake of a specific food group. Cytokine concentrations are expressed as pg/ml. CRP concentration is expressed as mg/L. The figure reports only factors with statistically significant values. p = Brown-Forsythe ANOVA or Kruskal Wallis test. *$p < 0.05$; **$p < 0.01$.

TABLE 3 | Multivariate linear regression analyses between risk factors and food groups for MCP-1, IL-1b, and CRP.

ANOVA model comparisons				Intercept		Risk factor		Food group		Interaction	
Model 1	**Model 2**	***p*-Value**	**Best fitting model**	**Estimate (95% CI)**	***p*-Value**	**Estimate (95% CI)**	***p*-Value**	**Estimate (95% CI)**	***p*-Value**	**Estimate (95% CI)**	***p*-Value**
MCP-1 ~ age + grains	MCP-1 ~ age * grains	0.486	Model 1	16.76 (5.98 – 27.5)	0.0025	0.356 (0.130 –0.581)	0.0022	3.06 (−0.213 to 6.33)	0.07	–	–
IL-1b ~ age + smoke + shelled fruit	IL-1b ~ (age + smoke) * shelled fruit	0.158	Model 1	1.66 (1.48 –1.85)	$<2^{-16}$	Age 0.007 (0.0023–0.012)	Age 0.0029	3.06 (−0.219 to 6.34)	0.07	–	–
						Smoke 0.141 (0.03–0.252)	Smoke 0.014			–	–
CRP ~ BMI + shelled fruit+ eggs + red meat + greens	CRP ~ BMI * (shelled fruit + eggs + red meat + greens)	0.301	Model 1	−0.662 (−2.24 to 0.92)	0.413	0.356 (0.132–0.58)	0.022	Shelled fruit −0.32 (−1.07 to 1.05)	Shelled fruit 0.082	–	–
								Eggs −0.054 (−1.67 to 0.432)	Eggs 0.77	–	–
								Red meat −0.12 (−0.263 to 2.65)	Red meat 0.574	–	–
								Greens −0.243 (−0.577 to 0.92)	Greens 0.061	–	–

For each linear regression, two models have been evaluated and compared: Model 1 considering variables without interactions and Model 2 considering both variables and interactions. Models have been compared with an ANOVA test, whose output revealed if the differences among the two models were statistically significant (p-value < 0.05). The best-fitting model has been chosen accordingly. Coefficients for intercept, risk factor(s), and food group(s) have been shown for the best-fitting model.

IL-6, IL-8, and VEGF (32). At serum levels, data are sometimes conflicting since some studies have enrolled different aged people, without considering health status. Other studies have been performed on very old people, such as non-agenarians and centenarians (33). In this study, we have shown that 20–60 years old healthy subjects display an age-associated increase of IL-1b, eotaxin, MCP-1, and MIP-1α. Accordingly, eotaxin levels have been found to increase in 21–86 and in 7–17 years old subjects, compared with 1–6 years old children (26).

Finally, the contribution of physical activity has been evaluated. A general idea coming from recent literature data indicates that exercise stimulates both pro- and anti-inflammatory cytokines (IL-6, IL-8, IL-10, TNF-α), whose levels return to baseline sometime from 5 to 24 h after exercise (13). The dual response depends also on the type of exercise (aerobic or anaerobic) and whether it comes from short-term or long-term physical activity (34). In this study, we have observed that people who in the 7 days before the questionnaire consumed more *Metabolic EquivalenT (MET)*, a unit used to express energy spent and oxygen burned, had higher levels of MIP-1α, a protein mainly produced by macrophages and involved in leucocyte recruitment (35). Its increase may in part explain the mobilization of different leukocyte populations to the blood observed in diverse studies focused on the effect of exercise on blood cells and molecules (13).

Healthy diets are commonly considered protective for LGCI. Anti-inflammatory properties have been conferred to single foods (i.e., almonds, yogurt, nuts, dark chocolate, and extra virgin olive oil), functional foods (i.e., omega-3 fatty acids, polyphenols, and fibers), and whole diets, such as Mediterranean diet (1, 7, 36–38). To quantify the dietary quality, various dietary indices or scores have also been reported. For instance, the Dietary Inflammatory Index (DII®) is a literature-derived method based on 45 food parameters (39), the Healthy Eating Index-2015 (HEI-2015) is a measure of dietary quality according to the Dietary Guidelines for Americans (DGA) (15), the polyphenol antioxidant content (PAC) score is an index of the total content of diet in polyphenols (16), and the empirical dietary inflammatory pattern (EDIP) is a score based on the reduced rank regression approach, developed in the United States and validated across nations (40). Moreover, some indices have been conceived around specific dietary patterns, such as the Mediterranean diet and the Dietary Approaches to Stop Hypertension (DASH) (14, 41).

The main result of our work is the finding that 7-day consumption of particular food items is associated with increased levels of specific cytokines. All volunteers were from Southern Italy, a place where the food culture adheres to the Mediterranean diet. Indeed, the main components of the dietary intake of the volunteers were grains followed by greens, fresh fruit, milk and dairy, and white meat. The beneficial effect of this diet is represented by the result that in this population, no associations were detected between specific food consumption and CRP levels (when adjusted for BMI). At present, there are few studies and some discrepancies about the intake of grains. Some studies reported an anti-inflammatory effect of whole grains for their nutrients and fibers; other studies, performed with healthy subjects, displayed no effects (42, 43). Moreover,

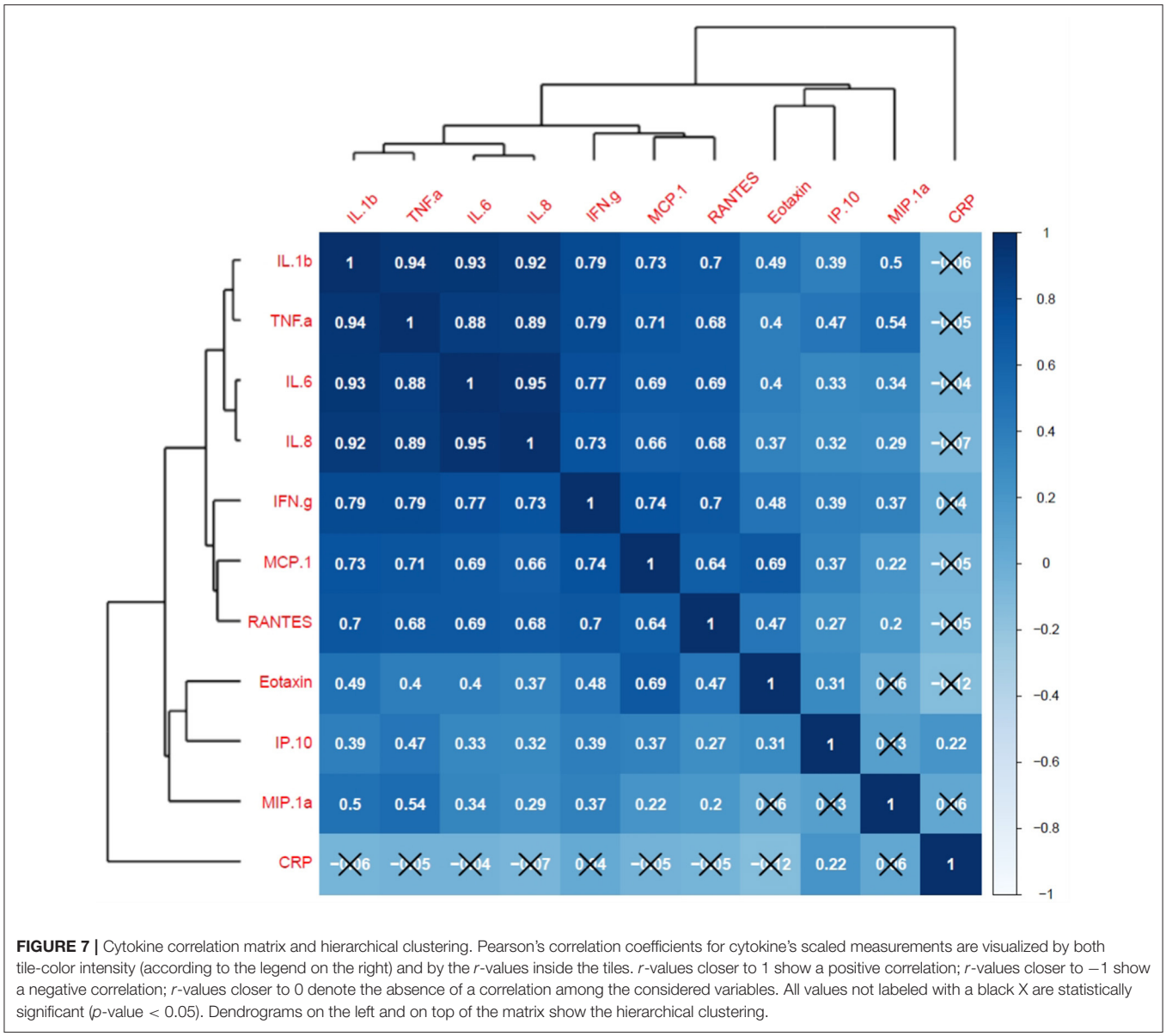

FIGURE 7 | Cytokine correlation matrix and hierarchical clustering. Pearson's correlation coefficients for cytokine's scaled measurements are visualized by both tile-color intensity (according to the legend on the right) and by the *r*-values inside the tiles. *r*-values closer to 1 show a positive correlation; *r*-values closer to −1 show a negative correlation; *r*-values closer to 0 denote the absence of a correlation among the considered variables. All values not labeled with a black X are statistically significant (p-value < 0.05). Dendrograms on the left and on top of the matrix show the hierarchical clustering.

the daily consumption of wheat products and cereals has been reported to contribute to chronic inflammation and autoimmune diseases (42). Our data indicate that volunteers with grain consumption over the 75th percentile had higher levels of TNF-α and IFN-γ, compared with those in the IQR range, representing 50% of the population. TNF-α increase may be associated with carbohydrate-related hyperglycemia, which has been shown to increase circulating cytokine concentrations by an oxidative mechanism (44). IFN-γ was increased also in subjects with grain consumption under the 25th percentile. This low inflammatory profile may be due to the large use of cereals and of whole-wheat pasta and bread. Red meat and shelled fruit, even though represented a small percentage of the whole dietary intake, showed the greatest influence on the correlation between foods and inflammatory cytokines. Subjects with red meat consumption over the 75th percentile displayed the most striking inflammatory pattern, characterized by the increase in IL-6 and IL-8. These data are in agreement with other reports showing that a higher intake of meat, as in Western-like diets, is associated with inflammation and detrimental health outcome (7).

Fruits and vegetables, rich in flavonoids and antioxidants, have been associated with a lower risk of LGCI-related diseases and low levels of some markers of inflammation (45, 46). Surprisingly, volunteers who had a large consumption of fresh fruits displayed higher circulating levels of IL-8, IFN-γ, and IP-10, compared to those with a moderate or low intake. Different studies suggest an anti-inflammatory role for polyphenols, elicited mainly through the modulation of NF-kB and MAPK pathways at multiple levels (46). However, clinical trials are somewhat contradictory, and *in vitro* studies have also reported an induction of IL-8 expression by

resveratrol, a polyphenol contained in many fruits, thus opening new questions (46, 47). Moreover, IL-8 and IP-10 release may be induced by IFN-γ, whose increase could suggest an immunostimulatory effect of the fruit. Interestingly, the intake of shelled fruits was associated with a significant decrease of the most pro-inflammatory molecules IL-1b and IL-6. These results are in line with previous studies showing an anti-inflammatory and antioxidant role for almonds and nuts (48, 49).

Thus, in a healthy population, age, BMI, smoke, physical activity, and certain dietary habits are associated with specific cytokines. These associations remained also upon the adjustment for BMI. However, CRP, the classical marker of acute inflammation, was associated only with the BMI and did not correlate with the other cytokines. Thus, although CRP is a valuable indicator of inflammation, other molecules may be useful as markers, particularly to identify the relative weight of specific factors involved in the progression of LGCI. This is of particular importance when considering dietary habits. In this study, we have identified IL-1b and IL-6 as the cytokines with the strongest relevance in the correlation between the cytokinome and LGCI risk factors. These molecules are considered inflammatory markers for multiple conditions (17, 50) and, together with TNF-α and IL-8, establish a cluster that may represent a potential LGCI-related cytokine biomarker.

In conclusion, within this study, we have provided evidence for the measurement of multiple cytokines in a well-defined and characterized healthy population and how lifestyle factors and aging affect specific cytokines.

Future research directions will expand the dietary analysis by considering the percentage of macronutrients, mainly the type of fats (i.e., omega 3 and omega 6) that have displayed a relevant impact on circulating cytokine concentrations (51, 52). Moreover, the impact of lifestyle/dietary factors in the framework of international studies on healthy and pathological populations will be addressed. Finally, intervention studies will further define the contributions of the examined factors on cytokine levels. Results will allow to envision new technological tools and digital platforms to early detect mediators and risk factors of LGCI and to encourage healthy lifestyle behaviors.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committee of the University of Naples Federico II. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization: PF and MLi. Software: MS and FC. Formal analysis: MDT and MS. Investigation: MLe, SC, GP, BC, and SM. Data curation: MDT, VD'E, MRA, and AP. Writing—original draft preparation: VD'E, MDT, and MLe. Writing—review and editing: VD'E, PF, MLi, and FC. Supervision: PF and MS. Project administration and funding acquisition: PF and VD'E. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.913176/full#supplementary-material

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Effect of red meat, vegetable, tobacco, and alcohol consumption on national cancer mortality index: Data from 1989 to 2013 in 37 developed countries

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This study aimed to examine the association between red meat (RM) and death from all types of cancer, as well as its association with the incidence of colon cancer in developed countries. We selected RM, vegetable, tobacco, alcohol consumption, and socioeconomic status as the dependent variables' risk factors and performed ordinary least squares (OLS) and a fixed-effect model (FEM) analysis. Data from 1989 to 2013 for 37 Organization for Economic Cooperation and Development (OECD) countries. According to the FEM, cancer death had statistically significant associations with education level (Coef = −0.022, P = 0.009), total health expenditure (Coef = −0.049, P = 0.000), aging rate (Coef = −0.178, P = 0.000), tobacco consumption (Coef = 0.096, P = 0.000), RM consumption (Coef = 0.107, P = 0.000), and vegetable consumption (Coef = −0.034, P = 0.000). A similar trend was also observed in the 3 and 5-year lagged models. RM consumption also demonstrated a significantly positive association with the incidence of colon cancer in the OLS. According to the scatter plots and fitted lines based on the recommended allowance RM consumption, cancer deaths and incidence of colon cancer increased as consumption increased in the excess consumption group. Regarding vegetable consumption, cancer deaths and incidence of colon cancer decreased as consumption increased in the group exceeding the recommended allowance level. RM consumption was found to be higher than the recommended allowance level. RM consumption increased cancer deaths and the incidence of colon cancer. There is justification for public health interventions to limit RM consumption in major developed countries.

KEYWORDS

red meat, vegetable, tobacco, alcohol, cancer, mortality, colon cancer

Introduction

Red meat consumption

From a nutritional perspective, red meat (RM) is rich in essential nutrients, such as proteins, vitamin B, heme iron, and zinc. Furthermore, fatty acids found in lean tissue, such as n_3 polyunsaturated and conjugated linoleic acids, are known for their health benefits (1, 2). Nevertheless, there have been several reports that inappropriate intake of RM is not good for health. Saturated fats found in RM increase levels of low-density lipoprotein (LDL) and cholesterol, which have adverse effects on cardiovascular health (3). Since the 1950s, the American Heart Association has recommended reducing the intake of dietary cholesterol and saturated fats to prevent cardiovascular disease. Current guidelines suggest that saturated fats should account for < 7–8% of the total daily calories, and the consumption of cholesterol should be < 300 mg per day (4). Furthermore, the genotoxicity and oxidative stress from RM consumption can induce the destruction of DNA and adenoma formation, which can lead to cancer (5). RM consumption increases the incidence of colon and rectal cancers and is associated with breast (6), prostate, and pancreatic cancers (7). In 2007, the World Cancer Research Fund (WCRF) recommended < 71 g of daily intake of RM (8), while the International Agency for Research on Cancer (IARC) officially named RM as a group 2A carcinogen in 2015. Particularly, heme iron from RM has been identified as a risk factor for colon carcinogenesis (9).

Overall, although RM is a major food category that provides essential nutrients, excessive consumption of RM has been recognized in modern society as posing negative effects on health, including its association with cancer. However, the effect of over-consumption of RM on cancer from a public health perspective is unclear. This is because most related studies thus far have presented findings at the individual level rather than at the population or national level. However, clinical or individual studies do not lead to population-level health outcomes due to a combination of ecological factors (10). Individual-level studies suggest that people who consume more meat are more likely develop cancer and die. However, they do not conclude that cancer death and incidence are higher in countries with high meat consumption. Therefore, it is not free from an individualistic fallacy to claim that RM should be restricted at the community or country level through studies using individuals as a unit (11, 12). As such, study findings can differ within the same variables, depending on the research methods and scope of analysis. Nevertheless, most findings thus far have been at the individual level (13), and additional research is needed to examine associations at a nationwide level. A study by Ranabhat. et al. examined the association between RM consumption and life expectancy in 164 countries. The findings demonstrated a positive association between RM consumption and life expectancy in developing countries but a negative association with high-income countries (11). Nonetheless, the limitation that life expectancy can be affected by many factors other than RM was discussed, suggesting the need for additional analysis of health indicators such as cancer, which are more closely related to RM, as a dependent variable.

Vegetable, tobacco, and alcohol consumption

Vegetables are known to reduce cancer, stroke, heart disease, cataracts, and hypertension, and have a positive effect on health outcomes (12, 14). Vegetable intake is considered an important aspect of cancer prevention through diet, especially that of colon cancer (15). Tobacco smoke, the most common cause of death, is a Group 1 carcinogen that causes 7 million premature deaths each year worldwide (16). Mortality from smoking alone is greater than the combined effect of all other causes, including alcohol consumption, traffic accidents, and acquired immune deficiency syndrome (AIDS) (17). While the effect of consumption of small amounts of alcohol on health is debatable, with regards to cancer, alcohol is known to increase the risk of liver, prostate, and several other types of cancers (18–20).

Aims and goals

This study aimed to examine the association between RM and death from all types of cancer, as well as the incidence of colon cancer in Organization for Economic Cooperation and Development (OECD) countries between 1989 and 2013. For this, diet-related behavioral variables of vegetable, tobacco, and alcohol consumption and socioeconomic status (SES) were set as control variables.

Methods and materials

Subject and data

Our study subjects were OECD countries. Statistics of OECD member countries are relatively well established. Therefore, to compare statistics for policymaking, member states are required to regularly submit statistics in various fields, such as economy, society, and health. In this context, the OECD publishes annual reports in each field, including health at a glance.

The Food Agriculture Organization (FAO) provides RM and other food-related statistics. We used data from 1989 to 2013 from 37 countries that had joined the OECD. In this study, data on RM and vegetable consumption were collected from FAO STAT (http://www.fao.org/faostat), and other data provided by OECD STAT (https://stats.oecd.org/ and https://data.oecd.org/)

were used. Both OECD and FAO data are provided by country and year.

Dependent variables

In this study, deaths due to all types of cancer were included. The number of cancer deaths per 100,000 people was selected as the main dependent variable. Among cancers, colon cancer has already been proven to be closely related to RM. In the case of colon cancer in the OECD STAT, only the incidence can be downloaded. Therefore, we selected the incidence rate of colon cancer as the dependent variable. In the OECD, cancer-related statistics are officially reported by the health ministries of each country, and cancer death is noted based on ICD-10 (C00-C97) and colon cancer based on the diagnosis of C18. The unit was the incidence per 100,000 people.

Explanatory variables

In FAO STAT, total meat consists of meat obtained from bovine animals, aquatic mammals, mutton, goat, pig, and poultry. Except for poultry, the rest were defined as RM (21). Total vegetable consumption was the sum of onions, peas, potatoes, roots, tomatoes, and others. RM and vegetable consumption were measured in grams per capita day. Tobacco consumption in grams per capita year and alcohol consumption in liters per capita year; both variables are at age $\geq$15 years.

Consumption is the starting stock plus imports and production, minus exports, seeds, animal feed, disposal, and other non-food uses and ending stock. FAO defines this as food available for consumption (22).

$$\begin{aligned} \textit{Food available for consumption} = {} & \textit{starting stocks} + (\textit{quantity imported} + \textit{quantity produced}) - (\textit{quantity exported} + \textit{seed} \\ & + \textit{animal feed} + \textit{waste} + \textit{other non-food uses}) - \textit{ending stocks} \end{aligned}$$

SES is closely related to cancer (23, 24) and is the factor that most affects health outcomes at the national level (25). The most widely used indicator at the national level is gross domestic product (GDP) per capita (USD). Health expenditure is known to positively affect population health, such as longevity and child mortality (25, 26). Education level is highly correlated with health (27), and we used the percentage of tertiary education completed in the 25–64-year-old population. Total health expenditure (THE) is a concept that encompasses investment costs of goods and health services, administration, and health, including medical treatment services, such as treatment, rehabilitation, and long-term care, and is widely used as a factor that determines health at a macroscopic level. We used THE per capita (USD). Finally, we selected the percentage of the older people over 65 years (aging rate), which affects the overall socioeconomic factors (28).

Data analysis

A descriptive analysis was conducted on the data pertaining to 24 years in 37 countries for all variables. Furthermore, some variables had missing values, and replacing them improved the predictive power of the model when analyzing panel data (29, 30). As such, multiple imputations using the Markov chain Monte Carlo were performed. Although there are no set standards for the proportion of missing data, substitution was not performed when the proportion of missing data was $<$50% in this study (29, 31). Particularly, substitution was difficult in this study as the proportion of missing data was higher in colon cancer than in other variables, and data from some countries were unavailable. A correlation analysis was conducted with cancer death, the incidence of colon cancer, and RM, vegetable, tobacco, and alcohol consumption. Moreover, pooled ordinary least squares (OLS) and a fixed-effect model (FEM) were used to determine whether independent variables affected dependent variables. FEM is a widely used model for panel data in units of countries. Furthermore, since independent variables are expected to affect cancer with some time lag, an additional analysis was conducted with a lag of 3 and 5 years. However, there were many missing values in the case of colon cancer, so FEM was not performed, and only OLS was analyzed. Lastly, the regression line may not necessarily be straight, and it may affect the dependent variable only when it is at a certain level. Therefore, we divided the recommended daily allowance into groups of 71 grams or less and excess in the RM (18). In the case of vegetables, the average daily consumption of $<$ 500 grams and excess (32). And scatter plots and quadratic curves were checked according to this subgroup. In the analysis, the entity is a country, and the time unit is a year. In addition, all variables were converted to natural logarithms.

Results

Descriptive statistics

The average number of cancer deaths per 100,000 was 229.5, of which Mexico (138.7) had the lowest and Hungary (320.3) had the highest number of deaths. The average incidence of colon cancer was 29.3 people per 100,000, with Hungary (43.4) having the highest and Mexico (7.3) having the lowest incidence. The average GDP per capita was $24,888, with Luxembourg (58,860.6) having the highest and Colombia (7,924.1) the lowest GDP. The average of education level was 24.0%, with Canada (40.8%) having the highest and Turkey (11.8%) having the lowest rate. The THE averaged $2,086.4, with the USA (5,308.0) having the highest and Turkey (481.6) the lowest THE. The average aging rate was 13.6%, with Italy (18.3%) having the highest

TABLE 1 Summary for dependent and explanatory variables of 37 OECD countries, average from 1989 to 2013.

	Cancer (SD)	Colon (SD)	GDP (SD)	Education (SD)	THE (SD)	Aging rate (SD)	Tobacco (SD)	Alcohol (SD)	RM (SD)	Vegetable (SD)
OECD	229.5 (36.9)	29.3 (8.3)	24,888.1 (13,349.7)	24.0 (9.4)	2,086.4 (1,390.5)	13.6 (3.7)	1,976.3 (588.0)	9.4 (3.1)	149.1 (49.4)	511.8 (170.4)
Australia	221.6 (17.4)	33 (3.6)	30,604.5 (9,595.2)	29.6 (6.8)	2,393.5 (954.1)	12.5 (0.9)	1,402.9 (315.1)	10.3 (0.3)	209.1 (14.7)	446.8 (20.2)
Austria	229.1 (21)	32.7 (2.8)	31,317.2 (9,117.7)	22.6 (5.6)	2,945.6 (1,060.4)	16 (1.1)	1,954.2 (302.2)	13.3 (0.9)	216.6 (21.7)	437.2 (35.3)
Belgium	244.5 (26.7)	34.3 (2.7)	29,355.2 (8,212)	27.2 (5.9)	2,589.9 (1,020.9)	16.5 (0.9)	2,023.4 (339.1)	11 (0.7)	165.3 (13)	622.2 (72.2)
Canada	233.8 (16.8)	35 (2.7)	30,808.8 (8,201.2)	40.8 (8.3)	2,822.2 (937)	12.7 (1.1)	1,437.8 (279.2)	7.9 (0.5)	168.5 (10.5)	589.1 (27.4)
Chile	220.9 (14.9)	24.5 (3.8)	11,502.7 (5,427.4)	21.3 (2.6)	758.2 (431)	7.9 (1.2)	2,188.8 (305.1)	7.3 (0.8)	103.9 (16.5)	448.1 (42.6)
Colombia	172.2 (14.8)	14.1 (2.5)	7,924.1 (2457.7)	18.0 (4.0)	683 (311.3)	5.9 (0.7)	2,277.5 (220.8)	5.4 (1.3)	59.5 (5)	286.5 (29.8)
Czechia	287.8 (29.2)	36.7 (4)	19,222.5 (6,767.4)	12.7 (3.3)	1,230.7 (622.2)	14 (1.2)	2,468.8 (276.7)	11.7 (0.3)	159.7 (17.5)	497 (101.1)
Denmark	275.1 (18.6)	39.2 (3)	30,541.6 (9,365.1)	26.2 (7.4)	2,665.5 (1,038.3)	15.6 (0.9)	1,605.4 (176.3)	11.7 (1.1)	188.5 (41.9)	501 (37.2)
Estonia	243.5 (11)	24 (4.2)	15,020 (7,106.8)	29.5 (6.1)	2,128.5 (2,424)	15.3 (2.1)	2,208.7 (251.2)	10.6 (2.2)	131.3 (31.9)	622.8 (69.8)
Finland	197.9 (16.4)	25.7 (2.5)	28,160.7 (8866.7)	28.1 (8.7)	2,208.9 (923)	15.4 (1.6)	1,090.1 (236.9)	9.2 (0.7)	144.6 (5.6)	435.5 (20.9)
France	231.4 (18.4)	33.3 (2.5)	27,173.6 (7,211.1)	23.8 (5.2)	2,833.5 (972.3)	15.7 (1.1)	1,718.5 (388.4)	13.8 (1.3)	180.9 (10)	511.1 (48.9)
Germany	232.3 (22.3)	33.4 (3.5)	29,887.3 (7,969.5)	23.5 (2.8)	3,096.7 (987.8)	17.5 (2.3)	2,074.3 (298.7)	12.5 (1.1)	215.5 (26.7)	479.6 (16.5)
Greece	207.2 (6.3)	20.8 (4)	21,249 (6,042.6)	18.6 (5.3)	1,696.6 (646.4)	17.1 (2)	3,060.6 (587.7)	9.1 (1)	168.5 (16.4)	958.5 (70.8)
Hungary	320.3 (23.3)	40.3 (3)	14,184 (5,893.6)	17.8 (3.6)	1,150.5 (418.1)	15.2 (1.2)	1,924.1 (257.9)	12.5 (0.9)	111.3 (18.9)	492.5 (59.4)
Iceland	226.2 (18.9)	32.8 (4.1)	31,933.8 (7,949.1)	27.6 (3.9)	2,668.4 (787)	11.6 (0.6)	1,761 (458.7)	6 (1)	147.9 (9.8)	335.6 (40.3)
Ireland	258.6 (19.8)	36.3 (2.1)	31,006.7 (12,499.1)	24.9 (9.4)	2,304.8 (1,288.8)	11.3 (0.3)	2,002.2 (470.5)	12.3 (1.3)	175.3 (14.4)	600.8 (34.4)
Israel	207.7 (15.7)	28.2 (4.3)	24,607.4 (4,031)	33.8 (9.8)	1,463 (529.4)	9.7 (0.4)	1,503.3 (247.8)	2.3 (0.4)	72.6 (14.5)	699.2 (93.8)
Italy	232.1 (19.9)	32.1 (2.2)	27,547.5 (6,175)	13.1 (5.9)	2,137.5 (677.1)	18.3 (2.1)	1,797.2 (262.9)	9 (1.2)	173.2 (5.8)	599.3 (53.4)
Japan	200.7 (11.3)	27.5 (3.8)	28,137.8 (6,028.4)	34.4 (8.5)	2,156.1 (931.3)	18 (4.1)	2,709 (539.1)	8.3 (0.7)	76.8 (5)	388.8 (22.6)
Latvia	243.5 (6.5)	16.6 (7.5)	9,918 (7,556.8)	18.1 (7.3)	1,215 (954.8)	15.4 (2.3)	2,547.2 (484.6)	8.9 (1.5)	106.1 (28.9)	670.4 (56.8)
Lithuania	236.8 (5.6)	20.4 (3)	11,361 (6,000)	23.2 (10.8)	1001.4 (368.7)	12.6 (1.5)	2494.7 (280.7)	11.6 (2.5)	129.5 (19)	673.5 (52)
Luxembourg	235.2 (29.3)	35.5 (2.6)	58,860.6 (2,2375.3)	28.1 (6.2)	3,656.6 (1,364.1)	13.9 (0.2)	1,121.8 (560.6)	13 (1.1)	198.2 (27.4)	468.9 (85.2)
Mexico	138.7 (13.7)	8.6 (2.7)	11,421.9 (3,268.5)	17.0 (4.7)	982.1 (316.1)	5.2 (0.8)	1,831.2 (384.5)	4.8 (0.5)	79.4 (7.2)	202.9 (27.4)
Netherlands	256.6 (16.9)	35.8 (3.3)	32,941.8 (10,349.3)	25.0 (6.6)	2,952.4 (1,237.6)	14.1 (1.2)	2,248.2 (519.5)	9.7 (0.4)	192.3 (25.1)	537 (36.5)
New Zealand	242.5 (20.4)	36.2 (3.6)	23,128.7 (6,648.6)	30.1 (6.6)	1,952.5 (784.3)	12 (0.8)	1,246.4 (338.4)	9.3 (0.4)	201.7 (24.7)	592.4 (95.1)
Norway	223.6 (11.6)	32.4 (4.1)	39,201.1 (16,567.9)	29.8 (7.1)	3,100.9 (1,378)	15.4 (0.6)	1,455 (341.6)	5.7 (0.7)	132.6 (6.4)	425.1 (13.1)
Poland	255.3 (9.5)	26.2 (4.2)	12,623.1 (5,964)	15.7 (4.8)	786.8 (436)	12.3 (1.3)	2,157.4 (366.8)	9.1 (0.9)	156 (14.7)	773.7 (53.1)
Portugal	206.5 (9.6)	25.3 (5)	19,613.6 (5,715.8)	13.8 (4.8)	1,610.2 (678.9)	16.3 (1.9)	2,446.3 (474.9)	12.3 (1.3)	157.9 (16.5)	787.1 (86.5)
Republic of Korea	200.5 (15.7)	20.8 (5.4)	20,550.2 (8,644)	25.9 (10.6)	997 (590.4)	7.9 (2.2)	2,695.9 (486.2)	9 (0.3)	94.7 (24.4)	625.7 (36.7)
Slovakia	264.9 (15.1)	30.1 (3.5)	15,048 (6,905.6)	13.6 (3.1)	1,106.3 (578.1)	11.5 (0.8)	2,337.7 (365.7)	11 (1.1)	135.6 (26.5)	470.9 (67.9)
Slovenia	261 (8.8)	34.6 (3.4)	21,884.8 (5,430)	18.8 (4.8)	1,711.3 (503.2)	14.1 (2.2)	2,068.1 (316.2)	11.7 (1.5)	168.7 (18.2)	414.7 (78)
Spain	213.9 (12.5)	27.4 (2.7)	23,185.4 (7,340.9)	23.4 (7.1)	1,770.9 (730.5)	16 (1.3)	2,089.3 (387.1)	11.3 (1.1)	203.8 (21.2)	697.9 (104.5)
Sweden	203.5 (8.9)	29.3 (2.4)	31,168.3 (9,038.4)	29.5 (5.0)	2,544.4 (1045.4)	17.7 (0.6)	1,521.7 (294.8)	6.6 (0.5)	158 (12.9)	419.1 (27)
Switzerland	208.7 (25.9)	30.3 (3.1)	38,986.1 (10,925.9)	26.9 (5.5)	3,676 (1,168.3)	15.6 (0.9)	2,341 (452.1)	11.1 (1)	162.4 (13.7)	417 (22.7)
Turkey	186.5 (24.5)	15 (4.5)	12,045.9 (4,180.6)	11.0 (3.8)	481.6 (279)	6.2 (1)	2,103 (274)	1.5 (0.1)	32.6 (5.4)	413 (24.5)
U.K	249.5 (19.4)	37.4 (2.3)	27,639 (7,862.1)	28.3 (7.7)	2,151.3 (1,036.8)	16 (0.4)	1,515.7 (460.1)	10.2 (0.7)	138.3 (7.2)	211.7 (9.4)
USA	224.4 (20.6)	34.7 (2.2)	37,455.9 (9,822.1)	35.9 (5.9)	5,308 (2,006.9)	12.7 (0.4)	1,826.8 (272.7)	8.5 (0.3)	196.1 (8.9)	226.7 (13.4)

The results were calculated after multiple imputations of missing values; SD: standard deviation; Cancer: death from cancer per 100,000; Colon: colon cancer incidence per 100,000; GDP: gross domestic product per capita (US dollars); Education: Percentage of tertiary education completed (25–64 year); THE: total health expenditure per capita (US dollars); Aging rate: rate in a 65+ population; Tobacco: tobacco consumption (grams per capita/year, +15), alcohol consumption (liters per capita/year, +15), RM: red meat consumption (grams per capita/day), Vegetable: vegetable consumption (grams per capita/day). All variables were converted to natural logarithms.

and Mexico (5.2%) having the lowest aging rate. The average tobacco consumption per capita was 1,976.3 grams per capita year, with Greece (3,060.6) having the highest and Luxembourg (1,121.) the lowest. The average alcohol consumption was 9.4 liters per capita/year, with France (13.8) having the highest and Turkey (1.5) the lowest. The average RM consumption was 149.1 g/capita/day. Austria had the highest RM consumption at 209.1 g/capita/day, and Turkey (32.6 g/capita/day) had the lowest. The average vegetable consumption was 511.8 g/capita/day. Greece had the highest vegetable consumption at 958.5 g/capita/day, with Mexico having the lowest at 202.9 g/capita/day (Table 1).

TABLE 2 Correlation matrix of cancer death, the incidence of colon cancer, GDP, education, THE, aging rate, tobacco, alcohol, RM, and vegetable consumption (coefficient and *P* value).

	Cancer	Colon	GDP	Education	THE	Aging rate	Tobacco	Alcohol	RM	Vegetable
Cancer	1									
Colon	0.592 (0.000)	1								
GDP	−0.076 (0.000)	0.541 (0.000)	1							
Education	0.164 (0.000)	0.369 (0.000)	0.417 (0.000)	1						
THE	−0.059 (0.074)	0.457 (0.000)	0.358 (0.000)	−0.369 (0.000)	1					
Aging rate	0.370 (0.000)	0.566 (0.000)	0.226 (0.000)	−0.410 (0.000)	0.580 (0.000)	1				
Tobacco	0.166 (0.000)	−0.296 (0.000)	−0.317 (0.000)	0.108 (0.000)	−0.455 (0.000)	−0.144 (0.000)	1			
Alcohol	0.488 (0.000)	0.441 (0.000)	0.105 (0.002)	−0.288 (0.000)	0.305 (0.000)	0.556 (0.000)	0.054 (0.101)	1		
RM	0.421 (0.000)	0.566 (0.000)	0.222 (0.000)	−0.197 (0.000)	0.541 (0.000)	0.629 (0.000)	−0.197 (0.000)	0.741 (0.000)	1	
Vegetable	0.290 (0.000)	0.094 (0.004)	−0.088 (0.007)	0.089 (0.007)	−0.135 (0.000)	0.294 (0.000)	0.204 (0.000)	0.198 (0.000)	0.192 (0.000)	1

Cancer: death from cancer per 100,000; Colon: colon cancer incidence per 100,000; GDP: gross domestic product per capita (US dollars); Education: Percentage of tertiary education completed (25–64 year); THE: total health expenditure per capita (US dollars); Aging rate: rate in a 65+ population; Tobacco: tobacco consumption (grams per capita/year, +15), alcohol consumption (liters per capita/year, +15), RM: red meat consumption (grams per capita/day), Vegetable: vegetable consumption (grams per capita/day). All variables were converted to natural logarithms.

Estimates from pearson's correlation, pooled OLS and fixed effect regression

Almost all the variables correlated with each other. The RM consumption was correlated with all variables. Cancer death had a statistically significant correlation with all other variables except THE, and colon cancer was statistically correlated with all variables (Table 2).

The pooled OLS data from 1989 to 2013 showed that the cancer death rate was associated with GDP [Coefficient (Coef) = −0.007, $P = 0.028$], THE (Coef = −0.092, $P = 0.000$), aging rate (Coef = 0.159, $P = 0.000$), alcohol consumption (Coef = 0.072, $P = 0.000$), and RM consumption (Coef = 0.123, $P = 0.000$). Colon cancer incidence was associated with GDP (Coef = 0.102, P = 0.000), education level (Coef = 0.081, $P = 0.003$), THE (Coef = −0.066, $P = 0.001$), aging rate (Coef = 0.366, $P = 0.000$), tobacco consumption (Coef = −0.107, $P = 0.001$), and RM consumption (Coef = 0.248, $P = 0.000$) (Table 3).

Longitudinal analysis was performed using FEM (non-lagged) and 3- and 5-year lagged analyses. Cancer death had statistically significant associations with education level (Coef = −0.022, $P = 0.009$), THE (Coef = −0.049, $P = 0.000$), the aging rate (Coef = −0.178, $P = 0.000$), tobacco consumption (Coef = 0.096, $P = 0.000$), RM consumption (Coef = 0.107, $P = 0.000$), and vegetable consumption (Coef = −0.034, $P = 0.000$). In the 3-year lagged model, education level (Coef = −0.030, P = 0.003), THE (Coef = −0.040, $P = 0.000$), aging rate (Coef = −0.157, $P = 0.000$), tobacco consumption (Coef = 0.094, $P = 0.000$), and RM consumption (Coef = 0.071, $P = 0.000$) were statistically related. In the 5-year lagged model, THE (Coef = −0.043, $P = 0.000$), aging rate (Coef = −0.077, $P = 0.015$), tobacco consumption (Coef = 0.083, $P = 0.001$), alcohol consumption (Coef = 0.068, $P = 0.001$), and RM consumption (Coef = 0.043, $P = 0.027$) were related (Table 4).

Scatter plots and fitted lines by sub-group analysis of red meat and vegetable consumption

In the case of RM, the slope did not change as consumption increased in the group below the recommended allowance. However, in the excess group, as consumption increased, cancer death also increased, and when it was above a certain level, the slope decreased. In the case of vegetables, the death rate increased as consumption increased in the group below the recommended level. However, the death rate decreased as consumption increased in the group above the recommendation, as was the incidence of colon cancer (Figure 1).

Discussion

Processed meat is a Group 1 carcinogen that should be avoided. In contrast, RM is considered a limiting food instead of an avoidable food (21). For this reason, whether the consumption of RM should be as restricted as processed meat remains debatable. In general, in developed countries, health problems caused by the overconsumption of meat receive more attention than those caused by a lack of nutrition. In this study, OECD countries were found to have consumed an average of 149 g of RM per day. This is approximately twice the daily recommended intake of 71 g (8), and a consumption rate higher than this was observed in all countries except Turkey.

TABLE 3 Association between cancer death and incidence of colon cancer and each independent variable by pooled OLS analysis, 1989–2013.

	Cancer death		Incidence of colon cancer	
	Coef. (T)	*P* value	Coef. (T)	*P* value
GDP	−0.007 (−2.20)	0.028	0.102 (15.49)	0.000
Education	−0.015 (−1.13)	0.257	0.081 (2.97)	0.003
THE	−0.092 (−9.30)	0.000	−0.066 (−2.97)	0.001
Aging rate	0.159 (8.08)	0.000	0.366 (9.20)	0.000
Tobacco	0.015 (1.00)	0.318	−0.107 (−3.49)	0.001
Alcohol	0.072 (4.96)	0.000	0.058 (1.95)	0.051
RM	0.123 (6.78)	0.000	0.248 (6.77)	0.000
Vegetable	0.010 (0.69)	0.489	−0.015 (−0.53)	0.595
F	75.51		148.29	
Adj. R-square	0.394		0.562	
Number of observations	918		918	

Cancer: death from cancer per 100,000; Colon: colon cancer incidence per 100,000; GDP: gross domestic product per capita (US dollars); Education: Percentage of tertiary education completed (25–64 years); THE: total health expenditure per capita (US dollars); Aging rate: rate in an 65+ population; Tobacco: tobacco consumption (grams per capita/year, +15), alcohol consumption (liters per capita/year, +15), RM: red meat consumption (grams per capita/day), Vegetable: vegetable consumption (grams per capita/day). All variables were converted to natural logarithms.

TABLE 4 Association between GDP, education, THE, aging rate, RM, vegetable consumption and cancer death by fixed-effect model, 1989–2013.

	Fixed effect		Lagged 3 year		Lagged 5 year	
	Coef. (T)	*P* value	Coef. (T)	*P* value	Coef. (T)	*P* value
GDP	0.001 (0.78)	0.437	−0.000 (−0.03)	0.977	−0.003 (−0.88)	0.380
Education	−0.022 (−2.62)	0.009	−0.030 (−3.00)	0.003	−0.019 (−1.58)	0.115
THE	−0.049 (−9.74)	0.000	−0.040 (−7.13)	0.000	−0.043 (−6.82)	0.000
Aging rate	−0.178 (−8.20)	0.000	−0.157 (−5.78)	0.000	−0.077 (−2.43)	0.015
Tobacco	0.096 (10.34)	0.000	0.094 (9.70)	0.000	0.083 (8.43)	0.000
Alcohol	0.016 (0.94)	0.349	0.034 (1.71)	0.087	0.068 (3.26)	0.001
RM	0.107 (7.40)	0.000	0.071 (3.91)	0.000	0.043 (2.22)	0.027
Vegetable	−0.034 (−1.80)	0.000	−0.014 (−0.53)	0.527	−0.021 (−1.00)	0.315
F	124.89		114.07		117.03	
Adj. R-square	0.539		0.432		0.396	
Number of observations	918		807		733	
Groups	37		37		37	

Cancer: death from cancer per 100,000; Colon: colon cancer incidence per 100,000; GDP: gross domestic product per capita (US dollars); Education: Percentage of tertiary education completed (25-64 year); THE: total health expenditure per capita (US dollars); Aging rate: rate in a 65+ population; Tobacco: tobacco consumption (grams per capita/year, +15), alcohol consumption (liters per capita/year, +15), RM: red meat consumption (grams per capita/day), Vegetable: vegetable consumption (grams per capita/day). All variables were converted to natural logarithms.

In OLS, RM was positively associated with cancer death, while non-lagged, 3-year, and 5-year lagged models of FEM showed a positive association with cancer death. Within OECD countries, RM demonstrated a positive association with nationwide cancer death rates, which suggests that RM is a health threat that requires appropriate control for cancer prevention and that public health control for RM has not been well implemented in major developed countries. However, an FEM analysis of colon cancer incidence was not performed in this study due to limitations in data collection. Nevertheless, RM also demonstrated a statistically positive association with the incidence of colon cancer in the pooled OLS analysis.

Moderate meat intake is not yet a matter of concern for increased cancer incidence, and the WCRF recommends consuming no more than 350 to 500 grams of RM per week for cancer prevention (13, 18). Although RM consumption in most countries included in this study exceeded this level, the average consumption was significantly lower than the 200 g per day considered as 'high consumption' and only 5 countries had most countries included. Nevertheless, it is clear that

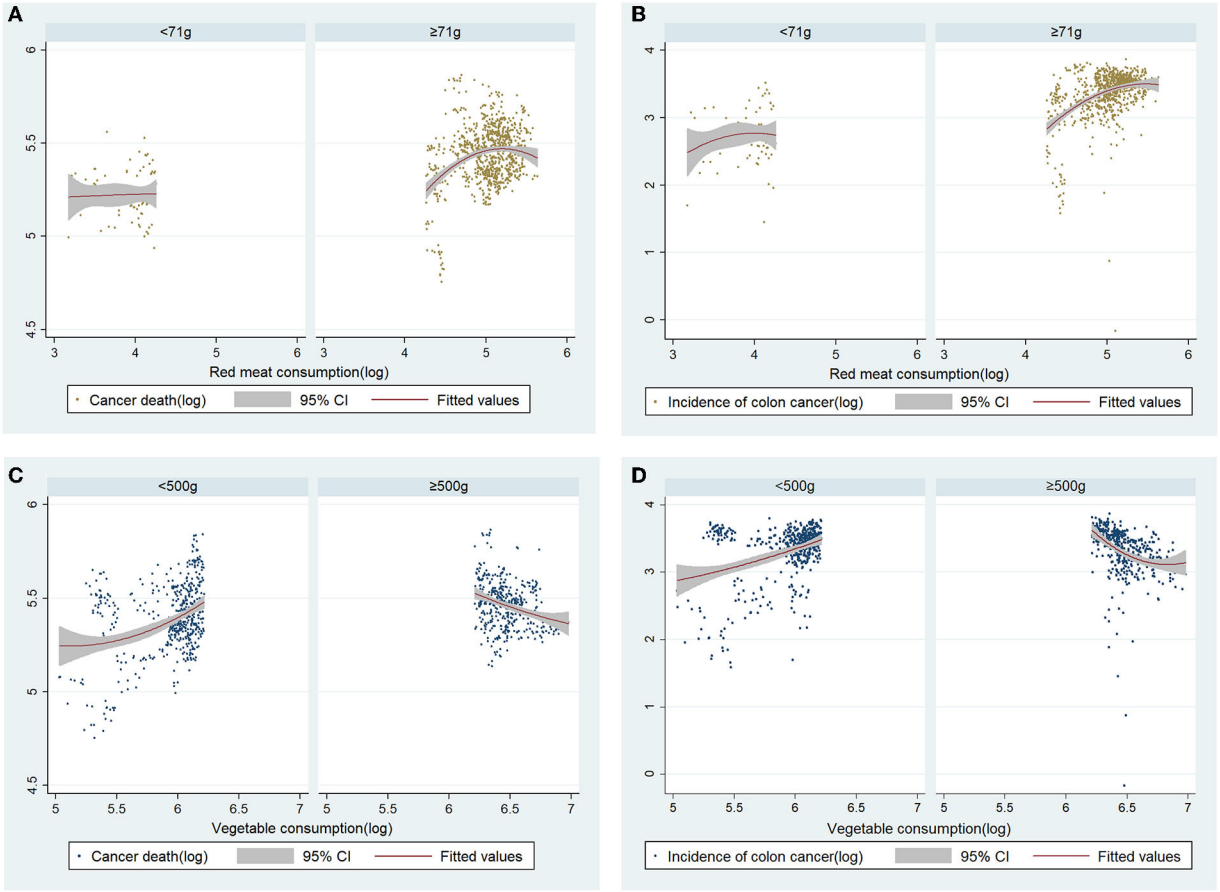

FIGURE 1
Correlation between red meat consumption, cancer death, and incidence of colon cancer; sub-group analysis by a recommended allowance of red meat a day (71 grams). Scatter plots and quadratic curves for red meat consumption and **(A)** cancer death, and **(B)** incidence of colon cancer. All variables were converted to natural logarithms. Correlation between vegetable consumption, cancer death, and incidence of colon cancer; sub-group analysis by recommended vegetable requirements a day (500 grams). Scatter plots and quadratic curves for vegetable consumption, **(C)** cancer death, and **(D)** incidence of colon cancer. All variables were converted to natural logarithms.

RM consumption increases cancer incidence (including colon cancer) and mortality.

We can obtain more information through sub-group analysis according to the consumption of RM. In the case of RM, the positive association between cancer death and incidence of colon cancer was more clearly confirmed in the group exceeding the recommended allowance. Except for a few countries, the recommended allowance was exceeded in many OECD countries. Therefore, to respond more sensitively to cancer prevention policies, it is necessary to implement a nutrition policy that restricts RM consumption in most OECD countries and high RM consuming countries. However, the implications of this study do not involve the restriction of RM consumption in developing countries. This study involved developed countries, and considering that RM remains important from a public health nutrition standpoint following previous research, there will be no need to control consumption in developing countries (11, 33, 34). In this study, vegetable consumption was negatively associated with cancer deaths in FEM, but it was not significant in OLS and lagged models. The association with vegetable consumption could be obtained more clearly through scatter plots through sub-groups. It was confirmed that the dependent variable decreased as the consumption of vegetables increased in the group above the recommended consumption amount.

This is consistent with previous findings that indicate the effectiveness of vegetable consumption above certain levels in reducing cancer incidence (35). However, although vegetable consumption has been demonstrated to reduce the risk of several types of cancer, including colon cancer (36), some studies have demonstrated no association (15, 36). Our findings suggest that a policy intervention to increase consumption of vegetables above the recommended intake may be necessary to prevent cancer, with vegetable consumption in OECD countries averaging 511.8 grams per day, indicating that vegetable consumption is still insufficient in half of the countries. The effects of vegetables on cancer may also vary depending on the type of vegetable

consumed (36). However, the type of vegetable is not considered in this study. More interesting findings may be derived from a more curated analysis of the amount of consumption and types of vegetables.

In the FEM and lagged 3 and 5-year model, the number of cancer deaths decreased as the level of education increased. It has been consistently studied that education level has a positive relationship with better health conditions at the individual as well as the national level. Although this mechanism is somewhat complicated, it may be because the higher the education level, the higher the social class or economic level (37) or the education may have affected health by increasing health literacy (27). Meanwhile, the incidence of colon cancer death decreased in both OLS and FEM among SES as the THE increased. THE has been reported to be positively associated with national health level in many studies (25), including a study on OECD countries, where it was deemed to have a negative association with cancer mortality (38, 39). Traditionally, colon cancer has been known as a major health problem in developed countries (40). In the OLS of this study, GDP demonstrated a positive association with the incidence of colon cancer, which is supported by a previous study on 11 Balkan countries (41). Furthermore, a positive association was observed between cancer death and the incidence of colon cancer in the aging rate. Aging is the most well-known cause of cancer, and the aging population is a common cause of increased cancer incidence in developed countries (39).

Smoking is another major risk factor for cancer. Although the incidence of colon cancer demonstrated a negative association with tobacco consumption in the OLS, a positive association was observed in the simple correlation analysis. This may be a problem caused by the lack of data and limitations of the analysis method. Tobacco smoking increases the incidence and mortality rates of colon cancer (42). In most studies thus far, a high level of association was observed between smoking and rectal cancer, but relatively lower or, in some cases, no level of association with colon cancer (42, 43). Furthermore, few studies have assessed this association at the country level. As such, additional research on tobacco consumption and colon and rectal cancer at the national level is needed with supplementary data.

Alcohol consumption was positively associated with cancer death and the incidence of colon cancer in OLS and the lagged 5-year model. Alcoholic beverages have been classified as group 1 carcinogens by the IARC and can cause various types of cancer, including breast, liver, and esophageal cancer (18). Avoiding excessive alcohol consumption is key to cancer prevention, not absolute abstinence (42). Nevertheless, it was clear throughout this study that the level of alcohol consumption in major developed countries contributed to increased cancer incidence and mortality. It is estimated that approximately 4% of the incidence of all cancers worldwide is due to alcohol consumption (44). A previous study stated that even small amounts of alcohol increase the risk of some cancers, and there is no safe level of alcohol consumption (18). Therefore, it is imperative that policies to reduce alcohol consumption be further strengthened for cancer prevention, regardless of the level of consumption.

It is possible to limit excessive intake of RM in terms of individual disease prevention through individual unit research. However, whether these interventions affect health outcomes at the population level is another matter and does not justify a policy to limit consumption at the national level. Our findings indicate that nutritional policies to limit RM consumption may be needed at the national level in OECD countries.

Limitations and future study

This study is one of the first to confirm the association between RM and national cancer incidence using panel data from 37 countries. Nevertheless, this study had several limitations. First, our research design could not explain the causal relationship between RM and cancer. Second, the findings of this study cannot be generalized at the individual level, as the study was conducted at the country level (ecological fallacy). Likewise, most previous studies have been on individual intake (individualistic fallacy), whereas this study focused on national level consumption, meaning that one must be wary of direct comparisons. Third, results may vary from country to country depending on consumption and food culture. To overcome this limitation, we provided supplementary figures showing the correlation with the dependent variable according to the consumption level by country. This may help understand the relationship between cancer and RM and vegetable consumption in each country. Finally, the reproducibility or reliability of the results is not high for colon cancer due to limitations in the available data. Thus, in the case of colon cancer, data should be sufficiently supplemented and analyzed using more advanced techniques than OLS. Furthermore, setting rectal cancer as a dependent variable will enable more robust research.

Conclusion

The RM consumption in 37 OECD countries was found to be higher than the recommended intake but lower than the "high consumption" level. The consumption of RM was positively related to deaths due to cancer and the incidence of colon cancer. This finding suggests that an increase in consumption of RM is highly likely to increase cancer death and incidence of colon cancer. Our results justify public health interventions to limit RM consumption in major developed countries. Moreover, the current level of alcohol consumption is likely to contribute to an increase in cancer, and policies to reduce its consumption are necessary. Vegetable consumption was not found to be related to

cancer in this study, but consumption above a certain level may effectively prevent cancer.

Data availability statement

Publicly available datasets were analyzed in this study. The data are available from FAO (http://www.fao.org/faostat) and OECD (https://stats.oecd.org/ and https://data.oecd.org/). If you need the processed data, please contact the author to request the data.

Author contributions

M-BP initiated the idea and led the formal analysis, reviewed, and edited the final draft of the article.

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Conflict of interest

The author declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.929553/full#supplementary-material

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Higher Levels of Urinary Thiocyanate, a Biomarker of Cruciferous Vegetable Intake, Were Associated With Lower Risks of Cardiovascular Disease and All-Cause Mortality Among Non-smoking Subjects

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Background: Epidemiologic studies on cruciferous vegetable (CV) intake and cardiovascular disease (CVD) were inconclusive.

Objective: To investigate the associations of urinary thiocyanate, a biomarker of CV intake, with CVD and all-cause mortality among non-smoking adults.

Methods: This prospective cohort study comprised 10,489 non-smoking adults (weighted mean age, 46.8 years; 43.4% male) from the National Health and Nutrition Examination Survey 2001–2014. Non-smokers were defined as subjects with serum cotinine < 3 ng/mL. Urinary thiocyanate was measured with ion chromatography tandem mass spectrometry at baseline, and CVD and all-cause mortality were identified through linkage to National Death Index until December 31, 2015. Cox proportional hazards model was applied to estimate the hazard ratios (HRs) with 95% confidence intervals (CIs) for CVD and all-cause mortality.

Results: A total of 800 deaths, of which 136 died of CVD, were ascertained within a median 7.8 years of follow-up. Urinary thiocyanate was positively correlated with total CV intake among non-smoking adults ($r_S = 0.088$, $P < 0.001$). Comparing extreme quartiles, the multivariate-adjusted HRs for CVD and all-cause mortality were 0.50 (95% CI: 0.29–0.85) and 0.75 (95% CI: 0.60–0.92), respectively. Each 1 μg/g creatinine increment of log-transformed urinary thiocyanate was associated with a 25% (HR: 0.75; 95% CI: 0.62–0.91) reduced CVD mortality risk and 12% (HR: 0.88; 95% CI: 0.81–0.96) reduced all-cause mortality risk. The documented inverse associations persisted in sensitivity analyses.

Conclusion: Increased levels of urinary thiocyanate, a candidate biomarker of CV intake, were associated with low risks of CVD and total mortality among non-smoking adults. This prospective biomarker-based study provided further evidence to support the cardiovascular benefits of CVs.

Keywords: thiocyanate, biomarker, cruciferous vegetable, cardiovascular disease, mortality, National Health and Nutrition Examination Survey

INTRODUCTION

Immense health and economic burdens are produced by cardiovascular disease (CVD) globally (1, 2). Despite the fact that fatalities attributable to CVD in United States declined during the 1980s to the 2010s, CVD remains the leading cause of death worldwide, and the global death toll of CVD was expected to exceed 23.6 million by 2030 (1). It was estimated by the American Heart Association in 2016 that nearly half of the American population would be affected by CVD to some extent and total costs of CVD would reach 1.1 trillion dollars by 2035 (1). Hence, much concern had been raised about developing effective strategies, such as healthy diets (3), to prevent CVD.

Cruciferous vegetables (CVs) are featured by their high glucosinolates contents (4) and had been recognized as part of healthy diet (5). Nonetheless, human studies on CV intake and CVD were inconclusive, with some prompting protective effects while others reporting no significant associations (**Supplementary Table 1**). Joshipura et al. (6) observed no significant association between CV intake and risk of ischemic CVD among 70,870 females from the Nurses' Health Study (NHS) and 38,918 males from the Health Professionals' Follow-Up Study (HPFS). However, a pooled analysis of two prospective cohort studies comprising 134,796 Chinese adults reported that higher CV intake was associated with a lower risk of mortality from CVD (7). Food frequency questionnaires (FFQs) were applied to estimate CV intake in previous studies (**Supplementary Table 1**), making the results susceptible to measurement error and misclassification. Hence, biomarker-based studies are anticipated to have a better understanding of the association between CV intake and risk of CVD.

Thiocyanate, a metabolite of cyanide from tobacco or glucosinolates from CVs (8), could be ubiquitously detected in urine samples (9). Elimination of thiocyanate occurs in the kidneys, and the half-life of thiocyanate is 3 days in individuals without renal insufficiency (8). Cigarette smoking makes considerable difference in the major source of thiocyanate, and thiocyanate primarily originates from tobacco for smokers and diet for non-smokers, respectively (10). Moreover, urinary thiocyanate levels vary between smokers and non-smokers, and smokers have much higher urinary thiocyanate measurements (11, 12). These facts provide a clue that urinary thiocyanate might be a biomarker of CV intake among non-smokers.

In this prospective cohort study of non-smoking adults, we aimed to investigate the associations of urinary thiocyanate with CVD and all-cause mortality. We hypothesized that higher levels of urinary thiocyanate, a biomarker of CV intake, may be associated with lower risks of CVD and all-cause mortality among non-smoking subjects.

MATERIALS AND METHODS

Study Population

National Health and Nutrition Examinations Survey (NHANES) is a series of nationally representative surveys enrolling approximately 5,000 non-institutional civilians in the United States each year (13). This program had been approved by the National Center for Health Statistics (NCHS) Ethics Review Board and gained informed consent from participants. Details on the program were described elsewhere (13).

We used data from NHANES 2001–2014 cycles, from which a total of 69,236 participants with medical examination data were preliminarily selected. 33,004 adults were left after participants aged < 20 years, without mortality data, or having CVD at baseline were excluded. A total of 14,500 participants with complete urinary thiocyanate and creatinine and serum cotinine measurements were further identified. Benowitz et al. (14) recommended 3 ng/mL as the cut-off point to distinguish smokers and non-smokers based on a US nationally representative sample of 3,078 smokers and 13,078 non-smokers. Hence, 10,489 adults with serum cotinine < 3 ng/mL were identified as non-smokers and included in this prospective cohort study (**Supplementary Figure 1**).

Urinary Thiocyanate Measurement

Spot urine specimens were collected into sterile 250-mL containers, following the instructions described in the NHANES Laboratory Procedures Manual (15). Urinary thiocyanate levels were determined with ion chromatography coupled with electrospray tandem mass spectrometry in the National Center for Environmental Health (16). Chromatographic separation of compounds was carried out in IonPac AS16 column with sodium hydroxide as the eluant (16). Details of laboratory methodology, quality control, and quality assurance were described elsewhere (16). The limit of detection (LOD) was 20 ng/mL for thiocyanate, and the detection limit divided by the square root of two was assigned as the corresponding value for the measurements below LOD.

Outcomes Assessment

The outcomes of interest in our study were CVD and all-cause mortality, which were identified through linkage to the National Death Index until December 31, 2015 (17). Causes of death had been ascertained according to the International Classification of

Diseases, Tenth Revision (ICD-10) by the NCHS. In the current study, CVD deaths were defined as deaths attributed to heart disease or cerebrovascular diseases (ICD-10 codes I00-I09, I11, I13, I20-I51, I60-I69). Follow-up duration was defined as the interval from the mobile examination center date to the date of death or December 31, 2015, whichever occurred first.

Covariates Assessment

Demographic, socioeconomic, lifestyle, and dietary information was collected with questionnaires by trained interviewers. Race/ethnicity was categorized as non-Hispanic white, non-Hispanic black, Mexican American, and others (10, 18). Family poverty income ratio (PIR), a measure of family income, was classified into three categories (<1.3, 1.3- < 3.5, ≥ 3.5). Participants who had less than 12 alcohol drinks in their lifetime were classified as never drinkers; those who had at least 12 alcohol drinks but avoided alcohol in the past 12 months were defined as former drinkers; and individuals who drank alcohol in the past 12 months when surveyed were categorized as current drinkers. Physical activity was classified into never, moderate, and vigorous according to replies of respondents to the items related to daily, recreational, and sedentary activities. Vigorous activities were defined as activities that cause large increases in breathing or heart rate for at least 10 min, and moderate activities were defined as activities that cause small increases in breathing or heart rate for at least 10 min (19). Dietary information was obtained with 24-h dietary recall interviews by trained dietary interviewers, and total energy intake was calculated with the automated multiple pass method. Healthy Eating Index-2015 (HEI-2015), which comprises nine adequacy and four moderation components, was used to indicate overall diet quality (20). HEI-2015 ranged from 0 to 100, and a higher score indicated a better diet quality (20). A 139-item FFQ, which was developed from the validated National Cancer Institute Diet History Questionnaire (DHQ) (21), was added to NHANES 2003–2004 and 2005–2006 to obtain information on food and food group consumption patterns during the past year. The FFQ contained two items related to CV, "Did you eat broccoli?" and "Did you eat cauliflower?" and the answers ranged from "never" to "two or more times per day." Total CV consumption (times/week) was calculated by summing the consumption of broccoli and cauliflower, two common *Brassica* species. The arithmetic mean of the upper and lower limits was used as the corresponding consumption. If the highest intake category interval was right-open (e.g., ≥ 7 times/week), the corresponding intake was set at 1.2 times the lower boundary (e.g., 8.4 times/week) (22). If the lowest intake category interval was left-open (e.g., < 1 time/week), the corresponding intake was set at half the upper boundary (e.g., 0.5 time/week) (23). Anthropometric information was collected by trained health technicians, and body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Cotinine, the metabolite of nicotine, has a half-life of 15–20 h in plasma, and was preferred as the biomarker of smoking in previous studies (14, 24, 25). Serum cotinine was measured with an isotope dilution-high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (26). The detection limit of serum cotinine was 0.015 and 0.011 ng/mL was assigned as the corresponding value for the results below the detection limit (26). Second-hand smoking was defined as serum cotinine between LOD and 3-ng/mL cut-off point in this study. Blood pressure measurements were collected by examiners who had been certified through a training program with mercury sphygmomanometers. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg, diastolic blood pressure (DBP) ≥ 90 mmHg, or currently taking prescribed medicine for hypertension (27). Urinary concentrations of creatinine were measured with an enzymatic method based on Jaffe rate reaction (28). Urinary iodine was measured with inductively coupled plasma-mass spectrometry (29) and categorized into low iodine excretion (<100 μg/L) and high iodine excretion (≥ 100 μg/L) according to the classification recommended by the World Health Organization (30).

Statistical Analysis

Missing values of covariates were imputed with medians and missing indicators for continuous and categorical variables, respectively. Number and proportion of missing covariates were shown in **Supplementary Table 2**, and a total of 4,827 missing values were imputed. Urinary thiocyanate was divided by creatinine concentration to adjust for urine dilution, and log-transformed to alleviate the skewed distribution. Geometric means of urinary thiocyanate measurements according to population characteristics were calculated. Taking the complex, multistage, and probability sampling design of NHANES into account, we applied sampling weights and sample design variables in formal analyses.

Participants were categorized according to quartiles of urinary thiocyanate. Continuous variables were expressed with weighted means and standard errors, and categorical variables were expressed with numbers and weighted proportions. The baseline characteristics across thiocyanate quartiles were compared with linear regression for continuous variables and logistic regression for categorical variables. Partial Spearman correlation coefficient between urinary thiocyanate and total CV intake was calculated in a pilot study among NHANES 2005–2006. Cox proportional hazards regression model was applied to investigate the associations of urinary thiocyanate with risks of CVD and total mortality. The proportional hazards assumptions were tested with Schoenfeld residuals method, and no violation was observed. Confounders, including age (years, continuous), sex (male, female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, others), secondhand smoking (yes, no), BMI (< 25, 25- < 30, ≥ 30 kg/m^2), education attainment (under high school, high school, above high school), family PIR (< 1.3, 1.3- < 3.5, ≥ 3.5), alcohol consumption (never, former, current), physical activity (never, moderate, vigorous), total energy intake (kcal, continuous), HEI-2015 score (continuous), urinary iodine (< 100, ≥ 100 μg/L), and hypertension (yes, no), were adjusted in the multivariate models. The *P*-value for linear trend was calculated by introducing medians of quartiles as continuous variables into the model. We additionally calculated the multivariate-adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) for CVD and total mortality associated with

each 1 μg/g increment in log-transformed urinary thiocyanate. Restricted cubic splines with 3 knots at the 5th, 50th, and 95th percentiles of log-transformed urinary thiocyanate distribution were further plotted to examine the log-linear dose–response relationships between urinary thiocyanate and CVD and total mortality, and the reference value was set at the 10th percentile.

Stratified analyses by age ($<$ 50 years, $\geq$ 50 years), sex (male, female), race/ethnicity (non-Hispanic white, others), obesity (yes, no), secondhand smoking (yes, no), current drinking (yes, no), hypertension (yes, no), and diet quality (lower, higher) were performed to examine whether these factors modified the association between urinary thiocyanate and CVD mortality. Potential interaction between urinary thiocyanate and stratification factor was evaluated by introducing a multiplicative term between urinary thiocyanate and stratification variable as continuous variables into the multivariate models, and testing whether the coefficient of the interaction term was equal to zero. Taking increased false positive in multiple hypothesis testing into account, we adjusted *P*-value with Bonferroni correction, and statistical significance was set at $P < 0.006$ (0.05/8 subgroups). We also calculated the false discovery rate (FDR) to identify as many significant interactions as possible while controlling a relatively low proportion of false positives, and FDR < 0.05 was considered as significant.

Moreover, several sensitivity analyses were conducted to evaluate the robustness of our results. First, we introduced urinary creatinine as a covariate into the multivariate models rather than divided thiocyanate by creatinine to adjust for urine dilution. Second, participants with daily energy intake $<$ 500 or $>$ 5,000 kcal were excluded to examine whether our results were sensitive to extreme daily energy intake. Third, we excluded the total vegetables component from the HEI-2015 score and precluded the adjustment of BMI to avoid potential over-adjustment, and introduced fasting plasma glucose into models. Fourth, dietary fiber, β-carotene, folate, vitamin K, total fruits, total dairy, and whole grains intake, rather than HEI-2015 score, were adjusted in multivariate models. Fifth, we adjusted SBP, DBP, and antihypertensive therapy rather than hypertension in models, considering the evidence that antihypertensive drug treatment could affect the cardiovascular outcomes (31, 32). Finally, multiple imputed data sets for missing covariates were generated under the missing-at-random assumption since single imputation did not reflect the uncertainty about the predictions of the missing values.

We used STATA 15.1 (StataCorp LLC, Texas, United States) and SAS 9.4 (SAS Institute, NC, United States) for statistical analysis. All tests were bilateral, and *P*-values lower than 0.05 were recognized as statistical significance unless otherwise stated.

RESULTS

Characteristics of Study Population

This cohort comprised 10,489 non-smoking subjects (weighted mean age, 46.8 years; 43.4% male). The mean urinary thiocyanate was 1.28 mg/L. The geometric mean of urinary thiocyanate was 0.94 and 0.95 mg/g creatinine in never and secondhand smokers (**Supplementary Table 3**), respectively. Higher urinary thiocyanate levels were observed among female and non-Hispanic white participants (**Supplementary Table 3**). Non-smoking subjects with higher urinary thiocyanate levels were more likely to be current drinkers, have high family incomes and education attainments, exercise regularly, and have higher intakes of total vegetables, total dairy, whole grains, fiber, β-carotene, folate, and vitamin K (**Table 1**). There was no significant difference in serum cotinine levels across thiocyanate quartiles ($P = 0.31$). Urinary thiocyanate was positively correlated with total CV intake ($r_s = 0.088$, $P < 0.001$) in this non-smoking population (**Supplementary Table 4**).

Associations of Urinary Thiocyanate With Cardiovascular Disease and All-Cause Mortality

During 78,095 person-years of observation (median follow-up, 7.8 years), a total of 800 deaths, of which 136 died of CVD, were ascertained. Comparing extreme thiocyanate quartiles, the multivariate-adjusted HRs for CVD and all-cause mortality were 0.50 (95% CI: 0.29–0.85, *P*-trend = 0.02) and 0.75 (95% CI: 0.60–0.92, *P*-trend = 0.009), respectively (**Table 2**). Inverse log-linear dose–response relationships between urinary thiocyanate and risks of CVD (*P*-non-linearity = 0.86) and total (*P*-non-linearity = 0.14) mortality were depicted in the restricted cubic splines (**Figure 1**). Each 1 μg/g creatinine increment of log-transformed urinary thiocyanate was associated with a 25% (HR: 0.75; 95% CI: 0.62–0.91) reduced risk of CVD mortality and 12% (HR: 0.88, 95% CI: 0.81–0.96) reduced risk of all-cause mortality (**Table 2**).

Subgroup and Sensitivity Analyses

The association of urinary thiocyanate with CVD mortality stratified by several important confounders was depicted, and a stronger inverse association between urinary thiocyanate and CVD mortality was observed among non-Hispanic white participants (*P*-interaction = 0.002, FDR = 0.016) (**Supplementary Figure 2**). After directly introducing urinary creatinine as a covariate into the multivariate model to adjust for urine dilution, we consistently observed the inverse associations (**Supplementary Table 5**). Moreover, neither excluding extreme values of daily energy intake nor excluding the total vegetables component from HEI-2015 score distorted the documented inverse associations of thiocyanate exposure with CVD and all-cause mortality (**Supplementary Tables 6**, **7**). The inverse associations persisted after excluding BMI from the multivariate models and further adjusting fasting plasma glucose in models (**Supplementary Table 7**). Comparing extreme quartiles, the HRs for CVD and all-cause mortality were 0.50 (95% CI: 0.29–0.85) and 0.75 (95% CI: 0.61–0.92) in the multivariate models where dietary fiber, β-carotene, folate, vitamin K, total fruits, total dairy, and whole grains intake were adjusted (**Supplementary Table 8**). In the multivariate model where SBP, DBP, and antihypertensive drug treatment were controlled, HRs (95% CIs) for CVD and all-cause mortality risk

TABLE 1 | Characteristics of study population according to quartiles of urinary thiocyanate[a].

Characteristics	Total	Quartiles of urinary thiocyanate				*P*-value
		1 (lowest)	2	3	4 (highest)	
No. of participants	10,489	2,623	2,624	2,620	2,622	
Age, years	46.8 ± 0.3	47.6 ± 0.5	45.6 ± 0.5	46.1 ± 0.5	47.8 ± 0.4	<0.001
Men, *n* (%)	4,519 (43.4)	1,296 (47.9)	1,148 (43.9)	1,045 (41.8)	1,030 (41.3)	<0.001
Race/ethnicity, *n* (%)						<0.001
Non-Hispanic white	4,640 (68.7)	809 (54.1)	1,034 (63.6)	1,254 (72.1)	1,543 (79.8)	
Non-Hispanic black	1,917 (9.7)	547 (12.6)	507 (11.2)	448 (9.1)	415 (7.0)	
Mexican American	2,212 (9.6)	730 (14.9)	588 (11.1)	528 (8.7)	366 (5.5)	
Others	1,720 (12.0)	537 (18.4)	495 (14.0)	390 (10.0)	298 (7.6)	
Family poverty income ratio, *n* (%)						<0.001
<1.3	2,448 (15.0)	758 (19.2)	646 (17.6)	539 (12.9)	505 (11.8)	
1.3- < 3.5	3,670 (32.3)	970 (35.4)	918 (32.6)	896 (30.5)	886 (31.5)	
>3.5	3,614 (47.1)	700 (39.9)	863 (43.7)	999 (51.4)	1,052 (51.0)	
Education attainment, *n* (%)						<0.001
Under high school	2,580 (14.4)	894 (21.2)	646 (15.3)	552 (11.8)	488 (11.2)	
High school	2,166 (20.5)	526 (20.7)	543 (20.5)	512 (19.8)	585 (21.1)	
Above high school	5,737 (65.0)	1,198 (57.8)	1,435 (64.2)	1,555 (68.4)	1,549 (67.7)	
Smoking status[b], *n* (%)						0.10
Never	3,058 (30.7)	718 (29.4)	784 (30.6)	829 (33.0)	727 (29.6)	
Secondhand	7,431 (69.3)	1,905 (70.6)	1,840 (69.4)	1,791 (67.0)	1,895 (70.4)	
Alcohol consumption, *n* (%)						<0.001
Never	1,741 (13.3)	535 (17.5)	452 (14.8)	367 (11.3)	387 (11.0)	
Former	1,769 (14.0)	501 (16.0)	423 (13.9)	423 (13.5)	422 (13.2)	
Current	6,235 (66.4)	1,362 (58.6)	1,560 (64.8)	1,648 (69.0)	1,665 (70.6)	
Physical activity, *n* (%)						<0.001
Never	3,485 (26.3)	1,027 (31.7)	881 (27.7)	801 (24.3)	776 (23.1)	
Moderate	3,337 (33.0)	765 (30.0)	818 (33.1)	851 (33.2)	903 (34.9)	
Vigorous	3,583 (40.0)	800 (37.0)	904 (38.6)	948 (41.8)	931 (41.6)	
Hypertension, *n* (%)	3,437 (28.8)	952 (32.5)	800 (27.7)	790 (26.2)	895 (29.4)	0.003
BMI, kg/m^2	28.7 ± 0.1	28.4 ± 0.2	28.8 ± 0.2	28.5 ± 0.2	28.9 ± 0.2	0.007
Urinary creatinine, mg/dL	115.5 ± 1.0	148.3 ± 2.3	129.5 ± 1.9	109.2 ± 1.8	87.0 ± 1.3	<0.001
Urinary thiocyanate, μg/L	1282.7 ± 23.1	458.4 ± 9.4	877.5 ± 12.7	1261.0 ± 20.2	2208.6 ± 43.6	<0.001
Urinary iodine, μg/L	331.6 ± 94.9	222.7 ± 8.7	268.1 ± 28.4	244.5 ± 29.2	538.1 ± 320.4	0.47
Serum cotinine, ng/mL	0.14 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.31
Total energy intake, kcal	2133.7 ± 13.3	1995.2 ± 25.5	2121.0 ± 24.0	2179.8 ± 24.1	2199.2 ± 21.9	<0.001
HEI-2015 score	54.8 ± 0.2	54.3 ± 0.3	54.5 ± 0.3	55.0 ± 0.3	55.3 ± 0.4	0.04
Total fruits[c]	2.67 ± 0.03	2.83 ± 0.05	2.65 ± 0.05	2.67 ± 0.05	2.57 ± 0.05	<0.001
Total vegetables[c]	3.42 ± 0.02	3.27 ± 0.04	3.39 ± 0.03	3.45 ± 0.03	3.52 ± 0.03	<0.001
Total dairy[c]	5.50 ± 0.06	5.15 ± 0.08	5.40 ± 0.10	5.56 ± 0.09	5.75 ± 0.08	<0.001
Whole grains[c]	2.77 ± 0.05	2.56 ± 0.08	2.68 ± 0.08	2.86 ± 0.08	2.92 ± 0.09	<0.001
Dietary fiber, g	17.2 ± 0.2	16.3 ± 0.3	16.9 ± 0.3	17.6 ± 0.3	17.8 ± 0.3	<0.001
β-carotene, mg	2201.6 ± 58.8	1872.1 ± 88.9	2132.7 ± 95.3	2267.3 ± 126.2	2428.4 ± 114.1	<0.001
Folate, μg	420.1 ± 3.8	394.2 ± 6.8	412.7 ± 7.7	429.9 ± 5.8	435.3 ± 5.9	<0.001
Vitamin C, mg	90.1 ± 1.4	89.3 ± 2.3	89.6 ± 2.6	92.9 ± 2.3	88.6 ± 2.0	0.32
Vitamin K, mg	107.9 ± 2.4	94.5 ± 4.2	104.4 ± 3.6	107.7 ± 3.8	120.4 ± 4.4	<0.001

[a]*Continuous variables were expressed as weighted means and standard errors and categorical variables were expressed as numbers and weighted percentages. The sums of percentages may not reach 100%, owing to the rounding of decimals and missing values. Baseline characteristics across thiocyanate quartiles were compared with linear regression for continuous variables and logistic regression for categorical variables.*

[b]*Participants with serum cotinine ≤ 0.015 mg/dL and 0.015- < 3 mg/dL were considered as never and secondhand smokers, respectively.*

[c]*Total vegetables, total fruits, total dairy, and whole grains intake were estimated with HEI-2015 total vegetables, total fruits, total dairy, and whole grains components, respectively.*

BMI, body mass index; HEI, Health Eating Index.

comparing extreme thiocyanate quartiles were 0.52 (0.30–0.91) and 0.75 (0.59–0.95), respectively (**Supplementary Table 9**). Similar results were observed after missing values of covariates were handled with multiple imputation (**Supplementary Table 10**).

DISCUSSION

In this prospective cohort of non-smoking adults, we observed inverse associations of urinary thiocyanate with CVD and total mortality. Comparing extreme quartiles, the

TABLE 2 | Associations between urinary thiocyanate and cardiovascular disease and all-cause mortality among non-smoking adults.

	Quartiles of urinary thiocyanate				Continuous[c]	*P*-value for trend
	1 (*n* = 2,623)	2 (*n* = 2,624)	3 (*n* = 2,620)	4 (*n* = 2,622)		
Range, mg/g	≤0.50	0.50–0.89	0.89–1.51	>1.51		
CVD mortality						
No. of death	46	28	37	25		
Model 1[a]	1.00 (reference)	0.71 (0.42–1.22)	0.89 (0.57–1.40)	0.51 (0.30–.57)	0.77 (0.64–0.92)	0.02
Model 2[b]	1.00 (reference)	0.68 (0.40–1.15)	0.89 (0.56–1.43)	0.50 (0.29–0.85)	0.75 (0.62–0.91)	0.02
All-cause mortality						
No. of death	262	166	187	185		
Model 1[a]	1.00 (reference)	0.78 (0.61–0.99)	0.83 (0.68–1.02)	0.70 (0.57–0.86)	0.86 (0.80–0.94)	0.001
Model 2[b]	1.00 (reference)	0.77 (0.61–0.97)	0.86 (0.70–1.05)	0.75 (0.60–.92)	0.88 (0.81–0.96)	0.009

[a]*Model 1: adjusted for age (years, continuous), sex (male, female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, others), secondhand smoking (yes, no).*

[b]*Model 2: further adjusted for body mass index (< 25, 25- < 30, ≥ 30 kg/m^2), education attainment (under high school, high school, above high school), family poverty income ratio (< 1.3, 1.3- < 3.5, ≥ 3.5), alcohol consumption (never, former, current), physical activity (never, moderate, vigorous), total energy intake (kcal, continuous), Healthy Eating Index-2015 score (continuous), urinary iodine (< 100 μg/L, ≥ 100 μg/L), and hypertension (yes, no).*

[c]*Per 1 μg/g creatinine increment in log-transformed urinary thiocyanate.*

CVD, cardiovascular disease; HR, hazard ratio.

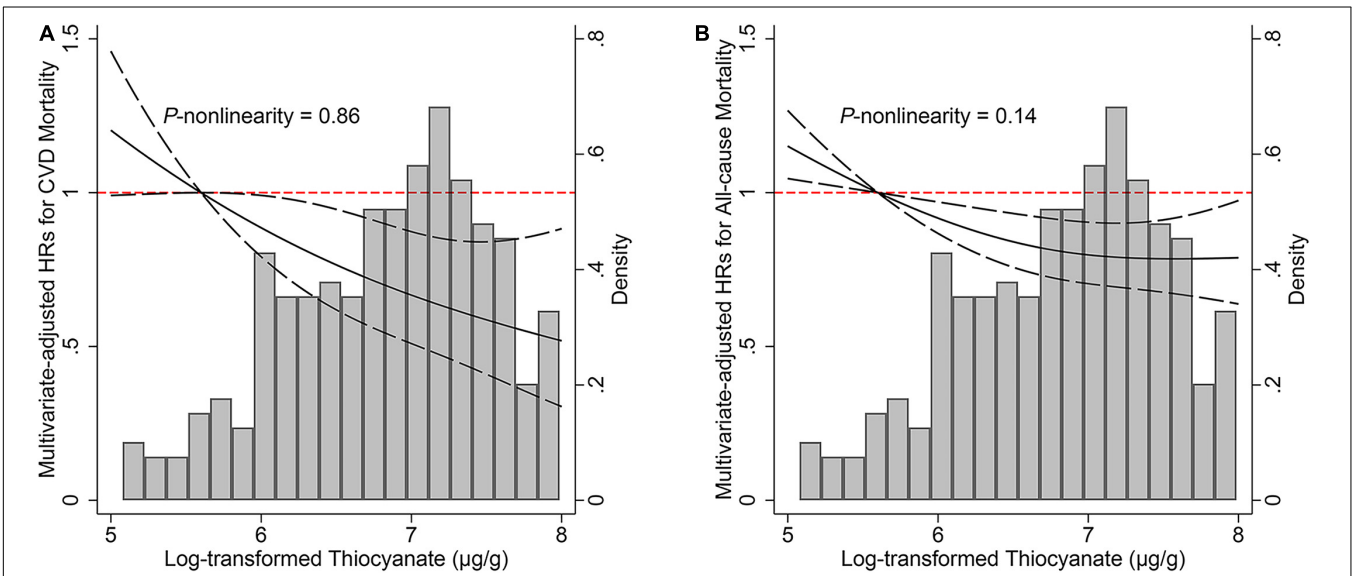

FIGURE 1 | Associations of urinary thiocyanate levels with **(A)** cardiovascular disease and **(B)** all-cause mortality among non-smokers. Hazard ratio was represented by solid line and 95% confidence intervals were represented by dashes. Model was adjusted for age (years, continuous), sex (male, female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, others), secondhand smoking (yes, no), body mass index (< 25, 25- < 30, ≥ 30 kg/m^2), education attainment (under high school, high school, above high school), family poverty income ratio (< 1.3, 1.3- < 3.5, ≥ 3.5), alcohol consumption (never, former, current), physical activity (never, moderate, vigorous), total energy intake (kcal, continuous), Healthy Eating Index-2015 score (continuous), urinary iodine (< 100 μg/L, ≥ 100 μg/L), and hypertension (yes, no). CVD, cardiovascular disease; HR, hazard ratio.

multivariate-adjusted HRs for CVD and all-cause mortality were 0.50 (95% CI: 0.29–0.85) and 0.75 (95% CI: 0.60–0.92), respectively. Moreover, each 1 μg/g creatinine increment of log-transformed urinary thiocyanate was associated with a 25% (HR: 0.75; 95% CI: 0.62–0.91) reduced CVD mortality risk and 12% (HR: 0.88; 95% CI: 0.81–0.96) lower all-cause mortality risk.

To our knowledge, this cohort represents the first biomarker-based study to examine the associations of CV intake with CVD and all-cause mortality. Our findings were consistent with emerging studies that suggested the cardiovascular benefits of CVs (5, 7, 33, 34). An inverse association between CV intake and incident ischemic stroke was observed in a pooled analysis of two prospective cohort studies, which comprised 75,596 females in the NHS and 38,683 males in the HPFS (34). Zhang et al. (7) reported that higher CV intake was associated with a lower risk of mortality from CVD in two large Chinese prospective cohorts. Moreover, two recent meta-analyses of prospective studies provided the evidence of inverse association between CV intake and CVD (5, 35).

Estimating CV intake with quantitative biomarkers has received increasing attention (36, 37). Urinary isothiocyanate, another decomposed product of glucosinolates, had been applied in previous studies (36–38). A positive correlation between urinary isothiocyanate and self-reported CV intake (r_s = 0.1149, $P < 0.0001$) was observed in 3,589 females and 1,015 males from Shanghai (37). It should be noted that nearly half of isothiocyanates were eliminated after 2–4 h of CVs administration (38). The reproducibility, namely the correlation between samplings within the same individual on independent occasions (39), of a biomarker is determined by its half-life and the stability of individual intake of certain food/nutrient (39). Hence, the reproducibility of isothiocyanate might be poor in populations where CVs are less frequently or stably consumed. Compared with isothiocyanates, thiocyanates have a half-life of 3 days in healthy individuals (8). However, the validity of the urinary thiocyanate biomarker was questionable among smokers, and we observed no significant correlation between urinary thiocyanate and CV intake in smoking adults. In the current study, smokers had much higher urinary thiocyanate measurements than non-smokers in US adults (data not shown), consistent with previous studies (11, 12). A cross-sectional sample of 2027 females from NHANES 2003–2008 suggested that urinary thiocyanate levels among smokers were approximately five times higher than among non-smokers (11). After controlling environmental tobacco smoke, urinary thiocyanate was positively correlated with self-reported CV intake (r_s = 0.086, $P < 0.001$) among those with serum cotinine < 3 ng/mL in this study.

The underlying mechanisms of the documented inverse associations remain unclear, however, there are several possible explanations. First, CVs are important sources of dietary fiber, vitamins, and various phytochemicals, such as flavonoids (40) and sulforaphane (41), and these components are likely to act synergistically to enhance the cardiovascular benefits. There was convincing evidence of inverse association between dietary fiber and CVD mortality (42). A pooled analysis of 21 prospective studies provided evidence of inverse association between dietary vitamin K consumption and risk of coronary heart disease (43). A meta-analysis of 11 prospective cohort studies suggested that higher dietary flavonoid intake was associated with a lower risk of stroke (44). Moreover, sulforaphane had been suggested to protect against CVD due to its antioxidant and anti-inflammatory properties (45). Second, thiocyanate had been validated to play an important role in the host defense and protect cells against hypochlorous acid (HOCl), a powerful oxidant (8, 46). Hypothiocyanous acid, a product of hydrogen peroxide and thiocyanate catalyzed by peroxidases, is a potent antimicrobial agent and has the capability to cross the bacterial cell wall and inhibit the activity of glycolytic enzymes and urease (8, 46). Moreover, a previous *in vitro* study found that thiocyanate could exert influence on the extent and nature of HOCl-induced macrophage damage, and reported a protective effect of thiocyanate intervention on the development of chronic inflammation (47). Third, higher CV intake had been suggested to be associated with a lower risk of diabetes (48), a major risk factor for CVD (1).

Strengths of our study included the large sample of non-smoking adults and prospective and biomarker-based study design. Compared with interviews or self-reported FFQs, the quantitative biomarker could minimize the measurement error and misclassification introduced by biased recall, limited food items in questionnaires, and inaccurate portion-size estimation. Moreover, variability in glucosinolates contents across *Brassica* species, storage conditions, and preparing methods was allowed for with the measure of internalized exposure to *Brassica* thiocyanates.

Limitations of this prospective cohort study should be also acknowledged. First, we limited our analyses to those that were eligible for mortality linkage. Hence, internal validity of estimates derived from this cohort study is threatened due to follow-up bias (49). Second, urinary thiocyanate was measured with spot urine specimens at baseline. Hence, measurement error and potential misclassification might arouse due to variable hydration of participants and temporal variability. Single measurement of urinary thiocyanate might not reflect long-term exposure due to within-individual variability. Moreover, reproducibility of the urinary thiocyanate biomarker could not be examined with single measurements. Nonetheless, it was burdensome to collect repeated 24-h urine samples in large-scale surveys, and we performed conventional creatinine standardization as well as covariate adjustment to adjust for urine dilution and observed consistent results. Further longitudinal studies with repeated measurements are expected to validate the reproducibility of the biomarker and replicate our results. Third, although total CV intake was obtained from the FFQ developed from the validated DHQ, the FFQ only included two commonly consumed *Brassica* species. A previous cross-sectional study conducted in Korea, where residents consume large amounts of CVs, revealed that daily intake of thiocyanate through CVs varied across species (50). Moreover, portion size information was not collected with the FFQ, hence, we failed to obtain a more accurate estimate of CV intake. Hence, further validation studies with species and portion-size considered are needed. Fourth, low levels of thiocyanate could also be found in milk and cassava (10, 51, 52), hence, urinary thiocyanate is not perfectly specific for CVs in this non-smoking population. However, the inverse associations were not significantly altered by adjusting the total dairy adequacy component. Moreover, considering previously reported no significant associations of starchy vegetable intake with CVD and total mortality (53), the observed inverse associations in the current study may be attenuated. Finally, our results should be interpreted with caution due to residual confounding. Although we have adjusted total energy intake in multivariate models, consistent with the isocaloric diet/disease relationship of greatest interest, the standard multivariate model failed to completely adjust for confounding from common causes of dietary intake and composition (54). In addition, there are numerous nutrients and non-nutrients in foods due to the complexity of human diet, and residual confounding of other dietary factors could not be eliminated, although we have adjusted some nutrients and foods intake in multivariate models and observed unaltered results.

CONCLUSION

This prospective cohort study suggested that higher levels of urinary thiocyanate, a biomarker of CV intake, were associated with lower risks of CVD and all-cause mortality among non-smoking adults. Our findings supported recommendations to increase CV consumption to promote cardiovascular health, and further studies are warranted to validate our results.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. These data can be found here: https://www.cdc.gov/nchs/nhanes/index.htm.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the NCHS Research Ethics Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

QW contributed to the conception and design of the study and drafted the manuscript. LK contributed to the analysis and interpretation of data. PW, GJ, and CD contributed to the eacquisition and interpretation of data. JY and ZS contributed to the study design. YH, JY, ZS, JX, and LL critically revised the manuscript for important intellectual content. LL was the guarantor of the work and had full access to the data underlying the article. All authors gave approval to the final manuscript and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.919484/full#supplementary-material

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Healthy Eating Index-2015 and Predicted 10-Year Cardiovascular Disease Risk, as Well as Heart Age

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Background and Aims: Dietary factor plays an important role in the prevention of cardiovascular disease (CVD). The healthy eating index-2015 (HEI-2015), an indicator of the overall dietary quality, has been introduced to reflect adherence to the 2015–2020 Dietary Guidelines for Americans (DGA). This study aims to explore the associations of the HEI-2015 with predicted 10-year CVD risk and heart age among United States adults aged 30–74 years old using data from the National Health and Nutrition Examination Survey (NHANES) 2011–2014.

Methods and Results: We conducted a cross-sectional analysis among 6,614 participants aged 30–74 years old. The HEI-2015 scores were calculated from 2-days 24-h dietary recall interviews. The 10-year CVD risk and heart age were derived from the sex-specific Framingham general cardiovascular disease risk score. We defined high cardiovascular disease risk as a predicted 10-year cardiovascular disease risk of > 20%. Multiple linear regression and binary logistic regression models were used to investigate the associations of the HEI-2015 with predicted 10-year CVD risk and heart age. Compared with participants in the lowest HEI-2015 quartile, those in the highest quartile had lower predicted 10-year CVD risk (β = −2.37, 95% CI: −3.09 to −1.65, $P < 0.0001$), lower heart age (β = −2.63, 95% CI: −3.29 to −1.96, $P < 0.0001$) and lower odds for high risk of CVD (OR = 0.62, 95% CI: 0.49 to 0.80, P-trend < 0.0001) after adjusting for multiple covariates.

Conclusion: Higher adherence to the 2015–2020 Dietary Guidelines for Americans is associated with lower predicted 10-year cardiovascular disease risk and lower heart age among United States adults.

Keywords: HEI-2015, 10-year CVD risk, heart age, cross-sectional study, NHANES

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Abbreviations: CVD, cardiovascular disease; HEI, healthy eating index; DGA, Dietary Guidelines for Americans; NHANES, National Health and Nutrition Examination Survey; CHD, coronary heart disease; AUC, Area Under Curve; US, United States; NCHS, National Center for Health Statistics; CDC, Centers for Disease Control and Prevention; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; HbA1c, glycated hemoglobin A1c; WWEIA, What We Eat in America; USDA, United States Department of Agriculture; DHHS, United States Department of Health and Human Services; AMPM, Automated Multiple-Pass Method; MEC, Mobile Examination Center; TC, total cholesterol; HHS, Department of Health and Human Services; BMI, body mass index; SE, standard error; OR, odds ratio; CI, confidence interval; SFA, saturated fatty acid; MedDiet, Mediterranean diet; DASH, Dietary Approach to Stop Hypertension; USFA, unsaturated fatty acid; NHS, Nurses' Health Study; HPFS, Health Professionals Follow-up Study; MEC, Multiethnic Cohort; HDI, Healthy Diet Indicator; GDR, Global Dietary Recommendations; AHEI, Alternative Healthy Eating Index; WHO, World Health Organization.

INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of death in the United States in 2018, with coronary heart disease (CHD) leading the list (42.1%), followed by stroke (17%), hypertension (11%), heart failure (9.6%), and arterial disease (2.9%) (1). Age, sex, high blood pressure, smoking, high cholesterol, and diabetes are recognized as risk factors for CVD (1–4). To help with the primary prevention of persons at high CVD risk, D'Agostino et al. developed a model based on the Framingham Heart Study to assess a person's absolute risk of developing CVD in the next 10 years. This general CVD risk prediction model was well-discriminative and calibrated, with a C statistic ranging from 0.76 to 0.79. The C statistic is similar to the area under the receiver operating characteristic curve (AUC) and it is usually used to reflect the predictive value for a prediction model (5). The predicted 10-year CVD risk score is useful for clinicians to provide treatment recommendations to patients with CVD and for patients to conduct self-cardiac assessments based on this model (6). As a new concept derived from the Framingham risk score, heart age was defined as the chronological age of a person with the same CVD risk score but other risk factors at the normal level (5). Compared with 10-year CVD risk, heart age is more likely to prompt and motivate people to understand CVD risk factors and improve life behaviors to arouse emotional resonance (7, 8).

According to the global burden of disease study, the health care cost of CVD continues to rise in recent years (9, 10). The direct cost of CVD in the United States was estimated to be as high as $216 billion in 2016–2017, while cancer costs only $105.6 billion (11). Improving unhealthy dietary behaviors provides crucial new insights into reducing the risk of CVD and alleviating the healthcare burden caused by CVD (12, 13). The Healthy Eating Index (HEI) is updated every 5 years as a comprehensive measure of diet quality. HEI-2015, the latest version of the HEI, was developed to evaluate adherence to the 2015–2020 Dietary Guidelines for Americans (DGA) (14). To date, few studies have investigated the association between the HEI-2015 score and CVD risk in the general population (15–17). In this study, we provide the most recent estimates of the associations between HEI-2015 and predicted 10-year CVD risk, as well as heart age, in a representative United States population aged 30–74 years old, based on combined data from the 2011–2012 and 2013–2014 National Health and Nutrition Examination Surveys (NHANES).

MATERIALS AND METHODS

Study Population

National Health and Nutrition Examination Surveys is a nationally representative cross-sectional survey conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC) (18). The Framingham general CVD risk score was recommended for use in the 30–74 age range (5). Of the participants who completed the interview (19,931 in the NHANES 2011–2014), we included 8,240 participants aged 30–74 in this study. We further excluded participants with missing HEI-2015 data (n = 1,043), missing data for variables for the construction of 10-year CVD risk score [high-density lipoprotein cholesterol (HDL-C), systolic blood pressure (SBP), and glycated hemoglobin A1c (HbA1c)] (n = 508), and missing data on covariate (n = 75). A total of 6,614 participants were included in the final analysis. A flow chart of the sample selection is shown in **Figure 1**.

Healthy Eating Index-2015 Assessment

The dietary interview component of the National Health and Nutrition Examination Survey was collected for What We Eat in America (WWEIA). WWEIA is conducted as a partnership between the United States Department of Agriculture (USDA) and the United States Department of Health and Human Services (DHHS). DHHS is responsible for the survey sample design and data collection, while USDA is responsible for the dietary data collection methodology, maintenance of the databases used to code and process the data, and data review and processing. The USDA Automated Multiple-Pass Method (AMPM) is used for collecting 24-h dietary recalls in WWEIA (19). The first day was conducted face-to-face in the dietary interview room of the Mobile Examination Center (MEC) and the second day was collected *via* telephone 3–10 days later. The food group of the diet calculation was determined by the USDA Food Patterns Equivalence Database, and energy or nutrient content was determined by the USDA Food and Nutrient Database for Dietary Studies.

The calculation of the HEI-2015 score was not based on the absolute amount of ingredients but was based on the energy density per 1,000 kcal (except fatty acids). Fatty acids were scored as unsaturated fatty acids divided by saturated fatty acids. The index with a total score ranging from 0 to 100 consists of 13 components: total fruits, whole fruits, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant proteins, fatty acids (nine recommended components to include in a healthy diet) and refined grains, sodium, added sugars, and saturated fats (four components that should be consumed sparingly). Each component is scored separately and added together to obtain the HEI-2015 total score. Adequacy components intake is proportional to score, while the moderation components are the opposite.

Estimation of the 10-Year Cardiovascular Disease Risk and Heart Age

The endpoint used in the Framingham Heart Study for predicting CVD risk was a composite outcome including CHD (coronary death, myocardial infarction, coronary insufficiency, and angina), cerebrovascular events (including ischemic stroke, hemorrhagic stroke, and transient ischemic attack), peripheral artery disease (intermittent claudication), and heart failure (20). The sex-specific Framingham general CVD risk score is based on age, sex, total cholesterol (TC), HDL-C, SBP, hypertension treatment or not, diabetes mellitus, and smoking status (5). Regarding the CVD risk, the population was further stratified into two risk categories: low (predicted 10-year CVD risk score of $\leq$ 20%) and high (predicted 10-year CVD risk score of $>$ 20%) (5).

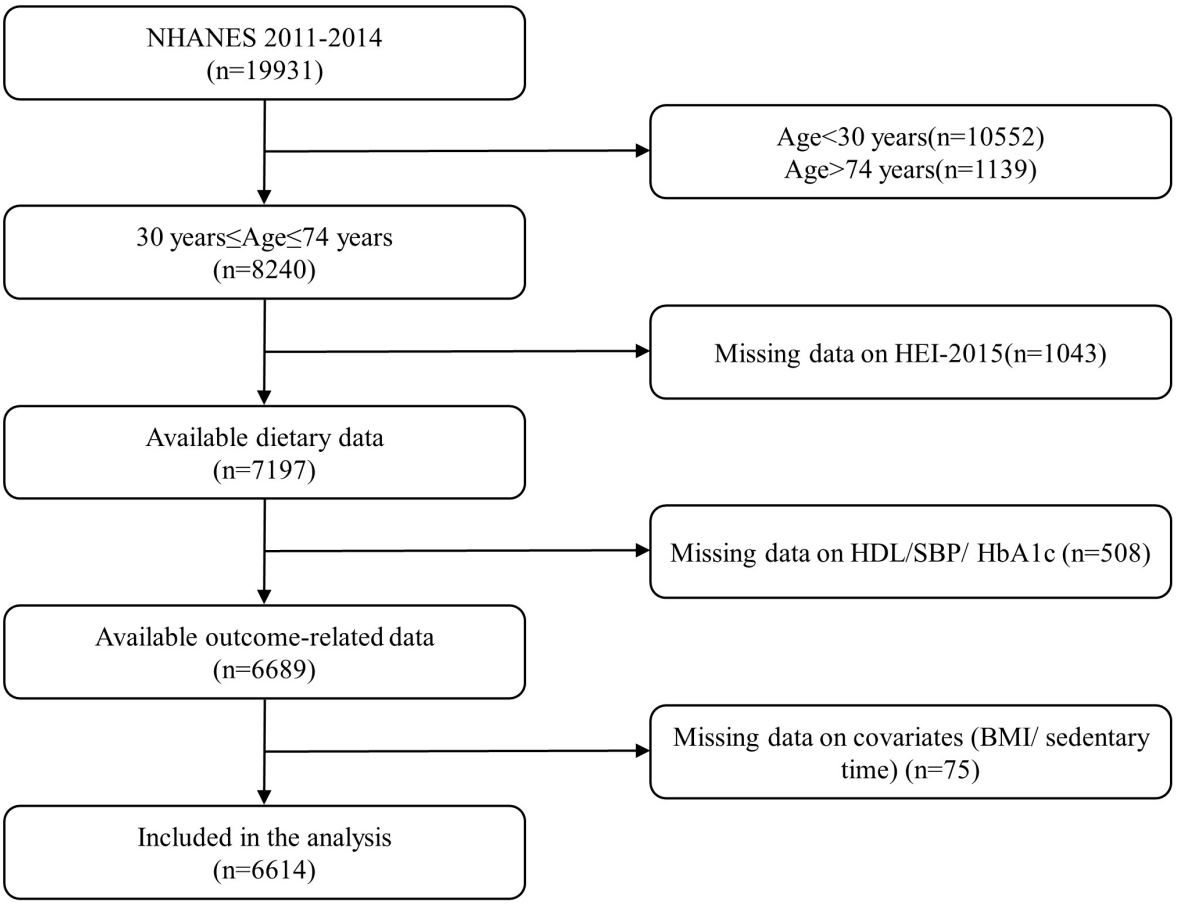

FIGURE 1 | Flow diagram of the study participant selection. NHANES, National Health and Nutrition Examination Survey; HEI-2015, Healthy Eating Index-2015; HDL, high-density lipoprotein; SBP, systolic blood pressure; HbA1c, glycated hemoglobin A1c; BMI, body mass index.

Meanwhile, heart age, defined as the age of a person with the same predicted risk of CVD but with all other risk factors at the normal level, was also estimated using the sex-specific-based Framingham general CVD risk score, and the risk factors used in the calculation were fully consistent with the predicted 10-year CVD risk. Briefly, we first calculated the CVD points for men and women based on the CVD risk factors mentioned above. Then, the predicted heart age was determined by converting the CVD points according to the "heart age" sheet published previously (5).

Definition of Covariates

Sociodemographic, lifestyle factors, and physical examination data were obtained through household questionnaires by bilingual trained study staff. The self-reported demographic covariates included sex (male or female), educational level (<high school, high school, or > high school), ethnicity (Hispanic, non-Hispanic White, non-Hispanic Black, or others), and family economic situation (income-to-poverty ratio ≤ 1.30, 1.31–1.85, and > 1.85). The family monthly poverty level index (income-to-poverty ratio) represents household income as a ratio of total family income to the poverty level defined by the Department of Health and Human Services (DHHS).

As for lifestyle factors, smokers were defined as those who had smoked at least 100 cigarettes in life. Drinkers were defined as participants who consumed alcohol 12 or more times in any given year. Sedentary time is an indicator of sedentary behavior refers to the amount of time spent sitting during the day other than sleep.

All anthropometric data were measured by highly trained medical personnel to minimize errors in the measurement process. SBP and diastolic blood pressure were the averages of three consecutive readings of measurements in a sitting position after a 5-min quiet rest. A fourth blood pressure measurement was taken if one reading was incomplete. Standing height was measured by a stadiometer and body weight was measured by a digital weight scale requiring participants to wear a standard examination gown and no jewelry. Body mass index (BMI) (kg/m^2) was defined as weight (kg) divided by height squares (m^2).

Diabetes was diagnosed as HbA1c ≥ 6.5% or currently using insulin or diabetes medications. Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 was used to determine the content of % HbA1c in whole blood (21). The analysis of serum HDL-C and serum TC was carried out by Roche/Hitachi Modular P Chemistry Analyzer, for measurement of HDL-C

by a magnesium/dextran sulfate method and serum TC by a completely enzymatic method (22, 23).

Statistical Analysis

Considering the complex sampling design and multi-year data of NHANES 2011–2014, the sample weights for both cycles were used for statistical analysis. The results are presented as weighted mean ± SE for continuous variables and weighted percentages for categorical variables. To compare characteristics by HEI-2015 quartile groups, we conducted weighted one-way analyses of variance for continuous variables and weighted chi-square tests for categorical variables. The associations of HEI-2015 with 10-year CVD risk and heart age were estimated using multivariable linear regression models according to quartiles of the HEI-2015, in which the lowest quartile was used as the reference category. The binary logistic regression models were also used to determine the odds ratio (OR) and 95% confidence interval (CI) for the high predicted 10-year risk of CVD, with adjusting for potential confounders. We also used the HEI-2015 score as a continuous variable in the analysis. Model 1 adjusted for age and sex. Model 2 additionally adjusted for ethnicity, drinking status, education level, family monthly poverty level, and sedentary time. In Model 3, we adjusted for all the covariates in Model 2 plus BMI as a continuous variable. To test the overall trend, we used the median of HEI-2015 in each quartile as a continuous variable in the regression model. In addition, the interactions between HEI-2015 and various covariates have also been tested, and stratified analyses were performed. Statistical significance for all analyses was two-tailed $P < 0.05$. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, United States).

RESULTS

Characteristics of Study Participants

We identified 6,614 participants with sufficient information to predict the 10-year CVD risk (3,211 men and 3,403 women). The general characteristics of the participants are shown in **Table 1**. Participants with higher HEI-2015 scores were more likely to be older, female, more likely to have higher educational level and family monthly poverty level index, and less likely to be a drinker. In contrast, weighted mean BMI was higher among participants who had a lower HEI-2015 score. No statistically significant differences were found between groups for the variable of sedentary time.

The Association of the HEI-2015 Score With Predicted 10-Year Cardiovascular Disease Risk and Heart Age

Results of multivariable linear regression analyses for the association of HEI-2015 score with 10-year CVD risk and heart age are shown in **Table 2**. There were significant negative associations of HEI-2015 with 10-year CVD risk and heart age after adjusting for age, sex, ethnicity, drinker, education level, family monthly poverty level, sedentary time, and BMI. The corresponding regression coefficients of the highest quartile group of the HEI-2015 score were −2.05 (95% CI: −2.76 to −1.33, $P < 0.0001$) for 10-year CVD risk and −2.19 (95% CI: −2.85 to −1.53, $P < 0.0001$) for heart age, with the lowest quartile group as the reference. In addition, linear trends were also observed in the associations of HEI-2015 with 10-year CVD risk (P-trend<0.05) and heart age (P-trend<0.05).

The Association of HEI-2015 With High 10-Year Cardiovascular Disease Risk Among United States Adults Aged 30–74 Years Old

The ORs and 95% CIs for high 10-year CVD risk (predicted 10-year risk > 20%) are presented in **Table 3**. In the age and sex-adjusted model (model 1), participants who had the highest quartile of HEI-2015 score had lower odds of high 10-year CVD risk compared to those who had the lowest quartile of HEI-2015 score (OR: 0.51; 95%CI: 0.40–0.64; P-trend<0.0001). The significant association remained when further it is adjusted for ethnicity, drinking status, education level, family monthly poverty level, sedentary time, and BMI, and the corresponding OR (95% CI) was 0.62 (0.49–0.80). In addition, the interactions between other covariates and HEI-2015 scores, except for BMI (P for interaction = 0.0335), were not significant (**Figure 2**). The variables adjusted for subgroup analysis in **Figure 2** were consistent with Model 3 in **Table 3**, except for the subgroup variables that were not included in the model. A subgroup analysis according to normal or wasting (BMI < 25 kg/m^2), overweight (25 ≤ BMI < 30 kg/m^2), and obese (BMI ≥ 30 kg/m^2) showed that participants with normal BMI and the highest HEI-2015 score had 61% lower odds of high 10-year CVD risk (OR = 0.39, 95% CI: 0.22–0.69) compared with those with the lowest HEI-2015 score.

DISCUSSION

In this cross-sectional study, we found that a higher HEI-2015 score was associated with a significantly lower predicted 10-year CVD risk and lower heart age in United States general population aged 30–74 years old.

Numerous studies have shown that some foods and dietary ingredients were associated with the risk of CVD (24–27), and those studies usually draw inconsistent conclusions. For example, several studies found that saturated fatty acid (SFA) intake was positively associated with cardiovascular events (28–30), but a recent review reported that there is a lack of association between SFA consumption and non-communicable diseases (31). Although the research on the effect of a single food or nutrient on CVD risk has extensive and profound significance, people's daily diet is a mixture of multiple foods and nutrients. In recent years, the impact of complete dietary patterns on CVD has aroused widespread interest, such as the Mediterranean diet (MedDiet), the Dietary Approach to Stop Hypertension (DASH) diet, the vegetarian dietary pattern, etc. The Mediterranean diet, which is rich in whole grains, vegetables, fruits, and olive oil (replace SFA with USFA), etc., has strong relevance for reducing the incidence

TABLE 1 | Characteristics of study participants according to HEI-2015 quartile (weighted analysis).[1]

Characteristic	Quartile of HEI-2015				*P*-value[2]
	Q1 (19.0–44.8)	Q2 (44.8–54.4)	Q3 (54.4–64.4)	Q4 (64.4–95.8)	
N	1,653	1,654	1,654	1,653	
Age (years)	47.5 ± 0.3	50.0 ± 0.3	50.5 ± 0.3	52.9 ± 0.3	<0.0001
Sex, n (%)					<0.0001
Male	889 (54.4)	840 (52.7)	789 (47.3)	693 (40.5)	
Female	764 (45.6)	814 (47.3)	865 (52.7)	960 (59.5)	
Ethnic, n (%)					<0.0001
Hispanic	306 (12.5)	367 (14.0)	413 (14.8)	382 (12.5)	
Non-Hispanic white	778 (69.7)	665 (68.4)	628 (67.9)	605 (70.0)	
Non-Hispanic black	431 (12.8)	415 (11.5)	376 (10.4)	295 (7.5)	
Other races	138 (5.1)	207 (6.2)	237 (6.9)	371 (9.9)	
Education, n (%)					<0.0001
<High school	434 (20.2)	371 (15.2)	361 (14.9)	253 (8.9)	
High school	428 (25.8)	415 (24.7)	315 (16.3)	262 (14.0)	
>High school	791 (54.0)	868 (60.1)	978 (68.8)	1,138 (77.1)	
Family monthly poverty level category, n (%)					<0.0001
≤1.30	720 (31.9)	580 (24.9)	510 (20.1)	371 (14.5)	
1.31–1.85	274 (14.5)	253 (11.9)	285 (14.1)	252 (10.6)	
>1.85	659 (53.6)	821 (63.2)	859 (65.8)	1030 (74.9)	
Drinker, n (%)					<0.0001
Yes	1,214 (77.7)	1,172 (77.4)	1,148 (77.1)	1,103 (75.2)	
No	439 (22.3)	482 (22.6)	506 (22.9)	550 (24.9)	
BMI (kg/m^2)	30.4 ± 0.2	30.4 ± 0.2	29.3 ± 0.2	27.9 ± 0.1	<0.0001
Sedentary time (h/day)	6.6 ± 0.1	6.7 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	0.3891

[1]Values are presented as weighted mean ± SE or number (weighted %); HEI-2015, healthy eating index-2015; BMI, body mass index; Q, quartile; Q1 refer to the unhealthiest diet quality; Q4 refer to the healthiest diet quality. [2]Weighting factors were used in calculating P-values to account for the complex survey design of NHANES. One-way analyses of variance were used for continuous variables and the chi-square test was used for categorical variables.

TABLE 2 | Regression coefficients and 95% confidence intervals of HEI-2015 for 10-year CVD risk and heart age.[1]

HEI-2015	Predicted 10-year CVD risk			Heart age		
	Unstandardized coefficients B (95%CI)	*P*-value	*P* trend[5]	Unstandardized coefficients B (95%CI)	*P*-value	*P* trend
Model1[2]						
HEI-2015[6]	−0.09 (−0.10, −0.07)	<0.0001		−0.10 (−0.11, −0.08)	<0.0001	
Q1	0 (reference)	–	<0.0001	0 (reference)	–	<0.0001
Q2	−0.44 (−1.14, 0.25)	0.2110		−0.85 (−1.49, −0.21)	0.0096	
Q3	−1.46 (−2.15, −0.76)	<0.0001		−1.76 (−2.41, −1.12)	<0.0001	
Q4	−3.13 (−3.83, −2.43)	<0.0001		−3.48 (−4.14, −2.83)	<0.0001	
Model2[3]						
HEI-2015	−0.07 (−0.08, −0.05)	<0.0001		−0.07 (−0.09, −0.06)	<0.0001	
Q1	0 (reference)	–	<0.0001	0 (reference)	–	<0.0001
Q2	−0.18 (−0.87, 0.51)	0.6160		−0.57 (−1.21, 0.07)	0.0791	
Q3	−1.08 (−1.78, −0.38)	0.0023		−1.31 (−1.95, −0.66)	<0.0001	
Q4	−2.37 (−3.09, −1.65)	<0.0001		−2.63 (−3.29, −1.96)	<0.0001	
Model3[4]						
HEI-2015	−0.06 (−0.07, −0.04)	<0.0001		−0.06 (−0.08, −0.04)	<0.0001	
Q1	0 (reference)	–	<0.0001	0 (reference)	–	<0.0001
Q2	−0.15 (−0.83, 0.53)	0.6664		−0.54 (−1.17, 0.09)	0.0934	
Q3	−0.92 (−1.61, −0.23)	0.0093		−1.09 (−1.72, −0.45)	0.0008	
Q4	−2.05 (−2.76, −1.33)	<0.0001		−2.19 (−2.85, −1.53)	<0.0001	

[1]HEI-2015, healthy eating index-2015; Q, quartile; Q1 refers to the unhealthiest diet quality; Q4 refers to the healthiest diet quality; B, unstandardized regression coefficient; CI, confidence interval. Unstandardized coefficients B and 95% CI from Multivariable linear regression models with adjustment as follows: [2]Model 1 adjusted for age, and sex. [3]Model 2 adjusted for variables in model1 + ethnicity, drinker, education level, family monthly poverty level, and sedentary time. [4]Model 3 adjusted for variables in model 2 + BMI. [5]P trend, Test for trend was a sequential test of the quartiles of dietary quality scores. [6]HEI-2015 as a continuous variable.

TABLE 3 | Odds ratios (OR) and 95% confidence intervals (CI) of HEI-2015 for high predicted 10-year CVD risk.[1]

	HEI-2015					*P* trend[5]
	Continuous[6]	Q1	Q2	Q3	Q4	
Total	6,614	1,653	1,654	1,654	1,653	
No of participants at high risk (>20%)	1,474	362	412	356	344	
Model 1[2]	0.98 (0.97, 0.99)	1 (reference)	1.08 (0.86, 1.36)	0.69 (0.54, 0.86)	0.51 (0.40, 0.64)	<0.0001
Model 2[3]	0.984 (0.98, 0.99)	1 (reference)	1.14 (0.91, 1.44)	0.73 (0.57, 0.92)	0.59 (0.47, 0.76)	<0.0001
Model 3[4]	0.986 (0.98, 0.99)	1 (reference)	1.13 (0.89, 1.42)	0.75 (0.59, 0.95)	0.62 (0.49, 0.80)	<0.0001

[1]HEI-2015, healthy eating index-2015; Q, quartile; Q1 refer to the unhealthiest diet quality; Q4 refer to the healthiest diet quality. OR and 95% CI from a binary logistic regression model with adjustment as follows: [2]Model 1 adjusted for age, and sex. [3]Model 2 adjusted for variables in model 1 + ethnicity, drinker, education level, family monthly poverty level, and sedentary time. [4]Model 3 adjusted for variables in model 2 + BMI. [5]P trend, Test for trend was a sequential test of the quartiles of dietary quality scores. [6] HEI-2015 as a continuous variable.

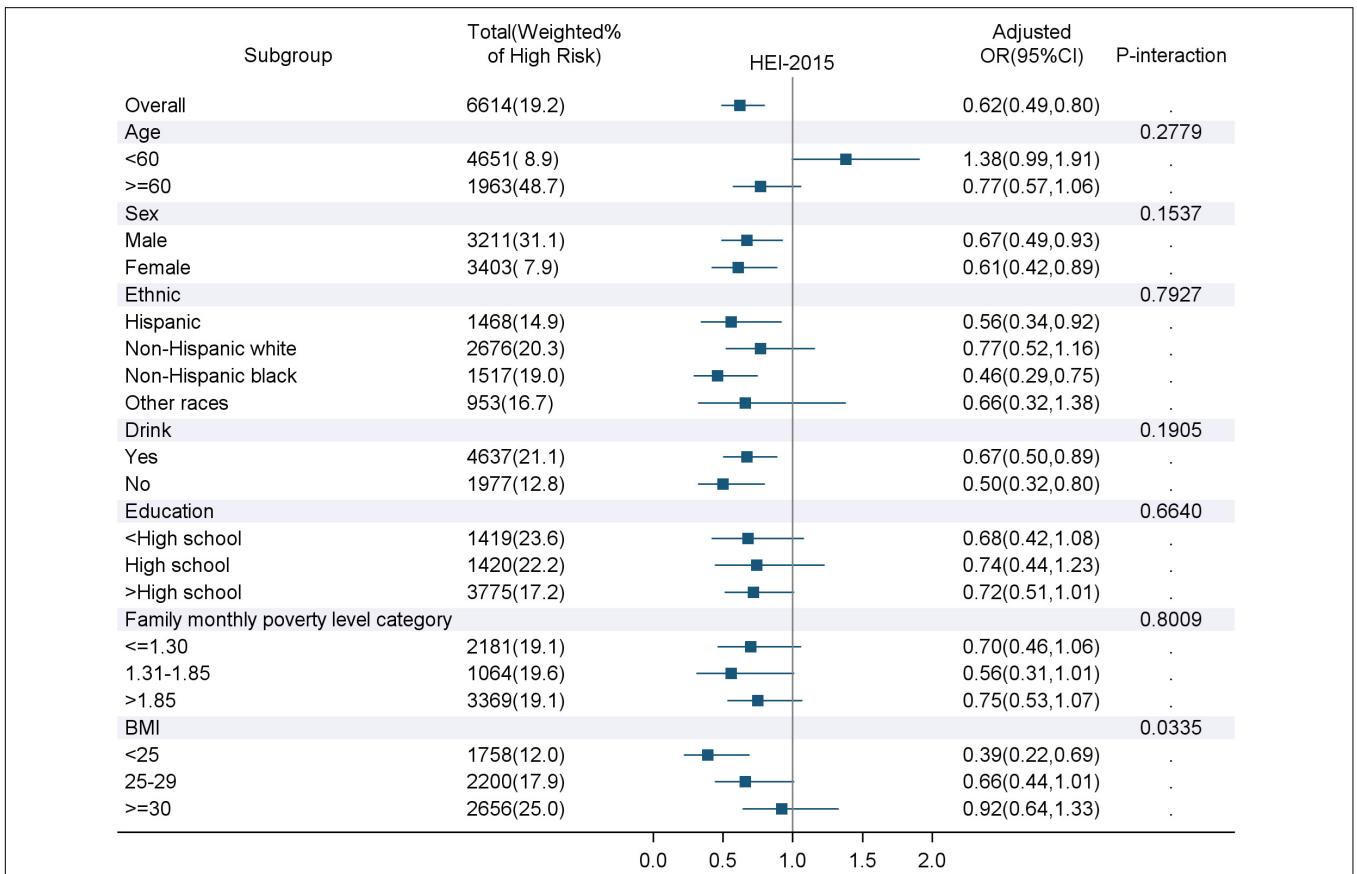

FIGURE 2 | Associations of the highest quartile of HEI-2015 score with high predicted 10-year CVD risk for subgroups. HEI-2015, Healthy Eating Index-2015; BMI, Body Mass Index; OR, odds ratio; CI, confidence interval. The variables adjusted for in the subgroup analysis were consistent with Model 3 in **Table 3**, except for the subgroup variables that were not included in the model. P-interaction refers to the interaction analysis between HEI-2015 and various covariates.

and mortality of CVD and preventing the occurrence of various chronic diseases (32–34). The DASH diet is generally used to reduce high blood pressure, one of the risk factors for CVD, and, thus, it also makes a significant contribution to the prevention of CVD (34–36). A systematic review and meta-analysis of the DASH diet have shown that there was a significant inverse linear relationship between DASH diet consumption and CVD risk, and the changes in blood pressure and cholesterol concentration caused by the DASH diet would reduce the predicted 10-year CVD risk by approximately 13% (37, 38). Some previous studies have examined the relationship between dietary patterns based on dietary guidelines and CVD, but most of them have focused on revealing the occurrence of specific cardiovascular outcome events (such as CHD, stroke) and CVD mortality (15–17). A recent analysis of the relationship between HEI-2015 and CVD based on the Nurses' Health Study (NHS), NHS II, and Health Professionals Follow-up Study (HPFS) has shown that higher diet scores were significantly associated with a lower risk of

cardiovascular events (15), which is similar to our findings. Our study population was more comprehensive and representative of the United States population of all races and genders than the study population, which included only female nurses and male health professionals. Our study of the HEI-2015 in the NHANES 2011–2014 is consistent with previous findings in the Multiethnic Cohort, linking HEI-2015 with reduced CVD risk (39). HEI-2015 has also demonstrated the predictive criterion validity for all-cause, cancer, and CVD mortality in the United States Participants, with the highest diet quality had a 13% to 21% decreased risk of CVD mortality compared with the lowest diet quality (40).

Apart from the aforementioned studies on CVD, only two studies focused on the relationship between dietary guideline-based dietary indices and predicted 10-year CVD risk and showed mixed results. A randomized study of Iranian male military personnel found no significant relationship between 10-year CVD risk and the Healthy Diet Indicator (HDI)-2020 (41). HDI-2020, based on the Global Dietary Recommendations (GDR), is the latest version of the WHO publication for the prevention of chronic diseases, and its components are calculated as a percentage of energy (42). Results from the randomized sample study of Iranian employees also showed no significant association between the Alternative Healthy Eating Index (AHEI) and predicted 10-year CVD risk (43). The AHEI is also designed to reduce food and nutrient intake associated with chronic disease risk (44). Different from the above two studies, our study used HEI-2015, an index reflecting the dietary guidelines for United States residents, in a nationally representative United States population. We found that there was a significant inverse association between HEI-2015 and the predicted 10-year CVD risk (OR: 0.62; 95%CI: 0.49–0.80; *P*-trend<0.0001). Furthermore, similar results were also found when we replaced predicted 10-year CVD risk with heart age as the outcome in the analysis. Compared with 10-year CVD risk, the psychological impact of the concept of heart age on the general population will be more intuitive.

There are several strengths in our study. First, we used the latest version of HEI to explore the relationship between the overall diet quality and the predicted 10-year CVD risk. HEI-2015, a comprehensive dietary index, can better reflect Americans' compliance with the 2015–2020 DGA. Second, NHANES provides a large and nationally representative sample of United States adults. However, our study has several limitations. First, two self-reported 24-h dietary interviews recall data maybe not be a good indicator to reflect the long-term diet consumption. Second, dietary data were subject to recall bias as participants self-reported their diet through 2 days of 24-h dietary recall interviews. Third, cross-sectional studies cannot accurately reflect the causal relationship between the two variables. Fourth, although we adjusted for many potential confounders, we could not rule out the effect of confounders not measured in this study.

CONCLUSION

In conclusion, we found that HEI-2015 total scores following the 2015–2020 Dietary Guidelines for Americans were negatively associated with predicted 10-year CVD risk and heart age. Our study adds to the evidence of an association between the HEI-2015 total score and the risk of CVD and demonstrates the importance of following dietary guidelines for the prevention of CVD in the general United States population.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: National Center for Health Statistics (NHANES): https://wwwn.cdc.gov/nchs/nhanes/Default.aspx.

AUTHOR CONTRIBUTIONS

YY, LZ, YZ, and CL contributed to the study design. LZ, YZ, and CL performed the data cleaning and analysis. YZ drafted the manuscript. LZ, XL, and YF critically revised and edited the manuscript for important intellectual content. All authors approved the final manuscript to be published.

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Association between dried fruit intake and pan-cancers incidence risk: A two-sample Mendelian randomization study

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Background: Observational studies have revealed that dried fruit intake may be associated with cancer incidence; however, confounding factors make the results prone to be disturbed. Therefore, we conducted a two-sample Mendelian randomization (MR) study to explore the causal relationship between dried fruit intake and 11 site-specific cancers.

Materials and methods: Forty-three single nucleoside polymers (SNPs) with robust genome-wide association study (GWAS) evidence, strongly correlated with dried fruit intake, were used as instrumental variables (IVs) in this study. The summary-level genetic datasets of site-specific cancers were obtained from the Oncoarray oral cavity and oropharyngeal cancer consortium, International Lung Cancer Consortium, Breast Cancer Association Consortium (BCAC), Ovarian Cancer Association Consortium, PanScan1, and GWAS of other scholars. We analyzed the causality between dried fruit intake and 11 site-specific cancers using the inverse-variance-weighted (IVW) and weighted median (WM) methods. For the results of the MR analysis, Cochran's *Q* test was used to check for heterogeneity, and multiplicative random effects were used to evaluate the heterogeneity further. Gene pleiotropy was tested using MR-Egger regression and MR-PRESSO methods. In addition, the main results of this study were validated by using the summary statistical data from the FinnGen and UK Biobank databases, and adjusted body mass index (BMI), years of education, fresh fruit intake, and vitamin C using multivariable MR analysis to ensure the stability of the research results.

Results: The evidence from IVW analyses showed that each increase of dried fruit intake by one standard deviation was statistically significantly associated with 82.68% decrease of oral cavity/pharyngeal cancer incidence risk (P = 0.0131), 67.01% decrease of lung cancer incidence risk (P = 0.0011),

77% decrease of squamous cell lung cancer incidence risk (P = 0.0026), 53.07% decrease of breast cancer incidence risk ($P = 4.62 \times 10^{-5}$), 39.72% decrease of ovarian cancer incidence risk (P = 0.0183), 97.26% decrease of pancreatic cancer incidence risk (P = 0.0280), 0.53% decrease of cervical cancer incidence risk (P = 0.0482); however, there was no significant effect on lung adenocarcinoma (P = 0.4343), endometrial cancer (P = 0.8742), thyroid cancer (P = 0.6352), prostate cancer (P = 0.5354), bladder cancer (P = 0.8996), and brain cancer (P = 0.8164). In the validation part of the study results, the causal relationship between dried fruit intake and lung cancer (P = 0.0043), squamous cell lung cancer (P = 0.0136), and breast cancer (P = 0.0192) was determined. After adjusting for the potential impact of confounders, the causal relationship between dried fruit intake and lung cancer (P = 0.0034), squamous cell lung cancer (P = 0.046), and breast cancer (P = 0.0001) remained. The sensitivity analysis showed that our results were stable and reliable.

Conclusion: The intake of dried fruits may have a protective effect against some site-specific cancers. Therefore, health education and a reasonable adjustment of dietary proportions may help in the primary prevention of cancer.

KEYWORDS

dried fruit intake, site-specific cancers, causal relationship, Mendelian randomization, incidence risk

Introduction

Cancer is a major global health problem and the second leading cause of morbidity and mortality, resulting in a heavy disease burden (1). In recent years, significant progress has been made in cancer treatment (2), early detection (3, 4), and control of specific risk factors, such as smoking (5), polycyclic aromatic hydrocarbon (6), cyclophosphamide (7), and carcinogenic infection (8, 9); however, the harm of cancer to human health and quality of life still exists. Generally, cancer prevention focuses on specific risk factors, such as tobacco use, diet, living habits, and carcinogen infection, which is determined by its high complexity and heterogeneity (10). Studies have shown that more than 30% of cancers are caused by dietary factors (11, 12). Therefore, adjusting dietary patterns and changing dietary habits can effectively prevent cancer development.

Traditional observational epidemiological studies have shown that tumor incidence is associated with insufficient intake of fruits and vegetables. For instance, increased dietary fiber consumption may have additional benefits in patients with colorectal cancer after diagnosis (13). Fruits and vegetables are considered protective factors in the etiology of lung cancer, even though the confounding effects of smoking cannot be ruled out (14). The risk of prostate cancer is significantly reduced when the intake of fruits and vegetables is high (15). However, most observational studies on the relationship between diet and cancer do not distinguish between dried fruit and raw fruit or do not mention the impact of dried fruit on cancer. Dietary guidelines in many countries also encourage people to choose non-juice-form fruits as much as possible, including dried fruit (16). Dried fruit is a stable form of fruit that remains fresh through drying technology; however, it mainly appears in the human diet as a snack, accounting for a relatively small proportion. Some clinical and laboratory studies have reported that the intake of dried fruit is related to the progression or occurrence of some cancers. Nevertheless, the discussion on the relationship between dried fruit intake and the risk of cancer is only based on animal models or laboratory data (17, 18), and there is a lack of reliable epidemiological causality assessments.

Traditional observational studies may lead to deviation and even misjudgment of the research results due to various observable and unobservable residual confounding factors, reverse causality, and bias; meanwhile, they mainly focus on the correlation between exposure factors and outcomes rather than the actual causal relationship. Mendelian randomization (MR) is a new epidemiological method that imitates the design of randomized controlled studies (19). It uses single nucleotide polymorphisms (SNPs) as instrumental variables (IVs) to infer causality between the

risk factors and outcomes of interest. SNP is randomly assigned to individuals with gametes during meiosis (20), which is similar to the requirements of randomized controlled trials. Simultaneously, genetic variation precedes the occurrence of diseases, which avoids the potential impact of reverse causality.

Therefore, MR is an ideal method to explore the causal relationship between dried fruit intake and pan-cancer. This study used a two-sample MR design to investigate whether dried fruit intake has a causal relationship with 11 site-specific cancers and estimate its effect to provide scientific evidence for cancer primary prevention.

Materials and methods

Study design

A two-sample MR design was used to evaluate the causal effect of dried fruit intake on cancer risk (**Figure 1**). The MR design is based on the following three core assumptions: First, genetic IVs must be closely related to dried fruit intake (Assumption 2). Second, confounding factors cannot affect the selected IVs that influence the association between dried fruit intake and cancer (Assumption 1). Third, IVs can only affect cancer risk through dried fruit intake (Assumption 3).

Dried fruit intake exposure data source

The genome-wide association study (GWAS) summary statistics of dried fruit intake were obtained from the UK Biobank, which is a large cohort of approximately 500,000 individuals aimed at collecting the genotype and various phenotypic data and was approved by the Research Ethics Committee (21, 22) (REC reference is 11/NW/0382). All participants in the cohort were invited to the local evaluation center to obtain corresponding data using a touch-screen questionnaire or anthropometry with standardized procedures. Dried fruit intake, as an exposure factor was obtained by questioning the frequency of dried fruit intake in the questionnaire. Participants were asked, "how many pieces of dried fruit would you eat per day?" (Count one prune, one dried apricot, and ten raisins as one piece; put "0" if you do not eat any). Answer with the average (integer) of participants' intake in the past year. Other options are "10," "1," or "3," representing less than one, do not know, and prefer not to answer, respectively. Finally, 421,764 participants of European ancestry obtained dried fruit intake as the exposure factor through the questionnaire's frequency of dried fruit intake.

Site-specific cancers outcome data sources

We considered 11 site-specific cancers as the outcomes of this study (lung cancer selected three datasets: lung cancer, lung adenocarcinoma, and squamous cell lung cancer). The sources and corresponding information for all the aggregated statistical datasets used in this study are listed in **Supplementary Table 1**.

The GWAS summary statistics of oral/pharyngeal cancer were obtained from the Oncoarray oral cavity and oropharyngeal cancer consortium, included 2,342 cases and 2,329 controls mainly from the International Head and Neck Cancer Epidemiology Consortium (INHANCE), as well as a European cohort study (EPIC) and the United Kingdom case-series (HN5000) (23); GWAS summary data for lung cancer (27,209 participants including 11,348 patients and 15,861 controls) squamous cell lung cancer (18,313 participants with 3,275 cases and 15,038 controls) and lung adenocarcinoma (18,336 participants with 3,442 cases and 14,894 controls) were from the International Lung Cancer Association (ILCCO), and the patient data were based on previously reported GWAS: IARC-GWAS, NCI-GWAS, ICR-GWAS, and MDACC-GWAS (24–27); the GWAS summary data of breast cancer on 33,832 participants (15,748 breast cancer patients and 18,084 controls) came from the Breast Cancer Association Consortium (BCAC), included 8 (C-BCAC) and a subset of BPC3 GWAS (CGEMS) (28–31); participants in epithelial ovarian cancer were from the Ovarian Cancer Associations Consortium (OCAC, including 25,509 population-based patients and 40,941 controls) (32); the pancreatic cancer summary data of PanScan1 consortium included 1,896 cases and 1,939 controls, this is a GWAS based on 12 prospective cohort (33); the summary statistics of endometrial cancer were obtained in meta-GWAS of O'Mara et al. (34), including 12,906 cases of endometrial cancer and 108,979 country-matched controls; the controls were from 17 studies of the Endometrial Cancer Association Consortium (ECAC), the Epidemiology of Endometrial Cancer Consortium (E2C2), and the UK Biobank; the summary data of thyroid cancer were from Kohler et al. (35), a GWAS based on 649 patients with thyroid cancer and 431 controls; the summary data of prostate cancer generated in GWAS by Schumacher et al. (36), including 79,148 patients and 61,106 controls; the summary statistics for bladder cancer included 1,279 patients and 372,016 controls; summary statistics for brain cancer included 606 patients and 372,016 controls; and summary statistics for cervical cancer included 563 patients and 198,523 controls.

All of the above cancer datasets were acquired from individuals of European ancestry. All consortiums obtained informed consent from the participants and the approval

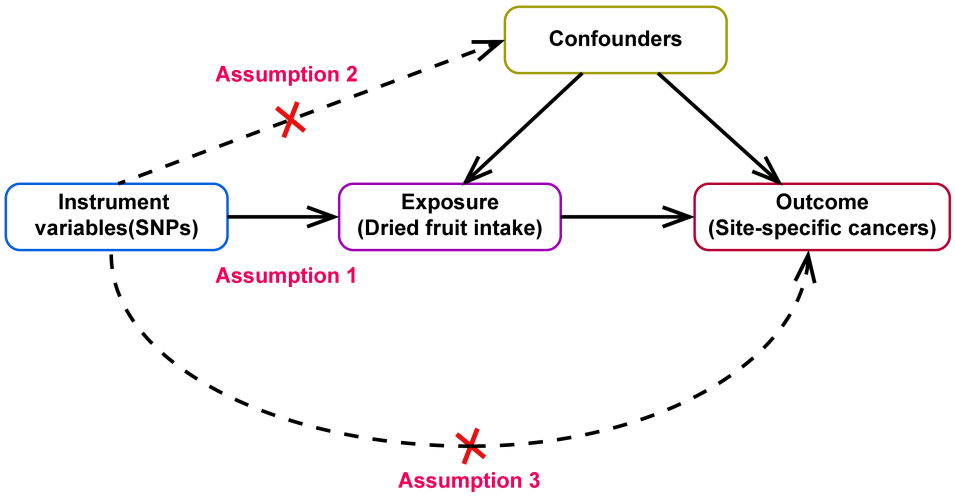

FIGURE 1
Directed acyclic graph of Mendelian randomization (MR) framework showing the hypothesis of dried fruit intake on site-specific cancers, the dotted line indicates that there has pleiotropic or direct causal relationship between exposure and outcome.

of the relevant ethics committees when participants participated in the study.

Selection of instrumental variables

To explore the causal relationship between dried fruit intake and site-specific cancers better, SNPs were used as IVs. The criteria selected for SNPs were as follows: (i) the SNPs were highly correlated with dried fruit intake, which is significant for whole-genome research, that is, $P < 5 \times 10^{-8}$. (ii) SNPs are independent of each other to avoid offset caused by linkage disequilibrium (LD). When R^2 of the LD was greater than 0.001, one of them was eliminated. (iii) Genetic distance refers to the length of the region, considering LD. Here, we set the genetic distance as 10,000 kb; within 10,000 kb, remove the SNP with R^2 greater than 0.001 with the most significant SNPs. SNPs characteristics of dried fruit intake were extracted, including SNP number, chromosome location, effective allele, effective allele frequency (EAF), effect value, standard error, and *P*-value of the effective allele and dried fruit intake.

The *F*-statistic of each SNP was used to judge the correlation strength and avoid bias caused by weak IVs to ensure a strong correlation between IVs and exposure factors. When the *F* value was greater than 10, it was considered that there was no bias in weak IVs. The following formula was used to calculate the *F*-statistic for each SNP:

$$F = \frac{N - K - 1}{K} \times \frac{R^2}{1 - R^2}$$

where N is the sample size of the exposure dataset, K is the number of SNPs, and R^2 represents the proportion of variation explained by IVs in the exposure dataset; specifically, the calculation formula of R^2 is:

$$R^2 = 2 \times (1 - \text{MAF}) \times \text{MAF} \times (\frac{\beta}{\text{SD}})^2 \qquad (1)$$

here, MAF is the secondary allele frequency, equivalent to EAF when calculating R^2, β is the allele effect value, and SD is the standard deviation.

In addition, IVs identified for inclusion in this study were searched on the PhenoScanner website[1] to detect pleiotropic effects for the selected IVs, if there was any SNP correlated with the outcomes, they should be excluded from the IVs prior to perform MR analysis. The detailed results of the search are presented in **Supplementary Table 2**. We found that IVs were strongly associated with body mass index (BMI) and years of education; therefore, these two factors were also included in the multivariate MR analysis to exclude their effects on the causal relationship between exposure and outcome.

Univariate two-sample Mendelian randomization analysis

This study used two MR methods to estimate the relationship between dried fruit intake and cancer: inverse-variance-weighted (IVW) and weighted median (WM). The premise of the IVW method is that all the IVs are effective. If any SNP does not meet the assumption of IV, the result will be biased. Multiple SNPs can enhance the statistical ability of MR analysis. However, due to the existence of pleiotropy, when some

1 http://www.phenoscanner.medschl.cam.ac.uk/

SNPs do not meet the hypothesis of IV, the causal relationship between dried fruit intake and cancers will deviate. However, when 50% of SNPs are effective IV, the estimation obtained by the WM method should be consistent with the actual effect (37, 38).

The intercept term of the MR-Egger regression model was used to test whether there was gene pleiotropy. If the intercept term was close to 0 ($P < 0.05$), it was considered that the influence of genetic pleiotropy was small. IVs were not directly related to outcome events, then Assumption 3 is valid. The MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) method, which corrects the estimate by eliminating outliers, was also used to detect the existence of gene pleiotropy. Cochran's *Q* test was used to assess IV heterogeneity to evaluate further the impact of heterogeneity on causal estimation. When heterogeneity was present in the results, a multiple random effects model was used to re-estimate causality. Simultaneously, SNPs are removed one by one, and the remaining SNPs continue to be analyzed by MR, using the leave-one-out analysis method to investigate the sensitivity of the results.

Multivariate Mendelian randomization analysis

According to the results of the search on the PhenoScanner website and possible confounding factors between dried fruit intake and outcomes (fresh fruit intake and vitamin C), we used a multivariate MR analysis with the addition of fresh fruit intake, vitamin C, BMI, and years of education to adjust for causal effects between exposure and outcome in five rounds of adjustment: (i) fresh fruit intake alone, (ii) vitamin C alone, (iii) BMI alone, (iv) years of education alone, and (v) a combination of fresh fruit intake, vitamin C, BMI, and years of education.

Mendelian randomization in validation datasets

To validate the main findings, 12 cancer datasets from the FinnGen database and two cancer datasets from the UK Biobank database (endometrial cancer and oral cavity/pharyngeal cancer were not available in the FinnGen database, and summary statistics from the UK Biobank database were used) were used as outcomes for the two-sample MR analysis. As the summary statistics for dried fruit intake were also obtained from the UK Biobank database, the results of the IVW method were corrected for endometrial cancer and oral cavity/pharyngeal cancer using the *MRlap* function to avoid any possible overlap of samples affecting the causality. The sources of all datasets used in the validation are listed in **Supplementary Table 1**.

Statistical analyses

All analyses were performed using R software (version 4.0.5) under the Windows environment. The R packages used for all MR-related analyses and image plotting included "*vcfR*," "*TwoSampleMR*," "*MR-PRESSO*," "*MRlap*," and "*forestplot*." A two-sided $P < 0.05$ was considered a statistically significant difference.

Results

Details of instrumental variables

After screening, 43 SNPs that were closely related to dried fruit intake ($P < 5 \times 10^{-8}$) and independent of each other ($R^2 < 0.001$) were identified (**Supplementary Table 3**). A Manhattan plot of these 43 SNPs is shown in **Figure 2**. The average *F*-statistic was 24.7464 (range, 17.4989–47.9013), indicating that the results are less likely to deviate owing the influence of weak IVs, consistent with Assumption 1. Based on the search results on the PhenoScanner website, there were no cancer-associated SNPs among these 43 IVs; therefore, we used these 43 SNPs as IVs to estimate the causal effects of dried fruit intake and 11 site-specific cancers in the subsequent analysis.

Causal effect analysis between dried fruit intake and site-specific cancers

Causal correlation analysis of dried fruit intake and 11 site-specific cancers used inverse variance weighting (IVW) and weight median (WM) methods. The results of the IVW method supported the causal relationship between dried fruit intake and oral cavity/pharyngeal, lung, squamous cell lung, breast, ovarian, pancreatic, and cervical cancers. The higher the dried fruit intake, the lower the cancer incidence risk. The risk of oral cavity/pharyngeal cancer decreased by 82.68% (OR = 0.1732, 95% CI: 0.0433–0.6922, $P = 0.0131$) for every increase of dried fruit intake by one standard deviation; lung cancer risk was reduced by 67.01% (OR = 0.3299, 95% CI: 0.1695–0.642, $P = 0.0011$); the risk of squamous cell lung cancer was reduced by 77.00% (OR = 0.2300, 95% CI: 0.0884–0.5986, $P = 0.0026$); the risk of breast cancer was reduced by 53.07% (OR = 0.4693, 95% CI: 0.3261–0.6753, $P = 4.62 \times 10^{-5}$); the risk of ovarian cancer was reduced by 39.72% (OR = 0.6028, 95% CI: 0.3960–0.9177, $P = 0.0183$); the risk of pancreatic cancer was reduced by 97.26% (OR = 0.0274, 95% CI: 0.0011–0.6784, $P = 0.0280$); the risk of cervical cancer was reduced by 0.53% (OR = 0.9947, 95% CI: 0.9897–0.9998, $P = 0.0482$). The WM method also supported a causal relationship between dried fruit intake and lung, squamous cell lung, breast, and pancreatic cancers. However, for lung adenocarcinoma, endometrial, thyroid, prostate, bladder,

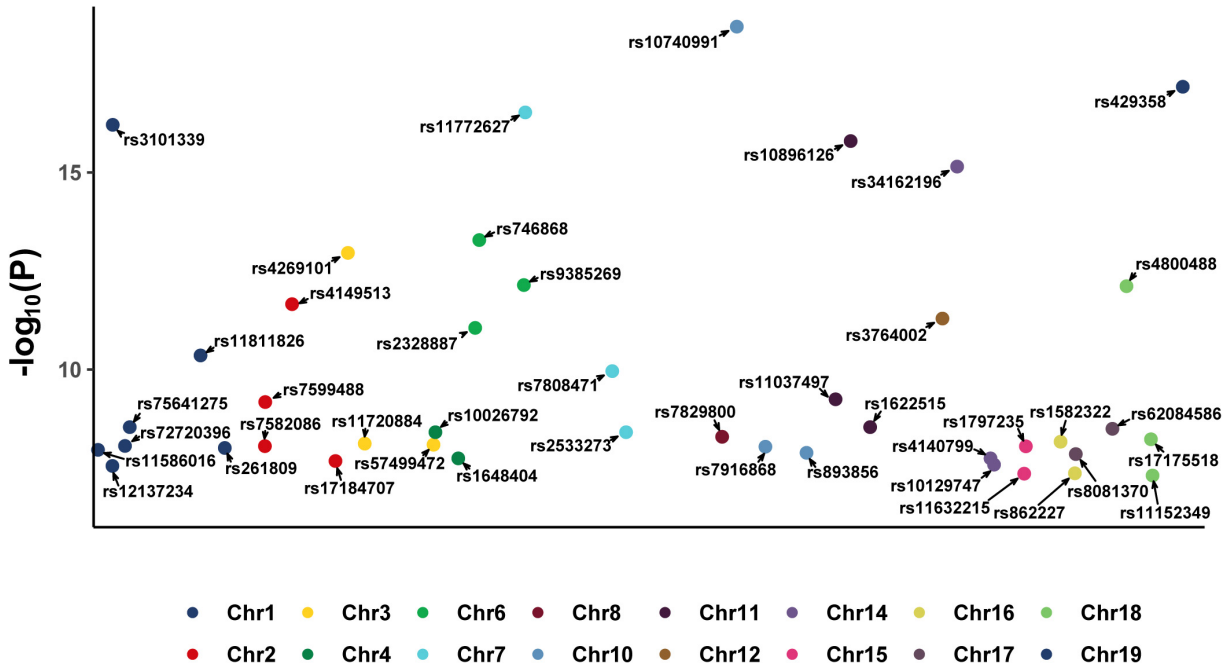

FIGURE 2
Manhattan plot of 43 SNPs identified as instrumental variables (IVs) from exposure dataset. SNP, single nucleotide polymorphism.

and brain cancers, neither the IVW nor WM method showed statistical significance. The details of the results are presented in Figure 3 and Table 1.

The scatter plots show the estimated effect of IVs on exposure and outcomes, and the rising slope in the plot indicates a negative correlation between dried fruit intake and the risk of site-specific cancer (Supplementary Figure 1). In addition, because of the results extracted from different outcome datasets and the deletion of palindrome SNPs with intermediate allele frequencies, the number of IVs used in the causal analysis between dried fruit intake and various types of cancer was not equal.

The funnel plot (Supplementary Figure 2) shows that when a single SNP was used as an IV, the causal effects were symmetrically distributed, indicating that the results were less likely to be affected by potential bias and that the results were stable and reliable.

Sensitivity analysis on results of univariate two-sample Mendelian randomization

The existence of gene pleiotropy was tested using MR-Egger regression analysis. Among the 11 site-specific cancers (oral cavity/pharyngeal, lung, squamous cell lung, breast, ovarian, cervical, lung adenocarcinoma, pancreatic, endometrial, thyroid, prostate, bladder, and brain), all of the intercept term was close to zero ($P > 0.05$), indicating that the results may be less affected by potential bias. At the same time, the MR-PRESSO method also obtained results consistent with the MR-Egger regression; that is, gene pleiotropy did not exist. Although in lung, squamous cell lung, breast, endometrial, and prostate cancers, the *P*-values of the MR-PRESSO analysis were less than 0.05, the results of the MR-PRESSO destruction test were supported by the absence of horizontal pleiotropy ($P > 0.05$). The MR-PRESSO distortion test here refers to whether there is a difference between the results after removing outlier SNP and the initial results (39). The detailed results are presented in Table 2.

Leave-one-out analysis was used to analyze the results of the IVW method. After removing each SNP individually, the results were consistent with the IVW method in causal effect analysis, indicating that no single SNP affected the causal estimation results. The results are presented in Supplementary Figure 3. Cochran's statistical test showed no statistically significant heterogeneity effect (Q-value > 0.05) of the SNP related to dried fruit intake between oral cavity/pharyngeal, ovarian, pancreatic, cervical, lung adenocarcinoma, thyroid, bladder, and brain cancers. Although the results for lung, squamous cell lung, breast, endometrial, and prostate cancers showed heterogeneity (Q-value < 0.05), the results of the multiple random effects model were consistent with MR estimates, indicating that there was a causal effect between dried fruit intake and lung, squamous cell lung, and breast cancers ($P < 0.05$); while no association with endometrial cancer, and prostate cancer

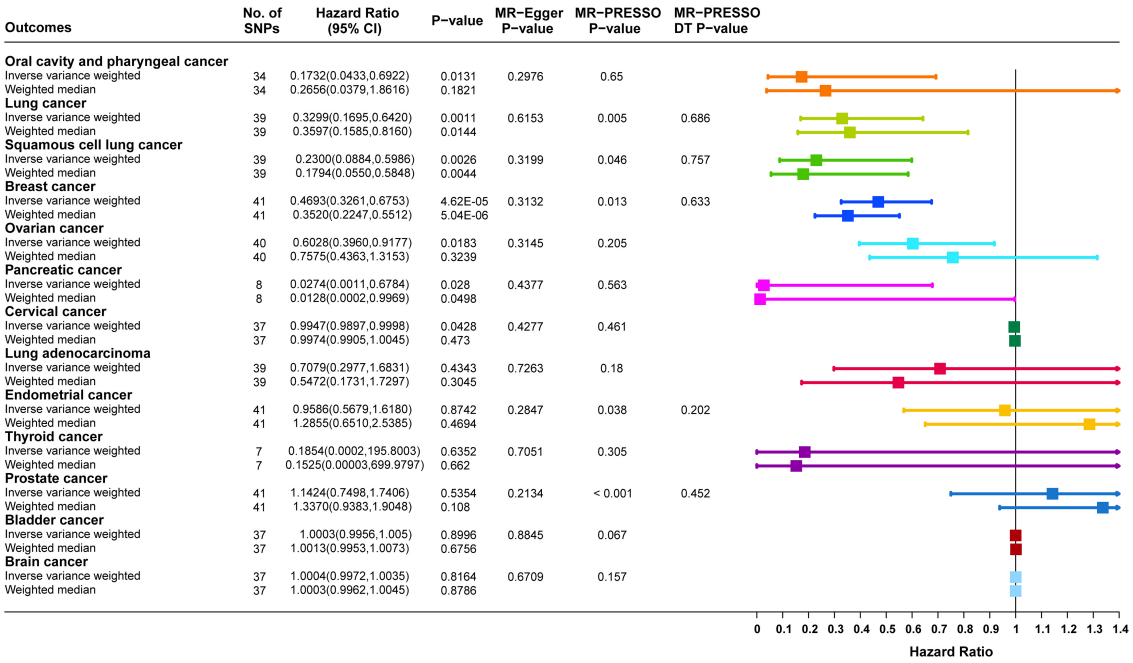

FIGURE 3
Forest plot of two-sample Mendelian randomization (MR) estimation of the association between dried fruit intake and cancer risk. No. of SNPs, number of single nucleotide polymorphisms; CI, confidence interval.

($P > 0.05$). The reliability of the results of this study can be explained further. The specific results are listed in Table 3.

Multivariate Mendelian randomization analysis

Multivariate MR analysis for each cancer found that: for lung cancer, after adjusting for fresh fruit intake (OR = 0.2383, 95% CI: 0.1085–0.5233, $P = 0.0004$), vitamin C (OR = 0.3958, 95% CI: 0.1794–0.8732, $P = 0.0217$), BMI (OR = 0.3419, 95% CI: 0.1858–0.6291, $P = 0.0006$), years of education (OR = 0.2946, 95% CI: 0.1295–0.6701, $P = 0.0036$), and all of these four (OR = 0.2931, 95% CI: 0.129–0.6659, $P = 0.0034$), dried fruit intake remained causally associated with lung cancer, and the effect size for causality was slightly enhanced in multivariate MR compared with univariate MR (Figure 4A); for squamous cell lung cancer, after adjusting for fresh fruit intake (OR = 0.2586, 95% CI: 0.0841–0.7954, $P = 0.0183$), vitamin C (OR = 0.2278, 95% CI: 0.0727–0.7135, $P = 0.0111$), BMI (OR = 0.285, 95% CI: 0.1145–0.7094, $P = 0.007$), years of education (OR = 0.2156, 95% CI: 0.0665–0.6993, $P = 0.0106$), and all of these four (OR = 0.2804, 95% CI: 0.0804–0.9778, $P = 0.046$), though the effect of causality was slightly attenuated in multivariate MR compared to univariate MR, dried fruit intake and squamous cell lung cancer remained causally associated (Figure 4B); for breast cancer, after adjusting for fresh fruit intake (OR = 0.4911, 95% CI: 0.3166–0.7618, $P = 0.0015$), vitamin C (OR = 0.5601, 95% CI: 0.3129–0.8185, $P = 0.0055$), BMI (OR = 0.3393, 95% CI: 0.229–0.5028, $P = 7.12 \times 10^{-8}$), years of education (OR = 0.574, 95% CI: 0.365–0.9029, $P = 0.0163$), and all of these four (OR = 0.339, 95% CI: 0.1971–0.5829, $P = 0.0001$), dried fruit intake remained causally associated with breast cancer, and the effect size for causality was slightly enhanced in multivariate MR compared to univariate MR (Figure 4C). However, for oral cavity/pharyngeal, ovarian, cervical, pancreatic, lung adenocarcinoma, endometrial, thyroid, prostate, bladder, and brain cancers, after adjustment for multivariate MR, the causal relationship between dried fruit intake and outcome was not statistically significant (Supplementary Figure 4).

Validation

In the validation cohort, the results of the IVW method supported a causal relationship between dried fruit intake and lung, squamous cell lung, and breast cancers. The higher the dried fruit intake, the lower the cancer incidence risk. For every increase in dried fruit intake by one standard deviation, lung cancer risk was reduced by 76.86% (OR = 0.2314, 95% CI: 0.0847–0.6323, $P = 0.0043$); squamous cell lung cancer risk was reduced by 93.11% (OR = 0.0689, 95% CI: 0.0082–0.5773, $P = 0.0136$); and breast cancer risk was reduced by 45.24% (OR = 0.5476, 95% CI: 0.3307–0.9067,

TABLE 1 Two-sample Mendelian randomization (MR) analyses of the association between dried fruit intake and eleven site-specific cancers.

Outcome	IVW method			WM method		
	OR	95% CI of OR	*P*-value	OR	95% CI of OR	*P*-value
Oral cavity and pharyngeal cancer	0.1732	(0.0433, 0.6922)	**0.0131***	0.2656	(0.0379, 1.8616)	0.1821
Lung cancer	0.3299	(0.1695, 0.6420)	**0.0011***	0.3597	(0.1585, 0.8160)	**0.0144***
Squamous cell lung cancer	0.2300	(0.0884, 0.5986)	**0.0026***	0.1794	(0.0550, 0.5848)	**0.0044***
Breast cancer	0.4693	(0.3261, 0.6753)	**4.62×10^{-5}***	0.3520	(0.2247, 0.5512)	**5.04×10^{-6}***
Ovarian cancer	0.6028	(0.3960, 0.9177)	**0.0183***	0.7575	(0.4363, 1.3153)	0.3239
Pancreatic cancer	0.0274	(0.0011, 0.6784)	**0.0280***	0.0128	(0.0002, 0.9969)	**0.0498***
Cervical cancer	0.9947	(0.9897,0.9998)	**0.0428***	0.3045	(0.1731, 1.7297)	0.3045
Lung adenocarcinoma	0.7079	(0.2976, 1.6831)	0.4342	1.2855	(0.6510, 2.5385)	0.4694
Endometrial cancer	0.9586	(0.5679, 1.6180)	0.8742	0.1525	(3.32×10^{-5}, 699.9797)	0.6620
Thyroid cancer	0.1854	(0.0001, 195.8003)	0.6352	1.3370	(0.9383, 1.9048)	0.1080
Prostate cancer	1.1424	(0.7498, 1.7406)	0.5354	1.3370	(0.9383, 1.9048)	0.1080
Bladder cancer	1.0003	(0.9956,1.0050)	0.8996	1.0013	(0.9953,1.0073)	0.6756
Brain cancer	1.0003	(0.9972,1.0035)	0.8164	1.0003	(0.9961,1.0045)	0.8786

OR, odds ratios; 95% CI, 95% confidence interval; IVW, inverse-variance-weighted; WM, weighted median.
*Indicate $P < 0.05$. The bold values represent the statistically significant *P*-values.

TABLE 2 Horizontal pleiotropic test between dried fruit intake and eleven site-specific cancers.

Outcome	Horizontal pleiotropy test			MR-PRESSO	
	Intercept	*SE*	*P*-value	*P*-value	DT *P*-value
Oral cavity/pharyngeal cancer	0.041	0.0388	0.2976	0.65	
Lung cancer	−0.0095	0.0187	0.6153	0.005	0.686
Squamous cell lung cancer	−0.0268	0.0265	0.3199	0.046	0.757
Breast cancer	−0.0103	0.0101	0.3132	0.013	0.633
Ovarian cancer	0.0118	0.0116	0.3145	0.205	
Pancreatic cancer	0.1324	0.1593	0.4377	0.563	
Cervical cancer	−0.0001	0.0001	0.4277	0.461	
Lung adenocarcinoma	−0.0085	0.0244	0.7263	0.18	
Endometrial cancer	0.0159	0.0146	0.2847	0.038	0.202
Thyroid cancer	0.1141	0.2848	0.7051	0.305	
Prostate cancer	0.0148	0.0117	0.2134	<0.001	0.452
Bladder cancer	−0.00001	0.0001	0.8845	0.067	
Brain cancer	0.00004	0.0001	0.6709	0.157	

SE, standard error; DT, distortion test.

P = 0.0192); however, for oral cavity/pharyngeal, ovarian, cervical, pancreatic, lung adenocarcinoma, endometrial, thyroid, prostate, bladder, and brain cancers, neither the IVW nor WM methods showed statistical significance. The details of the results are shown in Figure 5. The MR-Egger regression analysis and MR-PRESSO method excluded the effect of horizontal multiplicity on causality to some extent (Figure 5). Cochran's statistical test found no significant statistical effect of heterogeneity on causality estimates, ensuring the robustness of the results (Supplementary Table 4). For endometrial cancer (corrected P value = 0.4793) and oral cavity/pharyngeal cancer (corrected P value = 0.9565), the analysis results of "*MRlap*" showed that after adjusting for the impact of sample overlap, the causal effects between dried fruit intake and these two cancers were consistent to the results of two-sample MR.

Discussion

This study used a two-sample MR method to explore the relationship between dried fruit intake and 11 site-specific cancers in the European population. The results showed a causal relationship between dried fruit intake and

oral cavity/pharyngeal, lung, squamous cell lung, breast, ovarian, pancreatic, and cervical cancers. However, no causal relationship was observed with lung adenocarcinoma, endometrial, thyroid, prostate, bladder, and brain cancers. In addition, the causal relationships between dried fruit intake and lung, squamous cell lung, and breast cancers were validated using the validation datasets. To our knowledge, this is the first study to focus on the causal relationship between dried fruit intake and cancer by using MR analysis.

Dried fruits are favored because of their sweet taste, stability, and ease of preservation. By drying fresh fruit, it retains as many nutrients as possible from the original food,

TABLE 3 Heterogeneity test between dried fruit intake and eleven site-specific cancers.

Outcome and method	Cochran's *Q* test			Multiplicative random effects		
	Q	**Q_df**	***Q*-value**	**Beta**	***SE***	***P*-value**
Oral cavity/pharyngeal cancer						
MR-Egger	28.012	32	0.6688			
IVW	29.1333	33	0.6602			
Lung cancer				−1.1091	0.3397	0.0011
MR-Egger	62.9339	37	0.0049			
IVW	63.3708	38	0.006			
Squamous cell lung cancer				−1.4697	0.488	0.0026
MR-Egger	54.303	37	0.033			
IVW	55.7951	38	0.0313			
Breast cancer				−0.7566	0.1857	4.62×10^{-5}
MR-Egger	61.4347	39	0.0124			
IVW	63.0793	40	0.0114			
Ovarian cancer						
MR-Egger	45.9324	38	0.1765			
IVW	47.1886	39	0.1727			
Pancreatic cancer						
MR-Egger	5.6025	6	0.4692			
IVW	6.2934	7	0.5059			
Cervical cancer						
MR-Egger	36.2438	35	0.4104			
IVW	36.9106	36	0.4266			
Lung adenocarcinoma						
MR-Egger	45.6275	37	0.1562			
IVW	45.7808	38	0.1805			
Endometrial cancer				−0.0423	0.2671	0.8742
MR-Egger	56.3945	39	0.0353			
IVW	58.0962	40	0.032			
Thyroid cancer						
MR-Egger	7.2731	5	0.2011			
IVW	7.5067	6	0.2765			
Prostate cancer				0.1331	0.2148	0.5354
MR-Egger	128.1553	39	1.9813×10^{-11}			
IVW	133.4129	40	5.5721×10^{-12}			
Bladder cancer						
MR-Egger	48.6025	35	0.063			
IVW	48.6322	36	0.0778			
Brain cancer						
MR-Egger	45.3404	35	0.1132			
IVW	45.5783	36	0.1316			

Q, Cochran's *Q* statistic; df, degrees of freedom; SE, standard error; IVW, inverse-variance-weighted.

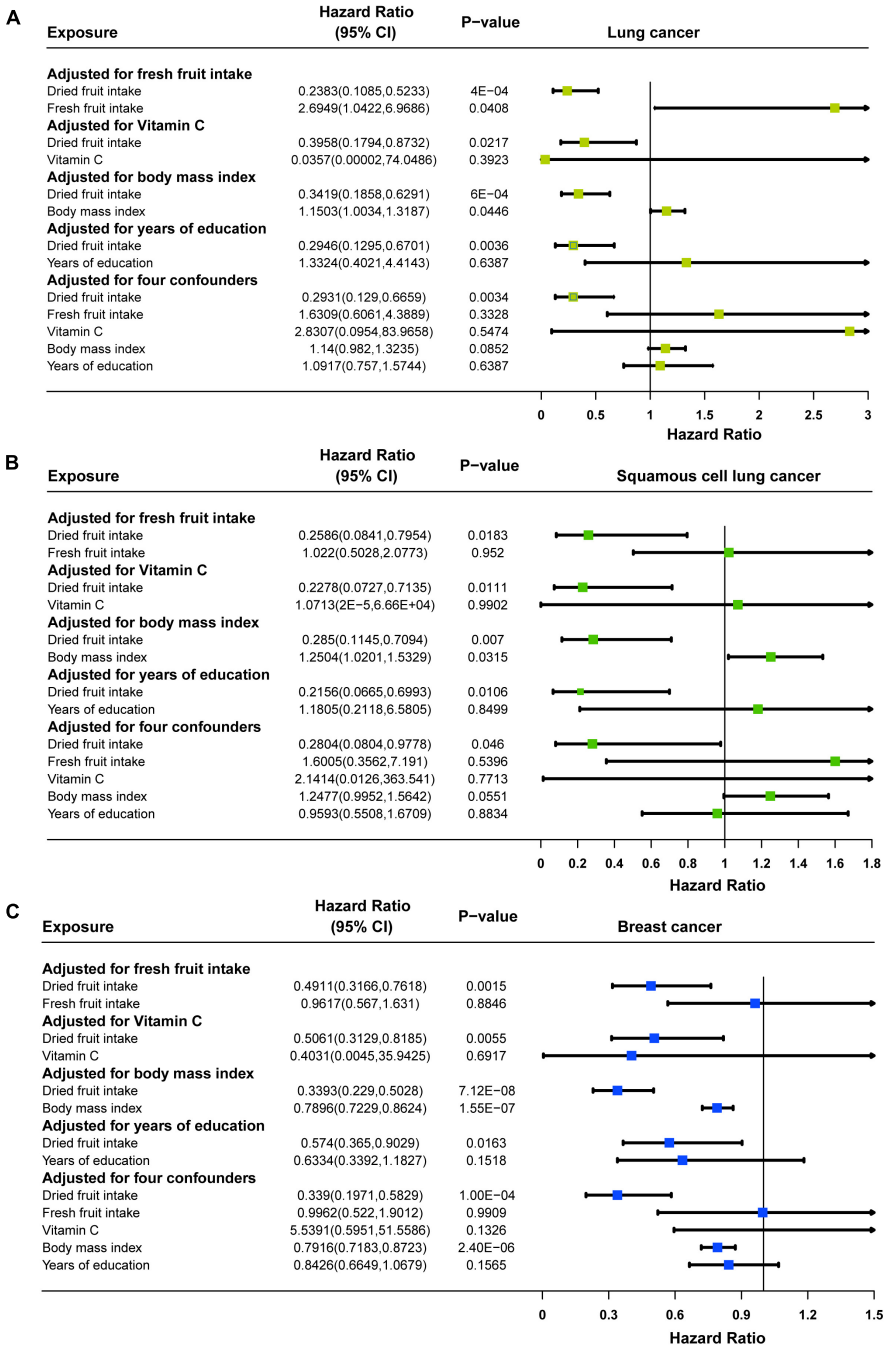

FIGURE 4
Forest plots of multivariable Mendelian randomization (MR) in **(A)** lung cancer, **(B)** squamous cell lung cancer, and **(C)** breast cancer. Adjusted for fresh fruit intake, vitamin C, body mass index, years of education or fresh fruit intake, vitamin C, body mass index, and years of education.

which is also why it is a good source of fiber and some trace elements (40, 41). In addition, dried fruits are rich in a wide range of bioactive components and phytochemicals. Because these compounds are not necessary to maintain life, they are not designated as traditional nutrients; however, the benefits of plant compounds may exceed human cognition. They can affect the occurrence and development of many chronic diseases by affecting metabolic pathways and cellular reactions and play a role in promoting health and longevity (42). However, people have preferred other processed fruits in recent years, such as pickled and fermented fruits; the consumption of dried fruits is even lower than that of canned

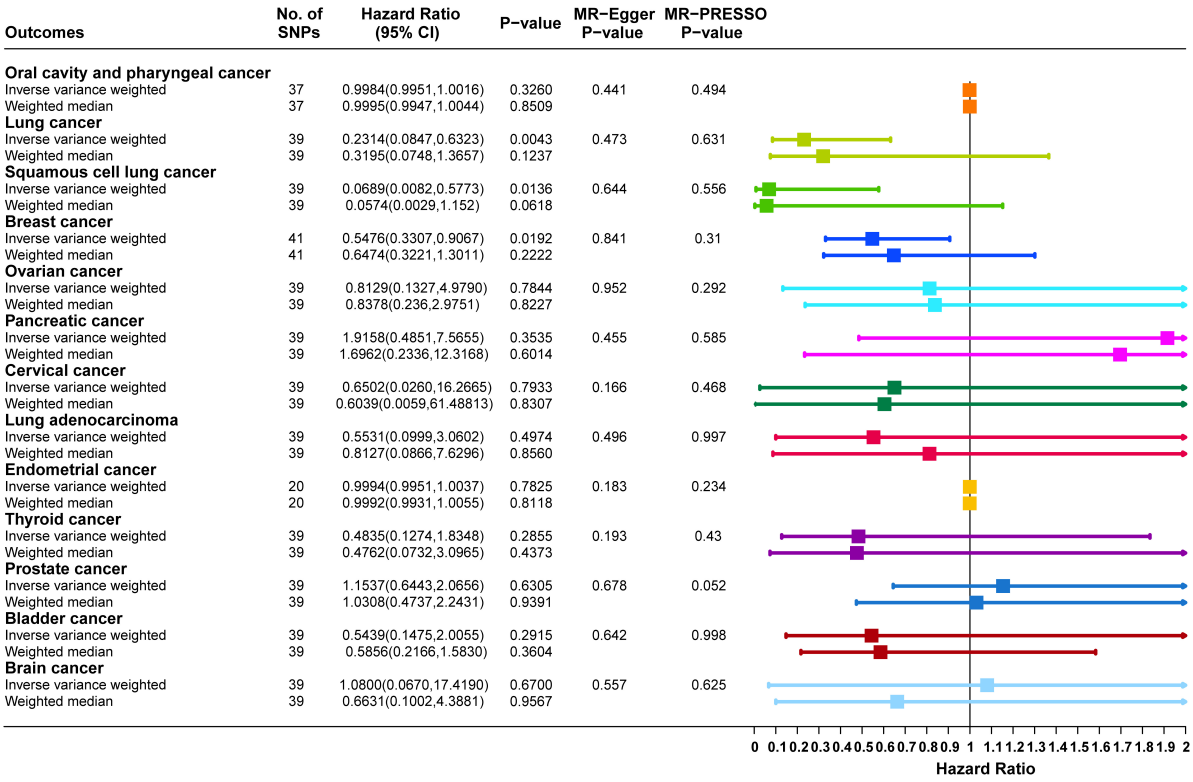

FIGURE 5
Forest plot of two-sample Mendelian randomization (MR) estimation of the association between dried fruit intake and cancer risk in validation datasets. No. of SNPs, number of single nucleotide polymorphisms; CI, confidence interval.

fruits (43, 44). Therefore, this study focused on dried fruit as an exposure factor to explore the causal relationship between dried fruit intake and cancer and provide a new entry point for cancer prevention.

A cohort study of 61 lung cancer patients in the United States showed that there was a statistically significant protective association between dried fruit intake and lung cancer (dried fruit intake less than three times per week, RR = 1.0; greater than or equal to three times per week, RR = 0.89) (45); After adjusting for age and sex, dried fruit intake was also a protective association with pancreatic cancer (dried fruit intake was less than one times per month, RR = 1; greater than or equal to three times per week, RR = 0.35) (46). The results of two prospective cohorts and one case-control study showed a protective trend for prostate cancer; however, only one study was statistically significant (47–49). These studies are consistent with the conclusion of this study. The MR method has its unique advantages because genes have been determined at human birth, SNP as an IV will not be affected by various confounding factors, and the reasonable temporal sequence in causal inference guaranteed the reliability of the conclusion.

As mentioned, causal estimation is effective when the three assumptions in the MR model are satisfied. First, 43 significantly correlated and independent SNPs loci were selected that were closely related to dried fruit intake. At the same time, the F-statistic for each SNP was greater than 10, indicating that the selected SNPs were robust IVs. Second, the data in this study were from the European population, which avoided the bias caused by different populations to a certain extent. Third, to evaluate the bias caused by pleiotropy in MR, we used the MR-Egger regression method and found that the intercept was close to 0 ($P > 0.05$), indicating that unknown factors caused no pleiotropy. Additionally, no pleiotropy was observed in the results of the MR-PRESSO method. Third, the heterogeneity test results support the lack of heterogeneity in our results. Therefore, the selected IVs and study results were reliable.

It is widely believed that the intake of fresh fruit can reduce the risk of cancer. Some studies have shown that fresh fruit intake had a significant protective effect on the oral cavity/pharyngeal (50), lung (51), and breast cancers (52, 53), but no significant effect on ovarian (54), pancreatic (51), endometrial (51), thyroid (55), prostate (56), bladder (57), and cervical cancers (58). In this regard, we performed a two-sample MR analysis between fresh fruit intake as exposure and 11 site-specific cancers as outcome. The evidence from IVW analysis showed that each increase of fresh fruit intake by one standard development was statistically significantly associated with 35.06% decrease of breast cancer incidence

risk (P = 0.0365); however, there was no significant effect on oral cavity/pharyngeal (P = 0.0533), lung (P = 0.8809), squamous cell lung (P = 0.2163), ovarian (P = 0.0969), pancreatic (P = 0.0734), lung adenocarcinoma (P = 0.5806), endometrial (P = 0.5982), thyroid (P = 0.7896), prostate (P = 0.1772), bladder (P = 0.41), cervical (P = 0.4315), and brain cancers (P = 0.0703), this is basically consistent with the research conclusion of other researchers. The further details of the results are shown in **Supplementary Figure 5** and **Supplementary Table 5**.

Our study suggests that intake of dried fruit has potential preventive value against some site-specific cancers. Interventions in dried fruit intake may help reduce the risk of some cancers. Active health education based on dried fruit intake and reasonable adjustment of the diet ratio may help improve human quality of life. Besides, the protective effect of dried fruit intake on site-specific cancers is no less than that of fresh fruit intake. Fresh fruit consumption is usually affected by seasonal factors (59), so intake of dried fruit can be another good choice.

The impact of dried fruit intake on cancer, the relevant research is not perfect at present, but some studies mentioned that, numerous beneficial phytochemicals are conserved even after processing of fruits to be dried fruits, therefore, intake of dried fruits can help prevent cancer (60). From another point of view, the potential mechanisms of the effects of both fresh and dried fruit on cancer need to be further explored. Research on the potential mechanism behind the protective effect of dried fruits on some cancers may support the pharmacological development of cancer prevention and treatment.

However, our study has some limitations. First, the participants in this study were all of European ancestry; extrapolation of the conclusion to other populations has certain limitations. Nevertheless, we have tried our best to ensure that the research results are not disturbed by other populations and increase the possibility of extrapolation. Second, we analyzed only 11 eleven site-specific cancers, which is the site-specific cancer data that we can obtain to the greatest extent. If possible, we will continue exploring other site-specific cancers to understand the relationship between dried fruit intake and cancers fully. Third, the MR method can only analyze the causal relationship but cannot explain the mechanism behind the protective effect of dried fruit intake on some cancers. Further experimental studies are needed to explore the mechanism of the impact of dried fruit intake on the risk of cancer.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

Author contributions

YW and GC conceptualized and designed the study and had full access to all the data in the study and had responsibility for the integrity of the data, the accuracy of the analyses, and the final decision to submit the manuscript for publication. CJ, RL, TD, ZL, HL, YaY, QS, JiW, YiY, and JuW collected the data and performed the analysis. CJ drafted the initial version of the manuscript. All authors contributed to results interpretation, critically reviewed many revisions of the manuscript, and contributed to important intellectual content.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.899137/full#supplementary-material

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Effects of oily fish and its fatty acid intake on non-alcoholic fatty liver disease development among South Korean adults

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Background: The benefits of fish fatty acid intake for non-alcoholic fatty liver disease (NAFLD) are rarely reported, although a previous study assessed the relationship between oily fish consumption and the prevalence of NAFLD.

Aims: We investigated whether oily fish and fish-based monounsaturated fatty acids, polyunsaturated fatty acids, and omega-3 fatty acids affect the development of NAFLD in South Korean adults.

Methods: In this large-scale cohort study, 44,139 participants of the Health Examinees study were selected for analysis after 5 years of follow-up. NAFLD is diagnosed with a non-invasive index, the fatty liver index. Using multivariable Cox proportional hazards models, adjusted for age, body mass index, total energy intake, education, physical activity, smoking status, and drinking (alcohol) status, we calculated the hazard ratios and 95% confidence intervals.

Results: For men, NAFLD had no statistically significant associations with quartiles of total oily fish or its fatty acid intake. However, among women, an inverse association was observed (all p for trend <0.05). Regarding the standard deviation (SD) increment of total oily fish or its fatty acid intake by one, all fatty acids from oily fish showed inverse associations for NAFLD in both men and women. After stratified analyses, we found that drinking status and menopause status were independent risk factors for NAFLD. Oily fish or its fatty acid intake has the same benefit pattern on metabolic dysfunction-associated fatty liver disease as NAFLD.

Conclusion: Oily fish and its fatty acid intake showed a preventative benefit for NAFLD and metabolic dysfunction-associated fatty liver disease, especially in South Korean women.

KEYWORDS

oily fish consumption, omega-3 fatty acid, non-alcoholic fatty liver disease – NAFLD, cohort study (or longitudinal study), South Korean adults

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; CIs, confidence intervals; FFQ, food frequency questionnaire; FLI, fatty liver index; HRs, hazard ratios; MUFAs, monounsaturated fatty acids; NAFLD, non-alcoholic fatty liver disease; PUFAs, polyunsaturated fatty acids; SDs, standard deviations.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a condition characterized by predominant macro-vesicular steatosis of the liver, progressing from insulin resistance, and it can lead to steatohepatitis, fibrosis, or cirrhosis (1–3). NAFLD has become a major worldwide public health concern due to the increased risk of chronic diseases, such as type 2 diabetes mellitus and cardiovascular disease (4, 5). In the Western countries, nearly 25% of adults suffer from NAFLD, and this condition will become the most frequent indication of the need for liver transplantation by 2030 (4, 6). The overall prevalence of NAFLD is approximately 30% among South Korean adults and is twice as high in men as in women (7).

As per the existing knowledge, there are no specific drugs or therapeutic methods against NAFLD, although lifestyle (physical activity) and diet or nutrition management appear to be mainly responsible for preventing and treating NAFLD (8–10). Findings from the previous studies confirmed that a high fructose diet or a high-fat diet could accelerate NAFLD development (8, 11, 12). Fructose consumption increases insulin resistance and visceral fat and affects the lipoprotein lipase activity, leading to increased lipid uptake into the hypertrophied adipocytes (12, 13). Similarly, a high-fat diet, especially one rich in trans-fatty acid, leads to NAFLD by inducing obesity and insulin resistance (11, 14). However, not all fat in the diet is harmful to the liver; some dietary fatty acids, such as monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), are considered to be beneficial to the liver (15). MUFAs and PUFAs can reduce lipid accumulation in the liver by affecting the activity of antioxidative enzymes (16). Oily fishes are a good source of MUFAs and PUFAs, especially omega-3 PUFAs. The most frequently consumed oily fishes in South Korea are mackerel, Pacific saury, and Spanish mackerel (17, 18).

This study aimed to investigate whether the intake of oily fish or its fatty acids affected NAFLD development among general South Korean adults, focusing on fish-based MUFAs, PUFAs, and omega-3 fatty acids. Additionally, we examined differences based on sex, age, body mass index (BMI), smoking status, drinking (alcohol) status, and menopausal status.

Materials and methods

Study population

The Health Examinees study is a large-scale prospective cohort study investigating epidemiologic characteristics, genomic features, and gene–environment interactions of major chronic diseases, such as cancer, in South Korea (19). The study protocol has been described in detail elsewhere (19). The baseline survey was conducted among adults aged 40–69 years between 2004 and 2013, and the first follow-up survey was initiated between 2012 and 2016 (N = 65,642). Among these participants, those with liver-related diseases (fatty liver disease, acute liver disease, chronic hepatitis, cirrhosis, cholelithiasis, cholecystitis, and thyroid disease) at baseline (n = 10,268), with missing outcome measures (blood biomarkers) (n = 5517), without sensible dietary information (energy intake $<800/\geq4{,}000$ kcal/day for men and $<500/\geq3{,}500$ kcal/day for women) (n = 859), with alcohol abuse (alcohol intake >210 g/week for men and >140 g/week for women) (n = 4824), with missing dietary information (n = 484), or with implausible BMI value (n = 35) were excluded in the current analysis, resulting in a final sample of 43,655 adults (women, 73.26%) (20–22). Detailed information on participant selection is shown in **Supplementary Figure 1**.

All participants voluntarily signed an informed written consent form before enrollment. This study was performed in accordance with the guidelines specified in the Declaration of Helsinki, and the study protocol was approved by the local Institutional Review Board (IRB) of the Ethics Committee of the Korean Genome and Epidemiology Study of the Korea National Institute of Health (IRB No. E-1503-103-657).

Dietary assessment

Dietary data were collected using a 106-item semi-quantitative food frequency questionnaire (FFQ) developed for estimating food and nutrient consumption in South Korea. The reliability and validity of this questionnaire for South Koreans were established in a previous study assessed by four 3-day dietary records over four seasons (23). The dietary missing values were processed by imputation methods. Participants were asked about the average quantity and frequency of oily fish (mackerel/Pacific saury/Spanish mackerel) consumption during the past year at the survey time. Nine responses for frequency were ranged from "never or less than once per month" to "three times per day." Moreover, the average portion sizes were estimated by photographs.

Estimation of fatty acid intake

Data on each food item's fatty acid content were obtained from the Korean Food Composition database 9.3 (24). First, food items of the FFQ containing more than one food component were separated according to their consumption ratios in each FFQ item, and consumption of each food item was converted into grams by multiplying each FFQ item's daily consumption. Subsequently, daily fatty acid intake was calculated.

Definition of non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease was identified using the fatty liver index (FLI), a well-established non-invasive method to rule out patients with NAFLD (21, 25, 26). The FLI was calculated according to the following formula: FLI = $1/[1 + \exp(-x)] \times 100$, $x = 0.953 \times \ln$ (triglycerides) (mg/dl) + $0.139 \times$ BMI (kg/m^2) + $0.718 \times \ln$ (γ-glutamyl transferase) (U/L) + $0.053 \times$ waist circumference (cm) − 15.745. The cut-off value for non-FLI-NAFLD was set to 30 (25–27).

For each participant, fasting venous blood was collected and processed by professionals (19). Weight (kg) and height (m) were also measured at the survey time. BMI was calculated as weight divided by the square of height.

Statistical analyses

All analyses were stratified by sex and performed using SAS 9.4 (SAS Institute, Cary, NC, United States). Participants were divided into quartiles based on their intake of total oily fish and its fatty acid. Q1 represented the lowest consumption group, and Q4 represented the highest consumption group. General characteristics are presented as means and standard deviations (SDs) for continuous variables and frequencies (*n*, %) for categorical variables across quartiles of oily fish or its fatty acid intake. Differences between categories were tested by the general linear model for continuous variables and the Chi-square test for categorical variables.

Linear trends across the quartiles were tested by assigning each participant the median of the category and modeling this value as a continuous variable in models. Multivariable Cox proportional hazards models were performed to assess the relationship between FLI-NAFLD and oily fish or fatty acid ratio consumption. Results from the models were presented as hazard ratios (HRs) and 95% confidence intervals (CIs). The proportional hazard assumption was tested by including time-dependent covariates in the Cox model ($P = 0.5116$).

Sociodemographic and lifestyle characteristics, such as age, sex, level of education, physical activity, smoking, and alcohol drinking habits, were collected using standardized questionnaires. Educational level was categorized as low (under middle school), medium (high school), and high (college and above). Physical activity was determined based on participants' participation in any sports, to the point of sweating for over 30 min, at least twice a week (21). Individuals were categorized according to their smoking status as a non-smoker, past-smoker, and current smoker. Similarly, they were categorized based on drinking status as non-drinker and current-drinker after excluding alcohol abusers.

Stratified analyses were also performed to test whether the associations relied on the confounder of interest. In the stratified analysis, multivariate Cox models (adjusted for continuous and categorical confounders) were applied to assess the association between the highest consumption quartile and FLI-NAFLD, separately for age categories (age $<$ or $\geq$ median of age, 56-years for men and 52-years for women), BMI categories (BMI $<$ 25 kg/m^2 or BMI $\geq$ 25 kg/m^2), smoking status (non-smoker, past-smoker, and current-smoker), drinking status (non-drinker and current-drinker), and menopause status (yes or no). For male participants, the analyses were stratified by age, BMI, smoking status, and drinking status, respectively, while in the case of female participants, menopausal status was added other than the aforementioned interests. Substitution analysis used the leave-one-out to calculate the associations with NAFLD by substituting certain fatty acid for another type (28).

Model 1 was adjusted for categorical confounders (education, physical activity, smoking status, and drinking status) and continuous variables (age, BMI, and total energy intake). Model 2 was adjusted for the same covariates as model 1 except for altering BMI to waist circumference. Model 3 was adjusted for the same covariates as model 1 plus energy percent from daily carbohydrate, protein, and fat intake. The statistical significance was set at $P \leq 0.05$.

Results

Baseline general characteristics and NAFLD incidence of the 43,655 participants included in the analysis across the quartiles of oily fish are given in **Table 1** and **Supplementary Table 1**. Compared with the lowest consumption group (Q1), the highest consumption group (Q4) had lower age and higher waist circumference, higher BMI level, high educational level, higher physical activity, and were current-drinkers with higher alcohol consumption. Moreover, in terms of blood biomarkers, Q4 showed a lower level of serum triglycerides (all *P*-values < 0.05).

The 1-SD increment analysis of the association between FLI-NAFLD and oily fish and its fatty acid intake revealed an inverse association among female participants. The HRs of oily fish intake, ratio of total fatty acid from oily fish and total daily diet, ratio of MUFA from oily fish and total daily diet, ratio of PUFA, and ratio of omega-3 fatty acid were 0.935 (95% CI: 0.910–0.961), 0.927 (95% CI: 0.903–0.951), 0.934 (95% CI: 0.910–0.958), 0.935 (95% CI: 0.911–0.959), and 0.935 (95% CI: 0.912–0.959), respectively (**Table 2**). However, the association between oily fish consumption and FLI-NAFLD was absent for male participants (**Table 2**).

The quartile analysis revealed that female participants consuming higher quantities of oily fish were less likely to have FLI-NAFLD (highest *vs.* lowest quartile, HR: 0.839; 95% CI: 0.780–0.902; *P* for trend <0.05; **Table 2**). The multivariate-adjusted analysis also revealed that the highest quartile of fatty acid ratio was significantly associated with FLI-NAFLD (**Table 2**). However, these significances were only present in

TABLE 1 Baseline general characteristics according to quartiles of oily fish consumption.

	Total	Q1	Q2	Q3	Q4	*P*-value
Men	11,672	1,137 (38.39%)	995 (38.06%)	1,213 (37.97%)	1,162 (40.06%)	
Age, years	55.65 ± 8.49	56.07 ± 8.65	55.27 ± 8.48	55.56 ± 8.40	55.64 ± 8.42	**0.0300**
BMI, kg/m^2	23.74 ± 2.26	23.59 ± 2.32	23.70 ± 2.28	23.76 ± 2.22	23.89 ± 2.22	<**0.0001**
Waist circumference, cm	83.76 ± 6.51	83.28 ± 6.72	83.64 ± 6.54	84.00 ± 6.40	84.11 ± 6.37	<**0.0001**
Smoking status						0.4173
Non-smoker	4,097 (35.10%)	1,069 (36.09%)	937 (35.85%)	1,109 (34.71%)	982 (33.85%)	
Past-smoker	4,908 (42.05%)	1,239 (41.83%)	1,063 (40.67%)	1,359 (42.54%)	1,247 (42.99%)	
Current-smoker	2,667 (22.85%)	654 (22.08%)	614 (23.49%)	727 (22.75%)	672 (23.16%)	
Educational level						<**0.0001**
Under middle school	2,388 (20.63%)	743 (25.39%)	548 (21.13%)	626 (19.76%)	471 (16.33%)	
High school	4,575 (39.53%)	1,161 (39.68%)	1,030 (39.71%)	1,260 (39.77%)	1,124 (38.96%)	
College or above	4,610 (39.83%)	1,022 (34.93%)	1,016 (39.17%)	1,282 (40.47%)	1,290 (44.71%)	
Physical activity						<**0.0001**
Inactive	8,864 (77.77%)	2,332 (80.22%)	1,998 (78.17%)	2,469 (79.16%)	2,065 (73.33%)	
Active	2,534 (22.23%)	575 (19.78%)	558 (21.83%)	650 (20.84%)	751 (26.67%)	
Drinking status						<**0.0001**
Non-drinker	3,997 (34.24%)	1,154 (38.96%)	886 (33.89%)	1,034 (32.36%)	923 (31.82%)	
Current-drinker	7,675 (65.76%)	1,808 (61.04%)	1,728 (66.11%)	2,161 (67.64%)	1,978 (68.18%)	
Alcohol consumption, g/day	6.31 ± 7.89	5.45 ± 7.48	6.49 ± 8.09	6.61 ± 8.01	6.69 ± 7.92	**0.0013**
Total energy intake, kcal/day	1,831.96 ± 534.95	1,672.17 ± 500.07	1,751.89 ± 500.90	1,867.44 ± 482.14	2,028.19 ± 585.25	<**0.0001**
Carbohydrate, E%	72.86 ± 7.00	75.04 ± 6.54	73.12 ± 6.38	71.65 ± 6.88	69.52 ± 7.31	<**0.0001**
Protein, E%	13.29 ± 2.37	12.21 ± 1.87	12.89 ± 1.87	13.41 ± 2.09	14.30 ± 2.44	<**0.0001**
Fat, E%	13.85 ± 5.10	12.75 ± 5.06	13.99 ± 4.90	14.94 ± 5.18	16.18 ± 5.31	<**0.0001**
Oily fish intake, g/day	6.30 ± 6.17	1.50 ± 0.73	3.47 ± 0.62	6.08 ± 0.92	14.01 ± 7.81	<**0.0001**
FLI	27.73 ± 18.07	27.52 ± 18.06	27.76 ± 18.29	27.58 ± 17.99	28.07 ± 17.98	0.7764
AST, IU/L	23.56 ± 10.81	23.07 ± 6.97	23.59 ± 8.61	23.59 ± 8.25	23.97 ± 16.68	**0.0164**
ALT, IU/L	23.35 ± 12.38	22.80 ± 11.70	23.30 ± 12.32	23.34 ± 11.71	23.97 ± 13.74	**0.0019**
γ-GTP, IU/L	31.04 ± 22.79	30.39 ± 23.23	31.43 ± 25.00	31.10 ± 21.57	31.29 ± 21.52	0.6248
TG, mg/dL	120.70 ± 61.45	124.60 ± 64.02	120.84 ± 61.42	118.82 ± 60.60	118.64 ± 59.54	**0.0002**
Women	31,983	1,614 (18.71%)	1,254 (18.72%)	1,563 (18.75%)	1,543 (18.54%)	
Age, years	52.69 ± 7.67	53.24 ± 7.94	52.24 ± 7.69	52.53 ± 7.63	52.63 ± 7.38	<**0.0001**
BMI, kg/m^2	23.32 ± 2.62	23.22 ± 2.63	23.28 ± 2.64	23.34 ± 2.62	23.43 ± 2.59	<**0.0001**
Waist circumference, cm	77.40 ± 7.47	77.23 ± 7.57	77.25 ± 7.49	77.47 ± 7.39	77.61 ± 7.42	<**0.0001**
Smoking status						0.3490
Non-smoker	31,293 (97.84%)	8,429 (97.72%)	6,544 (97.72%)	8,170 (98.01%)	8,150 (97.91%)	
Past-smoker	279 (0.87%)	75 (0.87%)	58 (0.87%)	64 (0.77%)	82 (0.99%)	
Current-smoker	411 (1.29%)	122 (1.41%)	95 (1.42%)	102 (1.22%)	92 (1.11%)	
Educational level						<**0.0001**
Under middle school	11,116 (35.10%)	3,493 (40.90%)	2,376 (35.80%)	2,783 (33.75%)	2,464 (29.88%)	
High school	14,073 (44.44%)	3,502 (41.00%)	2,884 (43.45%)	3,775 (45.79%)	3,912 (47.44%)	
College or above	6,481 (20.46%)	1,546 (18.10%)	1,377 (20.75%)	1,687 (20.46%)	1,871 (22.69%)	
Physical activity						<**0.0001**
Inactive	25,342 (80.83%)	6,992 (82.42%)	5,381 (81.46%)	6,653 (81.47%)	6,316 (78.00%)	
Active	6,010 (19.17%)	1,491 (17.58%)	1,225 (18.54%)	1,513 (18.53%)	1,781 (22.00%)	
Drinking status						**0.0010**
Non-drinker	23,035 (72.02%)	6,345 (73.56%)	4,755 (71.00%)	5,936 (71.21%)	5,999 (72.07%)	
Current-drinker	8,948 (27.98%)	2,281 (26.44%)	1,942 (29.00%)	2,400 (28.79%)	2,325 (27.93%)	
Alcohol consumption, g/day	0.97 ± 2.62	0.89 ± 2.49	1.02 ± 2.73	1.00 ± 2.63	0.97 ± 2.67	0.5169

(Continued)

TABLE 1 (Continued)

	Total	Q1	Q2	Q3	Q4	*P*-value
Total energy intake, kcal/day	1,701.87 ± 566.57	1,537.54 ± 494.25	1,621.75 ± 505.69	1,716.52 ± 516.94	1,921.95 ± 652.05	<**0.0001**
Carbohydrate, E%	72.83 ± 7.42	74.21 ± 7.20	72.46 ± 7.15	71.32 ± 7.24	69.04 ± 7.62	<**0.0001**
Protein, E%	13.52 ± 2.53	12.61 ± 2.12	13.25 ± 2.15	13.67 ± 2.22	14.58 ± 2.53	<**0.0001**
Fat, E%	13.65 ± 5.37	13.18 ± 5.49	14.28 ± 5.39	15.00 ± 5.41	16.38 ± 5.56	<**0.0001**
Oily fish intake, g/day	6.48 ± 6.80	1.37 ± 0.77	3.49 ± 0.65	6.11 ± 0.92	14.54 ± 8.75	<**0.0001**
FLI	17.89 ± 15.48	17.98 ± 15.55	17.84 ± 15.45	17.84 ± 15.52	17.89 ± 15.41	0.9001
AST, IU/L	22.04 ± 10.55	22.16 ± 14.85	21.95 ± 9.11	21.93 ± 8.27	22.09 ± 8.01	0.6095
ALT, IU/L	18.93 ± 14.06	18.96 ± 16.88	18.69 ± 12.81	18.92 ± 13.61	19.10 ± 12.10	0.3479
γ-GTP, IU/L	19.97 ± 15.24	20.08 ± 15.30	19.77 ± 15.60	19.90 ± 14.70	20.09 ± 15.39	0.4464
TG, mg/dL	107.3 ± 62.11	113.41 ± 67.62	106.62 ± 61.29	106.14 ± 59.57	102.66 ± 58.72	<**0.0001**

AST, aspartate transaminase; ALT, alanine aminotransferase; BMI, body mass index; FLI, fatty liver index; NAFLD, non-alcoholic fatty liver disease; Q, quartile; TG, triglyceride; WC, waist circumference; γ-GTP, γ-glutamyl transpeptidase. Values are shown as means ± standard deviations or *n* (%).
Comparisons were performed using a Chi-square test for categorical variables and a generalized linear model for continuous variables. Boldface indicates statistical significance ($P < 0.05$). FLI = $1/[1 + \exp(-x)] \times 100$, $x = 0.953 \times \ln$ (TG) (mg/dL) + $0.139 \times$ BMI (kg/m^2) + $0.718 \times \ln$ (γ-GTP) (U/L) + $0.053 \times$ WC (cm) − 15.745; (cut-off value, 30).

TABLE 2 Hazard ratios (HRs) for fatty liver index – non-alcoholic fatty liver disease (FLI-NAFLD) according to the quartiles of oily fish and its fatty acid intake.

	Q1	Q2	Q3	Q4	*P* for trend	1-SD increment
Men						
Oily fish	Ref	0.898 (0.824, 0.978)	0.958 (0.883, 1.040)	0.965 (0.886, 1.050)	0.9399	0.974 (0.946, 1.002)
Total fatty acid/fat	Ref	0.974 (0.898, 1.058)	0.941 (0.866, 1.022)	0.901 (0.828, 0.981)	**0.0116**	0.942 (0.911, 0.973)
MUFA/total dietary MUFA	Ref	0.947 (0.872, 1.028)	0.942 (0.867, 1.023)	0.947 (0.872, 1.030)	0.3524	0.957 (0.926, 0.989)
PUFA/total dietary PUFA	Ref	0.997 (0.918, 1.083)	0.983 (0.905, 1.068)	0.976 (0.898, 1.061)	0.5293	0.964 (0.935, 0.994)
Omega-3/dietary omega-3	Ref	0.993 (0.914, 1.079)	1.017 (0.936, 1.104)	0.956 (0.879, 1.038)	0.2831	0.962 (0.934, 0.990)
Women						
Oily fish	Ref	0.925 (0.859, 0.996)	0.962 (0.897, 1.032)	0.839 (0.780, 0.902)	<**0.0001**	0.935 (0.910, 0.961)
Total fatty acid/fat	Ref	0.890 (0.828, 0.957)	0.878 (0.817, 0.944)	0.806 (0.750, 0.867)	<**0.0001**	0.927 (0.903, 0.951)
MUFA/total dietary MUFA	Ref	0.918 (0.853, 0.988)	0.907 (0.843, 0.975)	0.834 (0.776, 0.896)	<**0.0001**	0.934 (0.910, 0.958)
PUFA/total dietary PUFA	Ref	0.953 (0.886, 1.025)	0.916 (0.853, 0.985)	0.858 (0.799, 0.922)	<**0.0001**	0.935 (0.911, 0.959)
Omega-3/dietary omega-3	Ref	0.939 (0.873, 1.009)	0.948 (0.882, 1.018)	0.853 (0.794, 0.917)	<**0.0001**	0.935 (0.912, 0.959)

BMI, body mass index; FLI, fatty liver index; HRs, hazard ratios; MUFA, monounsaturated fatty acid; NAFLD, non-alcoholic fatty liver disease; PUFA, polyunsaturated fatty acids; Q, quartile; SD, standard deviation; WC, waist circumference. Oily fish: Mackerel/Pacific saury/Spanish mackerel. Total fatty acid/fat: ratio of total fatty acid from oily fish and total dietary fatty acid. MUFA/total dietary MUFA: ratio of total MUFA from oily fish and total dietary fatty acid. PUFA/total dietary PUFA: ratio of total PUFA from oily fish and total dietary fatty acid. Omega-3/dietary omega-3: ratio of total omega-3 PUFA from oily fish and total dietary fatty acid. Model was adjusted by age, BMI, total energy intake, smoking status, drinking status, physical activity level, and educational level. Boldface indicates statistical significance ($P < 0.05$). NAFLD is defined by an FLI of >30. FLI = $1/[1 + \exp(-x)] \times 100$, $x = 0.953 \times \ln$ (TG) (mg/dL) + $0.139 \times$ BMI (kg/m^2) + $0.718 \times \ln$ (γ-GTP) (U/L) + $0.053 \times$ WC (cm) − 15.745.

female participants, except that the association with the total fatty acid ratio was significant in both sexes (highest vs. lowest quartile, HR: 0.901; 95% CI: 0.828–0.981; HR: 0.806; 95% CI: 0.750–0.867 in male and female participants, respectively, both *P* for trend <0.05; Table 2). After further adjusted analyses (models 2 and 3, Supplementary Table 2), the results stayed consistent.

The stratified analysis of participants revealed almost no differences in the effect of various groups toward FLI-NAFLD (Figures 1, 2). Among female participants, the association between the various dietary exposure groups and FLI-NAFLD was maintained regardless of age, BMI, and drinking status, whereas smoking status and menopause status were weakly associated with FLI-NAFLD (Figure 2). Among non-smokers and post-menopausal participants, oily fish or its fatty acid intake resulted in a significantly lower risk of FLI-NAFLD development (Figure 2). Age and drinking status were weakly associated with FLI-NAFLD among male participants (Figure 1).

The results from a leave-one-out substitution analysis are shown in Supplementary Table 3. Among female participants, after replacing fatty acid intake (from other food sources) with a fatty acid intake predominantly from oily fish, a one-unit increment of fatty acid was associated with a lower risk of FLI-NAFLD. The converse was also true. All adjusted models showed the same association pattern. However, there was no significant association for male participants.

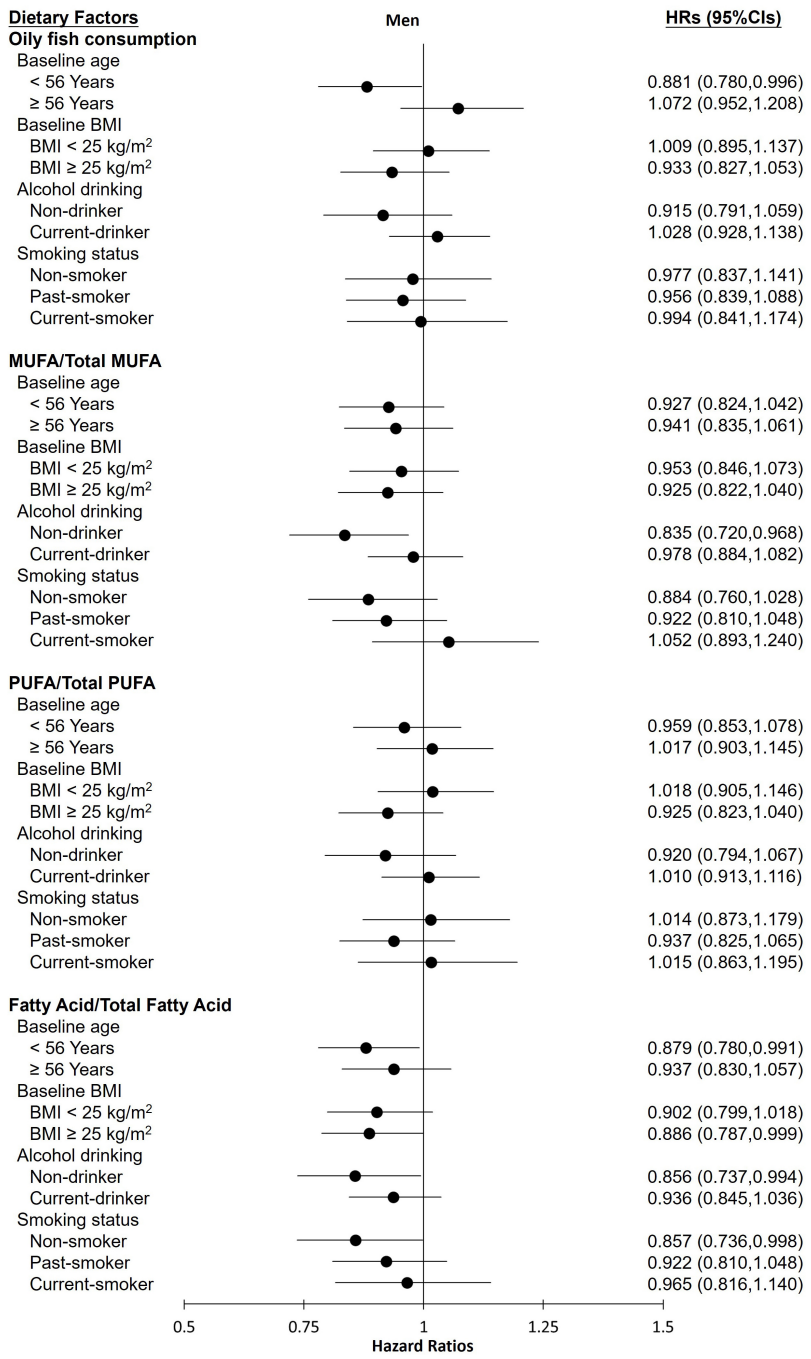

FIGURE 1
Hazard ratios (HRs) of fatty liver index (FLI)-non-alcoholic fatty liver disease for the highest categories compared with the lowest categories of oily fish and its fatty acid intake among male participants in the current cohort study. Analyses were stratified by body mass index (BMI), age, smoking status, and drinking status. HRs, hazard ratios; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

Discussion

Here, we evaluated the association between oily fish intake and its fatty acid consumption and FLI-defined NAFLD in a large-scale cohort of general adults recruited from 38 sites of South Korea.

A significant inverse association between high oily fish intake and FLI-NAFLD among female participants was found. The analysis also revealed that oily fish-sourced fatty acids, such as MUFAs, PUFAs, and omega-3 PUFAs, have preventative benefits for NAFLD. Moreover, the association continued to exist after being stratified by age, BMI, smoking status, drinking

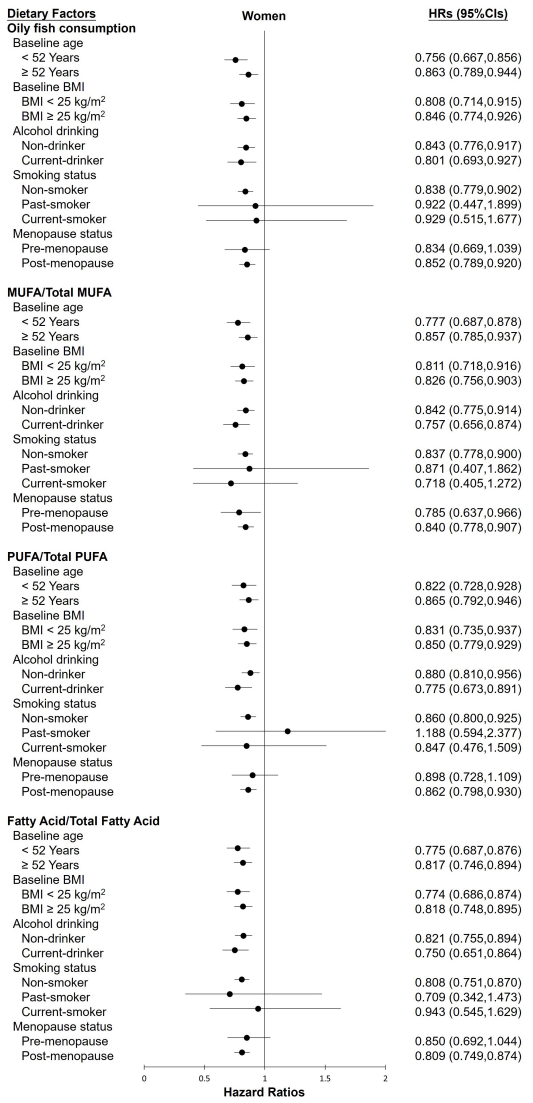

FIGURE 2
The HRs of FLI-non-alcoholic fatty liver disease for the highest categories compared with the lowest categories of oily fish and its fatty acid intake among female participants in the current cohort study. Analyses were stratified by body mass index (BMI), age, smoking status, drinking status, and menopause status. HRs, hazard ratios; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

status, and menopausal status. Although covariates, such as smoking status and menopausal status, impact the effects of oily fish and its fatty acid intake on FLI-NAFLD after stratified analysis, in most ways, total intake of oily fish and its fatty acids resulted in preventative effects independently, regardless of age, BMI, and drinking alcohol status.

Non-alcoholic fatty liver disease is a common chronic disease wherein triglycerides accumulate excessively in the liver without alcohol abuse (29). Moreover, NAFLD is closely associated with diabetes and metabolic syndrome, which are both related to the pathophysiology of inflammation and insulin resistance (30, 31). Dietary MUFAs have been reported to improve lipid profile through their anti-inflammatory characteristics (30, 32). Oily fish is protective against NAFLD owing to its omega-3 PUFA contents, which impact the lipid profile (33–35). Mackerel and Pacific saury are the types of oily fishes that have been reported to be enriched in various fatty acids, especially omega-3 PUFA (36–40).

Omega-3 PUFA has been previously associated with reducing the risk of NAFLD development in various epidemiological studies (41–46). A dietary intervention study suggested that patients with NAFLD showed a lower level of circulating liver enzymes and triglycerides, with a significant improvement of adiponectin after long-term (1 year) consumption of omega-3 PUFA (42). Furthermore, a cross-sectional study revealed that fish and omega-3 PUFA intake was associated with decreasing portal and lobular inflammation and a lower risk for hepatic inflammation among children (43). In Japan, a cross-sectional study conducted in adults showed that omega-3 PUFA was not an independent risk factor for NAFLD. However, dietary eicosapentaenoic acid and eicosapentaenoic acid + docosahexaenoic acid were preventive nutrients for NAFLD and improved inflammatory change in adipose tissue in men (47, 48). These findings are partially in line with our results that oily fish and its omega-3 PUFA content are associated with a lower incidence of FLI-NAFLD, while the effect of omega-6 PUFA content has not yet been fully elucidated.

Our findings also suggest that menopausal status is an independent risk factor for FLI-NAFLD among female participants. This result is partially in line with that of a previous study, which demonstrated that menopausal status change was correlated with NAFLD through altering sex hormones, and dietary factors could exacerbate the relationship (29). The previous study convinced that smoking and drinking were associated with higher prevalence of NAFLD (49) and in current study, men and women also showed gender differences in smoking and drinking habits shown in **Table 1**. Less current smokers and alcohol drinkers in women than men participants maybe another explanation to significant results only found in women. Further, it is important to consider sex difference, which may be another risk factor leading to different results of this study. A previous review research reported that adipose tissue distribution, gut microbiota, and innate immune response showed some sex differences (50). Adipose tissue and innate immune response play an important role in regulating insulin resistance and inflammatory reaction (51, 52). The gut microbiota could regulate lipid/glucose metabolism by activating the farnesoid X receptor (53).

The strength of the current study is that we conducted a large-scale cohort study in South Korea, and the result is partly adapted to the general population. Moreover, in PUFA and MUFA, we focused on oily fish-sourced fatty acids instead of its supplements or other dietary sources. However, this study has

certain limitations. First, the diagnosis of NAFLD was not based on a liver biopsy. However, the FLI used in the current study has been evaluated and verified in a previous study and is considered an appropriate tool for large nutritional epidemiological studies (21, 25, 26, 54). Second, rather than the exact time that FLI-NAFLD occurred, the endpoint was set on the time conducting follow-up survey. Considering soft endpoints more common in observational study, and it has little impacts on large-scale cohort study, this limitation could be negligible (55). Third, the fish-sourced fatty acid contents were not measured directly, but through linking the Korean Food Composition Database 9.3 to FFQ data, and different cooking or storage methods may lead to possible bias. Future studies should consider these factors. Finally, the lifestyle of participants was assessed by a self-reported questionnaire such as smoking, drinking, and physical activity, which may be overreported or under reported. So we grouped them as categorical variables when adjusting model to minimize the reporting bias.

Conclusion

In conclusion, we have demonstrated that the intake of oily fish and its fatty acid contents, such as MUFAs, PUFAs, and omega-3 PUFAs, is associated with a lower incidence of NAFLD. As a result, although we did not study their precise molecular mechanisms, oily fish may be considered effective preventative strategies for NAFLD development among South Koreans, especially for women. These findings may provide a basis for revising middle-aged and older adults' dietary guidelines in South Korea.

Data availability statement

The datasets presented in this article are not readily available because the datasets analyzed for this study can be available from National Genome Research Institute, Korea Centers for Disease Control and Prevention. Restrictions apply to the availability of these data, which were used under license for this study. Data described in the manuscript, codebook, and analytic code are available from the authors with the permission of National Genome Research Institute, Korea Centers for Disease Control and Prevention. Requests to access the datasets should be directed to National Genome Research Institute, Korea Centers for Disease Control and Prevention; https://kdca.go.kr/contents.es?mid=a40504060100.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board (IRB) of the Ethics Committee of the Korean Genome and Epidemiology Study of the Korea National Institute of Health (IRB No. E-1503-103-657). The patients/participants provided their written informed consent to participate in this study.

Authors contributions

SS designed and conducted the research and reviewed and revised the manuscript critically. L-JT analyzed the data, performed the statistical analysis, and wrote the first draft of the manuscript. SS and L-JT had primary responsibility for the final content. Both authors approved the final version of the article.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.876909/full#supplementary-material

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Higher dietary inflammatory index is associated with increased all-cause mortality in adults with chronic kidney disease

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Background: Diet property grounded on inflammatory potential, evaluated by the dietary inflammatory index (DII), has been proven to be connected with mortality, while studies of adults with chronic kidney disease (CKD) are scarce.

Objective: The purpose of this research was to evaluate the interrelationships between DII and all-cause mortality among adults with CKD.

Methods: In the National Health and Nutrition Examination Survey (NHANES) 2001–2006, we identified and evaluated data of 4,554 adults with CKD. DII scores were calculated from 24 h of dietary consumption at baseline. Vital status was followed through 31 December 2015. The association of all-cause mortality with DII score was assessed using the Kaplan–Meier curve and the Cox regression analysis.

Results: After an average follow-up of 132.103 months, a total of 1,246 (27.36%) deaths were recorded. The death rates in the DII tertile categories were 24.04, 26.81, and 31.23%, respectively. The Kaplan–Meier curve showed increased death risks for the high DII tertile as compared with the low DII tertile. After we adjusted for a broad range of possible confounders, the estimation between extreme tertiles of DII scores presented a positive and significant association with all-cause mortality [hazard ratio (*HR*): 1.21, 95% *CI*: 1.05–1.39].

Conclusion: Our results confirm the hypothesis that proinflammatory diets contribute to the increased all-cause mortality in adults with CKD.

KEYWORDS

dietary inflammatory index (DII), chronic kidney disease, mortality, nutrition, national health and nutrition examination survey (NHANES)

Introduction

Despite ongoing progress in the therapeutic regimen for chronic kidney disease (CKD), the mortality rate of this group of patients is still high (1). Chronic systemic inflammation often occurs in people with CKD and limits overall survival (2). Diet may have a key role in the regulation of chronic inflammation, as specific food ingredients have various anti- or proinflammatory properties that can influence immunology. On this basis, the inflammatory potential of diverse nutrients was quantified using the dietary inflammatory index (DII). The DII scoring system was independent of specific population means, which contributed to its superiority (3).

Previous studies have found a link between the DII score and mortality. The MONICA/KORA Cohort demonstrated a 41% higher risk of all-cause death comparing the highest to lowest DII category (4). Another cohort with meta-analysis reported an increased risk of all-cause, cardiovascular, and cancer mortality in a population with higher DII scores (5). As a prospective cohort reported, in survivors after coronary artery bypass grafting surgery, a pro-inflammatory diet was positively associated with mortality (6). After comparing the highest to the lowest DII category in the Japanese population, a 13% increased risk of all-cause mortality was identified (7). Therefore, the association between a pro-inflammatory diet and a higher death risk was confirmed by recent reports.

To the best of our knowledge, the relationship between DII and death risk in the CKD population has not yet been stated. Therefore, the investigation of dietary quality, especially in patients suffering from CKD who may be restricted in dietary consumption, is crucial to improve the inflammatory profile, along with its impact on the death risk. Generally, the purpose of the current study is to explore the relationship between the dietary factor and all-cause mortality among the adult CKD population, which is grounded on the large U.S. nationally representative sample with a retrospective design. It is proposed that DII may be positively associated with death risk in the CKD population.

Materials and methods

Data collection

Study sample

The study data originating from the National Health and Nutrition Examination Survey (NHANES) (RRID:SCR_013201) 2001–2006 were combined to increase the sample size. The NHANES was sponsored by the National Center for Health Statistics (NCHS), which used a stratified, multistage architecture to get a nationally representative sampling from the U.S. population.

In the current study, the urinary albumin-creatinine ratios (ACRs) and estimated glomerular filtration rate (eGFR) criteria were used to define CKD. ACRs were acquired from the urinary testing results and classified as less than 30, 30–300, or greater than 300 mg/g. The eGFR was calculated using the isotope dilution mass spectrometry 4-variable Modification of Diet in Renal Disease Study equation (MDRD)(8). CKD stages were categorized according to the following scale: stage 1, ACR $\geq$ 30 mg/g, along with eGFR $>$ 90 mL/min/1.73 m^2; stage 2, ACR $\geq$ 30 mg/g, along with 60 $\leq$ eGFR $<$ 89 mL/min/1.73 m^2; stage 3, 30 $\leq$ eGFR $<$ 59 mL/min/1.73 m^2; stage 4, 15 $\leq$ eGFR $<$ 29 mL/min/1.73 m^2; and stage 5, eGFR $<$ 15 mL/min/1.73 m^2 (9).

There were a total of 31,509 samples included from 2001 to 2006. According to the criteria, 6,330 CKD participants were selected. We identified 5,131 adults after excluding 1,199 participants less than 18 years old. After the exclusion of 570 participants with missing dietary consumption information, 5 participants with unknown death data, and 2 participants with both data missing, the final analysis enrolled 4,554 samples. The major characteristics between the final sample and participants with unavailable DII were compared (**Supplementary Material 1**). The NCHS's Institutional Review Board (IRB) authorized the NHANES protocol. The ethics review from the IRB committee of our center was exempted because this study relied on publicly used, de-identified secondary data.

Dietary inflammatory index and mortality data

The scoring system of DII was proposed by Shivappa to assess the inflammatory gradations of 45 food parameters, which were standardized to dietary intake from representative populations around the world (3). The overall DII is calculated from the sum of individual nutrient scores from the food taken in 24 h, which includes the score coming from both the anti-inflammatory and pro-inflammatory diets. Each nutrient parameter was scored according to whether it increased (+ 1), decreased (–1), or had no effect (0) on the inflammatory biomarkers. These scores were weighted based on the study design and were called inflammatory effect scores. To avoid the arbitrariness resulting from simply using raw consumption amounts, intakes of foods and nutrition were standardized to a representative range of dietary intakes based on actual human consumption in 11 populations living in different countries across the world that provided an estimate of a mean and standard deviation (SD) for each parameter. By adding each DII score, we can achieve an individual "overall DII score."

In this analysis, we used only observed intakes from the first 24-h dietary recall. The first 24-h dietary consultation was held in a private space in the NHANES mobile examination center. A series of measuring tools (various circles, bean bags, glasses, thickness sticks, bowls, mugs, a ruler, household spoons, measuring cups, and spoons) were accessible in the interview room for addressing the quantity of dietary intake.

In total, thirty-three available kinds of food components from the NHANES dataset were used to obtain the overall DII, which includes energy, ethanol, vitamin B12/B6, fiber, magnesium, total fat, monounsaturated fatty acids, b-carotene, caffeine, niacin, docosapentaenoic (22:5), eicosatetraenoic (20:4), octadecatetraenoic (18:4), octadecatrienoic (18:3), eicosapentaenoic (20:5), docosahexaenoic (22:6), octadecadienoic (18:2), protein, polyunsaturated fatty acids, folic acid, selenium, iron, thiamin, carbohydrate, cholesterol, vitamins A/C/D/E, riboflavin, saturated fat, and zinc. The more negative DII level represents a more anti-inflammatory dietary intake, whereas the increased score reflects a more proinflammatory dietary intake.

Mortality was defined as a binary variable for alive or dead. The mortality data were obtained from the National Death Index (NDI) files, which were linked to the NHANES dataset through 31 December 2015. A total of 12 characteristics (such as date of birth, sex, and social security number) were acquired to connect the NHANES samples with the NDI to confirm survival status. The follow-up period was calculated from the interview to the happening of the death event or the end of 2015. Due to massive data missing regarding the specific cause of death, we use only all-cause mortality in our analysis.

Covariates

If the covariates could shift the estimates of DII on mortality exceeding 10% or had previously reported association with mortality in the CKD population, they were adopted in the fully adjusted models. A series of covariates were acquired accordingly: basic demographic information, such as age at baseline, sex, and race; personal characteristics and underlying diseases, such as waist circumference, body mass index (BMI), physical activity, congestive heart failure, coronary heart disease, stroke, chronic kidney disease, and hypertension; laboratory detection data, such as urinary albumin, urinary creatinine, albumin, phosphorus, serum glucose, serum vitamin B12, hemoglobin, uric acid, and inferred data eGFR. The race was designated as Mexican American, Non-Hispanic white, Non-Hispanic black, other Hispanic, and other races. Physical activity was categorized into four grades according to the level of activity intensity: sit during the day, stand/walk a lot, light load/climb stairs often, and heavy work/load. Respondents who answered yes to the following questions were classified as being diagnosed with the corresponding disease: "Have you ever been informed by a health professional or a doctor that you had hypertension/congestive heart failure/stroke/chronic kidney disease/coronary heart disease?" The missing values regarding waist circumference ($n = 224$), BMI ($n = 167$), urinary albumin ($n = 85$), urinary creatinine ($n = 85$), hemoglobin ($n = 176$), serum vitamin B12 ($n = 246$), albumin ($n = 264$), serum glucose ($n = 264$), phosphorus ($n = 264$), and uric acid ($n = 264$) were imputed with median.

Statistical analysis

Continuous variates are indicated as mean ± standard deviation (SD) or median (range) according to data distribution, while categorical variates given as frequencies and percentages. The Kruskal–Wallis H-test (skewed distribution), one-way ANOVA test (normal distribution), and χ^2 (categorical variables) were applied to evaluate variance among different DII (tertiles). The Kaplan–Meier method was used to estimate the survival among different DII levels, and any differences in survival were evaluated with a stratified log-rank test. The study population was censored on 31 December 2015 if they survived the thorough follow-up cycle. The univariate and multivariate Cox proportional-hazards regression models were applied to analyze the relationship between DII and all-cause mortality with three different models. Therefore, no covariates were adjusted in model 1, and only socio-demographic variables were adjusted in model 2, while the covariates presented in **Table 1** were fully adjusted in model 3. To test the robustness of our results, we converted DII into a categorical variable according to tertile and calculated the p for trend to confirm the results of DII as the continuous variable, along with the examination of non-linear possibility.

All the statistical analyses were performed with software EmpowerStats (X&Y Solutions, Inc., Boston, MA)[1] and software R (The R Foundation, RRID:SCR_001905).[2] The results were affirmed statistically significant if p-values were less than 0.05 (two-sided).

Results

The median DII score was 1.464 (0.152–2.382). After analyzing 4,554 participants, we found a mean follow-up period of 132.103 months with a maximum of 181 months. In total, 1,246 (27.36%) death events were recorded. The baseline characteristics of the study cohort are presented in **Table 1**. Globally, samples with higher DII scores are more prone to be female and to have lower levels of physical activity. The prevalence of hypertension and stroke was higher in participants in the high DII tertile, as these basic diseases were correlated with the DII score. Besides, samples with higher DII levels are more prone to have higher values in urinary albumin, urinary creatinine, with lower values in eGFR, hemoglobin, albumin, and serum vitamin B12. Moreover, the composition of race is also different among the tertiles.

To illustrate the propensity of mortality hazards across the levels of DII per time, the Kaplan–Meier survival functions stratified by different levels of DII were presented in **Figure 1**.

1 http://www.empowerstats.com
2 http://www.R-project.org

TABLE 1 Baseline characteristics of the study participants with chronic kidney disease (CKD) (n = 4,554).

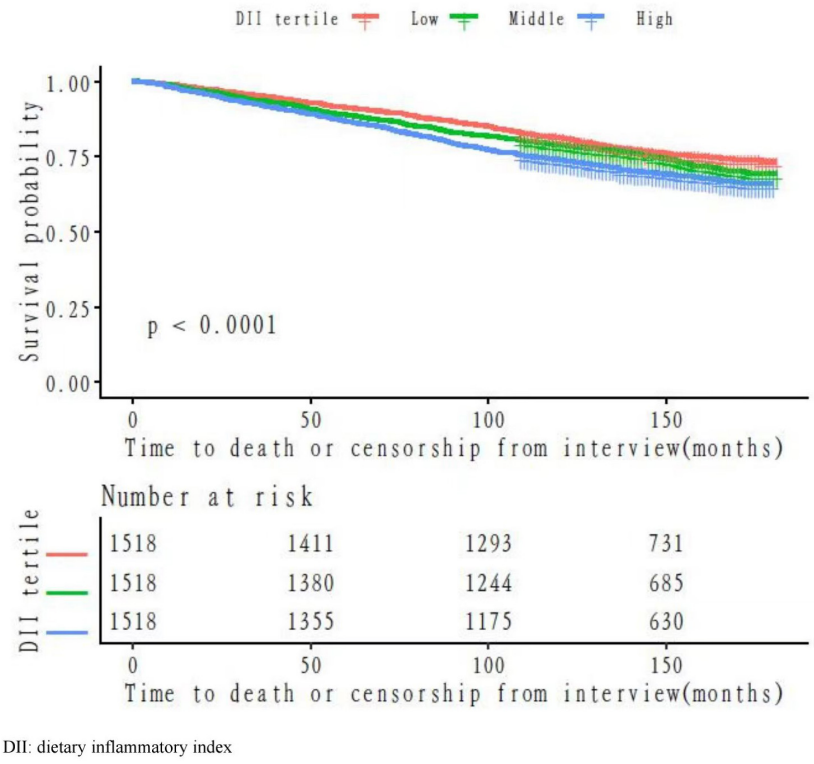
Characteristics	Dietary inflammatory index			*P*-values
	Low (−4.55 to 0.63)	Middle (0.64–2.07)	High (2.08–4.44)	
N	1,518	1,518	1,518	
Age (year)	53.93 (19.48)	54.47 (19.72)	55.62 (19.99)	0.055
BMI (kg/m^2)	28.35 (6.49)	28.83 (7.05)	28.77 (6.76)	0.102
Waist circumference (cm)	97.52 (15.87)	97.99 (15.03)	97.60 (15.20)	0.671
eGFR (mL/min/1.73 m^2)	57.75 (24.88)	57.76 (24.28)	55.80 (21.54)	0.031
Urinary albumin (μg/mL)	9.60 (4.30–33.38)	11.45 (5.30–44.80)	11.80 (5.50–40.88)	< 0.001
Urinary creatinine (g/L)	0.99 (0.53–1.49)	1.06 (0.63–1.56)	1.04 (0.63–1.63)	< 0.001
Hemoglobin (g/dL)	14.02 (1.51)	13.88 (1.52)	13.73 (1.47)	< 0.001
Albumin (g/dL)	4.17 (0.35)	4.12 (0.36)	4.12 (0.34)	< 0.001
Serum VitB12 (pg/mL)	487.50 (375.25–666.50)	472.00 (355.25–609.50)	472.00 (348.25–592.75)	< 0.001
Serum glucose (mg/dL)	101.34 (38.18)	103.32 (42.23)	102.23 (40.37)	0.399
Phosphorus (mg/dL)	3.80 (0.55)	3.79 (0.53)	3.80 (0.52)	0.531
Uric acid (mg/dL)	5.21 (1.46)	5.31 (1.50)	5.30 (1.48)	0.151
Time to death or censorship from interview (months)	135.97 (41.18)	132.53 (44.15)	127.82 (46.60)	< 0.001
Gender				< 0.001
Female	960 (63.24%)	1,048 (69.04%)	1,164 (76.68%)	
Male	558 (36.76%)	470 (30.96%)	354 (23.32%)	
Stroke				< 0.001
Yes	65 (4.28%)	70 (4.61%)	117 (7.71%)	
No	1,392 (91.70%)	1,377 (90.71%)	1,322 (87.09%)	
No records	61 (4.02%)	71 (4.68%)	79 (5.20%)	
Coronary heart disease				0.477
Yes	88 (5.80%)	101 (6.65%)	94 (6.19%)	
No	1,360 (89.59%)	1,330 (87.62%)	1,338 (88.14%)	
No records	70 (4.61%)	87 (5.73%)	86 (5.67%)	
Congestive heart failure				0.253
Yes	68 (4.48%)	71 (4.68%)	88 (5.80%)	
No	1,382 (91.04%)	1,367 (90.05%)	1,346 (88.67%)	
No records	68 (4.48%)	80 (5.27%)	84 (5.53%)	
Hypertension				0.017
No	920 (60.61%)	865 (56.98%)	838 (55.20%)	
Yes	593 (39.06%)	642 (42.29%)	674 (44.40%)	
No records	5 (0.33%)	11 (0.72%)	6 (0.40%)	
Physical activity				< 0.001
Sit during the day	340 (22.40%)	400 (26.35%)	523 (34.45%)	
Stand/walk a lot	383 (25.23%)	430 (28.33%)	398 (26.22%)	
Light load/climb stairs often	258 (17.00%)	265 (17.46%)	201 (13.24%)	
Heavy work/load	447 (29.45%)	337 (22.20%)	330 (21.74%)	
No records	90 (5.93%)	86 (5.67%)	66 (4.35%)	
Race				< 0.001
Black	241 (15.88%)	309 (20.36%)	369 (24.31%)	
Mexican_American	335 (22.07%)	315 (20.75%)	283 (18.64%)	
Other_Hispanic	48 (3.16%)	55 (3.62%)	64 (4.22%)	
Other_race, ethnicity	894 (58.89%)	839 (55.27%)	802 (52.83%)	

(Continued)

TABLE 1 (Continued)

Characteristics	Dietary inflammatory index			*P*-values
	Low (−4.55 to 0.63)	Middle (0.64–2.07)	High (2.08–4.44)	
Chronic kidney disease				0.430
Stage1	137 (9.03%)	134 (8.83%)	108 (7.11%)	
Stage2	184 (12.12%)	207 (13.64%)	202 (13.31%)	
Stage3	1,144 (75.36%)	1,130 (74.44%)	1,155 (76.09%)	
Stage4	41 (2.70%)	35 (2.31%)	45 (2.96%)	
Stage5	12 (0.79%)	12 (0.79%)	8 (0.53%)	
Mortality				< 0.001
Alive	1,153 (75.96%)	1,111 (73.19%)	1,044 (68.77%)	
Death	365 (24.04%)	407 (26.81%)	474 (31.23%)	

Values are presented as mean (standard deviation, SD) or median (Q1–Q3) for continuous variables, and as number (percentage) for categorical variables.
BMI, body mass index; eGFR, estimated glomerular filtration rate.



DII: dietary inflammatory index

FIGURE 1
Survival probability by the level of the dietary inflammatory index (DII).

The high tertile of DII had the lowest overall survival benefit when compared with the other tertiles ($p < 0.05$ for the log-rank test).

In this retrospective cohort, we observed an association between the DII score and all-cause death risk (Table 2). The analyses on continuous DII score revealed a 9% (95% *CI*: 1.05–1.13) higher hazard of death with each 1-unit growth of the DII score, without other confounders adjusted. The death risk persisted when confounders were fully adjusted (*HR*: 1.05, 95% *CI*: 1.01–1.09). The death rates in the DII tertile categories were 24.04, 26.81, and 31.23%, respectively. Participants with the highest DII score were prone to gain greater risk of death as compared with those with the lowest tertile (*HR*: 1.38, 95% *CI*: 1.21–1.59), without other confounders adjusted. The association persisted after potential confounders were further controlled (*HR*: 1.21, 95% *CI*: 1.05–1.39). Additionally, analyses with DII

TABLE 2 Multivariate analysis of the dietary inflammatory index associated with all-cause mortality, stratified by potential effect modifiers.

	Total individuals (No. of deaths)	HR (95% CI), P	
Dietary inflammatory index			
Low	1,518 (366)	1.0	
Middle	1,518 (407)	1.13 (0.98, 1.31) 0.0847	
High	1,518 (473)	1.21 (1.05, 1.39) 0.0077	
Ptrend		0.0081	
Continuous (1-unit increment)	4,554 (1,246)	1.05 (1.01, 1.09) 0.0104	
Stratification analysis			P interaction
Age (year)			0.0278
< 65	2,909 (306)	1.14 (1.05, 1.23) 0.0008	
≥65	1,645 (940)	1.03 (0.99, 1.08) 0.1267	
Gender			0.8377
Female	3,172 (701)	1.05 (1.00, 1.11) 0.0440	
Male	1,382 (545)	1.05 (0.99, 1.10) 0.0016	
Body mass index (kg/m^2)			0.6876
< 25	1,383 (380)	1.03 (0.96, 1.10) 0.4542	
≥25, < 30	1,657 (514)	1.07 (1.01, 1 .13) 0.0316	
≥30	1,514 (352)	1.05 (0.98, 1.13) 0.1287	
Waist circumference tertile (cm)			0.5844
< 91.2	1,515 (314)	1.02 (0.95, 1.09) 0.5864	
≥ 91.2, < 102.6	1,515 (499)	1.05 (0.98, 1.11) 0.1586	
≥102.6	1,524 (433)	1.07 (1.01, 1.14) 0.0287	
Hemoglobin tertile (g/dL)			0.1251
<13.4	1,508 (435)	1.04 (0.97, 1.11) 0.2810	
=13.4, < 14.5	1,514 (404)	1.01 (0.95, 1.08) 0.6975	
=14.5	1,532 (407)	1.11 (1.04, 1.18) 0.0013	
Albumin tertile (g/dL)			0.0528
<4.0	1,145 (382)	1.00 (0.93, 1.07) 0.9262	
≥4.0, < 4.3	1,739 (484)	1.11 (1.05, 1.18) 0.0007	
≥4.3	1,670 (380)	1.03 (0.96, 1.10) 0.3970	
Serum glucose (mg/dL)			0.5434
≥108	3,699 (872)	1.05 (1.01, 1.10) 0.0198	
>108	855 (374)	1.03 (0.96, 1.10) 0.4408	
Urinary albumin tertile (μg/mL)			0.9455
<6.8	1,512 (249)	1.04 (0.97, 1.13) 0.2560	
≥6.8, < 23.2	1,524 (401)	1.06 (0.99, 1.13) 0.0990	
≥23.2	1,518 (596)	1.04 (0.99, 1.10) 0.1210	
Urinary creatinine tertile (g/L)			0.7725
<0.74	1,495 (442)	1.07 (1.01, 1.14) 0.0241	
≥0.74, < 1.35	1,530 (497)	1.04 (0.98, 1.10) 0.1954	
≥1.35	1,529 (307)	1.06 (0.98, 1.14) 0.1587	
Serum VitB12 tertile (pg/mL)			0.9092
<399	1,514 (418)	1.06 (0.99, 1.13) 0.0968	
≥399, < 560	1,522 (408)	1.06 (0.99, 1.13) 0.0826	
≥560	1,518 (419)	1.04 (0.98, 1.11) 0.1747	
Phosphorus tertile (mg/dL)			0.4681
<3.6	1,380 (401)	1.04 (0.98, 1.12) 0.1865	
≥3.6, < 4.0	1,561 (434)	1.09 (1.02, 1.16) 0.0113	
≥4.0	1,613 (411)	1.03 (0.96, 1.09) 0.3981	

(Continued)

TABLE 2 (Continued)

	Total individuals (No. of deaths)	HR (95% CI), P	
Uric acid tertile (mg/dL)			1.0000
<4.6	1,469 (250)	1.03 (0.97, 1.08) 0.3170	
≥4.6, <5.7	1,502 (388)	1.06 (1.00, 1.12) 0.0507	
≥5.7	1,583 (608)	1.03 (0.98, 1.08) 0.1936	
Race			0.3041
Black	919 (225)	1.09 (0.99, 1.20) 0.0658	
Mexican_American	933 (176)	0.99 (0.90, 1.10) 0.9175	
Other Hispanic	167 (24)	0.87 (0.63, 1.21) 0.4066	
Other race, ethnicity	2,535 (821)	1.08 (1.03, 1.13) 0.0019	

Models were adjusted for race, waist circumference, stroke, coronary heart disease, congestive heart failure, eGFR, urinary albumin, urinary creatinine, hemoglobin, serum vitamin B12, albumin, serum glucose, phosphorus, uric acid, hypertension, physical activity, chronic kidney disease, except for the stratification factor itself.

tertile as a continuous variable showed a significant *p* for the trend in the adjusted model ($p_{trend} = 0.0081$).

The subgroup analyses, stratified by potential effect modifiers, did not reveal prominent differences regarding the impact of DII on all-cause death, except for in the categories of age (< 65 vs. ≥ 65 years) (**Table 2**). DII contributed more to the death risk in adults less than 65 years of age than in the elderly population (*HR*: 1.14, 95% *CI*: 1.05–1.23 vs. 1.03, 95% *CI*: 0.99–1.08), with *p* interaction < 0.05.

Discussion

In this large retrospective cohort with an average follow-up of 132.03 months, we observed a link between DII and all-cause death risk in a large CKD population of U.S. adult. As the result presented, persons with higher DII scores are at a more obvious risk of death. These associations are still obtained after adjusting for potential confounders, especially in populations less than 65 years. The difference between age stratifications can be partially attributable to the higher incidence of various comorbidities and shorter follow-up duration (146.89 ± 31.55 vs. 105.96 ± 50.66 m) in the elder subgroup.

Previous reports had illustrated the link between dietary-induced inflammation and the growing risk of various physical disorders, such as cardiovascular disease (10, 11), diabetes (12), cancer (13), and CKD (14). There was evidence that pro-inflammatory components in dietary intake might have an impact on CKD development, by promoting tissue-specific and systemic metabolic dysfunction (15).

Although the relation between dietary inflammatory potential and the risk of new-onset renal dysfunction had been reported in a series of studies (16, 17), the link between DII and mortality in the CKD population is a relatively novel area of research. The results obtained in this study provide support to the hypothesis that inflammatory injury may address the association between poor dietary consumption and increased death risk in the CKD population.

Studies with respect to DII, which had been carried out in other populations, address similar conclusions to our results. Supporting our findings, a prospective cohort study with the U.S. population demonstrated that individuals who consumed a more pro-inflammatory diet were at an increased risk of dying from all-cause (34%), cardiovascular disease (46%), and cancer (46%) compared with individuals who consumed a more anti-inflammatory diet (18). Similar to our cohort, the study was based on a single self-reported 24 h recall, which may not be an adequate reflection of the usual diet. In a meta-analysis including 38 studies, a higher level of DII was associated with a higher risk for mortality caused by all types of cancer by 16% [odds ratio (OR): 1.16; 95% *CI*: 1.01–1.32] (19). Although the reviewed studies were heterogeneous in terms of population characteristics, design, and duration of follow-up periods.

To date, the potential mechanisms remain uncertain about the issue that higher DII contributes to increased mortality. However, some feasible considerations with biological plausibility have been proposed. The underlying mechanisms include increased incidence of obesity and metabolic syndrome (20), the turbulence of glucose and insulin metabolism (21), and shortening of telomere length (22) accompanied by higher DII levels.

Recently, the pieces of evidence have proposed that intestinal microbiota and their metabolites may influence the progression and prognosis of CKD (23, 24). For example, as the degradative product of choline and L-carnitine by intestinal microbiota, trimethylamine oxide is proven to be highly associated with the death risk in CKD (25). In addition, the composition of intestinal microbiota may be noticeably impacted by the dietary intake (26). The DII derived from dietary intake may influence the mortality in CKD by modulating the composition of intestinal microbiota. We may achieve some valuable information if we focus on the dietary inflammatory potential of patients with CKD and its relation to microbiota.

This cohort has the largest sample focusing on the association of DII with mortality in the adult CKD population.

The results can be extended to the general U.S. population due to the randomly sampling design. Since data acquisition was carried out on all days of the week from the NHANES dataset, the possibility of day-specific information bias is fairly low. There are some limitations to our study as well. First, the major limitation is the lack of a specific cause of death, which does not allow to come to accurate inferences on the issue. Second, merely a single 24-h dietary recall is not sufficient in reflecting a person's long-term conventional eating habits (27). Within-individual random measurement errors cannot be excluded when using only observed intakes from a 24-h dietary recall. Third, the calculation of DII scores was based on the accessible 33 out of 45 dietary components, which brings potential interference with our findings. Fourth, in the setting of an observational study, we cannot exclude the possibility that some of our results were affected by residual confounding, even with various covariates adjusted. Finally, according to the data presented in **Supplementary Material 1**, populations with missing DII were more prone to lose information on waist circumference and BMI, with a large portion of laboratory tests undetected, which have a higher risk of death (46.84 vs. 27.36%) in shorter follow-up duration (136 vs. 145 m). Given the consideration of various different characteristics between the final and the withdrawn samples, the exclusion of the withdrawn samples may lead to potential bias in the results.

Conclusion

In summary, a healthy and appropriate dietary consumption with a lower DII score was inversely associated with all-cause mortality among the adult CKD population. These findings support recommendations for the adult CKD population to follow a balanced diet with lower inflammatory potential. Dietary instruction might provide a modifiable measure for CKD management, while more investigations are necessary to figure out the potential mechanism.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://www.cdc.gov/nchs/nhanes/index.htm.

Author contributions

L-JY, F-RZ, and YZ designed the research and wrote the article. L-JY and YZ conducted the research. F-RZ and C-SM analyzed the data. F-RZ and YZ had primary responsibility for the final content. All authors have read and approved the final version of the manuscript for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.883838/full#supplementary-material

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Association between dietary acid load and cancer risk and prognosis: An updated systematic review and meta-analysis of observational studies

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Epidemiological studies have suggested that dietary acid load (DAL) might be related to the risk and prognosis of cancer, whereas the evidence is contentious. Several high-quality observational studies have been published following a prior systematic review with only one study included. Consequently, we conducted an updated systematic review and meta-analysis to comprehensively investigate the relationship between DAL and cancer risk and prognosis. A systematic literature search was conducted in the PubMed, Embase, and Web of Science databases from inception to 26 October 2021. Summary relative risks (RRs) with 95% CIs were calculated using a random-effects model. Publication bias, subgroup, meta-regression, and sensitivity analyses were also conducted. Ten observational studies (six cohorts and four case–control studies) with 227,253 participants were included in this systematic review and meta-analysis. The summary RRs revealed a statistically significant associations between DAL and cancer risk (RR = 1.58, 95% CI = 1.23–2.05, I^2 = 71.9%, n = 7) and prognosis (RR = 1.53, 95% CI = 1.10–2.13, I^2 = 77.1%, n = 3). No evidence of publication bias was observed in the current analysis. Positive associations were observed in most subgroup analyses stratified by predefined factors, including region, study design, study quality, study population, participants' gender, age of participants, cancer type, DAL assessment indicator, and adjustment of potential confounding parameters. No evidence of heterogeneity between subgroups was indicated by meta-regression analyses. The high DAL

might be associated with an increased risk of cancer, as well as a poor prognosis of cancer. More high-quality prospective studies are warranted to further determine the associations between DAL and risk and prognosis for specific cancers.

KEYWORDS

dietary acid load, prognosis, risk, systematic review, cancer, meta-analysis

Introduction

Cancer is a leading cause of death and an important barrier to prolonging life (1). Globally, more than 19 million new cases of cancers were diagnosed, and nearly 10 million deaths from cancer occurred in 2020 (2). Most cancers were caused by a complex etiology such as environment, genetics, and lifestyle factors (3), and evidence had suggested that over 40% of cancer deaths could be prevented through changes in lifestyles, including diet (4). Due to the potential interaction between food and nutrients, the studies of dietary patterns or overall diet quality may better measure the impact of diet on health outcomes (5).

Dietary acid load (DAL) is one of the indexes to evaluate the quality of the whole diet, which provides more comprehensive information about the dietary intakes of subjects (6). It has been recently proposed that higher DAL, representing the consumption of diets characterized by a higher intake of meat and eggs and a lower intake of vegetables and fruits, could lead to changes or imbalances in blood pH and acid-base balance (7). DAL could be calculated through the potential renal acid load (PRAL), the net endogenous acid production (NEAP), the protein to potassium (Pro:K) ratio, and the net acid excretion (NAE), which are validated methods to assess DAL from dietary composition data (8, 9). Negative values of PRAL and lower values of NEAP, Pro:K, and NAE reflect alkaline-forming potential, whereas positive values of PRAL and higher values of NEAP, Pro: K, and NAE indicate acid-forming potential.

Experimental evidence has indicated that an acidic environment had a benign effect on the survival of cancer cells and promoted the invasion and metastasis of tumors (10, 11). The alkaline environment had the opposite effect on cancer cell survival compared with acidic environments (12). Several observational studies have also suggested that DAL is positively associated with some chronic diseases, such as metabolic syndrome (13) and type 2 diabetes (14). In 2016, Fenton and Huang (15) conducted a systematic review and found only one study focused on the association between DAL and cancer risk, which suggested null results. Interestingly, several epidemiological studies have published their results in recent years, but the findings have been controversial (16–25). For example, a large cohort study with 43,570 participants showed that consumption of high DAL food increased the risk of breast cancer (19). In contrast, a cohort study of 27,096 male smokers suggested a significant relationship between high DAL and an increased risk of bladder cancer (23).

To the best of our knowledge, there has been no updated systematic review and meta-analysis comprehensively verifying whether DAL plays a vital role in cancer risk and prognosis after the study of Fenton and Huang (15). Therefore, given the controversial findings as well as the current lack of high-level evidence of this issue, we conducted the present study to further understand and investigate the aforementioned topic.

Methods

Search strategy

This systematic review and meta-analysis were reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (26) and the Meta-analysis of Observational Studies in Epidemiology group (27). PubMed, Embase, and the Web of Science databases were searched systematically to obtain studies published up to 26 October 2021 by two independent investigators (RW and ZYW). The following search keywords were utilized: (diet or dietary or diet dependent) and (acid or acid-base or NEAP or potential renal net acid load or DAL) and (cancer or neoplasms or oncology). Our search was completed by an additional manual search of reference lists of all the retrieved articles.

Dietary acid load definitions

There were four ways to estimate DAL: (i) PRAL, which considered the absorption rates for dietary proteins and minerals, ionic dissociation, and sulfur metabolism (28); (ii)

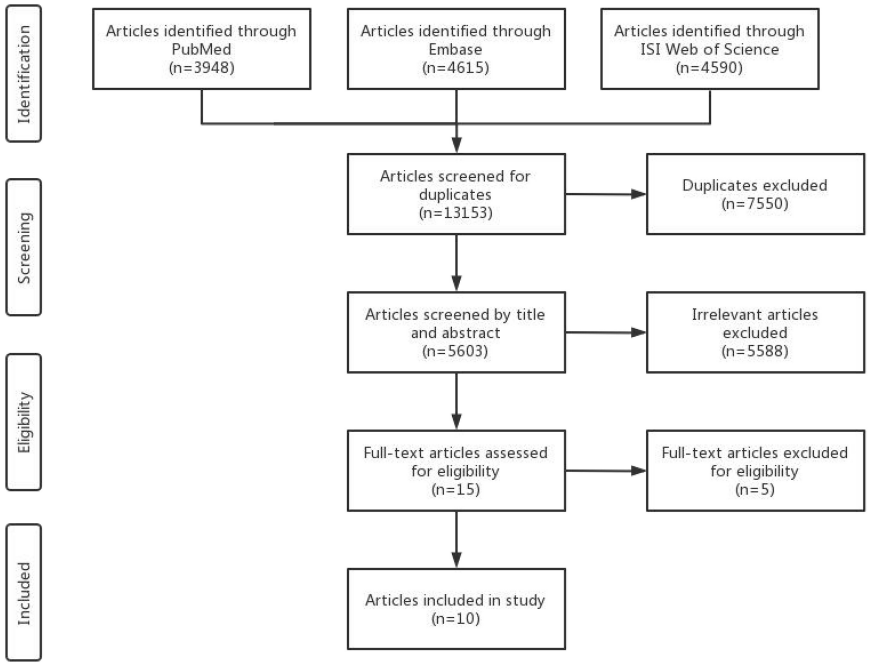

FIGURE 1
Flowchart of study selection.

TABLE 1 Characteristics of studies included in the systematic review and meta-analysis.

First author (ref), year, Country	Study design	Type of cancer	No. of case/event	No. of participants	Dietary assessment/ index	Exposure categories	Risk estimates (95%CI)
Hejazi et al. (16), Iran	Cohort study	NA	1,502	48,691	FFQ/PRAL	Q5 vs. Q2 PRAL	HR: 1.04 (0.89–1.22)
Milajerdi et al. (18), Iran	Case-control study	Glioma	128	384	FFQ/Pro: K	T3 vs. T1 Pro: K	OR: 3.05 (1.04–8.91)
Ronco et al. (20), Uruguay	Case-control study	Lung	843	2,309	FFQ/PRAL, NEAP	Q4 vs. Q1 PRAL NEAP	OR: 0.99 (0.64–1.52) OR: 2.22 (1.52–3.22)
Shi et al. (22), United States	Cohort study	Pancreatic	337	95,708	DHQ/PRAL, NEAP	Q4 vs. Q1 PRAL NEAP	HR: 1.73 (1.21–2.48) HR: 1.64 (1.14–2.36)
Nasab et al. (17), Iran	Case-control study	Colorectal	259	499	FFQ/PRAL, NEAP, Pro: K	T3 vs. T1 PRAL	OR: 4.82 (2.51–9.25)
Wu et al. (24), United States	Cohort study	Breast	295	2,950	24-h dietary recalls/PRAL, NEAP	Q4 vs. Q1 PRAL NEAP	HR: 1.30 (0.87–1.94) HR: 1.54 (1.04–2.29)
Wu et al. (25), United States	Cohort study	Breast	517	3,081	24-h dietary recalls/PRAL, NEAP	Q4 vs. Q1 PRAL NEAP	HR: 2.15 (1.34–3.48) HR: 2.31 (1.42–3.74)
Park et al. (19), United States	Cohort study	Breast	1,882	43,570	FFQ/PRAL, NEAP, Pro: K, NAE	Q4 vs. Q1 PRAL	HR: 1.21 (1.04–1.41)
Safabakhsh et al. (21), Iran	Case-control study	Breast	150	300	FFQ/PRAL, NEAP	T3 vs. T1 PRAL NEAP	OR: 1.00 (0.29–3.36) OR: 0.92 (0.25–3.36)
Wright et al. (23), Finland	Cohort study	Bladder	446	27,096	FFQ/NAE	Q5 vs. Q1 NAE	RR: 1.15 (0.86–1.55)

CI, confidence interval; DHQ, diet history questionnaire; FFQ, food frequency questionnaire; HR, Hazard Ratio; NA, not report; NAE, renal net acid excretion; NEAP, net endogenous acid production; OR, Odds Ratio; PRAL, potential renal acid load; Pro:K, Protein:Potassium (K); Q, quartile or quintile; RR, Relative Risk; and T, tertile.

NEAP, which took into account the acidification of proteins and the alkalization of potassium (8); (iii) Pro:K that also involved animal proteins and potassium (8); and (iv) NAE that similar to PRAL, which further included estimated excretion of organic acids (12).

Study selection and exclusion

To be included in this review, the following criteria were used for inclusion: (i) studies had an observational design, including cross-sectional, case–control, and cohort studies; (ii) studies assessing the relationship between DAL and cancer risk and prognosis; and (iii) studies recommending relative risk (RR), hazard ratio (HR), odds ratio (OR), or required data for an estimate. The studies were excluded for the following reasons: (i) studies that were not original research, including editors, case reports, and reviews; (ii) studies with randomized controlled or ecological design; and (iii) studies published in other languages instead of English.

Data extraction and quality assessment

The studies that fulfilled all the inclusion criteria were qualitatively evaluated by two investigators (RW and ZYW), and any disagreements were settled by a discussion with a third investigator (QJW). The extracted data included the first author, the year of publication, country, design of studies included, number of cases, dietary assessment index, exposure categories, risk estimates, and adjusted variables. We assessed the quality of the articles according to the Newcastle–Ottawa Scale (NOS; 29). The NOS consisted of three fields: selection, comparability, and outcome. These studies received full marks in at least two categories of selection, comparability, or outcome assessment and were classified as low-risk bias (30, 31).

Statistical analysis

In the meta-analysis, effect sizes for DAL were extracted from original studies, including standardized incidence ratio, HR, and RRs. The OR estimate and HR estimate were considered an approximation of the RR estimate (31). We calculated RR and 95% CI with a random-effects model (32) as a measure of the effect size for all the studies. A random-effects model accounted for variation between studies, as this can provide more conservative results than a fixed-effects model (33).

Heterogeneity in the relationship between DAL and cancer risk and prognosis across studies was quantified using I^2 statistics. Cutoff points of $\leq$25, $\leq$50, $\leq$75, and $>$75% were used to indicate no, small, moderate, and substantial levels of heterogeneity, respectively, (34). To explore the sources of heterogeneity among studies, we conducted subgroup analyses and sensitivity analyses. Subgroup analyses were conducted

TABLE 2 Adjustment potential confounders of included studies.

First author (ref), year	Adjustment for potential confounders in the primary analysis
Hejazi et al. (16)	Age, sex, BMI, smoking, alcohol, opium, wealth score, physical activity, dietary fat, carbohydrate, fiber intake, history of CVD, COPD, renal failure, diabetes
Milajerdi et al. (18)	Age, sex, energy intake, marital status, smoking, family history of cancer, physical activity, supplement use, disease duration, high-risk residential area, history of exposure to the radiographic X-ray, history of head trauma, duration of cell phone use, history of allergy, history of hypertension, exposure to chemicals, drug use, frequent fried food intake, frequent use of barbecue, canned foods and microwave, high-risk occupation, dietary intakes of polyunsaturated fatty acids, sodium, calcium, selenium, vitamin C, vitamin E, vitamin B6, folic acid, BMI
Ronco et al. (20)	Age, residence, family history of cancer in first degree, BMI, smoking intensity, alcohol status, "Mate" intake, tea intake, energy, total fiber, total carotenoids, lignans, flavonols, glutathione, vitamin C, vitamin E, animal-based iron, total heterocyclic amines
Shi et al. (22)	Age, sex, smoking status, history of diabetes, alcohol intake, BMI, family history of pancreatic cancer, dietary fiber, carbohydrate, energy intake from diet
Nasab et al. (17)	Age, comorbidity, cancer family history, common ways of cooking, level of salt intake, physical activity, calcium supplement use
Wu et al. (24)	Age at diagnosis, race/ethnicity, education level, intervention group, menopausal status at baseline, total calorie intake, alcohol intake, physical activity, BMI, number of comorbidities, tumor stage, tumor size, estrogen and progesterone receptor status, tamoxifen use, radiotherapy, chemotherapy
Wu et al. (25)	Age at diagnosis, race/ethnicity, education level, intervention group, menopausal status at baseline, total calorie intake, alcohol intake, smoking status, pack-years, physical activity, BMI, tumor stage, tumor size, estrogen and progesterone receptor status, tamoxifen use, radiotherapy, chemotherapy
Park et al. (19)	Age, race, household income, physical activity, pack-years of smoking, BMI, alcohol consumption, total energy intake, recent mammogram screening, stronger family history of breast cancer, breastfeeding history, parity, postmenopausal hormone therapy, age at menopause, multivitamin use
Safabakhsh et al. (21)	BMI, education, marital status, menopause status, socioeconomic status, alcohol use, smoking, vitamin supplements and medication uses, medical history, history of hormone replacement therapy, time of oral contraceptive use, age at first menarche, time since menopause in postmenopausal women, weight at age 18 years old, number of children, length of breastfeeding, family history of breast cancer, energy intake
Wright et al. (23)	Age, energy intake, number of years of smoking, cigarettes/day, intervention assignment

BMI, body mass index; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; "Mate" is the name of the staple infusion in Uruguay, made from the Ilex paraguariensis herb.

TABLE 3 Methodological quality of cohort studies included in the systematic review and meta-analysis.

First author, reference, publication year	Selection				Comparability	Outcome			Risk of bias[d]
	Representativeness of the exposed cohort	Selection of the unexposed cohort	Ascertainment of exposure	Outcome of interest not present at start of study	Control for important factor or additional Factor[a]	Assessment of outcome	Follow-up long enough for outcomes to occur[b]	Adequacy of follow-up of Cohorts[c]	
Hejazi et al. (16)	*	*	*	*	*	*	*	*	Low risk
Shi et al. (22)	*	*	*	*	**	*	*	–	Low risk
Wu et al. (24)	*	*	*	*	*	*	*	*	Low risk
Wu et al. (25)	*	*	*	*	*	*	*	*	Low risk
Park et al. (19)	*	*	*	*	**	*	*	*	Low risk
Wright et al. (23)	*	*	*	*	*	*	*	–	High risk

*A study could be awarded a maximum of one star for each item except for the item Control for important factor or additional factor. The definition/explanation of each column of the Newcastle-Ottawa Scale is available from (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp).

[a]This project receives a maximum of two stars. One star can be obtained by adjusting for total energy intake and another star can be obtained by adjusting for other important confounding factors.

[b]A cohort studies with follow-up > 5 years or cohort studies with prognosis > 1 year were eligible for one star.

[c]A cohort study with a follow-up rate > 75% is assigned one star.

[d]Studies that obtained full scores in at least two domains were considered to have a low risk of bias, other situations were considered as high risk.

TABLE 4 Methodological quality of case–control studies included in the systematic review and meta-analysis.

First author, reference, publication year	Selection				Comparability	Exposure			Risk of bias[c]
	Adequate definition of cases	Representativeness of cases	Selection of control subjects	Definition of control subjects	Control for important factor or additional Factor[a]	Exposure assessment	Same method of ascertainment for all subjects	Non-response Rate[b]	
Milajerd et al. (18)	*	*	–	*	**	*	*	–	High risk
Ronco et al. (20)	*	*	–	*	**	*	*	–	High risk
Nasab et al. (17)	*	*	–	*	*	*	*	*	High risk
Safabakhsh et al. (21)	*	*	*	*	**	*	*	*	Low risk

*A study could be awarded a maximum of one star for each item except for the item Control for important factor or additional factor. The definition/explanation of each column of the Newcastle-Ottawa Scale is available from (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp).

[a]This project receives a maximum of two stars. One star can be obtained by adjusting for total energy intake and another star can be obtained by adjusting for other important confounding factors.

[b]One star is assigned if there is no significant difference in the response rate between control subjects and cases by using the chi-square test ($P > 0.05$).

[c]Studies that obtained a full scores at least two domains were considered to have a low risk of bias, other situations were considered as high risk.

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based on region, study design, study quality, study population, gender, age, cancer type, DAL assessment indicator, and adjustments made for potential confounders, including body mass index, cigarette smoking, alcohol consumption, and physical activity. We also made a meta-regression model to identify potential sources of heterogeneity between subgroups. Sensitivity analysis was performed in which each study was eliminated from the study to evaluate the influence of that study (35). Publication bias was assessed by Begg's test (36), Egger's test (37), and visual inspection of funnel plots. A probability (P) value of <0.05 was considered statistically significant. All the analyses were conducted using Stata version 11.2 software (StataCorp, College Station, TX, United States).

Results

Search results, study characteristics, and quality assessment

The search strategy retrieved 13,153 articles from databases, of which 5,605 articles remained after removing the 7,550 duplicate articles. After the initial screening based on titles or abstracts, 5,588 studies were excluded, leaving 17 studies included. Of these, 5 articles (38–42) were further eliminated because of the duplicated study population and incomplete results. The final selection yielded 10 articles (16–25; 7 studies for cancer risk and 3 studies for cancer prognosis; **Figure 1**) included in the meta-analysis.

Seven studies focused on cancer risk were published between 2005 and 2021 (**Table 1**). Among them, four were case–control studies (17, 18, 20, 21), and three were cohort studies (19, 22, 23). Three studies were performed in Asia (17, 18, 21), two in North America (19, 22), one in South America (20), and one in Europe (23), respectively. DAL had been assessed using the PRAL and NEAP methods in five studies (17, 19–22), NAE in two studies (22, 23), and Pro:K in three studies (17, 20, 22). The included articles were assessed by dietary intake through the Food Frequency Questionnaire (FFQ) and the Diet History Questionnaire (DHQ). Potential confounders were adapted for age ($n = 6$), energy intake ($n = 6$), family history of cancer ($n = 6$), smoking status ($n = 6$), and body mass index ($n = 5$; **Table 2**). Five studies (lung, glioma, colorectal, breast, and pancreatic cancers) indicated a relationship between higher DAL intake and an increased risk of cancer (17–20, 22), whereas two studies (breast and bladder cancers) demonstrated a null association (21, 23).

Table 1 demonstrates the characteristics of the cancer prognosis studies (16, 24, 25), which were referred to as cohort studies. Of them, two studies were undertaken in North America (24, 25) and one study was undertaken in Asia (16). PRAL was assessed in all the studies, whereas NEAP was applied in two studies (24, 25). Dietary intake was evaluated through FFQ and 24-h dietary recall in all the included studies. Risk estimates were adjusted for body mass index ($n = 3$), smoking status ($n = 3$), physical activity ($n = 3$), and age at diagnosis ($n = 2$; **Table 2**). Two cohort studies indicated a significant relationship between higher DAL (represented by NEAP) intake and poor survival among patients with breast cancer (24, 25), whereas one cohort study demonstrated a null association (16).

The information on quality assessment is given in **Tables 3**, **4**. Five cohort studies (16, 19, 22, 24, 25) were graded as low risk, whereas only one cohort study (23) was graded as high risk (**Table 3**). For the item of "control for important factor or additional factor," four studies (16, 23–25) were not awarded two stars since these studies adjusted for less than two important confounder factors. For the classification of "outcome," two studies (22, 23) were not assigned full stars because of the inadequacy of the follow-up rate of cohorts. Most included case–control studies (75%) were at high risk (**Table 4**). For the "selection" classification, three studies (17, 18, 20) were not assigned full stars. For the item of "control for important factor or additional factor," one study (17) was not awarded two stars since these studies had adjusted for less than two important confounder factors in their analysis. For the classification of "exposure," two studies (18, 20) were not assigned full stars because there was a significant difference in the response rate between cases and controls.

Association of dietary acid load with cancer risk

Higher DAL was associated with a 58% increased risk of cancer (RR = 1.58, 95% CI = 1.23–2.05, I^2 = 71.9%; **Figure 2**). No publication bias was discovered (**Supplementary Figure 1**; Egger's $P = 0.21$ and Begg's $P = 0.47$).

Positive associations were found in most subgroup analyses (**Table 5**). Notably, in the stratified analysis, we observed significant positive associations in studies in non-Asia (RR = 1.41, 95% CI = 1.14–1.76), age of participants ≥50 years (RR = 1.60, 95% CI = 1.21–2.11), breast cancer (RR: 1.20, 95% CI = 1.03–1.40), and pancreatic cancer (RR = 1.69, 95% CI = 1.31–2.18). Furthermore, the risk of cancer incidence increased by 57% (RR = 1.57, 95% CI = 1.03–2.41) and 83% (RR = 1.83, 95% CI = 1.36–2.47) by high PRAL and NEAP, respectively. Additionally, meta-regression analysis revealed that there was no evidence of heterogeneity between these subgroup analyses.

In sensitivity analyses, we sequentially removed one study; in turn, the pooled RR did not change substantially. Our sensitivity analysis showed that the RR for cancer ranged from a low of 1.50 (95% CI = 1.16–1.95, I^2 = 68.4%) after removing the study by Ronco et al. (20) to a high of 1.68 (95% CI = 1.23–2.29, I^2 = 69.5%) after removing the study by Park et al. (19; **Supplementary Figure 2**).

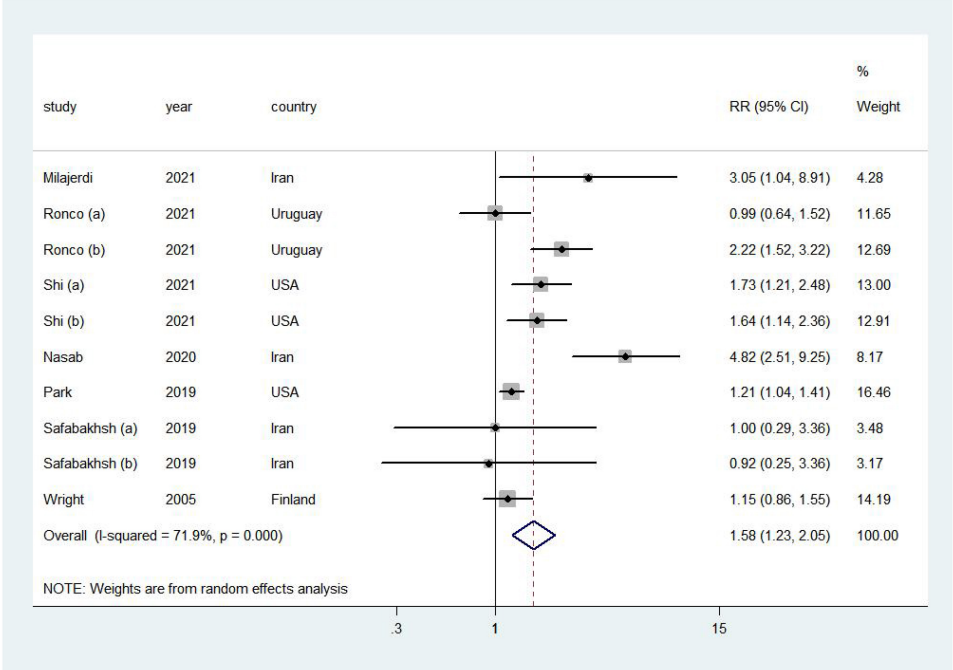

FIGURE 2
Forest plot (a random-effects model) of the association between DAL and cancer risk (highest vs. lowest). Squares indicate study-specific relative risk (RR), where the size of the square reflects the study-specific statistical weight; horizontal lines indicate the 95% CI; and diamonds denote the summary RR with 95% CI.

Association of dietary acid load with cancer prognosis

Higher DAL was associated with a poor prognosis of cancer (RR = 1.53, 95% CI = 1.10–2.13, I^2 = 77.1%; **Figure 3**). No publication bias was discovered (**Supplementary Figure 3**; Egger's P = 0.02 and Begg's P = 0.09). In sensitivity analyses, we sequentially removed one study; in turn, the pooled RR did not change substantially. Our sensitivity analysis showed that the RR for cancer ranged from a low of 1.41 (95% CI = 1.01–1.98, I^2 = 74.6%) after removing the study by Wu et al. (25) to a high of 1.62 (95% CI = 1.05–2.48, I^2 = 82.7%) after removing the study by Wu et al. (24; **Supplementary Figure 4**).

Discussion

To the best of our knowledge, the present review is the most comprehensive study reporting the relationship between DAL and cancer risk and prognosis. Findings from this systematic review and meta-analysis indicated that higher DAL might be an unfavorable factor for cancer risk and prognosis. These findings were consistently detected in numerous subgroups and sensitivity analyses.

Our findings are inconsistent with the previous systematic review, which included articles published before April 2015, and concluded that DAL was overall not significantly associated with an increased risk of cancer (15). However, this systematic review included only one study comprising 27,542 participants and 446 bladder cancers (23). Our systematic review and meta-analysis further included nine studies involving 227,253 participants published during the last 3 years (16–22, 24, 25). Of note, six low-risk studies were included in the present systematic review and meta-analysis (16, 19, 21, 22, 24, 25). Furthermore, numerous subgroup analyses and meta-regression analyses were conducted based on study characteristics and confounding factors.

In the subgroup analysis stratified by region, we only observed positive associations in studies carried out in the non-Asia region. This phenomenon could partly be attributed to the different DAL scores in patients with cancer from diverse regions. For example, when investigating 1,882 patients with breast cancer in the United States, it was found that the mean value was 2.25 for the PRAL score (19), whereas Safabakhsh et al. (21) reported that the mean value was –26.1 for the PRAL score based on 150 patients with breast cancer in Iran. Furthermore, a Western dietary pattern characterized by a high score of PRAL was associated with an increased risk of patients with cancer (43, 44).

The subgroup analyses suggested that DAL was positively associated with the risk of cancer in participants of age ≥50 years. Indeed, Frassetto et al. found that increasing age

TABLE 5 Summary risk estimates of the association between dietary acid load and risk of cancer (highest vs. lowest).

	No. of study	RR (95%CI)	I^2 (%)	P^1	P^2
Overall	7	1.58 (1.23, 2.05)	71.90	<0.01	
Subgroup analyses					
Region					0.149
Asia	3	2.16 (0.92, 5.06)	63.50	0.042	
Non-Asia	4	1.41 (1.14, 1.76)	66.60	0.012	
Age					0.869
<50	2	1.51 (0.68, 3.32)	24.10	0.268	
≥50	5	1.60 (1.21, 2.11)	79.50	<0.01	
Sex					0.152
Men	2	1.36 (0.86, 2.18)	79.70	<0.01	
Women	2	1.20 (1.03, 1.40)	0.00	0.880	
Both	3	2.30 (1.45, 3.66)	67.90	0.025	
Cancer type					0.858
Breast cancer	2	1.20 (1.03, 1.40)	0.00	0.880	
Pancreatic cancer	2	1.69 (1.31, 2.18)	0.00	0.838	
Glioma	1	3.05 (1.04, 8.91)	N/A	N/A	
Lung cancer	1	1.49 (0.68, 3.30)	N/A	N/A	
Bladder cancer	1	1.15 (0.86, 1.54)	N/A	N/A	
Colorectal cancer	1	4.82 (2.51–9.25)	N/A	N/A	
Study design					0.372
Cohort study	3	1.86 (1.05, 3.28)	75.10	<0.01	
Cross-sectional study	4	1.35 (1.12, 1.62)	45.50	0.138	
Study population*					0.149
<Median	3	2.16 (0.92, 5.06)	63.50	0.042	
≥Median	4	1.41 (1.14, 1.76)	66.60	0.012	
Study quality					0.382
Low risk	3	1.91 (1.12, 3.24)	83.60	<0.01	
High risk	4	1.38 (1.13, 1.67)	24.40	0.259	
DAL assessment indicator					0.812
PRAL	5	1.57 (1.03, 2.41)	80.50	<0.01	
NEAP	3	1.83 (1.36, 2.47)	18.00	0.295	
Pro: K	1	1.15 (0.86, 0.55)	N/A	N/A	
NAE	1	3.05 (1.04, 8.91)	N/A	N/A	
Adjust body mass index					0.429
Yes	5	1.49 (1.17, 1.89)	57.30	0.022	
No	2	2.28 (0.56, 9.29)	93.50	<0.01	
Adjust alcohol drinking					0.429
Yes	5	1.49 (1.17, 1.89)	57.30	0.022	
No	2	2.28 (0.56, 9.29)	93.50	<0.01	
Adjust cigarette smoking					0.241
Yes	4	2.46 (0.86, 7.07)	88.30	<0.01	
No	3	1.44 (1.14, 1.83)	57.80	0.027	
Adjust physical activity					0.233
Yes	3	2.49 (0.88, 7.02)	89.30	<0.01	
No	4	1.44 (1.14, 1.83)	52.00	0.051	

CI, confidence interval; NA, not applicable; RR, relative risk.

[1] *P*-value for heterogeneity within each subgroup.

[2] *P*-value for heterogeneity between subgroups with meta-regression analysis.

*The median study population for the analysis of DAL (highest vs. lowest) is 1,404.

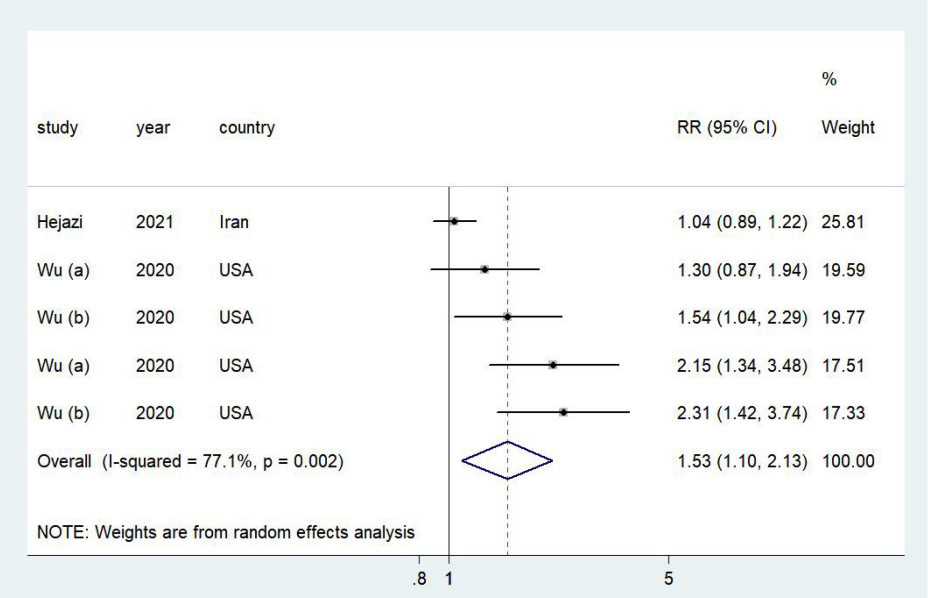

FIGURE 3
Forest plot (a random-effects model) of the association between DAL and cancer prognosis (highest vs. lowest). Squares indicate study-specific relative risk (RR), where the size of the square reflects the study-specific statistical weight; horizontal lines indicate the 95% CI; and diamonds denote the summary RR with 95% CI.

was associated with indicative of a progressively worsening low-level metabolic acidosis, and the changes seemed to be the most striking starting at about age 50 years (45). In addition, potential long-term effects of acidogenic diets are further compounded by the reduction of renal function typically from aging (45, 46). However, for the risk of cancer in participants at the age ≥50 years, more studies are warranted, mainly due to the presence of the high heterogeneity of these results.

Compared to the results of PRAL, the risk of cancer was considered to be substantially higher in NEAP. Both the PRAL and NEAP are approximate to DAL and highly correlated (r = 0.9; 8). However, the assessment of PRAL may be imprecise due to the error in the measurement of minerals or the protein intakes with low or high ranges (9, 47). In fact, Ronco et al. proposed that NEAP was found to be a better predictor of breast cancer risk than PRAL (20). One explanation is that PRAL relies on more information from the dietary database, which means that it may be more susceptible to confounding factors. Therefore, future studies should focus more on the accuracy of PRAL calculations.

Results of our subgroup analyses demonstrated that DAL increased the risk of breast and pancreatic cancers. PRAL is inversely correlated with the consumption of vegetables, while phytochemicals contained in vegetables may contribute to decreasing the level of the epidermal growth factor receptor (48, 49), which is known to be a major growth-stimulating factor exclusively in breast cancer (50). Furthermore, metabolic acidosis is found to reduce circulating adiponectin levels by inhibiting the transcription of the adiponectin gene (51); both the experimental and epidemiological studies have suggested a high level of adiponectin against the risk of pancreatic cancer (13, 52). In addition, we have previously found that a higher intake of red meat and dairy was statistically related to an increased risk of breast and pancreatic cancers (53–55). However, due to the limited number of studies, we yielded a null association between DAL and other cancers. Therefore, more prospective cohort studies of a specific cancer are needed to clarify these issues.

Several studies have indicated that consumption of high DAL dietary might be linked with a worse prognosis among patients with cancer (24, 25). Wu et al. (24) indicated that higher DAL was related to breast cancer-specific mortality and total mortality. Furthermore, Wu et al. (25) also found the same trend among 3,081 United States patients with breast cancer. Hejazi et al. (16), however, suggested that DAL was unrelated to the overall survival of cancer. They might miss an association between DAL and cancer survival because of unmeasured confounding and dietary changes. Of note, since dietary information was not updated during follow-up, they could not account for any changes in dietary consumption over time.

Although there was no evident mechanism to explain the relationship between DAL and cancer risk, several consensuses have been proposed. First of all, metabolic acidosis caused by DAL could promote cancer. Acid-base imbalance had been shown to regulate molecular activities, including insulin growth factor-1 (IGF-1; 56, 57) and osteoclast activation (58, 59),

which may serve as intermediaries for cancer occurrence and promotion (60–62). In addition, acid-producing diets were often high in animal and processed proteins and low in fruits and vegetables, which were associated with a higher carcinogenic effect (63–65). The evidence also showed that DAL reduces circulating adiponectin (66), and both the experimental and epidemiological studies (13, 51, 52, 67) had shown that it played a role in the occurrence of cancer.

Regarding cancer prognosis, several studies existed to interpret this phenomenon. Metabolic acidosis had been shown to stimulate cancer metastasis in cell and animal models (68–70). In addition, metabolic acidosis depleted endogenous bicarbonate levels, which neutralize acids. A cross-sectional study showed that lower bicarbonate levels were associated with loss of muscle mass and reduced body function (32). As the precursors of bases, potassium (71), magnesium (72), and calcium (73) could inhibit the metastasis and the growth of cancer cells.

The principal strengths were that the present study was the most comprehensive systematic review to estimate the relationship between DAL and the risk and prognosis of cancer. We conducted a rigorous literature search to include all the pertinent studies. In consideration of study features and main adjustments for confounding variables, subgroup, sensitivity, and meta-regression analyses were conducted to probe into possible sources of heterogeneity. In addition, most of the selected articles had a low risk after using the NOS to evaluate the quality of all the included literature. Nevertheless, some limitations of this study should be recognized. First of all, measurement and recall bias in the assessment of dietary intake were inevitable. The calculation of PRAL, NEAP, NAE, and Pro:K based on self-reported data was collected by FFQ, DHQ, and 24-h dietary recalls. However, the majority of the included studies used valid and reliable FFQ, and it had been proved that FFQ could be more precise in assessing the association between diet and diseases (74). Second, the estimation methods of DAL had not been unified (8, 9). PRAL and NEAP were widely recognized and used, while Pro:K and NAE were seldom used, suggesting that one of the estimation methods could be used as the main calculation and the other three methods could be used as a sensitivity analysis in future studies. Third, we only located observational studies that fitted inclusion criteria, which means a large space for future research on DAL and cancer incidence and prognosis, especially in terms of prognosis. The studies on cancer prognosis were mainly concentrated on breast cancer, while other types of cancer had not been covered. Fourth, even though several confounding factors were considered, the included studies cannot rule out the possibility that unmeasured factors might have contributed to these associations.

In summary, the current systematic review and meta-analysis revealed that a higher DAL was associated with an increased risk and poor prognosis for cancers. Further large-scale prospective studies were warranted to explore the role of DAL in different cancers.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

RW, H-HW, and Q-JW conceived the study. RW, F-HL, Y-HZ, T-TG, and Q-JW contributed to the design. RW, Z-YW, and Q-JW collected the data, cleaned the data and checked the discrepancy, and analyzed the data. RW, Z-YW, Y-FW, H-LX, M-LS, Y-HZ, T-TG, H-HW, and Q-JW interpreted the data. All the authors have interpreted the data, read the manuscript, and approved the final vision of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.891936/full#supplementary-material

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Risk of inflammatory bowel disease appears to vary across different frequency, amount, and subtype of alcoholic beverages

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Objective: Inflammatory bowel disease (IBD) and alcohol use has become a significant and growing public health concern. Alcohol use has been reported to be the most-avoided diet item among IBD patients. However, knowledge regarding the impact of different classes of alcoholic beverages on the management of IBD is limited. Our study aims to evaluate the association of different frequencies, amounts, and subtypes of alcoholic beverages with IBD risk.

Methods: The UK Biobank comprised 7,095 subjects with IBD and 4,95,410 subjects without IBD. Multivariate Logistic regression, stratifying analysis, and interaction terms were used to estimate the odds ratios (ORs) and 95% confidence intervals (95% CIs) of IBD. A generalized additive model was used to evaluate the linearity associations of the total amount of all alcoholic beverages or that of each of five alcoholic beverages with IBD risk.

Results: Compared with non-drinkers, the IBD risk was 12 to 16% lower in red wine consumers (1–2 glasses/week, OR [95%CI], 0.88 [0.80, 0.97]; 3–4 glasses/week, 0.84 [0.76, 0.93]; ≥5 glasses/week, 0.86 [0.78, 0.95]), whereas 12% higher in white wine and champagne consumers (1–2 glasses/week, 1.12 [1.03, 1.22]). Stratifying analysis showed low-frequency red wine consumers were associated with a lower IBD risk (0.85 [0.74, 0.97]), whereas spirits consumers were associated with a higher risk (1.28 [1.03, 1.59]). High doge of red wine consumers were associated with a lower IBD risk (above guidelines, 0.80 [0.67, 0.97]; double above, 0.83 [0.71, 0.97]), whereas high doge white wine and champagne (1.32 [1.09, 1.61]) and beer and cider (1.26 [1.02, 1.54]) consumers were associated with a higher IBD risk. White wine and champagne showed a significant interaction effect with high doge alcohol consumption (1.27 [1.03–1.58], $p = 0.029$). The dose-response association showed an increased IBD risk with more number of alcohol consumption of white wine and champagne, beer and cider, or the total amount of all alcoholic beverages. However, red wine is at low risk across the whole dose cycle.

Conclusions: The IBD risk appears to vary across different frequencies, amounts, and subtypes of alcoholic beverages. Overall, alcohol intake is not recommended.

KEYWORDS

inflammatory bowel disease, Crohn's disease, ulcerative colitis, alcohol consumption, prospective cohort, risk factor, UK Biobank, alcoholic beverage

Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are collectively known as inflammatory bowel disease (IBD). IBD has become a significant and growing public health concern and has conveyed a high rate of morbidity and mortality (1, 2). IBD is progressive and immune-mediated inflammatory disease of the gastrointestinal tract. The established importance of environmental factors (e.g., diet and gut microenvironment) in the development of IBD has been widely reported (3, 4). Dietary triggers have aroused significant interest, as identifying modifiable dietary factors could help reduce relapse frequency, thereby limiting steroid use and decreasing hospitalizations (5). However, the exact etiological mechanism of IBD still remains unknown.

Alcohol use is a leading risk factor for disease burden worldwide, accounting for nearly 10% of global deaths among populations aged 15 to 49 years, and poses dire ramifications for future population health in the absence of policy action today (6). Alcohol use in patients with IBD is common (7), and even though its prevalence in patients with IBD appears to be similar to the general population, it has been reported to be the most-avoided diet item among IBD patients (8). Niccum et al. found that alcohol consumption is associated with an increased risk of microscopic colitis (9). Jowett et al. found that alcohol consumption is associated with a higher risk of relapse in patients with UC (10). Mantzouranis et al. reviewed several literature and found that alcohol consumption is associated with worse IBD symptoms among patients who consumed alcoholic beverages compared with those who did not consume alcoholic beverages (11). Patients with alcohol abuse disorder have a similar microbial signature to that of patients with IBD, and ethanol ingested from alcoholic beverages has been widely known to impair gut barrier permeability and function (1, 12–15), which may suggest that the alcohol consumption is involved in modulating the microbiome and facilitating intestinal inflammation, and therefore could facilitate IBD pathogenesis. However, beneficial effect on our health of some classes of alcoholic beverages have also been reported in recent years (16–18). However, knowledge regarding the impact of different classes of alcoholic beverages on the management of IBD are limited.

The observed relationships between consumption of alcoholic beverages and diseases are often non-linear, for example, with low-to-moderate alcohol consumption being protective and heavy alcohol consumption being harmful, or J-shaped risk of diseases with the amount of consumption of alcoholic beverages (18–22). There is limited high-quality epidemiologic evidence for the effect of different classes of alcoholic beverages on IBD. In the present large longitudinal observational study, we systematically investigated the associations of different classes of alcoholic beverages with IBD risk, as well as their associations among different frequencies and amounts of alcohol consumption. We further examined the "dose"-response association between alcohol consumption and IBD risk by drawing the risk trajectory of IBD with different amounts of alcohol consumption.

Materials and methods

Study population

The UK Biobank is a prospective cohort with a total of 5,02,505 subjects recruited from March 2006 to December 2010. We discovered 7,095 IBD cases, which consists of 2,027 cases who only have a diagnosis of Crohn's disease, 4,334 cases who only have a diagnosis of ulcerative colitis, and 734 cases who are both diagnosed with Crohn's disease and ulcerative colitis. Ethical approval of the UK Biobank was obtained from the National Health System Northwest Multicenter Research Ethics Committee (REC reference: 16/NW/0274).

Exposure and outcomes

IBD was ascertained by the International Classification of Diseases, Version 10 (ICD-10), terms from the UK Biobank data field 4,1270, which included Crohn's disease (code K50) and ulcerative colitis (code K51).

To assess the frequency of alcohol consumption (Field ID 1558), subjects were asked to answer the question: About how often do you drink alcohol? Seven responses were given: prefer not to answer, never, special occasions

only, one to three times a month, once or twice a week, three or four times a week, and daily or almost daily. The frequency of alcohol consumption was classified into three categories: (1) high frequency (≥3 times/week); (2) low frequency (<3 times/week); and (3) never/special occasions only (18).

The average weekly intake of red wine (ID 1568), champagne plus white wine (ID 1578), beer and cider (ID 1588), spirits (ID 1598), and fortified wine (ID 1608) were calculated, respectively. For example, to assess the weekly intake of red wine, subjects were asked to answer the question: In an average week, how many glasses of red wine would you drink? A response with an exact value (e.g., 2 glasses/week) should be given.

The total amount of alcohol consumption was quantified by summing the average weekly intake of red wine, champagne plus white wine, beer and cider, spirits, and fortified wine. As shown in our previous study (18), the weekly intake level of alcohol was converted into units for wines (1 standard glass = 2 units), beer and cider (1 pint =2 units), and spirits (1 shot = 1 unit), and was classified into four categories: (1) non-drinker, previous drinker, or special occasions only; (2) within recommended guidelines: <14 UK units/week; (3) above recommended guidelines: ≥14 units/week and <28 units/week; and (4) 2-fold or more above the recommended guidelines: ≥28 units/week.

Confounding factors

A wide range of sociodemographic factors, lifestyle factors, sleep phenotypes, and comorbidities was considered as covariates to adjust for any potential confounding. The covariates of sociodemographic factors included age, sex, ethnicity (white and non-white), education level (college or above, others), body mass index [BMI≥30 and <30 kg/m (2)], current employment status, Townsend deprivation index, and overall health rating. Current employment status was classified into employed (including those in paid employment or self-employed) and unemployed (including those in retired, looking after home and/or family, unable to work because of sickness or disability, doing unpaid or voluntary work, or being full- or part-time students, and unemployed).

The covariates of lifestyle factors included smoking status (never, previous, or current) and usually walking pace (normal, slow, and fast walking pace). The covariates of comorbidities included cerebrovascular diseases, cardiovascular diseases, diabetes, respiratory disease, and cancer. The covariates of sleep phenotype included sleep duration (normal, short, and long sleep duration), early awakening, napping during the day, daytime dozing/sleeping (narcolepsy), sleeplessness or insomnia, and snoring.

Statistical analyses

Continuous variables are presented as mean ± SD, and categorical variables are presented as a number (percentage). We used the unpaired *t*-test and χ^2 test to compare differences between groups where appropriate.

We used Logistic regression analysis to estimate the odds ratios (ORs) and 95% confidence intervals (95% CIs). Multivariate Logistic regression analysis was used to evaluate the associations between consumption of alcoholic beverages and IBD risk after adjusting for sociodemographic factors, lifestyle factors, sleep phenotypes, comorbidities, frequency of alcohol consumption, amount of weekly intake level of alcohol consumption, and alcoholic beverages. Stratifying analysis was further used to examine the association of different subtypes of alcoholic beverages with the risk of IBD, separated by frequency of alcohol consumption and the total amount of alcohol consumption, respectively.

Interaction terms were employed for the overall sample to explore potential interactions between different subtypes of alcoholic beverages on the risk of IBD in the final model. A generalized additive model was used to evaluate the linearity associations of the total amount of alcohol consumption or that of alcoholic beverages with the risk of IBD.

All analyses were conducted with SPSS version 24.0 and R Statistical Software version 4.0. A two-tailed *p*-value <0.05 was considered significant.

Results

Sample characteristics

The demographic characteristics of the study population are presented in Table 1. Compared with subjects without IBD, those with IBD had a poor socioeconomic status [a lower education level ($p < 0.001$), a poor employment status ($p < 0.001$), and a higher Townsend deprivation score ($p < 0.001$)], a poor overall health rating ($p < 0.001$), more sleep disorders (long and short sleep duration; $p < 0.001$, usually napping during the day, $p < 0.001$; narcolepsy, $p < 0.001$; sleeplessness, $p < 0.001$; snoring, $p < 0.001$), and more comorbidities (cerebrovascular diseases, $p < 0.001$; cardiovascular diseases, $p < 0.001$; diabetes, $p < 0.001$; respiratory disease, $p < 0.001$; and cancer, $p < 0.001$). Furthermore, they were more likely to be males ($p < 0.001$), white ethnicity ($p = 0.004$), smokers ($p < 0.001$), and slow walking pacers ($p < 0.001$), but they were less likely to be frequent alcohol drinkers ($p < 0.001$), heavy drinkers ($p < 0.001$), red wine drinkers ($p < 0.001$), white wine and champagne drinkers ($p < 0.001$), and beer and cider drinkers ($p < 0.001$), whereas they were more likely to be spirits drinkers ($p < 0.001$) and fortified wine drinkers ($p < 0.001$).

TABLE 1 Characteristics of UK Biobank cohort.

Characteristics	IBD related risk		*p*-value
	IBD($n = 7095$)	Non-IBD($n = 495{,}410$)	
Age to 2021 (years), mean ± SD	70.2 ± 8.0	69.5 ± 8.1	<0.001
Age categories, *N* (%)			<0.001
≤60 years	1,105 (15.6)	89,836 (18.1)	
61-70 years	2,076 (29.3)	154,325 (31.2)	
≥71 years	3,914 (55.2)	251,248 (50.7)	
Sex (male), *N* (%)	3,482 (49.1)	225,640 (45.5)	<0.001
Education (college or above), *N* (%)	1,827 (26.3)	159,336 (32.8)	<0.001
Ethnicity (white), *N* (%)	6,718 (95.3)	465,977 (94.5)	0.004
Obesity (BMI ≥30 kg/m [2]), *N* (%)	1,735 (24.6)	120,512 (24.5)	0.76
Overall health rating, *N* (%)			<0.001
Excellent or good	3,813 (54.1)	367,109 (74.6)	
Fair or poor	3,230 (45.9)	124,995 (25.4)	
Employment status (in paid), *N* (%)	3,579 (50.8)	283,570 (57.6)	<0.001
Smoking (previous or current), *N* (%)	3,881 (55.0)	222,153 (45.1)	<0.001
Townsend deprivation, mean ± SD	−1.1 ± 3.2	−1.3 ± 3.1	<0.001
Usually walking pace, *N* (%)			<0.001
Normal	3,720 (53.3)	259,126 (52.8)	
Slow	897 (12.9)	40,070 (8.2)	
Fast	2,361 (33.8)	191,770 (39.1)	
Sleep duration, mean ± SD			
Sleep duration categories, *N* (%)			<0.001
Short sleep duration (≤6 h)	1,898 (27.1)	121,354 (24.7)	
Normal sleep duration (7-8 h)	4,501 (64.2)	332,189 (67.6)	
Long sleep duration (≥9 h)	609 (8.7)	37,738 (7.7)	
Early awakening, *N* (%)			<0.001
Not very easy/Not at all easy	1,525 (21.8)	88,173 (18.0)	
Fairly easy	3,363 (48.0)	243,283 (49.7)	
Very easy	2,119 (30.2)	158,368 (32.3)	
Nap during day, *N* (%)			<0.001
Never/rarely	3,544 (50.1)	277,525 (56.2)	
Sometimes	3,036 (42.9)	189,619 (38.4)	
Usually	493 (7.0)	26,393 (5.3)	
Daytime dozing/sleeping (narcolepsy), N (%)			<0.001
Never/rarely	5,064 (72.0)	373,642 (76.0)	
Sometimes	1,693 (24.1)	104,276 (21.2)	
Often/ All of the time	281 (4.0)	13,814 (2.8)	
Sleeplessness or insomnia, *N* (%)			<0.001
Never/rarely	1,473 (20.8)	119,302 (24.2)	
Sometimes	3,182 (45.0)	235,655 (47.7)	
Usually	2,420 (34.2)	138,969 (28.1)	
Snoring, *N* (%)	4,177 (64.0)	287,915 (62.7)	0.03
Cerebrovascular diseases, *N* (%)	282 (4.0)	12,205 (2.5)	<0.001
Cardiovascular diseases, *N* (%)	858 (12.1)	37,528 (7.6)	<0.001
Diabetes, *N* (%)	794 (11.2)	30,496 (6.2)	<0.001

(Continued)

TABLE 1 Continued

Characteristics	IBD related risk		*p*-value
	IBD($n = 7095$)	Non-IBD($n = 495{,}410$)	
Respiratory disease, *N* (%)	2,239 (31.6)	88,521 (17.9)	<0.001
Cancer, *N* (%)	1,369 (19.3)	74,119 (15.0)	<0.001
Frequency of alcohol intake, *N* (%)			<0.001
Never and special occasions only	1,748 (24.7)	96,901 (19.6)	
Once a month-twice a week	2,559 (36.2)	182,588 (37.0)	
≥3 times a week	2,763 (39.1)	214,444 (43.4)	
Alcohol consumption (dosage), UK units/week, mean ± SD	13.5 ± 17.8	14.8 ± 18.1	<0.001
Alcohol consumption, dosage, *N* (%)			<0.001
Never drinker, previous-drinkers or special occasions only	2,623 (37.0)	152,651 (30.8)	
Within guidelines	1,800 (25.4)	137,247 (27.7)	
Above guidelines	1,512 (21.3)	115,304 (23.3)	
Double above the guidelines or more	1,160 (16.3)	90,208 (18.2)	
Red wine drinkers, *N* (%)			<0.001
Non-drinkersNon-drinkers	4,391 (61.9)	268,247 (54.1)	
1–2 glasses/week	866 (12.2)	70,495 (14.2)	
3–4 glasses/week	627 (8.8)	54,219 (10.9)	
≥5 glasses/week	1,211 (17.1)	102,449 (20.7)	
White wine and champagne drinkers			<0.001
Non-drinkers	4,735 (66.7)	307,747 (62.1)	
1–2 glasses/week	1,052 (14.8)	80,226 (16.2)	
3–4 glasses/week	537 (7.6)	44,041 (8.9)	
≥5 glasses/week	771 (10.9)	63,396 (12.8)	
Beer and cider drinkers, *N* (%)			<0.001
Non-drinkers	4,639 (65.4)	314,004 (63.4)	
1–2 glasses/week	952 (13.4)	75,681 (15.3)	
3–4 glasses/week	485 (6.8)	35,459 (7.2)	
≥5 glasses/week	1,019 (14.4)	70,266 (14.2)	
Spirits drinkers, *N* (%)			0.001
Non-drinkers	5,327 (75.1)	366,804 (74.0)	
1–2 glasses/week	821 (11.6)	65,500 (13.2)	
3–4 glasses/week	389 (5.5)	25,957 (5.2)	
≥5 glasses/week	558 (7.9)	37,149 (7.5)	
Fortified wine drinkers, *N* (%)			0.13
Non-drinkers	6,646 (93.7)	461,067 (93.1)	
1–2 glasses/week	329 (4.6)	26,092 (5.3)	
3–4 glasses/week	66 (0.9)	4,583 (0.9)	
≥5 glasses/week	54 (0.8)	3,668 (0.7)	

Association between different alcoholic beverages and IBD risk

The associations of different alcoholic beverages with the risk of IBD are shown in Table 2. In the final multivariate model, compared with non-drinkers, the risk of IBD was 12 to 16% lower in red wine consumers (1–2 glasses/week, OR [95%CI], 0.88 [0.80, 0.97]; 3–4 glasses/week, 0.84 [0.76, 0.93]; ≥5 glasses/week, 0.86 [0.78, 0.95]) regardless of the amount of red wine, whereas 12% higher in white wine and champagne consumers of 1–2 glasses/week (1.12 [1.03, 1.22]). Furthermore, the higher risk was not significant when the amount of white wine and champagne was higher (3–4 glasses/week, 1.04 [0.94, 1.16]; ≥5 glasses/week, 1.04 [0.93, 1.15]). Compared with non-drinkers, beer and cider consumers, spirits wine consumers, and fortified wine consumers were not

TABLE 2 Association of different subtypes of alcoholic beverages with IBD risk.

Variables	Model 1		Model 2		Model 3		Model 4	
	OR (95%Cl)	*p*-value	OR (95%Cl)	*p*-value	OR (95%Cl)	*p*-value	OR (95%Cl)	*p*-value
Red wine drinkers								
Non-drinkers	Reference		Reference		Reference		Reference	
1–2 glasses/week	0.86 (0.79–0.92)	<0.001	0.86 (0.80–0.93)	<0.001	0.87 (0.81–0.95)	0.001	0.88 (0.80–0.97)	0.007
3–4 glasses/week	0.82 (0.75–0.89)	<0.001	0.80 (0.73–0.88)	<0.001	0.82 (0.75–0.90)	<0.001	0.84 (0.76–0.93)	0.001
≥5 glasses/week	0.82 (0.76–0.87)	<0.001	0.79 (0.74–0.85)	<0.001	0.81 (0.76–0.87)	<0.001	0.86 (0.78–0.95)	0.004
White wine and champagne drinkers								
Non-drinkers	Reference		Reference		Reference		Reference	
1–2 glasses/week	0.98 (0.91–1.05)	0.47	1.00 (0.93–1.07)	0.92	1.01 (0.94–1.09)	0.80	1.12 (1.03–1.22)	0.008
3–4 glasses/week	0.93 (0.85–1.02)	0.13	0.92 (0.83–1.01)	0.08	0.93 (0.85–1.03)	0.17	1.04 (0.94–1.16)	0.47
≥5 glasses/week	0.93 (0.86–1.01)	0.07	0.91 (0.84–0.99)	0.026	0.93 (0.86–1.01)	0.08	1.04 (0.93–1.15)	0.51
Beer and cider drinkers								
Non-drinkers	Reference		Reference		Reference		Reference	
1–2 glasses/week	0.88 (0.82–0.95)	0.001	0.89 (0.82–0.96)	0.002	0.90 (0.83–0.97)	0.006	0.97 (0.89–1.05)	0.42
3–4 glasses/week	0.89 (0.81–0.98)	0.021	0.88 (0.80–0.98)	0.019	0.89 (0.81–0.99)	0.035	0.97 (0.86–1.08)	0.54
≥5 glasses/week	0.83 (0.77–0.90)	<0.001	0.83 (0.76–0.90)	<0.001	0.84 (0.77–0.91)	<0.001	0.92 (0.82–1.03)	0.16
Spirits drinkers								
Non-drinkers	Reference		Reference		Reference		Reference	
1–2 glasses/week	0.92 (0.85–0.99)	0.025	0.92 (0.85–0.99)	0.036	0.93 (0.86–1.01)	0.07	1.00 (0.92–1.08)	0.91
3–4 glasses/week	1.02 (0.92–1.14)	0.66	0.99 (0.88–1.11)	0.83	0.99 (0.89–1.11)	0.88	1.07 (0.95–1.20)	0.25
≥5 glasses/week	0.96 (0.88–1.05)	0.36	0.94 (0.86–1.04)	0.23	0.94 (0.86–1.03)	0.21	1.03 (0.93–1.14)	0.60
Fortified wine drinkers								
Non-drinkers	Reference		Reference		Reference		Reference	
1–2 glasses/week	0.95 (0.85–1.06)	0.37	0.96 (0.85–1.08)	0.52	0.97 (0.87–1.10)	0.67	1.03 (0.91–1.16)	0.64
3–4 glasses/week	1.04 (0.81–1.34)	0.75	0.95 (0.73–1.25)	0.73	0.96 (0.73–1.26)	0.78	1.02 (0.78–1.34)	0.89
≥5 glasses/week	1.01 (0.77–1.33)	0.93	1.02 (0.76–1.35)	0.92	1.02 (0.76–1.35)	0.92	1.10 (0.82–1.47)	0.53

Model 1 analyses were adjusted for age, sex, education, ethnicity, employment, and overall health rating.
Model 2 analyses were further adjusted for smoking, Townsend, BMI, usually walking pace, sleep duration, getting up easily in the morning, nap during the day, daytime dozing/sleeping, sleeplessness, and snoring.
Model 3 analyses were further adjusted for cardiovascular diseases, diabetes, respiratory disease, and cancer.
Model 4 Analyses were further adjusted for frequency of alcohol intake, amount of weekly intake level of alcohol consumption, red wine, champagne plus white wine, beer and cider, spirits, and fortified wine.

significantly associated with IBD risk regardless of the amount of alcohol.

Sensitivity analysis of the association between alcoholic beverages and IBD risk

We further analyzed the association of alcoholic beverages with IBD risk among those subjects who only consumed one alcoholic beverage (Table 3). Compared with subjects without IBD, those subjects with IBD were less likely to be red wine drinkers ($p < 0.001$) and white wine and champagne drinkers ($p < 0.001$). However, no significant differences were found in beer and cider drinkers ($p = 0.1$), spirits drinkers ($p = 0.49$), and fortified wine drinkers ($p = 0.89$) between subjects with and without IBD. In the final multivariate model, compared with non-drinkers, the risk of IBD was 24% lower in red wine consumers of 3–4 glasses/week (0.76 [0.59, 1.00]) and 23% lower in ≥5 glasses/week (0.77 [0.66, 0.91]). However, white wine and champagne drinkers were not significantly associated with the risk of IBD.

Stratification analysis

We further examined the association of different alcoholic beverages with IBD risk, separated by frequency of alcohol intake (Table 4), amount of alcohol consumption (Table 5), and overall health rating (Table 6), respectively. In the final multivariate model, sociodemographic factors, lifestyle factors, sleep phenotypes, comorbidities, frequency of alcohol consumption, amount of weekly intake

TABLE 3 Characteristics of those subjects who only consumed one alcoholic beverage.

Characteristics	IBD related risk		*p*-value
	IBD($n = 7,095$)	Non-IBD($n = 495,410$)	
Only red wine drinkers, *N* (%)			<0.001
Non-drinkers	2,623 (89.1)	152,651 (84.5)	
1–2 glasses/week	59 (2.0)	4,980 (2.8)	
3–4 glasses/week	66 (2.2)	5,904 (3.3)	
≥5 glasses/week	195 (6.6)	17,077 (9.5)	
Only white wine and champagne drinkers			0.001
Non-drinkers	2,623 (89.5)	152,651 (87.0)	
1–2 glasses/week	53 (1.8)	4,304 (2.5)	
3–4 glasses/week	57 (1.9)	4,551 (2.6)	
≥5 glasses/week	199 (6.8)	14,022 (8.0)	
Only beer and cider drinkers, *N* (%)			0.1
Non-drinkers	2,623 (83.9)	152,651 (82.5)	
1–2 glasses/week	81 (2.6)	5,738 (3.1)	
3–4 glasses/week	80 (2.6)	5,662 (3.1)	
≥5 glasses/week	344 (11.0)	21,039 (11.4)	
Only spirits drinkers, *N* (%)			0.49
Non-drinkers	2,623 (94.4)	152,651 (94.7)	
1–2 glasses/week	27 (1.0)	1,669 (1.0)	
3–4 glasses/week	38 (1.4)	1,733 (1.1)	
≥5 glasses/week	91 (3.3)	5,087 (3.2)	
Only fortified wine drinkers, *N* (%)			0.89
Non-drinkers	2,623 (99.4)	152,651 (99.3)	
1–2 glasses/week	6 (0.2)	456 (0.3)	
3–4 glasses/week	4 (0.2)	272 (0.2)	
≥5 glasses/week	6 (0.2)	397 (0.3)	

#0.05≤p < 0.1, *p < 0.05, **p ≤ 0.01, and ***p ≤ 0.001 indicated p-values between IBD and non-IBD in male participants or female participants.

level of alcohol consumption, and alcoholic beverages were adjusted.

When stratifying our analysis by frequency of alcohol intake (Table 4), we found that, among the subjects who usually reported consumption of alcohol at a low frequency, consumption of red wine was associated with a lower risk of IBD (1–2 glasses/week, 0.85 [0.74, 0.97]), whereas consumption of spirits was associated with a higher risk of IBD (≥5 glasses/week, 1.28 [1.03, 1.59]). Among the subjects who usually reported consumption of alcohol at a high frequency, consumption of red wine was still associated with a lower risk of IBD (3–4 glasses/week, 0.83 [0.73, 0.96]; ≥5 glasses/week, 0.88 [0.78, 0.98]), whereas consumption of white wine and champagne was associated with a higher risk of IBD (1–2 glasses/week, 1.18 [1.06, 1.33]).

When stratifying our analysis by the amount of alcohol consumption (Table 5), we found that, among those subjects who usually reported consumption of alcohol within guidelines, all alcoholic beverages were not associated with IBD risk. Among those subjects who usually reported consumption of alcohol above guidelines, consumption of red wine was associated with a lower risk of IBD (3–4 glasses/week, 0.80 [0.67, 0.97]). Among those subjects who usually reported consumption of alcohol double above the guidelines, consumption of red wine was still associated with a lower risk of IBD (≥5 glasses/week, 0.83 [0.71, 0.97]), whereas consumption of white wine and champagne (1–2 glasses/week, 1.32 [1.09, 1.61]) and consumption of beer and cider (1–2 glasses/week,1.26 [1.02, 1.54]) were associated with a higher risk of IBD, respectively.

We also examined the association of different alcoholic beverages with IBD risk when stratifying our analysis by overall health rating (Table 6). Among those subjects with excellent or good overall health rating, consumption of red wine was associated with 11% to 23% lower risks of IBD (1–2 glasses/week, 0.89 [0.79, 1.00]; 3–4 glasses/week, 0.77 [0.67, 0.87]; ≥5 glasses/week, 0.84 [0.74, 0.95]) regardless of the amount of red wine, whereas consumption of white wine and champagne (1–2 glasses/week, 1.12 [1.01, 1.24]) was associated

TABLE 4 Odds ratios and 95% CIs for the association between different alcoholic beverages and IBD risk, separated by frequency of alcohol intake.

Variables (a)	Frequency of alcohol intake, OR (95%CI)			
	Once a month-twice/week	*p*	≥3 times/week	*p*
Red wine drinkers				
Non-drinkers	Reference		Reference	
1–2 glasses/week	0.85 (0.74–0.97)	0.015	0.95 (0.83–1.09)	0.46
3–4 glasses/week	0.89 (0.76–1.05)	0.18	0.83 (0.73–0.96)	0.009
≥5 glasses/week	0.89 (0.70–1.12)	0.31	0.88 (0.78–0.98)	0.023
White wine and champagne drinkers				
Non-drinkers	Reference		Reference	
1–2 glasses/week	1.08 (0.96–1.23)	0.21	1.18 (1.06–1.33)	0.004
3–4 glasses/week	1.11 (0.92–1.33)	0.28	1.04 (0.91–1.19)	0.60
≥5 glasses/week	1.11 (0.86–1.43)	0.42	1.05 (0.94–1.19)	0.39
Beer and cider drinkers				
Non-drinkers	Reference		Reference	
1–2 glasses/week	0.93 (0.81–1.07)	0.32	0.99 (0.88–1.11)	0.82
3–4 glasses/week	0.97 (0.80–1.17)	0.75	0.94 (0.81–1.10)	0.44
≥5 glasses/week	0.93 (0.73–1.17)	0.52	0.90 (0.78–1.04)	0.15
Spirits drinkers				
Non-drinkers	Reference		Reference	
1–2 glasses/week	1.01 (0.88–1.15)	0.94	0.98 (0.87–1.09)	0.67
3–4 glasses/week	1.08 (0.89–1.33)	0.44	1.04 (0.90–1.20)	0.62
≥5 glasses/week	1.28 (1.03–1.59)	0.029	0.94 (0.84–1.06)	0.31
Fortified wine drinkers				
Non-drinkers	Reference		Reference	
1–2 glasses/week	1.08 (0.88–1.32)	0.46	1.02 (0.88–1.19)	0.81
3–4 glasses/week	1.27 (0.78–2.08)	0.34	0.95 (0.68–1.32)	0.75
≥5 glasses/week	0.19 (0.03–1.38)	0.10	1.24 (0.92–1.66)	0.16

Logistic regression analysis adjusted for age, sex, education, ethnicity, employment, overall health rating, smoking, Townsend, BMI, usually walking pace, sleep duration, getting up easily in the morning, nap during the day, daytime dozing/sleeping, sleeplessness, snoring, cardiovascular diseases, diabetes, respiratory disease, cancer, amount of weekly intake level of alcohol consumption, red wine, champagne plus white wine, beer and cider, spirits, and fortified wine.

with a 12% higher risk of IBD. For those subjects with poor overall health rating, only consumption of 1–2 glasses/week of red wine was associated with a 15% lower risk of IBD (1–2 glasses/week, 0.85 [0.73, 0.99]), whereas the higher risk was not significant among white wine and champagne consumers. Other alcoholic beverages were not associated with IBD risk among those subjects with both good and poor overall health ratings. Therefore, our study suggests that the association between alcoholic beverages and the risk of IBD are not affected by overall health rating.

Interaction effects on IBD risk

Tables 7–11 showed that there were significant interaction effects between different alcoholic beverages, overall health rating, and amount of alcohol consumption (alcoholic beverage × alcoholic beverage, alcoholic beverage × overall health rating, alcoholic beverage × amount of alcohol consumption) regarding the risk of IBD after adjusting for sociodemographic factors, lifestyle factors, sleep phenotypes, comorbidities, frequency of alcohol consumption, amount of alcohol consumption, and alcoholic beverages.

There is an approximate interaction effect between 1–2 glasses/week of red wine and 1–2 glasses/week of fortified wine (1.38 [1.00–1.92], $p = 0.05$). However, other interaction effects of red wine with overall health rating, amount of alcohol consumption, white wine and champagne, beer and cider, or spirits on IBD risk were not found (Table 7). Consumption of 1–2 glasses/week of white wine and champagne showed significant interaction effects with double above the guidelines of alcohol consumption (1.27 [1.03–1.58], $p = 0.029$) and more than five glasses/week of beer and cider (1.46 [1.20–1.79], $p < 0.001$) on

TABLE 5 Odds ratios and 95% CIs for the association between different alcoholic beverages and IBD risk, separated by the amount of alcohol consumption.

Variables (b)	Alcohol consumption, dosage					
	Within guidelines	*p*-value	Above guidelines	*p*-value	Double above the guidelines or more	*p*-value
Red wine drinkers						
Non-drinkers	Reference		Reference		Reference	
1–2 glasses/week	0.89 (0.78–1.01)	0.08	0.89 (0.75–1.07)	0.21	1.02 (0.80–1.29)	0.90
3–4 glasses/week	0.91 (0.77–1.08)	0.27	0.80 (0.67–0.97)	0.021	0.86 (0.67–1.12)	0.27
≥5 glasses/week	0.97 (0.74–1.27)	0.83	0.88 (0.73–1.06)	0.17	0.83 (0.71–0.97)	0.02
White wine and champagne drinkers						
Non-drinkers	Reference		Reference		Reference	
1–2 glasses/week	1.11 (0.98–1.26)	0.09	1.12 (0.96–1.31)	0.14	1.32 (1.09–1.61)	0.005
3–4 glasses/week	1.11 (0.92–1.34)	0.27	1.12 (0.94–1.34)	0.21	0.93 (0.73–1.19)	0.57
≥5 glasses/week	1.31 (0.99–1.72)	0.06	1.04 (0.85–1.27)	0.70	1.02 (0.87–1.19)	0.85
Beer and cider drinkers						
Non-drinkers	Reference		Reference		Reference	
1–2 glasses/week	0.92 (0.81–1.06)	0.25	0.88 (0.75–1.03)	0.11	1.26 (1.02–1.54)	0.03
3–4 glasses/week	0.93 (0.76–1.14)	0.50	0.87 (0.71–1.06)	0.17	1.19 (0.93–1.52)	0.17
≥5 glasses/week	0.82 (0.62–1.10)	0.18	0.84 (0.67–1.05)	0.12	1.07 (0.88–1.31)	0.48
Spirits drinkers						
Non-drinkers	Reference		Reference		Reference	
1–2 glasses/week	1.05 (0.93–1.20)	0.41	1.02 (0.89–1.17)	0.78	0.82 (0.67–1.00)	0.051
3–4 glasses/week	1.06 (0.86–1.30)	0.59	0.99 (0.81–1.20)	0.92	1.16 (0.94–1.43)	0.17
≥5 glasses/week	1.26 (1.00–1.58)	0.053	1.05 (0.87–1.26)	0.62	0.86 (0.73–1.02)	0.08
Fortified wine drinkers						
Non-drinkers	Reference		Reference		Reference	
1–2 glasses/week	1.03 (0.85–1.26)	0.75	1.10 (0.91–1.34)	0.32	0.99 (0.77–1.28)	0.93
3–4 glasses/week	1.32 (0.78–2.22)	0.30	1.01 (0.66–1.52)	0.98	0.89 (0.52–1.51)	0.66
≥5 glasses/week	0.45 (0.06–3.23)	0.43	1.25 (0.79–1.97)	0.34	1.08 (0.73–1.60)	0.71

Logistic regression analysis adjusted for age, sex, education, ethnicity, employment, overall health rating, smoking, Townsend, BMI, usually walking pace, sleep duration, getting up easily in the morning, nap during the day, daytime dozing/sleeping, sleeplessness, snoring, cardiovascular diseases, diabetes, respiratory disease, cancer, frequency of alcohol intake, red wine, champagne plus white wine, beer and cider, spirits, and fortified wine.

a higher risk of IBD, respectively (Table 8). A similar interaction pattern was observed between 3–4 glasses/week of white wine and champagne and 3–4 glasses/week of beer and cider (1.45 [1.05–2.01], p = 0.024). Several interaction effects between beer and cider and poor health rating (3–4 glasses/week× poor health, $p = 0.04$; ≥5 glasses/week × poor health, $p = 0.001$) or spirits (1–2 glasses/week beer and cider × 3–4 glasses/week spirits, $p = 0.026$; 1–2 glasses/week × ≥5 glasses/week, $p = 0.003$; 3-4 glasses/week ×1–2 glasses/week, $p = 0.023$) on higher risks of IBD were observed (Table 9). There is an interaction effect between ≥5 glasses/week of spirits and 3–4 glasses/week of fortified wine ($p = 0.032$) (Table 10). However, this interaction pattern was not observed between fortified wine and overall health rating and between fortified wine and amount of alcohol consumption, respectively (Table 11).

Non-linear associations of alcoholic beverages with the amount of alcohol consumption on IBD risk

The dose-response associations between the amount of alcohol consumption and alcoholic beverages on IBD risk are shown in Figures 1A–F. The total amount of alcohol consumption (Figure 1A) and white wine and champagne (Figure 1C) showed a curvilinear J-shaped correlation with the risk of IBD, respectively. Red wine, beer and cider, spirits, and fortified wine showed a linear correlation with IBD risk, respectively (Figures 1B,D–F), where alcohol consumers had an increased risk with a greater number of consumptions of beer and cider, and the IBD risk was lower among red consumers across the whole dose cycle.

TABLE 6 Odds ratios and 95% CIs for the association between different alcoholic beverages and IBD risk, separated by overall health rating.

Variables	Overall health rating, OR (95%CI)			
	Excellent or good	*p*	Fair or poor	*p*
Red wine drinkers				
Non-drinkers	Reference		Reference	
1–2 glasses/week	0.89 (0.79–1.00)	0.044	0.85 (0.73–0.99)	0.039
3–4 glasses/week	0.77 (0.67–0.87)	<0.001	0.98 (0.83–1.15)	0.77
≥5 glasses/week	0.84 (0.74–0.95)	0.006	0.88 (0.75–1.03)	0.12
White wine and champagne drinkers				
Non-drinkers	Reference		Reference	
1–2 glasses/week	1.12 (1.01–1.24)	0.035	1.12 (0.98–1.28)	0.11
3–4 glasses/week	1.11 (0.98–1.27)	0.11	0.91 (0.75–1.10)	0.33
≥5 glasses/week	1.04 (0.91–1.19)	0.56	1.03 (0.86–1.22)	0.78
Beer and cider drinkers				
Non-drinkers	Reference		Reference	
1–2 glasses/week	0.96 (0.86–1.07)	0.45	0.97 (0.84–1.11)	0.62
3–4 glasses/week	0.99 (0.86–1.14)	0.87	0.92 (0.77–1.10)	0.36
≥5 glasses/week	0.94 (0.81–1.09)	0.43	0.89 (0.74–1.06)	0.19
Spirits drinkers				
Non-drinkers	Reference		Reference	
1–2 glasses/week	0.98 (0.88–1.09)	0.70	1.01 (0.88–1.17)	0.85
3–4 glasses/week	1.07 (0.92–1.24)	0.38	1.06 (0.88–1.28)	0.53
≥5 glasses/week	1.01 (0.88–1.16)	0.92	1.05 (0.90–1.23)	0.56
Fortified wine drinkers				
Non-drinkers	Reference		Reference	
1–2 glasses/week	0.99 (0.85–1.16)	0.91	1.11 (0.91–1.36)	0.32
3–4 glasses/week	1.03 (0.73–1.45)	0.87	1.01 (0.64–1.60)	0.97
≥5 glasses/week	1.30 (0.92–1.84)	0.14	0.79 (0.46–1.35)	0.38

Logistic regression analysis adjusted for age, sex, education, ethnicity, employment, overall health rating, smoking, Townsend, BMI, usually walking pace, sleep duration, getting up easily in the morning, nap during the day, daytime dozing/sleeping, sleeplessness, snoring, cardiovascular diseases, diabetes, respiratory disease, cancer, amount of weekly intake level of alcohol consumption, red wine, champagne plus white wine, beer and cider, spirits, and fortified wine.

Discussion

In this prospective study of a large-sized UK Biobank cohort, we documented four novel findings. First, red wine was identified as an independent protective factor for IBD. It played the protective effect on those subjects who consumed alcohol above or double above the guidelines and consumed alcohol both at high and low frequencies. Second, 1–2 glasses/week of white wine and champagne was identified as an independent risk factor for IBD, especially among those subjects who consumed alcohol double above the guidelines and who consumed alcohol at a high frequency. Third, consumption of ≥5 glasses/week of spirits at a low frequency and consumption of beer and cider double above the guidelines are associated with increased risk of IBD. Fourth, the dose-response associations showed an increased risk of IBD with more number of alcohol consumption. This association can be found in white wine and champagne, and beer and cider. However, red wine is still at a low risk across the whole dose cycle. Therefore, the IBD risk appears to vary across consumption of different subtypes, frequencies, and number of alcoholic beverages. Especially, our study suggests that the association between alcoholic beverages and the risk of IBD is not affected by overall health rating.

Our study found that alcohol intake was associated with a higher risk of IBD. The protective association of low-to-moderate alcohol consumption with lower risk of cataract (23), lower risk of myocardial infarction, (20) and better cognitive function (24) have been widely reported. Recent studies found that chronic alcoholism was not a risk or protective factor of IBD (25, 26). However, studies have shown that long-term alcohol abuse and acute binge drinking are associated with immunosuppression and increased susceptibility to both bacterial and viral infections (27, 28). The widely held view of the health benefits of alcohol needs revising, and Collaborators proposed that the safest level of drinking is none (6). Our results support this view. In our study, the total number of

TABLE 7 Odds ratios and 95% CIs for the interaction effect between red wine and risk of IBD.

	Red wine drinkers							
	Non-drinkers	**1–2 glasses/week**	**p**	**3–4 glasses/week**	***p***	**≥5 glasses/week**	***p***
Overall health rating							
Excellent or good	Reference	Reference		Reference		Reference	
Fair or poor	Reference	0.92 (0.78–1.09)	0.32	1.20 (1.00–1.44)	0.06	0.98 (0.85–1.13)	0.79
Alcohol consumption, dosage							
Never drinker, previous–drinkers or special occasions only	Reference	Reference		Reference		Reference	
Within guidelines	Reference	N/A		N/A		N/A	
Above guidelines	Reference	1.06 (0.87–1.30)	0.56	0.95 (0.77–1.17)	0.63	0.96 (0.74–1.25)	0.75
Double above the guidelines or more	Reference	1.26 (0.97–1.63)	0.08	1.01 (0.76–1.35)	0.93	0.92 (0.71–1.20)	0.54
White wine and champagne drinkers							
Non-drinkers	Reference	Reference		Reference		Reference	
1–2 glasses/week	Reference	1.21 (0.98–1.49)	0.08	0.93 (0.72–1.19)	0.55	1.16 (0.95–1.43)	0.15
3–4 glasses/week	Reference	1.31 (0.96–1.78)	0.09	1.05 (0.80–1.38)	0.73	1.25 (0.96–1.62)	0.09
≥5 glasses/week	Reference	1.20 (0.92–1.56)	0.18	1.02 (0.75–1.38)	0.91	1.02 (0.84–1.25)	0.82
Beer and cider drinkers							
Non-drinkers	Reference	Reference		Reference		Reference	
1–2 glasses/week	Reference	1.17 (0.95–1.45)	0.14	1.16 (0.91–1.47)	0.24	1.16 (0.95–1.41)	0.15
3–4 glasses/week	Reference	1.12 (0.84–1.50)	0.45	1.11 (0.80–1.52)	0.54	1.13 (0.89–1.45)	0.32
≥5 glasses/week	Reference	1.10 (0.88–1.39)	0.40	1.15 (0.88–1.49)	0.31	1.14 (0.95–1.38)	0.17
Spirits drinkers							
Non-drinkers	Reference	Reference		Reference		Reference	
1–2 glasses/week	Reference	1.16 (0.93–1.45)	0.18	1.12 (0.88–1.44)	0.36	1.07 (0.87–1.32)	0.53
3–4 glasses/week	Reference	1.20 (0.86–1.68)	0.29	1.31 (0.93–1.84)	0.12	1.13 (0.86–1.49)	0.39
≥5 glasses/week	Reference	1.22 (0.92–1.62)	0.18	1.34 (0.99–1.82)	0.06	0.86 (0.68–1.08)	0.19
Fortified wine drinkers							
Non-drinkers	Reference	Reference		Reference		Reference	
1–2 glasses/week	Reference	1.38 (1.00–1.92)	0.05	1.10 (0.76–1.59)	0.63	1.18 (0.85–1.64)	0.32
3–4 glasses/week	Reference	0.77 (0.35–1.69)	0.51	0.96 (0.45–2.04)	0.91	0.73 (0.36–1.48)	0.38
≥5 glasses/week	Reference	1.70 (0.78–3.72)	0.19	1.46 (0.57–3.71)	0.43	1.25 (0.62–2.53)	0.54

Logistic regression analysis adjusted for age, sex, education, ethnicity, employment, overall health rating, smoking, Townsend, BMI, usually walking pace, sleep duration, getting up easily in the morning, nap during the day, daytime dozing/sleeping, sleeplessness, snoring, cardiovascular diseases, diabetes, respiratory disease, cancer, frequency of alcohol intake, red wine, champagne plus white wine, beer and cider, spirits, and fortified wine.

alcohol consumption showed a curvilinear J-shaped correlation with IBD risk, and the recommended intake of alcohol is also none. This level conflicts with most health guidelines, which espouse health benefits associated with low-to-moderate alcohol consumption. However, subgroup analyses for alcoholic beverages reported a new finding, which shows that red wine is safe to drink throughout the whole dose cycle as it was associated with a lower risk of IBD. Therefore, it's worth noting that, although our findings suggest that alcohol intake is not recommended because it was associated with a higher risk of IBD, there is no direct evidence that red wine is not recommended for IBD high-risk population.

The most striking and interesting finding was that consumption of red wine was associated with a lower chance of developing IBD. More and more research are trying to explore the potentially beneficial properties of wine or its components on IBD, and studies have shown that the beneficial properties of wine are attributed to their polyphenolic content, but are not independent of the presence of alcohol (29, 30). Polyphenols are present in varying degrees in different subtypes of alcoholic beverages, particularly in red wine which has the highest concentrations of phenolic compounds (31) because all grape parts are used during the winemaking process of red wine (32). Red wine provides additional benefits compared to other subtypes of alcoholic beverages probably due to its higher polyphenolic content, by decreasing blood pressure, improving endothelial function, reducing inflammation and cell adhesion, inhibiting the oxidation of low-density lipoprotein particles, and other favorable effects on the cellular redox state, inhibiting platelet aggregation, and activating proteins that prevent cell

TABLE 8 Odds ratios and 95% CIs for the interaction effect between white wine and champagne and the risk of IBD.

	White wine and champagne drinkers					
	Non-drinkers	1–2 glasses/week	*p*	3–4 glasses/week	*p*	≥5 glasses/week	*p*
Overall health rating							
Excellent or good	Reference	Reference		Reference		Reference	
Fair or poor	Reference	0.99 (0.86–1.15)	0.94	0.86 (0.70–1.06)	0.16	0.97 (0.82–1.15)	0.69
Alcohol consumption, dosage							
Never drinker, previous–drinkers or special occasions only	Reference	Reference		Reference		Reference	
Within guidelines	Reference	N/A		N/A		N/A	
Above guidelines	Reference	1.04 (0.87–1.25)	0.64	1.00 (0.79–1.25)	0.97	0.82 (0.62–1.08)	0.15
Double above the guidelines or more	Reference	1.27 (1.03–1.58)	0.029	0.86 (0.65–1.14)	0.30	0.78 (0.59–1.02)	0.07
Beer and cider drinkers							
Non-drinkers	Reference	Reference		Reference		Reference	
1–2 glasses/week	Reference	1.17 (0.97–1.41)	0.11	1.26 (0.98–1.61)	0.07	1.14 (0.91–1.42)	0.26
3–4 glasses/week	Reference	1.27 (0.98–1.64)	0.07	1.45 (1.05–2.01)	0.024	1.02 (0.74–1.41)	0.91
≥5 glasses/week	Reference	1.46 (1.20–1.79)	<0.001	1.18 (0.88–1.59)	0.27	1.00 (0.77–1.29)	0.98
d							
Spirits drinkers							
Non-drinkers	Reference	Reference		Reference		Reference	
1–2 glasses/week	Reference	1.13 (0.93–1.37)	0.23	1.27 (0.99–1.64)	0.06	1.11 (0.87–1.41)	0.40
3–4 glasses/week	Reference	1.30 (0.98–1.73)	0.08	1.12 (0.78–1.62)	0.53	1.14 (0.83–1.58)	0.42
≥5 glasses/week	Reference	1.23 (0.96–1.60)	0.11	1.28 (0.93–1.78)	0.13	1.06 (0.81–1.38)	0.68
Fortified wine drinkers							
Non-drinkers	Reference	Reference		Reference		Reference	
1–2 glasses/week	Reference	1.14 (0.86–1.53)	0.37	1.15 (0.80–1.65)	0.45	1.13 (0.79–1.62)	0.50
3–4 glasses/week	Reference	1.11 (0.57–2.14)	0.76	0.85 (0.38–1.93)	0.71	0.73 (0.31–1.71)	0.47
≥5 glasses/week	Reference	1.75 (0.89–3.46)	0.11	1.06 (0.39–2.84)	0.92	0.95 (0.41–2.18)	0.90

Logistic regression analysis adjusted for age, sex, education, ethnicity, employment, overall health rating, smoking, Townsend, BMI, usually walking pace, sleep duration, getting up easily in the morning, nap during the day, daytime dozing/sleeping, sleeplessness, snoring, cardiovascular diseases, diabetes, respiratory disease, cancer, frequency of alcohol intake, red wine, champagne plus white wine, beer and cider, spirits, and fortified wine.

death (31). Polyphenols comprised several chemical compounds that are generally classified into flavonoids and non-flavonoids (33), which are considered the main bioactive components in wine that positively affect health (31, 34). The beneficial effect included antioxidant, anti-inflammatory, anti-cancer, and anti-microbial (35). As known, polyphenols could inhibit the effects of several types of viruses, including the Epstein–Barr virus (36, 37), herpes simplex virus (38), enterovirus (39, 40), COVID-19 (18), influenza virus (41), and other viruses causing respiratory tract-related infections (42, 43). Many of the phenolic compounds in wine have low bioavailability, and hence, reach low concentrations in the bloodstream, while their high content present in the gut can produce a more significant effect on enterocytes and the bacterial flora (44, 45). Therefore, these findings support the notion of the strong beneficial properties of red wine against developing IBD risk.

Our study suggests that consumption of some alcoholic beverages were associated with higher risks of developing IBD. We discovered risk factors of low frequency of ≥5 glasses/week spirit, high frequency and double above dose of the guidelines of 1–2 glasses/week of white wine and champagne, and double above dose of the guidelines of beer and cider for developing IBD. Sanja Radonjić et al. have reported the differences between wine and beer in the presence and the concentrations of phenolic substances (33). Furthermore, spirits had the highest alcohol concentration and the lowest polyphenolic concentration. These findings have shown the differences between these alcoholic beverages and red wine. Evidence have shown that red wine extract and wine digested fluids played a protective effect on the cellular barrier (46, 47) and led to increased intestinal permeability (48). However, this is in stark contrast to the evidence by Asai et al. that a low and acute dose of ethanol leads to apoptotic cell death in confluent Caco-2 cells and, therefore, impairs intestinal barrier function (49). Therefore, it is probable that the polyphenolic content per se has a positive effect on intestinal permeability, while the alcoholic content potentially negates this effect (17). These findings may suggest that the specific class of

TABLE 9 Odds ratios and 95% CIs for the interaction effect between beer and cider and the risk of IBD.

	Beer and cider drinkers						
	Non-drinkers	1–2 glasses/week	*P*	3–4 glasses/week	*p*	≥5 glasses/week	*p*
Overall health rating							
Excellent or good	Reference	Reference		Reference		Reference	
Fair or poor	Reference	0.92 (0.79–1.07)	0.29	0.81 (0.66–0.99)	0.040	0.78 (0.67–0.90)	0.001
Alcohol consumption, dosage							
Never drinker, previous–drinkers or special occasions only	Reference	Reference		Reference		Reference	
Within guidelines	Reference	N/A		N/A		N/A	
Above guidelines	Reference	0.90 (0.75–1.08)	0.25	0.82 (0.64–1.04)	0.10	0.95 (0.72–1.25)	0.73
Double above the guidelines or more	Reference	1.22 (0.98–1.53)	0.08	1.01 (0.77–1.33)	0.95	1.12 (0.85–1.48)	0.43
Spirits drinkers							
Non-drinkers	Reference	Reference		Reference		Reference	
1–2 glasses/week	Reference	1.09 (0.89–1.33)	0.41	1.35 (1.04–1.76)	0.023	0.97 (0.77–1.22)	0.79
3–4 glasses/week	Reference	1.40 (1.04–1.89)	0.026	1.40 (0.98–2.00)	0.06	1.07 (0.79–1.45)	0.64
≥5 glasses/week	Reference	1.46 (1.14–1.88)	0.003	1.11 (0.80–1.55)	0.53	0.93 (0.73–1.18)	0.55
Fortified wine drinkers							
Non-drinkers	Reference	Reference		Reference		Reference	
1–2 glasses/week	Reference	1.12 (0.84–1.48)	0.44	0.90 (0.57–1.40)	0.63	0.89 (0.59–1.33)	0.57
3–4 glasses/week	Reference	1.67 (0.91–3.07)	0.10	0.55 (0.13–2.29)	0.41	0.38 (0.09–1.60)	0.19
≥5 glasses/week	Reference	0.71 (0.30–1.71)	0.45	0.83 (0.25–2.73)	0.76	0.57 (0.20–1.63)	0.30

Logistic regression analysis adjusted for age, sex, education, ethnicity, employment, overall health rating, smoking, Townsend, BMI, usually walking pace, sleep duration, getting up easily in the morning, nap during the day, daytime dozing/sleeping, sleeplessness, snoring, cardiovascular diseases, diabetes, respiratory disease, cancer, frequency of alcohol intake, red wine, champagne plus white wine, beer and cider, spirits, and fortified wine.

TABLE 10 Odds ratios and 95% CIs for the interaction effect between spirits and risk of IBD.

	Spirits drinkers						
	Non-drinkers	1–2 glasses/week	*p*	3–4 glasses/week	*p*	≥5 glasses/week	*p*
Overall health rating							
Excellent or good	Reference	Reference		Reference		Reference	
Fair or poor	Reference	0.97 (0.82–1.13)	0.67	0.88 (0.70–1.10)	0.26	0.88 (0.73–1.06)	0.17
Alcohol consumption, dosage							
Never drinker, previous–drinkers or special occasions only	Reference	Reference		Reference		Reference	
Within guidelines	Reference	N/A		N/A		N/A	
Above guidelines	Reference	0.94 (0.78–1.13)	0.53	0.91 (0.69–1.20)	0.51	0.87 (0.67–1.12)	0.27
Double above the guidelines or more	Reference	0.77 (0.61–0.97)	0.024	1.00 (0.75–1.34)	0.98	0.67 (0.52–0.87)	0.002
Fortified wine drinkers							
Non-drinkers	Reference	Reference		Reference		Reference	
1–2 glasses/week	Reference	1.01 (0.76–1.32)	0.97	0.77 (0.49–1.22)	0.27	0.91 (0.60–1.38)	0.67
3–4 glasses/week	Reference	1.62 (0.76–3.45)	0.21	2.01 (0.94–4.30)	0.07	2.24 (1.07–4.69)	0.032
≥5 glasses/week	Reference	0.99 (0.43–2.26)	0.98	0.29 (0.04–2.13)	0.22	0.67 (0.29–1.53)	0.34

Logistic regression analysis adjusted for age, sex, education, ethnicity, employment, overall health rating, smoking, Townsend, BMI, usually walking pace, sleep duration, getting up easily in the morning, nap during the day, daytime dozing/sleeping, sleeplessness, snoring, cardiovascular diseases, diabetes, respiratory disease, cancer, frequency of alcohol intake, red wine, champagne plus white wine, beer and cider, spirits, and fortified wine.

polyphenolic constituents may be responsible for the beneficial effect of alcoholic beverages on IBD, but not the alcohol concentration.

The major strengths of this study are the prospective and comprehensive study design, large UK population-based cohort, dose-response associations of alcohol consumption with IBD

TABLE 11 Odds ratios and 95% CIs for the interaction effect between fortified wine and risk of IBD.

	Fortified wine drinkers							
	Non-drinkers	1–2 glasses/week		3–4 glasses/week		≥5 glasses/week		
Overall health rating								
Excellent or good	Reference	Reference		Reference		Reference		
Fair or poor	Reference	1.11 (0.87–1.41)	0.41	0.99 (0.56–1.75)	0.98	0.59 (0.32–1.11)	0.10	
Alcohol consumption, dosage								
Never drinker, previous–drinkers or special occasions only	Reference	Reference		Reference		Reference		
Within guidelines	Reference	N/A		N/A		N/A		
Above guidelines	Reference	1.09 (0.83–1.43)	0.55	0.80 (0.41–1.53)	0.49	3.01 (0.40–22.51)	0.28	
Double above the guidelines or more	Reference	0.98 (0.71–1.34)	0.88	0.67 (0.32–1.40)	0.29	2.51 (0.34–18.62)	0.37	

Logistic regression analysis adjusted for age, sex, education, ethnicity, employment, overall health rating, smoking, Townsend, BMI, usually walking pace, sleep duration, getting up easily in the morning, nap during the day, daytime dozing/sleeping, sleeplessness, snoring, cardiovascular diseases, diabetes, respiratory disease, cancer, frequency of alcohol intake, red wine, champagne plus white wine, beer and cider, spirits, and fortified wine.

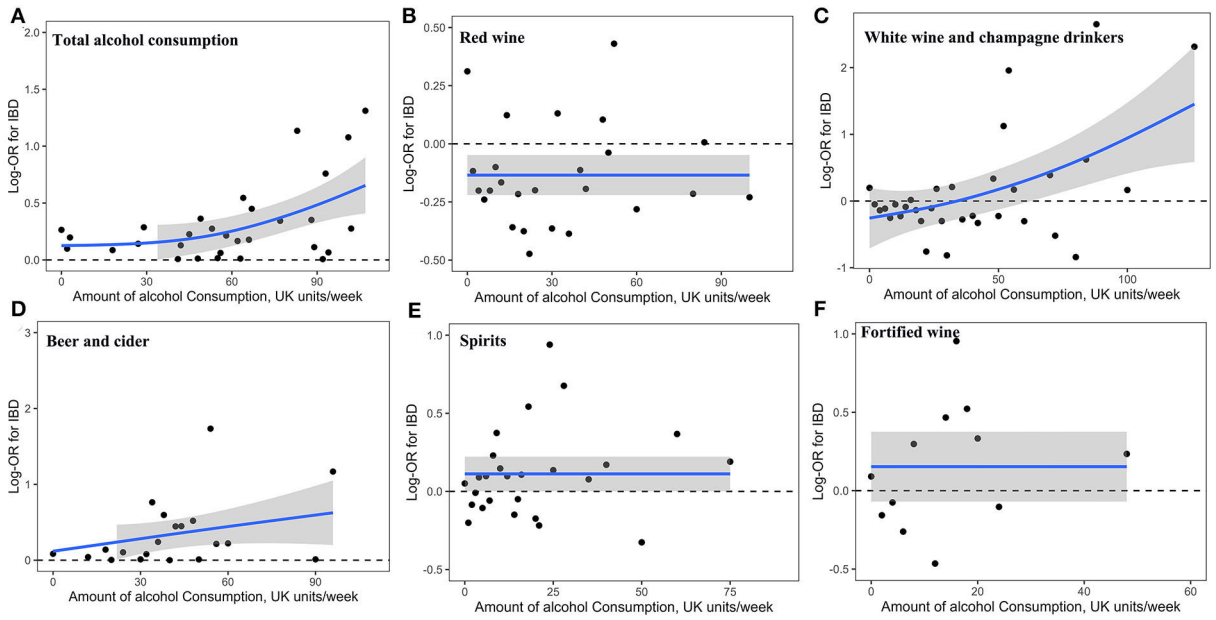

FIGURE 1
Association of alcohol consumption with risk of IBD. Linear and non-linear associations of the amount of total alcohol consumption **(A)** and that of alcoholic beverages (**B**, red wine; **C** white wine and champagne; **D**, beer and cider; **E**, spirits; **F**, fortified wine) with risk of IBD.

risk, and a focus on the association of different subtypes of alcoholic beverages with IBD risk. However, there are several limitations that should be addressed. First, subjects in the UK Biobank have a restricted age range, and therefore our data could not represent the whole population. Second, the data on alcohol drinking habits were derived from baseline, and we did not know about potential changes from baseline to outcome end-point. Third, recruiting heavy drinkers to test different alcoholic beverages for dose-response analyses is difficult. Fourth, although we adjusted for a wide range of potential confounders and did a series of rigorous statistical analyses, residual confounding factors may still exist, especially the interaction between different alcoholic beverages. Fifth, a relatively small number of IBD cases that only consumed one alcoholic beverage were included, which may reduce its reliability. Sixth, past trauma or stressful events may be a cause of alcohol intake, future studies should adjust for the effect of past trauma or stressful events.

Conclusions

In conclusion, our study suggests that the IBD risk appears to vary across different frequencies, amounts, and subtypes of alcoholic beverages. Overall, alcohol intake is not recommended because it was associated with a higher risk of IBD, and the safe level of drinking appears to be none. A focus on the association of different frequencies, amounts, and subtypes of alcoholic beverages with the risk of IBD provided an important addition to the existing research on alcohol intake and IBD risk. We found that consumption of red wine may reduce the risk of IBD, while high frequency and high dose of white wine and champagne, low frequency and acute dose of spirit, and high dose of beer and cider appear to increase the risk of IBD. Public health guidance should focus on reducing the risk of IBD by advocating healthy lifestyle habits and preferential policies among consumers.

Data availability statement

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

Ethical approval

Ethical approval of the UK Biobank was obtained from the National Health System Northwest Multicenter Research Ethics Committee (REC reference: 16/NW/0274). The patients/participants provided their written informed consent to participate in this study.

Author contributions

B-XL, CZ, X-JD, and YC had the idea for and designed this study. X-JD and B-XL had full access to all the data in this study, take responsibility for the integrity of the data and the accuracy of the data analysis, and drafted the paper. CZ, X-JD, and YC critically revised the manuscript for important intellectual content and gave final approval for the version to be published. JY, CZ, and YC take responsibility for double check of the data analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.918754/full#supplementary-material

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Effects of low-carbohydrate diets versus low-fat diets on metabolic risk factors in overweight and obese adults: A meta-analysis of randomized controlled trials

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Background and aims: Low-carbohydrate diets (LCD) and low-fat diets (LFD) have shown beneficial effects on the management of obesity. Epidemiological studies were conducted to compare the effects of the two diets. However, the results were not always consistent. This study aimed to conduct a meta-analysis to compare the long-term effects of LCD and LFD on metabolic risk factors and weight loss in overweight and obese adults.

Methods: We performed a systematic literature search up to 30 March, 2022 in PubMed, EMBASE, and Cochrane Library. The meta-analysis compared the effects of LCD (carbohydrate intake $\leq$ 40%) with LFD (fat intake $<$ 30%) on metabolic risk factors and weight loss for $\geq$6 months. Subgroup analyses were performed based on participant characteristics, dietary energy intake, and the proportions of carbohydrates.

Results: 33 studies involving a total of 3,939 participants were included. Compared with participants on LFD, participants on LCD had a greater reduction in triglycerides (−0.14 mmol/L; 95% CI, −0.18 to −0.10 mmol/L), diastolic blood pressure (−0.87 mmHg; 95% CI, −1.41 to −0.32 mmHg), weight loss (−1.33 kg; 95% CI, −1.79 to −0.87 kg), and a greater increase in high-density lipoprotein cholesterol (0.07 mmol/L; 95% CI, 0.06 to 0.09 mmol/L) in 6–23 months. However, the decrease of total cholesterol (0.14 mmol/L; 95% CI, 0.07 to 0.20 mmol/L) and low-density lipoprotein cholesterol (0.10 mmol/L; 95% CI, 0.06 to 0.14 mmol/L) was more conducive to LFD in 6–23 months. There was no difference in benefits between the two diets after 24 months. Subgroup analyses showed no significant difference in the reduction of total cholesterol, low-density lipoprotein cholesterol, and blood pressure between the two diets in participants with diabetes, hypertension, or hyperlipidemia.

Conclusion: The results suggest that LCD and LFD may have specific effects on metabolic risk factors and weight loss in overweight and obese adults over 6 months. At 24 months, the effects on weight loss and improvement of metabolic risk factors were at least the same. These indicated that we might choose different diets to manage the overweight and obese subjects. However, the long-term clinical efficacy and effects of various sources of carbohydrates or fat in the two diets need to be studied in the future.

KEYWORDS

low-carbohydrate diets, low-fat diets, overweight, metabolic risk factors, obesity

Introduction

Obesity is associated with an increased risk of hypertension, type 2 diabetes mellitus, dyslipidemia, metabolic syndrome, etc. The higher prevalence of obesity has become a significant global public health crisis issue. In 2016, more than 1.9 billion adults aged 18 years and older were overweight; of these, over 650 million were obese (1). The prevalence of obesity and its adverse consequences results in a heavy economic burden on the individual and on families and nations, including both developed and developing countries (2). Thus, improving the efficacy in preventing and controlling obesity worldwide is expected to have a great potential to reduce health costs and improve global health (3, 4).

Dietary factors play a vital role in the control of obesity. All methods of dietary intervention for obesity are based on reduced caloric diets (5). Among them, low-fat diets (LFD), especially reduced saturated fat intake, are the most widely used, which have been suggested in the dietary instruction for weight loss by the American Heart Association Nutrition Committee (6). However, in recent years, a number of studies have shown that other diets, such as low-carbohydrate diets (LCD), also have beneficial effects on significant weight loss, as well as increased energy expenditure, improved hyperinsulinemia and glycemic control, and decreased cardiometabolic risk (7–9). Thus, LCD has attracted more and more attention to the management of obesity.

Over the past 20 years, epidemiological studies have been conducted to compare the effects between LCD and LFD on metabolic risk factors and weight loss in overweight and obese adults (10–15); however, the results were not always consistent. These make people confused because both LFD and LCD have been suggested in the different dietary guidelines (6, 16, 17). Nadia et al. compared the effects of LCD and LFD on cardiovascular risk factors in healthy people (18). But this study only focused on persons without cardiometabolic diseases such as type 2 diabetes mellitus, myocardial infarction, stroke, etc., which are often accompanied by obesity and may benefit more from the two dietary patterns. Another earlier study by Hu et al. compared the effects of LCD versus LFD on metabolic risk factors in overweight and obese persons, indicating that LCD is at least as effective as LFD at decreasing weight and improving metabolic risk factors (19). After that, many new studies on this comparison are available (13–15, 20–24). The different effects on metabolic risk factors in overweight and obese persons between carbohydrate-restricted diets and fat-restricted diets still require further elucidation. Furthermore, the results of recent meta-analyses were usually conducted by medium- and short-term trials rather than separate analyses for longer-term studies, and they did not explore the effect on different populations such as patients with hyperlipidemia, diabetes, and hypertension. Therefore, we aimed to conduct the present study to compile the current evidence from all qualified randomized controlled trials to compare the long-term effects of the two diets on metabolic risk factors and weight loss in overweight and obese subjects.

Materials and methods

Literature search strategy

This meta-analysis was reported based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA). To evaluate the effects of low carbohydrate diets (LCD) versus low fat diets (LFD) on metabolic risk factors and weight loss. Studies that were published on or before 30 March 2022 were selected. Keywords or medical subject-heading terms were used to screen as follows: LCD, carbohydrate-restricted diet, diet, or ketogenic combined with blood glucose or blood pressure or triglycerides or total cholesterol or high-density lipoprotein or low-density lipoprotein. First, titles and abstracts were filtered to exclude irrelevant studies. The full contents of the remaining

literatures were next selected according to the pre-established criteria. Furthermore, references to the selected articles were also searched. The full search strategy is shown in the **Supplementary materials**.

Selection criteria

The inclusion criteria of this meta-analysis study were as follows: (1) the design of the study was a randomized controlled trial; (2) study participants were adults (at least 18 years old); (3) the subjects had a BMI $\geq$ 25 kg/m^2, including overweight and obese, or BMI $\geq$ 30 kg/m^2, including only obese (if the included study was from Asia, subjects with BMI $\geq$ 23 kg/m^2, or BMI $\geq$ 30 kg/m^2 were regarded as overweight or obese). (4) LCD and LFD were compared; (5) the intervention period was 6 months or longer; (6) both metabolic risk factors and body weight loss were included as the outcomes. Studies were excluded when other interventions such as drugs, surgery, and compulsory planned exercise were mentioned. The carbohydrate-restricted diets were defined as a prescribed intake of carbohydrates less than 40% of the total energy intake or a distinct reference to the Atkins diet, with an intake of only 20–40 g/d of carbohydrate in the first phase or carbohydrate intake of $<$ 20% of total energy intake (25). The LFD was defined as a prescribed fat intake of less than 30% of total energy intake (25–27).

Data extraction and quality assessment

Two investigators (LL and JH) independently searched and screened all the potential related studies. The following information from each eligible study was extracted: (1) the basic characteristics of the included studies, including author's name, year of publication, country of origin, duration of intervention, type of design, dietary composition, number, and rate of completion; (2) the characteristics of included persons, including sample size, gender, age, BMI, and health status such as basic diseases (diabetes, hypertension, cardiovascular diseases, and hyperlipemia); (3) the changes of metabolic risk factors compared with baseline, including triglycerides (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), fasting blood glucose, systolic blood pressure (SBP), diastolic blood pressure (DBP), and body weight loss.

Two investigators independently assessed the risk of bias in the included studies using the Cochrane Collaboration's tool (28), which contains the following criteria: (1) selection bias (random method); (2) performance bias; (3) detection bias (blind method for participants and results evaluation); (4) attrition bias (incomplete result data); (5) reporting bias (selective result reporting); and (6) other sources of bias. Studies were defined as having a high risk of bias: $\geq$1 item was a high risk of bias, and low risk of bias if all of the items were evaluated with a low risk of bias. The others were assessed as being at moderate risk of bias. Additionally, the quality of evidence for outcomes was evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE), which characterizes the evidence on the study limitations, imprecision, inconsistency, indirectness, and publication bias (29, 30).

Statistical analysis

Weighted mean differences (WMD) from baselines were calculated for the effects of LCD versus LFD on metabolic risk factors and weight loss and then a meta-analysis was performed. The baseline and outcomes values are shown in **Supplementary Table 1**. The heterogeneity was assessed by measuring the inconsistency (I^2 statistic) of treatment effects among the trials. If there was significant heterogeneity across studies ($I^2 > 50\%$), the random effect model was used. Otherwise, the fixed effect model was used. If data were missing or incomplete, complete cases were analyzed. It should be noted that due to the different blood lipid and blood glucose units reported in the included studies, the data in mg/dL of blood lipid were converted to mmol/L by multiplying 0.0259 of TC, 0.0258 of HDL-C, 0.0259 of LDL-C, and 0.0113 of TG. The blood glucose value in mg/dL was converted to mmol/L by dividing 18. In addition, to determine whether different intervention time has different effects, the duration of intervention was stratified into 6–11 months, 12–23 months, and 24 months.

The publication bias was judged by the funnel plot and Egger's regression test (31). Meta-regression and subgroup analyses were used to analyze the possible sources of heterogeneity, including hypertensive status, hyperlipidemia status, diabetic status, energy intake, and proportions of carbohydrates. Furthermore, sensitivity analyses were performed to explore the different potential influences by excluding each study in turn. All statistical analyses were performed using Stata statistical software (Version 14.0; Stata Corp.).

Results

Results of literature search

The flow diagram of the study screening procedure is shown in **Figure 1**. A total of 2,878 potentially relevant studies were retrieved. Based on the aforementioned criteria, 1921 articles were discharged after reviewing the titles and abstracts. After evaluating the full texts, 30 of the 63 studies did not meet the inclusion criteria and were removed. Finally, 33

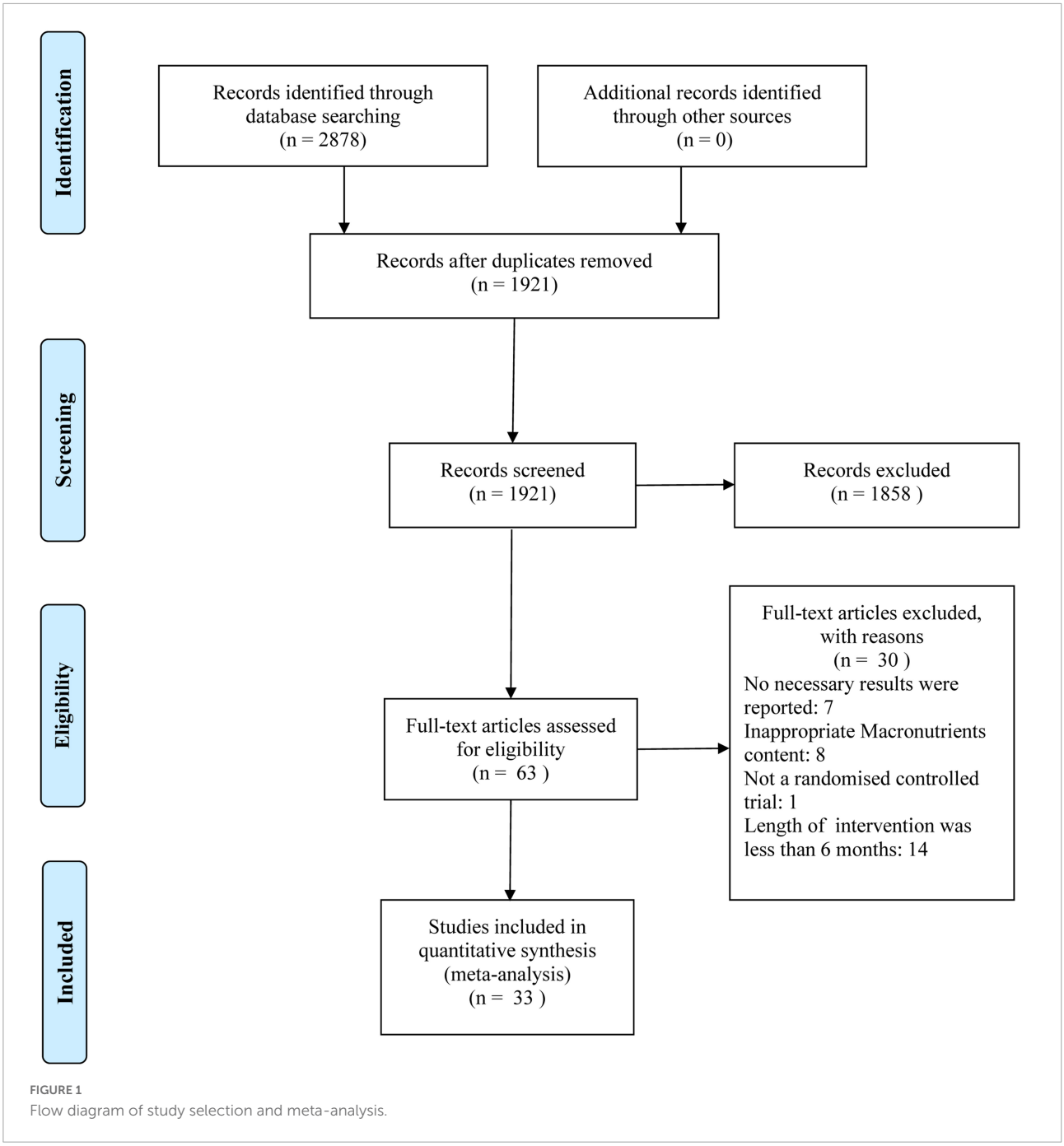

FIGURE 1
Flow diagram of study selection and meta-analysis.

studies met all inclusion criteria and were selected for further analysis in this study.

Characteristics of the included studies

The basic characteristics of 33 randomized controlled trials included in this meta-analysis are shown in **Table 1** (10–15, 20–24, 32–53). Nineteen of the included studies were conducted in North America, three in Asia, four in Oceania, and seven in Europe. A total of 3,939 participants, 1,978 on LCD and 1,961 on LFD, were included. The mean age of the participants at baseline ranged from 18 to 72 years. The follow-up period ranged from 6 to 24 months, eleven in 6 to 11 months, eighteen in 12 to 23 months, and four in 24 months. All studies were conducted among overweight or obese persons with or without basic diseases such as diabetes, hypertension, and hyperlipemia.

Although the carbohydrate-restricted diets were prescribed as intake of carbohydrates less than 40% of the total energy intake (E%), actual carbohydrate intakes ranged from 4 to 41.4

TABLE 1 Characteristics of randomized controlled trials included in the meta-analysis[1].

First author, (Reference no.)	Country	Duration of follow-up (months)	Design	No. of participants	Age (mean)	Male (%)	Population	Dietary composition		Completion %		Outcome measures
								LCD	LFD	LCD	LFD	
Bazzano, (15)	American	12	Parallel	148	46.8	12	Overweight/obese; BMI: 30–45; no T2DM or CVD	Carbohydrate intake <40 g/d, no set energy goal	<30% of daily energy intake from total fat (with <7% from saturated fatty acids), 55% carbohydrate	60 79%	59 82%	WL, SBP, DBP, TC, TG, LDL-C, HDL, BG
Brinkworth, (35)	Australia	12	Parallel	69	51.5	36	Overweight/obese; BMI = 21; metabolic syndrome risk factor	61% fat (20% saturated fatty acids), 4% carbohydrate, 35% protein	30% fat (8% saturated fatty acids), 46% carbohydrate, 24% protein	33 60%	36 69%	TC, TG, HDL-C, LDL-C, WL, SBP, DBP,BG
Brehm, (10)	American	6	Parallel	42	43.7	0	Obese; BMI: 30–35; no DM or CVD	Carbohydrate intake =20 g/d	30% fat, 55% carbohydrate, 15% protein	22 85%	20 74%	TC, TG, HDL-C, LDL-C, WL, SBP, DBP, BG
De Luis, (13)	Spain	9	Parallel	331	50.1	25.7	Obese; BMI: 35.4 ± 5.3; no DM or CVD	40% fat, 33% carbohydrate (86.1 g/day), 20% protein	27% fat, 53% carbohydrate, 20% protein	168 100%	163 100%	TG, TC, HDL-C, LDL-C, WL, SBP, DBP, BG
Davis, (37)	American	12	Parallel	105	55	21.9	Overweight/obese; BMI =25;T2DM	49% fat, 24% carbohydrate, 27% protein	25% fat, 53% carbohydrate, 22% protein	55 100%	50 100%	TC, TG, HDL-C, LDL-C, WL, SBP, DBP
Dansinger, (36)	American	12	Parallel	41	48	52.5	Overweight/obese; BMI: 27–42; at least one metabolic cardiac risk factors	Carbohydrate intake =20 g/d, and increasing up to 50 g/d	Vegetarian diet, 10% of calories from fat	21 53%	20 50%	TC, TG, HDL-C, LDL-C, WL, SBP, DBP, BG
Elhayany, (39)	Israel	12	Parallel	124	56.4	53.2	Obese; BMI: 27–34; T2DM	45% fat (50% monounsaturated fatty acid), 35% carbohydrate, 20% protein	30% fat, 50–55% carbohydrate, 15–20% protein	61 72%	63 71%	TC, TG, HDL-C, LDL-C, WL, BG
Ebbeling, (38)	American	18	Parallel	73	27.5	20.5	Obese; BMI: = 30; no DM	35% fat, 40% carbohydrate, 25% protein	20% fat,55% carbohydrate, 25% protein	28 78%	23 62%	TG, HDL-C, LDL-C, WL, SBP, DBP, BG
Foster, (50)	American	24	Parallel	307	45.5	32.2	Obese; BMI: 30–40; no DM	Carbohydrate intake 5 g/d per week	30% fat, 55% carbohydrate, 15% protein	153 58%	154 68%	TG, HDL-C, LDL-C, WL, SBP, DBP
Frisch, (41)	Germany	12	Parallel	200	47	31	Overweight/obese; BMI: = 27; no DM	>35% fat, <40% carbohydrate, 25% protein	<30% fat, >55% carbohydrate, <15% protein	100 85%	100 80%	TC, TG, HDL-C, LDL-C, WL, SBP, DBP, BG
Foster, (40)	American	6	Parallel	63	31.7	47	Overweight/obese; BMI: =21; no DM	Carbohydrate intake <20 g/d	25% fat, 60% carbohydrate, 15% protein	33 61%	30 57%	TC, TG, HDL-C, LDL-C, WL, SBP, DBP
Gardner, (23)	American	12	Parallel	609	40	43	Overweight/obese; BMI: 28–40; metabolic syndrome	The mean 12-month macronutrient distributions: 45% fat, 30% carbohydrate, 23% protein	The mean 12-month macronutrient distributions: 29% fat, 48% carbohydrate, 21% protein	218 74%	214 74%	TG, HDL-C, LDL-C, WL, SBP, DBP, BG

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TABLE 1 Continued

First author, (Reference no.)	Country	Duration of follow-up (months)	Design	No. of participants	Age (mean)	Male (%)	Population	Dietary composition		Completion %		Outcome measures
								LCD	LFD	LCD	LFD	
Guldbrand, (51)	Sweden	24	Parallel	61	62	44.3	Overweight/obese; BMI: 32; T2DM	50% fat, 20% carbohydrate, 30% protein	30% fat (<10 % saturated fatty acids), 55–60% carbohydrate, 10-15% protein	30 100%	31 100%	SBP, DBP, BG, LDL-C, HDL-C, TG
Gardner, (42)	American	12	Parallel	153	42	0	Overweight/obese; BMI: 32; no DM	Carbohydrate intake =50 g/d	<30% of total energy intake from fat	68 88%	59 78%	TG, HDL-C, LDL-C, WL, SBP, DBP, BG
Haufe, (20)	American	6	Parallel	170	44	20.6	Overweight/obese; BMI: 26.5–45.4, no T2DM	Carbohydrate intake =90 g/d	Fat intake of =20% of total energy	55 66%	56 64%	WL, TG, LDL-C, HDL-C, BG, TC
Hockaday, (43)	UK	12	Parallel	93	51	55.9	Weight: 76.4–82.2 kg	40% fat, 20% carbohydrate, 20% protein	26% fat, 54% carbohydrate, 20% protein	54 NR	39 NR	TG, BG
Jonasson, (14)	Sweden	6	Parallel	61	62	44.2	Overweight/obese; BMI: 33; DM	43% fat, 20% carbohydrate, 31% protein	30% fat, 55–60% carbohydrate	30 100%	31 100%	WL, TC, LDL-C, HDL-C, TG
Jenkins, (21)	Canada	6	Parallel	39	55	38.5	Overweight/obese; BMI: =27; hyperlipidemia	43% fat, 26% carbohydrate,31% protein	25% fat, 58% carbohydrate, 16% protein	13 68%	10 50%	LDL-C, HDL-C, TC, TG, BG, WL, SBP, DBP
Klemsdal, (44)	Norway	12	Parallel	202	50	42	Overweight/obese; BMI: 28–40; no DM or CVD	35%-40% fat (20% saturated fatty acids), 30%-35% carbohydrate, 25-30% protein	30% fat, 55%–60% carbohydrate, 15% protein	78 78%	86 84%	TC, TG, HDL-C, LDL-C, WL, SBP, DBP, BG
Lim, (45)	American	15	Parallel	60	48.5	22	Overweight/obese; BMI: 28–40; at least one CVD risk factor	60% fat (20% saturated fatty acids), 4% carbohydrate, 35% protein	10% fat (3% saturated fatty acids), 70% carbohydrate, 20% protein	17 63%	18 64%	TC, TG, HDL-C, LDL-C, WL, SBP, DBP, BG
Morgan, (11)	UK	6	Parallel	115	40.7	27	Overweight/obese; BMI: 27–40; no DM	Dr. Atkins' New Diet Revolution	Rosemary Conley's diet and fitness plan	33 58%	41 71%	TG, HDL-C, LDL-C, WL, BG
McAuley, (46)	New Zealand	12	Parallel	62	NR	0	Overweight; insulin resistance	Carbohydrate intake =20 g/d in the first 2 weeks, and increasing up to 50 g/day by 8 weeks	<30% fat (<10% saturated fatty acids), >55% carbohydrate, 15% protein	24 75%	24 75%	TC, TG, HDL-C, LDL-C, WL, SBP, DBP, BG
Saslow, (24)	American	12	Parallel	34	59.7	26.5	Overweight/obese; BMI: =25; T2DM	Carbohydrate intake <20–50 g/d	45%–50% carbohydrate	14 88%	15 83%	TG, HDL-C, LDL-C, WL, SBP, DBP

(Continued)

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TABLE 1 Continued

First author, (Reference no.)	Country	Duration of follow-up (months)	Design	No. of participants	Age (mean)	Male (%)	Population	Dietary composition		Completion %		Outcome measures
								LCD	LFD	LCD	LFD	
Shai, (53)	Israel	24	Parallel	214	52	94	Obese; BMI: 31; T2DM	Carbohydrate intake <20 g and later 120 g	30% fat (10 % saturated fatty acids), 55–60% carbohydrate, 10–15% protein	85 78%	84 90%	TG, HDL-C, LDL-C, WL, SBP, DBP, BG
Sacks, (52)	American	24	Factorial	403	51	34.5	Overweight/obese; BMI: 33; no DM or unstable CVD	40% fat, 35% carbohydrate, 25% protein	20% fat, 55% carbohydrate, 25% protein	168 83%	157 78%	TC, TG, HDL-C, LDL-C, WL, SBP, DBP, BG
Stern, (47)	American	12	Parallel	132	53.5	82.6	Obese; BMI =35; 83% DM or metabolic syndrome	Carbohydrate intake <30 g/d	To restrict caloric intake by 500 calories per day with <30% of calories from fat	44 69%	43 63%	TC, TG, HDL-C, LDL-C, WL, SBP, DBP, BG
Samaha, (32)	American	6	Parallel	132	54	82.6	Obese; BMI: =35; metabolic syndrome	Carbohydrate intake < 30 g/d	<30% of total energy intake from fat	43 67%	36 53%	TC, TG, HDL-C, LDL-C, WL, BG
Thomson, (33)	American	6	Parallel	43	56.2	0	Overweight/obese; BMI: 25–35; no DM or CVD.	35% Carbohydrate, 25–30% protein, 35-40% fat	55%–60% Carbohydrate,25% fat, 15%–20% protein	19 90%	21 95%	TC, TG, HDL-C, LDL-C, WL, SBP, DBP, BG
Tay, (12)	Australia	6	Parallel	88	50.6	35.2	Overweight/obese; BMI: 33.7; metabolic syndrome	61% fat (20% saturated fat), 4% carbohydrate, 35% protein	30% fat (< 8 % saturated fat), 46% carbohydrate, 24% protein	45 82%	43 80%	TC, TG, HDL-C, LDL-C, WL, SBP, DBP, BG
Wycherley, (49)	Australia	13	Parallel	49	50.0	34.7	Overweight/obese; BMI: 26–43; at least one metabolic syndrome risk factor	61% fat (20% saturated fat), 4% carbohydrate, 35% protein	30% fat (<8% saturated fat), 46% carbohydrate, 24% protein	26 46%	23 38%	TC, TG, HDL-C, LDL-C, WL, SBP, DBP, BG
Wolever, (48)	Canada	12	Parallel	110	59.6	43.3	Overweight/obese; BMI: 24–40; T2DM	40.1% fat, 39.3% carbohydrate, 20.6% protein	Low-glycemic-index (low-fat) diet: 26.5% fat, 51.9% carbohydrates, 21.6% protein,	53 98%	55 98%	TC, TG, HDL-C, LDL-C, WL, SBP, BG
Yamada, (22)	Japan	6	Parallel	24	63.3	50	Obese; BMI: 25.8; T2DM	Carbohydrate intake <70-130 g/d	<25% fat, 50–60% carbohydrate, <20% protein	12 100%	12 100%	TG, HDL-C, LDL-C, WL, SBP, DBP, BG
Yancy, (34)	American	6	Parallel	120	44.9	23.5	Obese; BMI:30–60; hyperlipidemia	Carbohydrate(<20 g/d) decreased to <5 g/d	<30% fat (<10% saturated fatty acids)	45 76%	34 57%	TC, TG, HDL-C, LDL-C, WL, SBP, DBP

[1]LCD, low carbohydrate diets; LFD, Low fat diets; DM, diabetes; T2DM, type 2 diabetes mellitus; CVD, cardiovascular disease; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; BG, blood glucose; WL, weight loss; NR, not report.

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E% in 6–11 months, from 4 to 43.5 E% in 12–23 months, and from 30 to 42.5 E% in 24 months. A total of 14 studies were very LCDs (VLCD) (carbohydrate intake ≤50 g/d) and 19 studies were moderate LCDs (MLCD) (carbohydrate intake >50 g/d). Similarly, although the LFD is prescribed as a fat intake of less than 30% of the total energy intake, actual fat intakes ranged from 2.8 to 33 E% in 6–11 months, from 20 to 30.8 E% in 12–23 months, and from 28.4 to 31 E% in 24 months. The completion rates of dietary interventions varied widely, ranging from 38 to 100%. It should be noted that though the intervention of the exercise program was discharged in our present study, a few studies also provided daily exercise volume.

Quality assessment

Two authors independently assessed the risk of bias in the included studies using the Cochrane Collaboration's tool. The results of the quality evaluation of the included 33 RCT studies are shown in **Supplementary Table 2**, which shows that the study qualities of the selected trials were diverse. According to the possibility of bias, the study was assessed as being low risk, moderate risk, or high risk. A total of six studies were evaluated as high risk of bias, three studies were assessed as low risk of bias, and the other studies had a moderate risk of bias. The quality of evidence for outcomes was evaluated as low or very low, and details for the evaluation of the GRADE framework are presented in **Supplementary Table 3**.

Effects of low-carbohydrate diets versus low-fat diets on blood lipids

The individuals assigned to LCD showed a significantly greater decrease in TG (WMD, −0.14 mmol/L; 95% CI, −0.18 to −0.10 mmol/L; **Figure 2**) and a significantly greater increase in HDL-C (WMD, 0.07 mmol/L; 95% CI, 0.06–0.09 mmol/L) than the individuals assigned to LFD (**Figure 3**). However, the pooled effect comparing LCD versus LFD in TC (WMD, 0.14 mmol/L; 95% CI, 0.07 to 0.20 mmol/L; **Figure 4**) and LDL-C (WMD, 0.10 mmol/L; 95% CI, 0.06 to 0.14 mmol/L) indicates a significantly greater reduction in LFD (**Figure 5**). It is noteworthy that LCD significantly decreased TG (6–11 months: WMD, −0.17 mmol/L; 95% CI, −0.25 to −0.09 mmol/L; 12–23 months: −0.16 mmol/L; 95% CI, −0.21 to −0.10 mmol/L) and significantly increased HDL-C (6–11 months: WMD, 0.08 mmol/L; 95% CI, 0.05 to 0.11 mmol/L; 12–23 months: 0.08 mmol/L; 95% CI, 0.02 to 0.09 mmol/L) when compared to LFD in 6–23 months, but the reduction effect of LDL-L (6–11 months: WMD, 0.12 mmol/L; 95% CI, 0.04 to 0.21 mmol/L; 12–23 months: 0.11 mmol/L; 95% CI, 0.06 to 0.17 mmol/L) and TC (6–11 months: WMD, 0.12 mmol/L; 95% CI, 0.01 to 0.24 mmol/L; 12–23 months: 0.15 mmol/L; 95% CI, 0.06 to 0.23 mmol/L) was in favor of LFD in 6–23 months. However, these outcomes were not significant differences between the two diets at 24 months. The heterogeneity test showed that four outcomes were of low heterogeneity (TG: I^2 = 21,3%, P = 0.14; LDL-C: I^2 = 35%, P = 0.03; HDL-C: I^2 = 35%, P = 0.03; TC: I^2 = 29%, P = 0.09).

Effects of low-carbohydrate diets versus low-fat diets on blood pressure

There was no difference in the effect of two diets on SBP (WMD, −0.73 mmHg; 95% CI, −1.55 to 0.09 mmHg; I^2 = 21%, P = 0.16; **Supplementary Figure 1**). However, compared with LFD, the decreased DBP was significantly greater in LCD (WMD, −0.87 mmHg; 95% CI, −1.41 to −0.32 mmHg; I^2 = 0%, P = 0.62; **Figure 6**). The difference in the decrease of −1.03 mmHg (95% CI, −1.73 to −0.33 mmHg) also exists in 12–23 months. However, the trend was not significant at 6–11 months or 24 months. The heterogeneity test showed that the results of both SBP and DBP were low heterogeneity.

Effects of low-carbohydrate diets versus low-fat diets on blood glucose

There was no difference in blood glucose between LCD and LFD (WMD, −0.01 mmol/L; 95% CI, −0.05 to 0.03 mmol/L; **Supplementary Figure 2**). The heterogeneity test showed that blood glucose was low heterogeneity (I^2 = 40%, P = 0.02).

Effects of low-carbohydrate diets versus low-fat diets on weight loss

Results indicated that the individuals assigned to LCD showed a greater reduction in weight loss than the individuals assigned to LFD (WMD, −1.33 kg; 95% CI, −1.79 to −0.87 kg; **Figure 7**). Compared with LFD, the levels of weight loss in LCD decreased by −2.10 kg (95% CI, −3.07 to −1.14 kg) in 6–11 months and −1.21 kg (95% CI, −1.79 to −0.63 kg) in 12–23 months. However, there was no difference in weight loss between the two diets at 24 months. The heterogeneity test showed that weight loss was low heterogeneity (I^2 = 20%, P = 0.16).

Subgroup and sensitivity analyses

Subgroup analyses for metabolic risk factors were carried out according to study variables and participant characteristics, including hypertensive status, hyperlipidemia status, diabetic status, energy intake, and proportions of carbohydrates (**Supplementary Tables 4–11**). Overall, we found that the TC

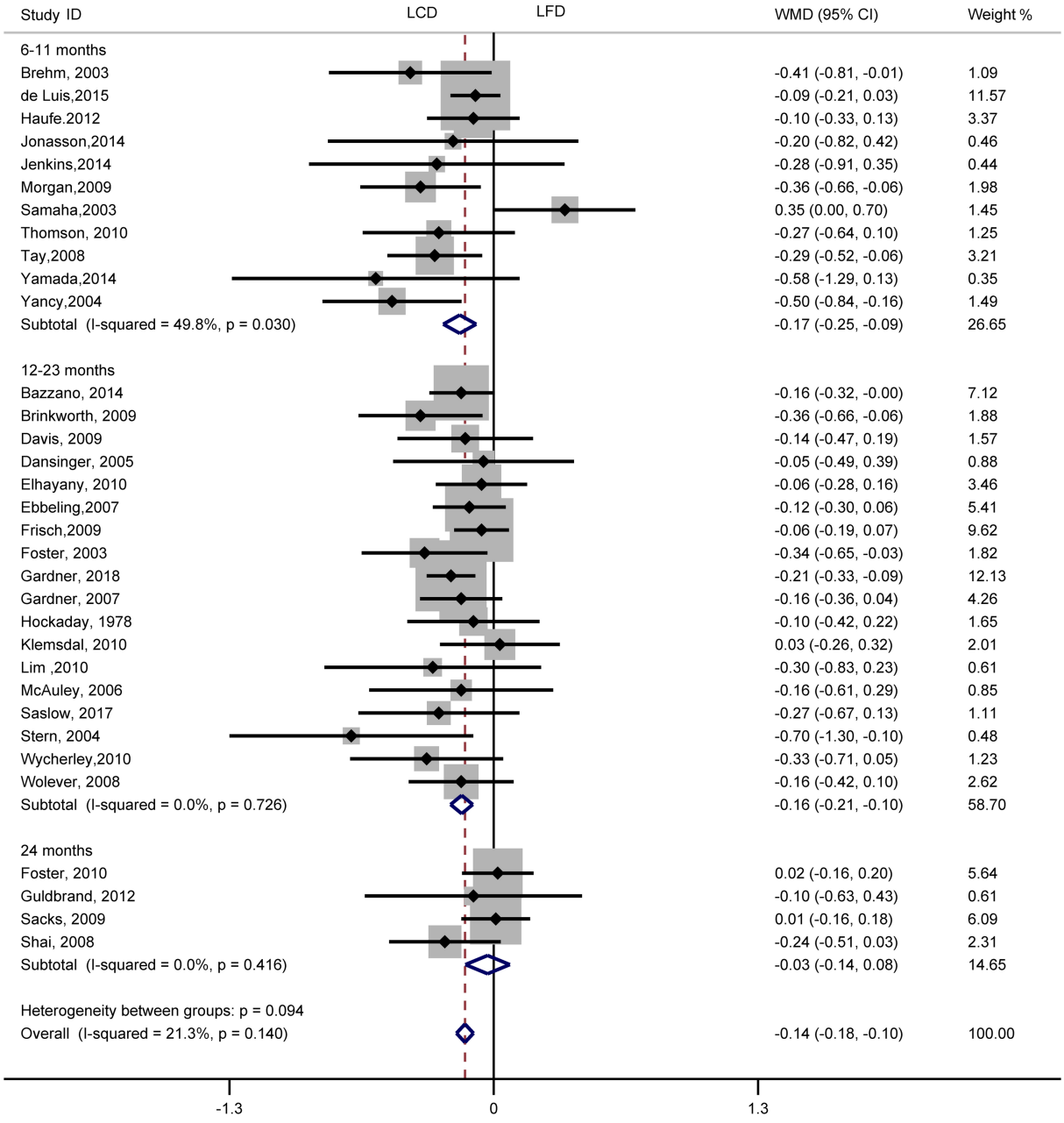

FIGURE 2
Forest plots showing weight mean differences (WMD) and 95% CI for triglyceride of the low-carbohydrate diets (LCD) in comparison with low-fat diets (LFD).

and LDL-C decreased more significantly in LFD. Still, SBP and DBP decreased more obviously in LCD in non-diabetic, non-hypertension, and non-hyperlipidemia participants. However, there were no significant differences between the two diets in participants with diabetes, hypertension, or hyperlipidemia. In identical caloric content and different caloric content subgroups, LCD had a stronger effect on SBP, DBP, and weight loss than LFD in identical caloric content subgroups, but no effect in different caloric content subgroups. When subgroup analyses were conducted based on proportions of carbohydrates, the reduction effect of DBP was favored in LCD in the moderate low carbohydrate subgroup, but not in the subgroup of very low carbohydrate. In the sensitivity analysis, the effects in the results remained unchanged after excluding one study at a time (**Supplementary Figures 3–10**). Meta-regression was used to explore heterogeneity, and we found that proportions of carbohydrates may be the source of heterogeneity (**Supplementary Table 12**).

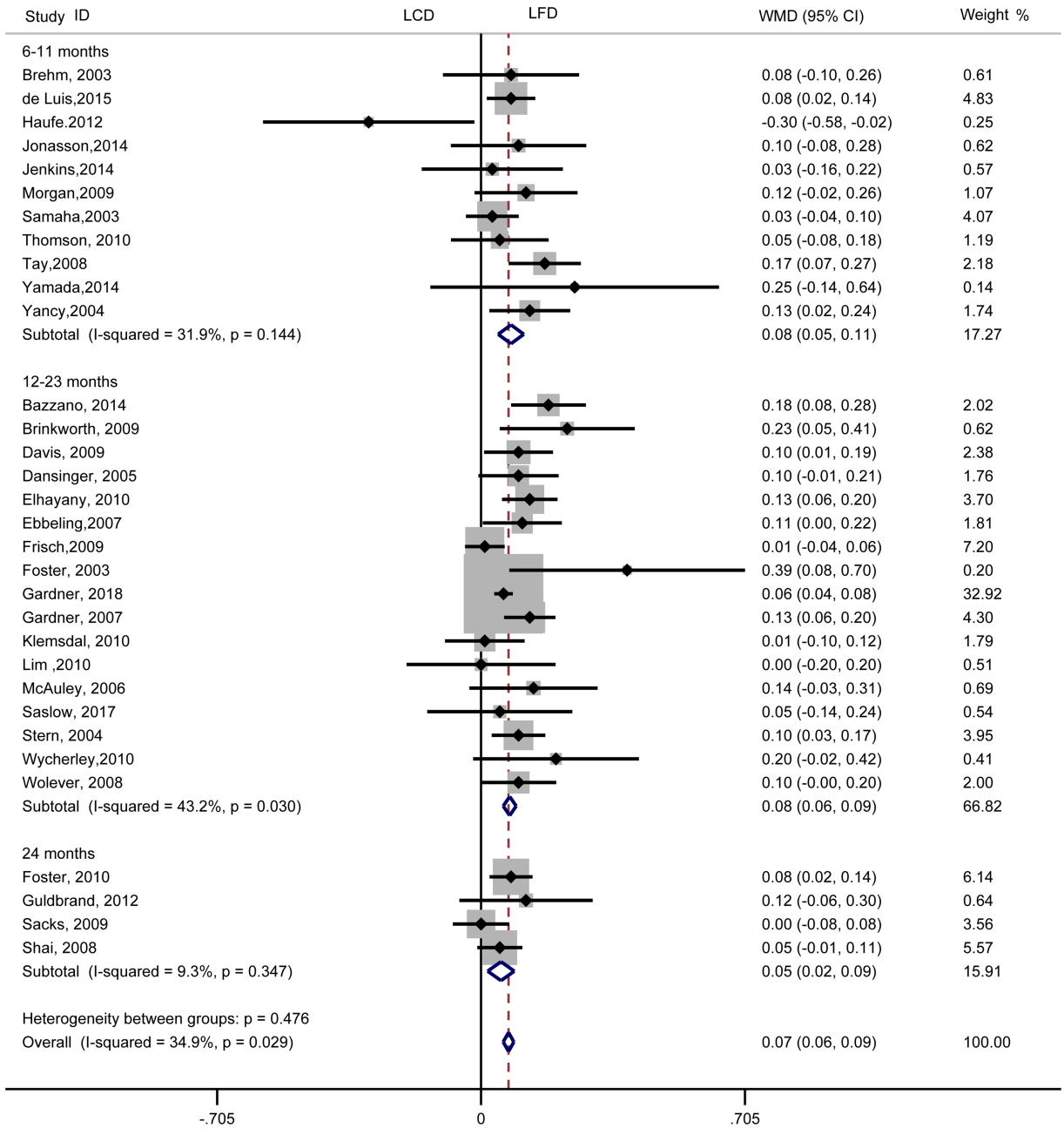

FIGURE 3
Forest plots showing weight mean differences (WMD) and 95% CI for HDL-cholesterol of the low-carbohydrate diets (LCD) in comparison with low-fat diets (LFD).

Publication bias

Results of funnel plots showed that TC, TG, LDL-C, HDL-C, SBP, DBP, blood glucose, and weight loss were symmetric (**Supplementary Figures 11–18**). Results of the Egger's tests also showed no significant publication bias (TC: $P = 0.41$; TG: $P = 0.09$; LDL-C: $P = 0.57$; HDL-C: $P = 0.08$; SBP: $P = 0.61$; DBP: $P = 0.69$; blood glucose: $P = 0.66$; weight loss: $P = 0.38$).

Discussion

Dietary intake, as a rule, follows a pattern of consumption and is one of the main factors that contribute directly to the impaired metabolic risk factors and obesogenic phenotype (54, 55). Numerous studies were performed to compare the effects on metabolic risk factors and weight loss in overweight and obese adults between LCD and LFD (10–15, 20–24, 32–53).

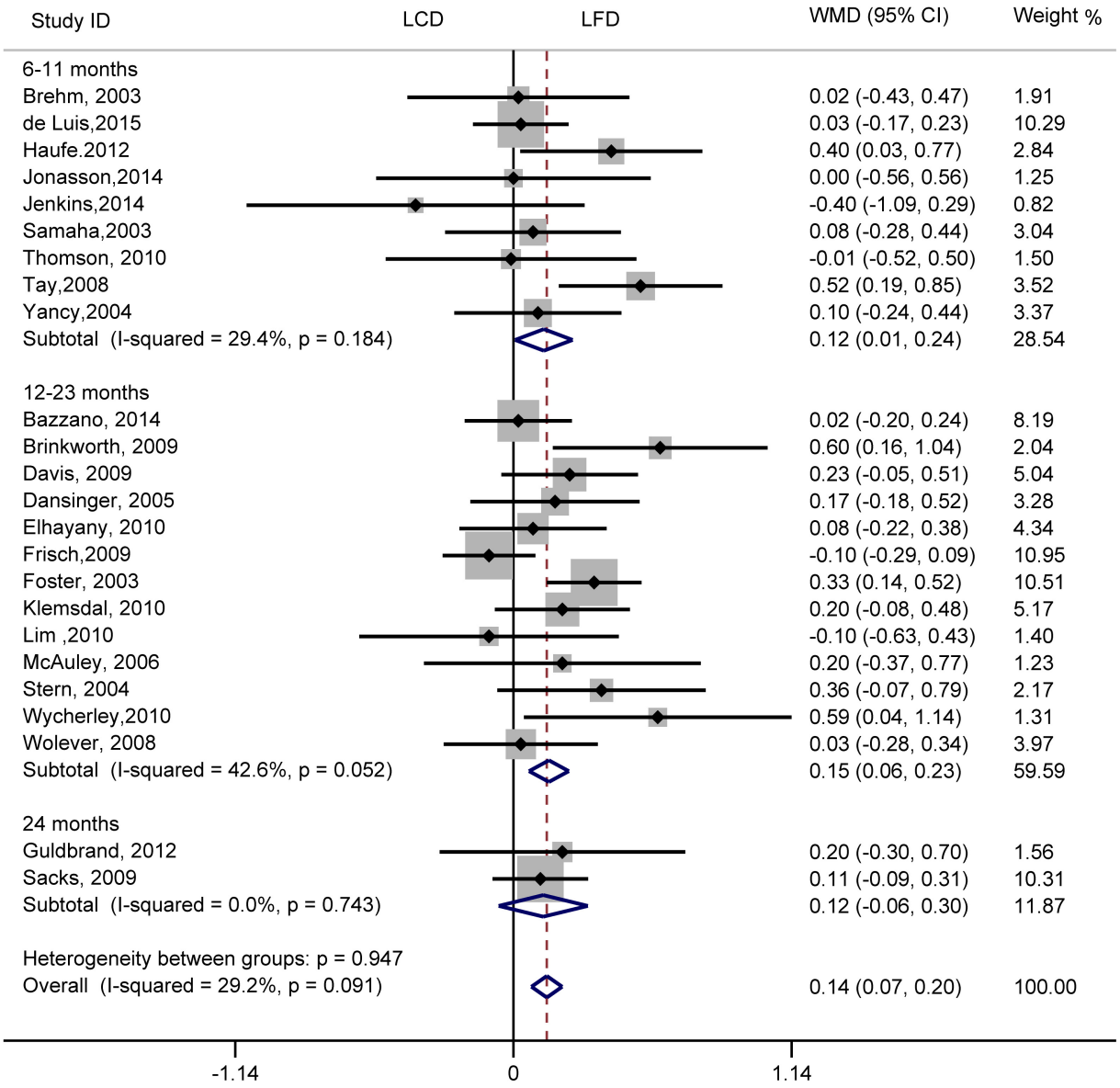

FIGURE 4
Forest plots showing weight mean differences (WMD) and 95% CI for total cholesterol of the low-carbohydrate diets (LCD) in comparison with low-fat diets (LFD).

However, the studies showed inconsistent results. In our present study, we performed a meta-analysis to the overall existing evidence from randomized controlled trials to compare LCD with LFD. Our results showed different effects on metabolic risk factors and weight loss in adults with overweight or obese between LCD and LFD. Compared with LFD, subjects on LCD had a greater reduction in TG, DBP, weight loss, and greater increases in HDL-C. However, participants on LFD had more decreases in LDL-C and TC. These indicated that we might choose different diets to manage the overweight and obese subjects according to their abnormal metabolic indicators and the need for weight loss.

Our results showed that LCD was more beneficial for improving TG and HDL-C, which was consistent with prior meta-analyses comparing the effect of the two diets in overweight and obese persons (56, 57). Even in healthy subjects in a meta-analysis including eleven randomized controlled trials with 1,369 participants, Nadia et al. also found that HDL-C and triglyceride levels had more favorable changes in LCD (18). The carbohydrate intake and macronutrient composition in LCD were related to the improvement of TG. The production of very low-density lipoprotein triglycerides in the liver is reduced in response to decreased carbohydrate substrate delivery (47). Moreover, the increase of HDL-C on the

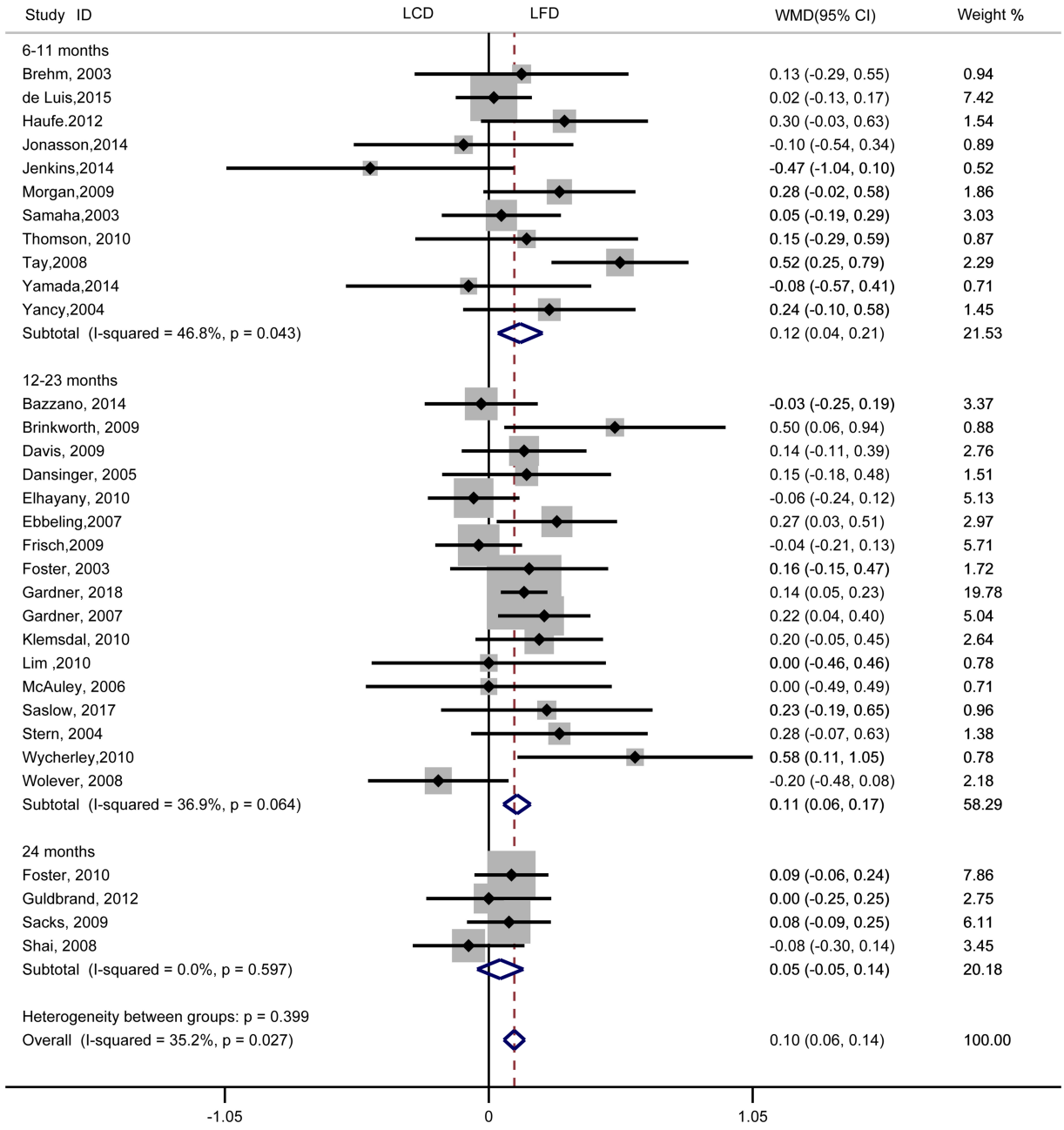

FIGURE 5
Forest plots showing weight mean differences (WMD) and 95% CI for LDL-cholesterol of the low-carbohydrate diets (LCD) in comparison with low-fat diets (LFD).

LCD may cause a greater decrease in TG via downregulation of hepatic scavenger receptor B1 levels because the receptor can bind HDL-C and promote the transportation of cholesterol to the liver (58). We also found that TC and LDL-C decreased significantly in LFD compared with LCD, which is different from previous reports (18, 19). The reasons may be that (1) we included more studies; (2) we included the overweight and obese subjects with or without basic diseases, but the other studies only included healthy persons. In this study, LCD did not cause a significant increase in TC and LDL-C, which may be related to our inclusion of more studies on MLCD. Our results were consistent with Hu et al. (19). However, Mansoor et al. and Lu et al. reported that LCD has adverse effects, which leads to an increase in TC and LDL-C (18, 59). Mansoor et al. only analyzed the effects of VLCD. Noteworthy, the VLCD caused higher levels of TC and LDL-C in many

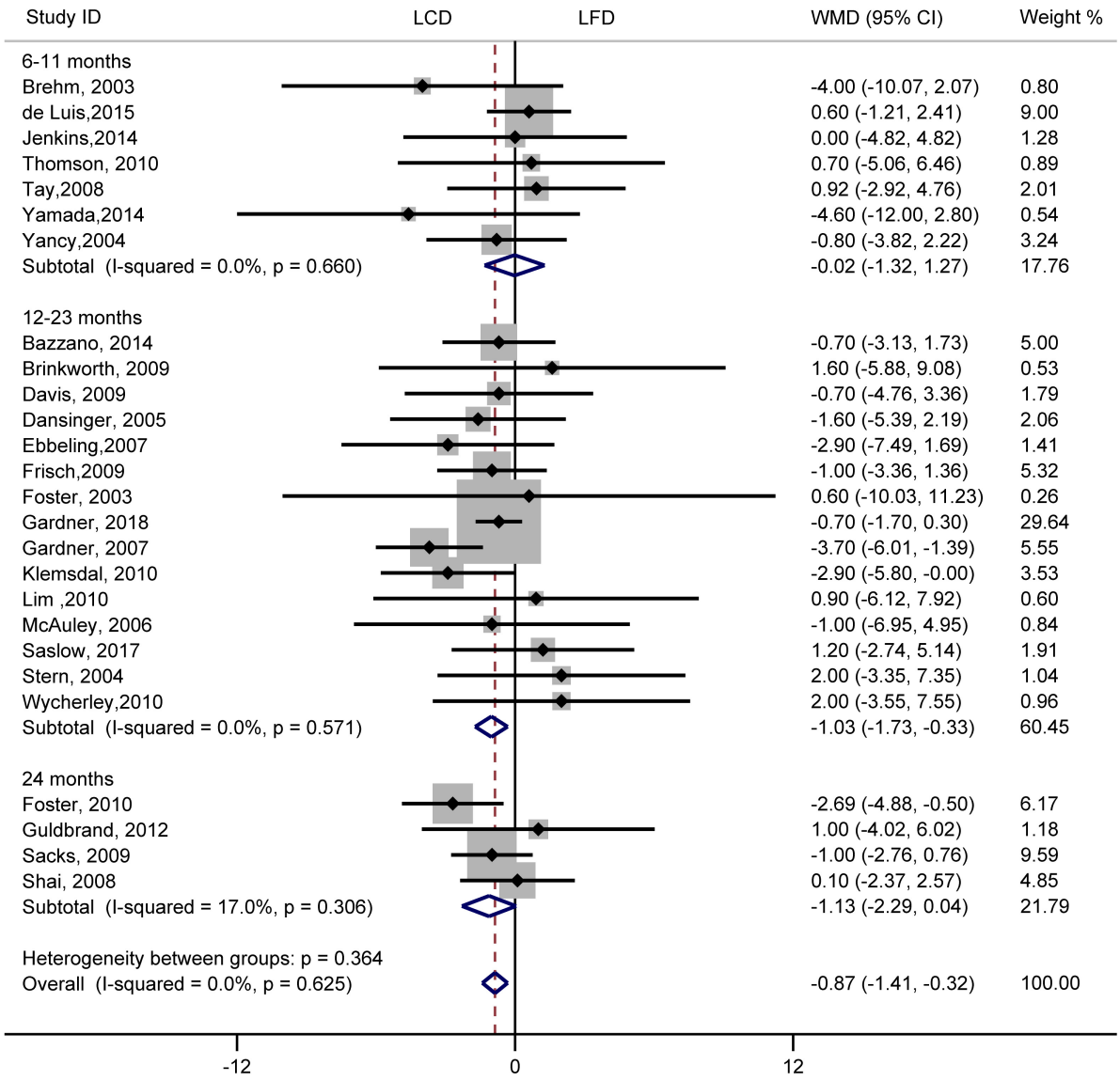

FIGURE 6
Forest plots showing weight mean differences (WMD) and 95% CI for diastolic blood pressure of the low-carbohydrate diets (LCD) in comparison with low-fat diets (LFD).

cases (60). The heterogeneity among the included studies was unavoidable. The source of heterogeneity was explored by meta-regression and subgroup analyses. The results found that the proportions of carbohydrates in LCD seem to be part of the source of heterogeneity. The research indicates that very low carbohydrate was related to better blood glucose control and greater weight loss (24), which means that the different content of carbohydrates in interventions may lead to heterogeneity. Moreover, subgroup analyses showed that LCD and LFD had different effects on blood pressure and blood lipids. Among participants with hypertension, hyperlipidemia, and diabetes, the reduction effect of blood pressure, TC, and LDL-C had no significant difference between the two diets. This would imply that the beneficial effects of LCD and LFD on blood pressure, TC, and LDL-C may be at least the same in participants with hypertension, hyperlipidemia, and diabetes. Subgroup analyses on energy intake indicated LCD had a significantly greater reduction of SBP, DBP, and weight loss in identical caloric content subgroups. Still, there was no significant difference in different caloric content subgroups. This difference between the subgroups may be due to the unequal dietary energy between the two diets in the studies because the energy intake of LCD was higher than that of LFD in three studies, and the energy intake of LFD was higher in three separate studies. It should be noted that LCD often increases the proportion of fat, which may cause a higher risk of some cardiovascular diseases or cancers

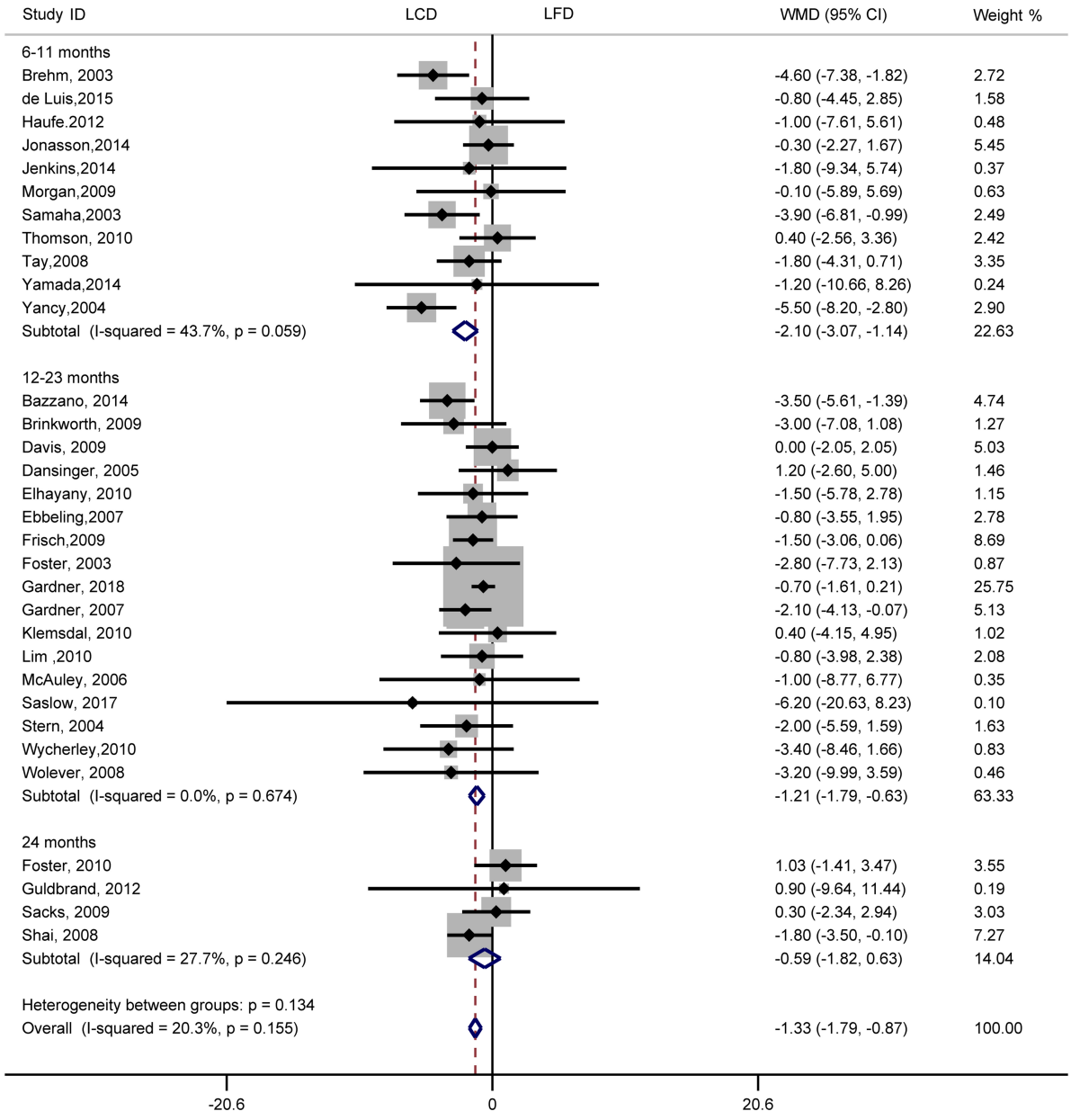

FIGURE 7
Forest plots showing weight mean differences (WMD) and 95% CI for weight loss of the low-carbohydrate diets (LCD) in comparison with low-fat diets (LFD).

(61). These causal relationships are long-term effects of high-fat diets, but studies included in our present and previous studies often persist from 6 to 24 months. Furthermore, it suggests that moderate replacement of carbohydrates with dietary fats may be a potential method to improve metabolic risk factors and simultaneously prevent increased risk for other diseases. On the other hand, some studies reduced dietary fats and replaced them with carbohydrates such as fruits and grains. However, this replacement did not affect atherogenic dyslipidemia among individuals with metabolic syndrome (62, 63).

Reducing dietary carbohydrates may produce clinical improvements in the management of blood pressure. We found that compared with LFD, individuals assigned to LCD showed a significantly greater reduction in diastolic blood pressure, not in systolic blood pressure, which is similar to a previous study (57). Studies have also shown that LCD with high monounsaturated fatty acids is beneficial for regulating blood pressure in some diseases such as type 2 diabetes (64). However, there was no difference in the improvement of blood pressure between the two diets in two earlier meta-analyses, including overweight and

obese subjects (19, 56). The reason for the difference in our results may, at least in part, be more studies were included in the current research.

Both LCD and LFD are beneficial for weight loss. Previous studies have shown that compared with participants on LFD, those on LCD experienced a greater weight loss reduction (56, 65). Moreover, Mansoor et al. found that even in healthy subjects, LCD is more effective for weight loss than LFD (19). Our results are consistent with the effects reported in the above studies, suggesting that the individuals assigned to LCD showed a significantly stronger reduction in weight loss than the individuals assigned to LFD. High fat in LCD can stimulate more secretion of peptide YY, a peptide mainly produced by endocrine L cells, which can reduce appetite and increase satiety (66). Most of the LCDs increase protein intake, thereby increasing subjects' satiety and reducing eating, which may be related to greater weight loss (33). A further study observed no difference in weight loss between the two diets lasting 24 months. It is similar to the results in an earlier study by Nordmann et al., which found that this different effect on weight loss between the two diets was no longer obvious after 12 months (56). However, the results were inconsistent. Some studies reported that the two diets are at least as effective in weight loss (19).

Reduction of carbohydrate intake has attracted more and more attention in recent years for its potential in health promotion and treatment of diseases, including decreasing body mass, improving fat and carbohydrate metabolism, producing clinical improvements in the management of type 2 diabetes mellitus, and reducing the predicted risk of atherosclerotic cardiovascular disease events (65, 67, 68). However, there are still controversial effects of LCD or the comparison between LCD and LFD (63, 65, 69). Some reasons that may be involved are as follows: (1) the criteria for included subjects are different. The criteria may include only overweight or obese persons or both, while in some studies, the criteria were the different BMI values. In addition, the included participants may be overweight and obese with or without basic diseases or healthy. (2) LCD often increases the percentage of energy from fat. Different fatty acids may have diverse effects. For instance, Abbasnezhad et al. found that LCD with high monounsaturated fatty acids benefits the regulation of blood pressure in some diseases such as type 2 diabetes (64). However, saturated fatty acids have been reported to increase both totals- and LDL-C (70). A study conducted by Sackner-Bernstein et al. showed that LCD is more significant in weight loss and in predicting ASCVD risk in overweight/obese subjects with health or dyslipidemia, but the outcomes were not stratified by follow-up time or different populations (68). Although Chawla et al. performed a stratified analysis of follow-up time, most of the studies were short-term trials (71). They found that LCD is more significant in improving weight loss, HDL-C, and TG within 12 months, but there is a lack of evidence to support the long-term effect of the two diets. This meta-analysis included more studies (over 12 months) and populations, and performed subgroup analyses of different populations and intervention durations to explore the short- and long-term effects of the two diets on metabolic risk factors.

It should be noted that LCD may have some adverse effects. First, LCD often increases the proportion of fat, which may cause a higher risk of some cardiovascular diseases or cancers (61). These causal relationships are long-term effects of high-fat diets, but studies included in our present and previous studies often persist from 6 to 24 months. Second, some meta-analyses based on observational studies have shown that long-term reduction of carbohydrate is related to a significantly increased risk of all-cause mortality (72, 73). Further, the study indicated that the source of food, especially the sources of protein and fat, notably modifies the association between carbohydrate intake and mortality (64). Third, some observational studies reported that in short-term interventional studies in humans, LCD has effects on mood and cognition, such as impaired cognitive function, attenuated performance on a memory-based task, and decreased cognitive processing speed (74–76). However, other studies have shown opposite effects, including having better sleep status, less involvement with mental disorders, and exerting a beneficial effect on depression (77, 78). A systematic review showed that reduction of carbohydrate intake has no stronger effect on psychosocial outcomes than diets of different macronutrient compositions, both in the short- and long-term (79). Thus, further studies are needed to investigate the effects of LCD on psychosocial outcomes.

Several potential limitations should be considered in our study. First, the definitions of LCDs are different. LCD is defined as a total carbohydrate intake of 20–60 g per day or less, or $\leq$45% of energy from carbohydrates. However, the definition of LFD is consistent, characterized as total fat intake $\leq$30% of energy from fat. Second, the duration is different, from 6 months to 2 years, and there is no trial lasting for more than 2 years. LCD may produce small short-term improvements in blood glucose control and weight loss, which are not sustained in the long term (80). Thus, the long-term effects of LCD on cardiovascular risk factors and weight loss require further research in the future. Third, only some studies reported the changed types and sources of carbohydrates or fat. Simple or complex carbohydrates have different effects on metabolic risk factors and weight loss (81, 82). Various fatty acids, including saturated, monounsaturated, or polyunsaturated fatty acids, also have diverse effects (83, 84). Therefore, further studies are needed to focus on the various types and sources of carbohydrates or fat in LCD and LFD in the future. Fourth, although we found significant differences in blood lipids, weight loss, and blood pressure between the two diets, most of the outcomes have weak differences, such as DBP, and lack of significant clinically significant. Large-scale clinical studies are needed to confirm the clinical effects of these metabolic risk makers in the future. Finally, the quality of evidence for outcomes ranges from low to very low in this study, not only because of study limitations and indirectness but also

because of inconsistency. The dietary trials in participants who are not blinded may be one of the reasons for the low certainty evidence. The quality of evidence for study needs to be improved by well-designed randomized trials in the future.

Conclusion

In summary, our present meta-analysis found that individuals assigned to LCD showed a significantly greater reduction in TG, diastolic blood pressure, and weight loss, as well as a significant increase in HDL-C. However, LFD was associated with a significantly greater decrease in TC and LDL-C. Moderate restriction of carbohydrate intake in LCD did not cause adverse effects on LDL-C and TC. We also found that LCD was as effective as LFD on weight loss, and metabolic risk factors improvement lasted up to 2 years. However, few large-scale and high-quality studies have analyzed the long-term effects of LCD and LFD on metabolic risk factors. Hence, the long-term clinical efficacy and effects of various sources of carbohydrates or fat in the two diets are still worth further clarification.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary materials**, further inquiries can be directed to the corresponding author.

Author contributions

LL and JY designed the research. LL and JH were responsible for data acquisition, statistical analysis, and the interpretation of the results. LZ and YH were responsible for providing information and advice on the data synthesis and analysis. SH and JY contributed to the concept and design of the study, provided guidance during study selection, data analysis, draft development, and final submission. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.935234/full#supplementary-material

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Association of time of breakfast and nighttime fasting duration with breast cancer risk in the multicase-control study in Spain

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Circadian nutritional behaviors, defined by the daily eating/fasting cycle, have been linked with breast cancer. This study aimed to further disentangle the association of nighttime fasting duration and time of breakfast with breast cancer risk. We analyzed data from 1,181 breast cancer cases and 1,326 population controls from the Spanish multicase-control study (MCC-Spain),

2008–2013. We collected circadian nutritional behaviors at mid-age *via* a telephonic interview. We applied logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CIs) for the association of nighttime fasting duration and time of breakfast with breast cancer risk in all women and stratified by menopausal status. Models were adjusted for age, center, education, family history of breast cancer, age at menarche, number of children, breastfeeding, age at first child, body mass index (BMI), contraceptive use, and hormonal replacement therapy (HRT). A later time of breakfast was associated with a non-significant increased risk of breast cancer (OR = 1.05, 95% CI: 0.95–1.16, per hour increase). This association was stronger among premenopausal women, among whom each hour later, the time of breakfast was associated with an 18% increase in breast cancer risk (OR = 1.18, 95% CI: 1.01–1.40). The association was not observed in postmenopausal women. We did not observe an association between nighttime fasting duration and breast cancer risk after adjusting for the time of breakfast. In this study, late breakfast was associated with increased breast cancer risk, especially among premenopausal women, compared with early breakfast. Aside from nutritional quality, circadian nutritional behaviors should be further studied in relation to cancer.

KEYWORDS

meal timing, circadian nutritional behaviors, nighttime fasting duration, breakfast, breast cancer risk, chrononutrition, circadian rhythms

Introduction

Female breast cancer was the most commonly diagnosed cancer and the fifth leading cause of cancer-related mortality worldwide in 2020 (1). It was first proposed during the 1970s that the disruption of circadian rhythms could influence breast cancer risk (2). On the basis of a growing body of evidence, the International Agency for Research on Cancer (IARC) classified circadian rhythm disruption, resulting from night shift work, as probably carcinogenic for cancer of the breast, prostate, and colon (3, 4). Circadian rhythms regulate multiple physiological activities including hormonal secretion, immune regulation, and cellular cycle (5). Several external factors or *zeitgebers* can synchronize the circadian rhythms including the daily light-dark and feeding-fasting cycles (5).

The emerging field of chrononutrition studies the relationship between the timing of nutritional behaviors, circadian rhythms, and health (6–8). Some studies have shown that circadian nutritional behaviors, or meal timings, may be associated with breast cancer risk and progression (9–11). A prolonged nightly period of fasting has been associated with reduced systemic inflammation (12), a putative risk factor for breast cancer. Fasting for less than 13 h overnight has been associated with increased odds of breast cancer recurrence compared with a longer nightly fasting period (11). Contrarily, a study from the French cohort NutriNet-Santé showed no association between the length of the nightly fasting period and the risk of breast cancer (10).

The nightly fasting interval can be elongated either by having an early dinner or by having a late breakfast. Results from the multicase-control study (MCC-Spain) and the NutriNet-Santé cohort showed that having an early dinner was associated with a reduced risk of breast cancer compared with a late dinner (9, 10). In contrast, previous studies indicate that skipping breakfast, or delaying the first meal, can lead to metabolic and inflammatory deregulation (13–17). It has also been inconsistently linked with weight gain (14, 18, 19). The omission of breakfast has also been associated with increased cancer-related and all-cause mortality (20). Finally, data from the NutriNet-Santé cohort shows a non-significant association between a later breakfast and a higher risk of breast cancer (10). These analyses were not stratified by menopausal status.

Some cross-sectional studies have suggested an association between a prolonged nightly fasting period and a reduction in potential breast cancer risk factors (12, 21). However, the evidence is scarce and inconclusive. Moreover, the association with the time of breakfast and with consideration of menopausal status remains unclear. This analysis builds on previous results within the MCC-Spain study in relation to the time of

dinner and the time interval between dinner and sleep (9) to build a more integrated understanding of the circadian nutritional behaviors as a whole. This study investigates whether circadian nutritional behaviors, specifically nighttime fasting duration and time of breakfast, are associated with breast cancer risk.

Materials and methods

Study design and population

The multicase-control MCC-Spain study[1] is a large population-based case-control study of 5 common tumors, which was conducted in Spain between 2008 and 2013 (22, 23). Histologically confirmed cancer cases were recruited from 23 collaborating hospitals in 12 Spanish provinces. Simultaneously, controls were randomly selected from the primary healthcare centers located within the catchment area and were frequency-matched to cases by age, sex, and region. All participants were aged 20–85 years and had resided in the catchment area for 6 months or more prior to recruitment. For each of the included centers, the ethics committees reviewed and approved the study protocol. Before being included in this study, participants signed an informed consent form (22).

In this analysis, only breast cancer was examined. A total of 3,648 women were eligible for this analysis, including 1,738 breast cancer cases and 1,910 population controls. We excluded 360 women who reported ever working on the night shift and 7 with missing menopausal status (**Figure 1**). We excluded night shift workers, to focus our analysis mainly on the circadian disruption specifically related to nutritional behaviors and to avoid potential confounding with this other source of circadian disruption. We considered night shift work as working entirely or partly between 00:00 and 6:00 for 3 nights or more per month (9). We also excluded 661 women who did not respond to the circadian questionnaire and 113 who had missing information on nighttime fasting (**Figure 1**). Finally, 1,181 breast cancer cases and 1,326 population controls were included in these analyses.

Data collection and variable assessment

Trained personnel administered an epidemiological questionnaire in a face-to-face interview. The questionnaire included information on socio-demographics, personal and family medical history, reproductive factors, medication, weight and height (corresponding to the year prior to study inclusion), recreational physical activity, and smoking (22). Using the Ainsworth classification (24), we assigned a physiological measure of energy expenditure (Metabolic Equivalent of Task, MET) to all recreational physical activities reported and we calculated the equivalent MET hour/week. We excluded data on physical activity corresponding to the 2 years before the interview to avoid any changes caused by the disease. We calculated body mass index (BMI) from self-reported weight and height. We also provided the participants with a previously validated food frequency questionnaire (FFQ) that was self-administered to evaluate nutritional behaviors over the previous year (22). The overall response rate was 88%. The questionnaire included an assessment of alcohol consumption between 30 and 40 years of age. Daily energy intake (kcal/day) and past daily consumption of ethanol (g/day) were estimated separately using the Centro de Enseñanza Superior de Nutrición y Dietética (CESNID) food composition table (25). As a proxy of a healthy diet, we also considered daily consumption of vegetables and fresh fruits (g/day).

Cases were classified into three subtypes based on pathology records, namely, (1) tumors with hormonal receptors either for estrogens or progesterone (labeled as positive hormonal receptors), (2) tumors with overexpression of the human epidermal growth factor 2 (HER2 +), and (3) tumors without hormonal receptors nor overexpression of HER2 (triple negative).

In total, 6 months to 5 years after enrollment in the study (median time 3 years), a telephonic interview was performed to assess circadian nutritional behaviors, timing of physical activity, and sleep patterns (questionnaire available on the study website; see text footnote 1). This interview also included a question on bedroom light during sleep assessed with a four-digit Likert scale (a) total darkness, (b) almost dark, (c) dim light, and (d) quite illuminated. Chronotype is the individual preference for the timing of circadian activity and has a genetic basis (26). This was also assessed in the circadian interview. Participants were asked to report their behaviors at mid-age (40 years of age) and the year before their inclusion in the study. Circadian nutritional behavior questions assessed the frequency of consumption of main meals and usual timing during weekdays and weekend days. We conducted the main analyses with behaviors at mid-age to avoid potential reverse causation from the more recent behaviors. We asked the participants about the frequency of breakfast consumption as never having breakfast, having breakfast only on weekends, having breakfast only on weekdays, and always having breakfast. Sleep duration was calculated as the difference between the time of turning off the lights and the time of awakening on weekdays and weekends. Nighttime fasting duration was calculated as

1 http://www.mccspain.org

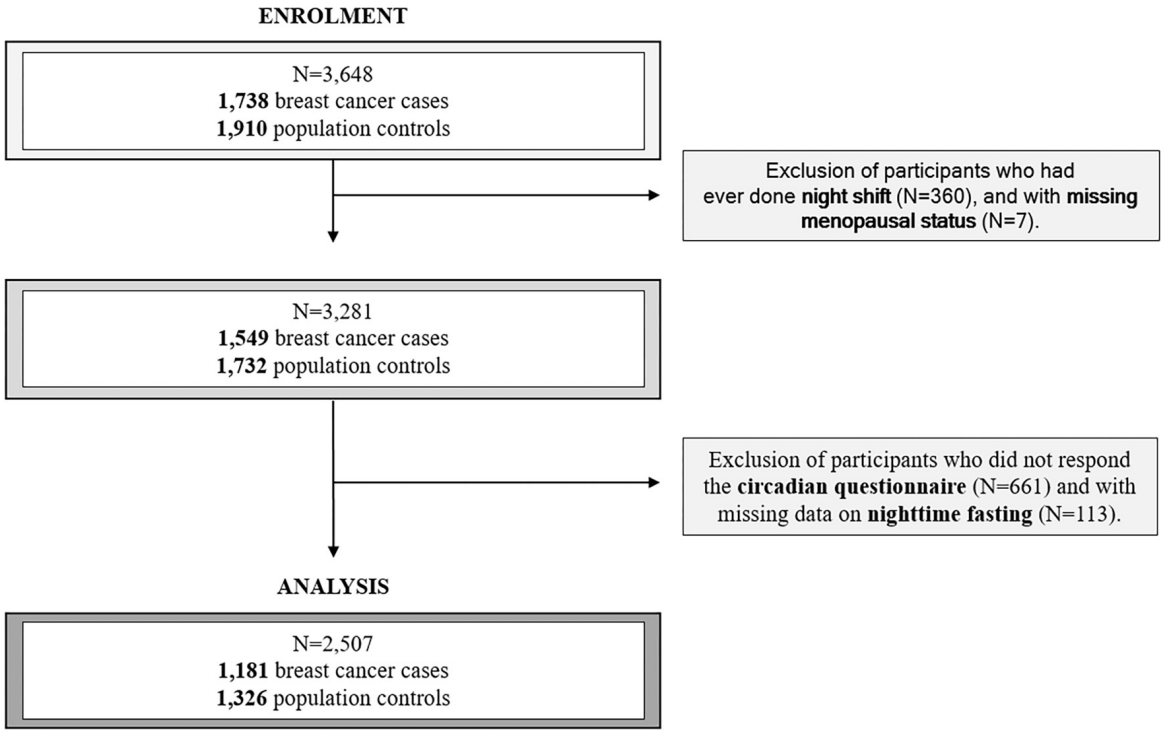

FIGURE 1
Participant flow diagram.

the time elapsed between the last meal and breakfast the following day. For those participants that reported never having breakfast (1%) or having it only on weekends (< 1%), the time of lunch was considered as breakfast, understood as the broader concept of the time when the nightly fast was broken.

Statistical analyses

We compared basic characteristics among cases and controls and in premenopausal and postmenopausal women separately.

To investigate the associations between nighttime fasting duration, time of breakfast, and breast cancer risk, we built logistic regression models and estimated odds ratios (ORs) and 95% confidence intervals (CIs). Models were adjusted for age (continuous, years), center (Madrid, Barcelona, Navarra, Gipuzkoa, Leon, Asturias, Huelva, Cantabria, Valencia, and Gerona), and educational level (less than primary school, primary school, secondary school, university). We also adjusted all models for well-established breast cancer risk factors: family history of breast cancer (no, yes), age at menarche (continuous, years), number of children (nulliparous, 1 or 2 children, 3 or more children), breastfeeding (parous women no breastfeeding, breastfeeding up to 6 months, breastfeeding 6–24 months, breastfeeding for more than 24 months, and nulliparous women), age at the first child (less than 20 years, from 20 to 35 years, more than 35 years, and nulliparous women), BMI 1 year before inclusion to the study (continuous, kg/m^2), contraceptive use (never, ever), and hormonal replacement therapy (HRT) and menopausal status (premenopausal women, postmenopausal women who ever used HRT, and postmenopausal women who never used HRT). All the covariates included in the main model had less than 3% of missing values; therefore, we applied a complete case analysis. We initially explored separate models for the two exposures (nighttime fasting duration and time of breakfast) and then a model mutually adjusting both circadian nutritional behaviors.

There are well-established molecular and etiological differences between premenopausal and postmenopausal breast cancer (27), a pattern that has been replicated also for the effect of night shift work (28). We checked whether there was evidence of effect modification of the association between time of breakfast and nighttime fasting duration with breast cancer risk, by menopausal status by including an interaction term in the adjusted model and conducting a likelihood ratio test. We did follow the same procedure for chronotype and for HRT history (ever vs. never) among postmenopausal women.

We inspected the linearity of the association between nighttime fasting duration, time of breakfast, and breast cancer risk by building generalized additive models (GAMs). To test for linearity, we conducted an ANOVA comparing two models with the exposure of interest included with or without the smoothing term. None of the models showed a significant departure from linearity; therefore, we considered exposure variables as continuous. We further categorized nighttime fasting duration and time of breakfast according to the median point in controls: 11.00 h (interquartile range, IQR 10.00–12.00) and 8:00 a.m. (IQR 7:30–9:00 a.m.), respectively. We explored the correlation among both exposures and also with other circadian behaviors including those already examined in the previous MCC-Spain study on mistimed eating patterns (9).

Finally, in a multinomial logistic regression, we investigated the association of nighttime fasting duration and time of breakfast with the risk of breast cancer subtype, reporting relative risk (RR) ratios and examining differences between subtypes with the Wald test.

In sensitivity analyses, we explored adjustment for other lifestyle factors (daily alcohol intake, physical activity, daily caloric intake, and daily consumption of fruits and vegetables) and other potential risk factors for breast cancer including socioeconomic status, smoking, and age at menopause. We also explored further adjustment with other circadian behaviors including time of dinner, interval between dinner and sleep, indoor light-at-night, sleep duration, and chronotype. To investigate the potential influence of recall bias, we examined the association between nighttime fasting duration and time of breakfast with breast cancer risk using data reported for the year previous to diagnosis (or enrollment for controls) and checked the correlation with behaviors at mid-age. Finally, we explored the joint effects of nighttime fasting and breakfast timing in a model combining both exposures.

The statistical package R 4.0.5 was used to perform these analyses (R Foundation for Statistical Computing, Vienna, Austria).[2]

Results

Study population

The characteristics of our study population are shown in Table 1. The mean age of cases was 55 years (11.6 *SD*) and of controls 58 years (12.5 *SD*). Overall, cases were more likely to have a family history of breast cancer, to be premenopausal

TABLE 1 Main characteristics of the study population.

	Controls ($N = 1,326$) mean (*SD*) or *N* (%)	Cases ($N = 1,181$) mean (*SD*) or *N* (%)
Age (years)	58.4 (12.5)	55.4 (11.6)
BMI (kg/m^2)	25.7 (4.7)	25.9 (0.187)
Education		
Less than primary school	193 (14.6)	137 (11.6)
Primary school	412 (31.1)	403 (34.1)
Secondary school	438 (33.0)	407 (34.5)
University	283 (21.3)	234 (19.8)
Score socioeconomic		
Low	357 (27.8)	334 (28.3)
Medium	696 (54.2)	660 (55.9)
High	232 (18.1)	187 (15.8)
Family history of breast cancer		
Yes	124 (9.4)	175 (14.8)
No	1,202 (90.6)	1,006 (85.2)
Diabetes		
No	1,222 (92.4)	1,104 (93.9)
Yes	100 (7.6)	72 (6.1)
Age at menarche (years)	12.8 (1.6)	12.7 (1.5)
Number of children		
Nulliparous	236 (17.8)	243 (20.6)
1–2 children	745 (56.3)	694 (58.8)
3 children or more	342 (25.9)	243 (20.6)
Age at first child		
First child < 20 years old	52 (4.8)	46 (4.9)
First child 20–35 years old	957 (88.1)	811 (86.9)
Parous ≥ 35 years old	77 (7.1)	76 (8.1)
Breastfeeding		
Parous without breastfeeding	166 (15.3)	141 (15.5)
Parous breastfeeding for less than 6 months	294 (27.2)	269 (29.6)
Parous breastfeeding for 6–24 months	496 (45.8)	423 (46.5)
Parous breastfeeding for more than 24 months	126 (11.6)	76 (8.4)
Contraceptive use		
Never	648 (48.9)	603 (51.1)
Ever	677 (51.1)	577 (48.9)
Menopausal status		
Premenopausal	386 (29.1)	436 (36.9)
Postmenopausal	940 (70.9)	745 (63.1)
Hormonal replacement therapy		
Never	1,178 (92.0)	1,071 (92.5)
Ever	102 (8.0)	87 (7.5)
Smoking		
Never smoker	778 (58.7)	662 (56.1)
Past smoker	292 (22.0)	311 (26.4)
Current smoker	256 (19.3)	207 (17.5)
Daily alcohol intake (g ethanol)	6.0 (10.1)	6.9 (12.7)
Daily caloric intake (Kcal)	1,717.8 (537.4)	1,831.6 (610.5)
Daily consumption of vegetables and fruits (g)	557.0 (264.4)	555.8 (300.2)
Physical activity[a]		
Inactive	516 (38.9)	500 (42.3)
Poorly active	255 (19.2)	203 (17.2)
Moderately active	167 (12.6)	147 (12.4)
Very active	387 (29.2)	331 (28.0)

(Continued)

2 http://www.R-project.org/

10.3389/fnut.2022.941477

TABLE 1 (Continued)

	Controls ($N = 1,326$) mean (SD) or N (%)	Cases ($N = 1,181$) mean (SD) or N (%)
Chronotype		
Morning	508 (38.8)	426 (36.5)
Intermediate	528 (40.3)	464 (39.7)
Evening	273 (20.9)	278 (23.8)
Sleep duration (hours)	6.9 (1.3)	7.1 (1.3)
Breakfast		
Never	9 (0.7)	21 (1.8)
Only weekends	10 (0.8)	6 (0.5)
Only weekdays	20 (1.5)	24 (2.0)
Always	1,279 (97.0)	1,129 (95.7)
Time of breakfast (start time, a.m.)	8.4 (1.4)	8.5 (1.4)
Nighttime fasting duration (hours)	11.0 (1.6)	11.1 (1.6)

BMI, body mass index; N, sample size; SD, standard deviation.
[d]Physical activity was classified according to the annual mean of METS h/week. Inactive = 0 METS h/week; poorly active = 0.0001–8 METS h/week; moderately active = 8.0001–16 METS h/week; very active = more than 16.0001 METS h/week.

women, to have less children, and to have a higher past consumption of alcohol and daily energy. Sleep differed between controls and cases with a duration of 6.9 h (1.3 *SD*) and 7.1 h (1.3 *SD*), respectively. Only 9 controls (0.7%) reported never having breakfast, whereas 21 cases (1.8%) skipped breakfast. Moreover, the time of breakfast was later for cases (8.5, 1.4 *SD*) compared with controls (8.4, 1.4 *SD*). Nighttime fasting duration was similar between both groups (11.0, 1.6 *SD* controls and 11.1, 1.6 *SD* cases).

We found no correlation among controls between the time of breakfast and the time of last meal (Spearman's correlation coefficient 0.09, **Supplementary Figure 1**) nor with the interval between dinner and time going to sleep (Spearman's correlation coefficient 0.06). We found a high correlation between nighttime fasting duration and time of breakfast (Spearman's correlation coefficient 0.8).

We explored characteristics of cases and controls by menopausal status (**Supplementary Table 1**). Postmenopausal cases tended to have a higher BMI compared with postmenopausal controls (27.1 vs. 26.3 kg/m^2). Among premenopausal women, BMI was similar among both groups. Premenopausal cases had a longer nighttime fasting duration and a later breakfast compared with controls (11.0 vs. 10.6 and 8.6 vs. 8.2, respectively). In postmenopausal women, there were no differences in neither of these two nutritional circadian behaviors.

Association of nighttime fasting duration and breast cancer risk

In all women, we observed no association between nighttime fasting duration and breast cancer risk after adjusting for the time of breakfast (**Table 2**, OR = 1.01, 95% CI: 0.93–1.11). For premenopausal women, we observed an association between nighttime fasting duration and breast cancer risk (OR = 1.11, 95% CI: 1.01–1.21), but no association was observed after adjusting for time of breakfast (OR = 0.99, 95% CI: 0.86–1.14). We observed the same tendency, in the GAMs (**Figures 2A–C**). Among postmenopausal women, we did not observe an association between nighttime fasting duration and breast cancer risk (**Table 2**, OR = 1.04, 95% CI: 0.93 – 1.17, model adjusted for time of breakfast). The absence of an

TABLE 2 Logistic regression models investigating the association between nighttime fasting and time of breakfast with breast cancer risk.

	All women			
	Controls N (%) or mean (SD)	Cases N (%) or mean (SD)	OR (95% CI)[a]	OR (95% CI)[b]
Nighttime fasting				
Continuous (hours)	11.0 (1.6)	11.1 (1.6)	1.05 (0.99–1.10)	1.01 (0.93–1.11)
≤11.00 h[c]	744 (60.4)	646 (57.9)	*Ref*	*Ref*
>11.00 h	488 (39.6)	470 (42.1)	1.12 (0.94–1.33)	1.02 (0.83–1.27)
Time of breakfast				
Continuous	8.4 (1.4)	8.5 (1.4)	1.06 (1.00–1.13)	1.05 (0.95–1.16)
≤8.00 a.m.[c]	648 (52.6)	518 (46.4)	*Ref*	*Ref*
>8.00 a.m.	584 (47.4)	598 (53.6)	1.27 (1.08–1.51)	1.25 (1.02–1.54)
Premenopausal women				
Nighttime fasting				
Continuous (hours)	10.6 (1.5)	11.0 (1.8)	1.11 (1.01–1.21)	0.99 (0.86–1.14)
≤11.00 h	265 (69.7)	262 (61.9)	*Ref*	*Ref*
>11.00 h	115 (30.3)	161 (38.1)	1.31 (0.96–1.78)	0.97 (0.66–1.43)
Time of breakfast				
Continuous	8.2 (1.3)	8.6 (1.6)	1.18 (1.06–1.31)	1.18 (1.01–1.40)
≤8.00 a.m.	227 (59.7)	205 (48.5)	*Ref*	*Ref*
>8.00 a.m.	153 (40.3)	218 (51.5)	1.53 (1.13–2.07)	1.40 (0.98–2.00)
Postmenopausal women				
Nighttime fasting				
Continuous (hours)	11.2 (1.7)	11.2 (1.5)	1.02 (0.95–1.09)	1.04 (0.93–1.17)
≤11.00 h	479 (56.2)	384 (55.4)	*Ref*	*Ref*
>11.00 h	373 (43.8)	309 (44.6)	1.06 (0.86–1.32)	1.07 (0.82–1.38)
Time of breakfast				
Continuous	8.5 (1.5)	8.5 (1.3)	1.01 (0.93–1.09)	0.97 (0.85–1.11)
≤8.00 a.m.	421 (49.4)	313 (45.2)	*Ref*	*Ref*
>8.00 a.m.	431 (50.6)	380 (54.8)	1.22 (0.98–1.52)	1.26 (0.97–1.62)

[a]Adjusted for age, center, education, family history of breast cancer, menarche, number of children, BMI, contraceptive use, hormonal replacement therapy, menopausal status, breastfeeding, and age of the first child.
[b]Same as a. Models for both exposures were mutually adjusted.
[c]Categorizations in both exposures were performed according to the median point among controls. N, sample size; OR, odds ratio; SD, standard deviation. The p-value for interaction between the time of breakfast and menopause = 0.021.

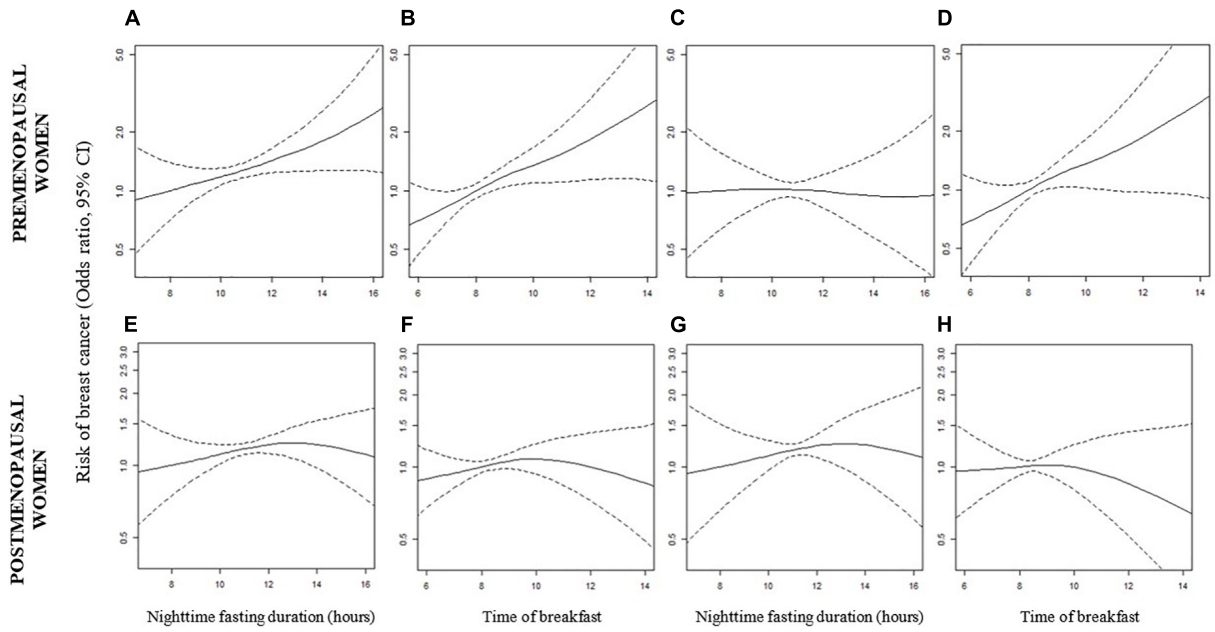

FIGURE 2
Generalized additive models showing the association between nighttime fasting duration, time of breakfast, and breast cancer risk in premenopausal **(A–D)** and postmenopausal women **(E–H)**. Models were adjusted for age, center, education, family history of breast cancer, menarche, number of children, BMI, contraceptive use, hormonal replacement therapy, breastfeeding, and age of the first child. In models **(C)**, **(D)**, **(G)**, and **(H)**, nighttime fasting duration and time of breakfast were mutually adjusted.

association was also observed in Figures 2E,G. There was no significant evidence of effect modification by menopausal status.

Association of time of breakfast and breast cancer risk

In all women, having the first meal after 8 a.m. was associated with a 25% increase in the risk of having breast cancer compared with breakfast before 8 a.m. (Table 2, OR = 1.25, 95% CI: 1.02–1.54) after adjusting for nighttime fasting. In the continuous model, this association was weaker and non-significant (OR = 1.05, 95% CI: 0.95–1.16). This pattern was stronger for premenopausal women: the OR for having breakfast after 8 a.m. was 1.40 (95% CI: 0.98–2.00), while each hour later in the time of breakfast was associated with an 18% increase in the risk of having breast cancer (OR = 1.18, 95% CI: 1.01–1.40). Both models were adjusted for nighttime fasting duration. This association was linear (Figure 2D, *p*-value from ANOVA test = 0.35). In postmenopausal women, the pattern was less clear with a slightly increased risk observed in the categorical analysis but without a clear dose response (OR per hour later in breakfast = 0.97, 95% CI: 0.85–1.11) (Table 2). Figures 2F,H showed the same pattern. Finally, in a model combining our main exposures, we observed that an early breakfast (8 a.m. or before) was associated with a slightly reduced risk independently of the nighttime fasting duration (Supplementary Table 2). This was observed in all women and stratified by menopausal status.

We found a statistically significant effect modification of the association between the time of breakfast and breast cancer risk by menopausal status (*p*-value for interaction = 0.021). We found no effect modification by chronotype (*p*-value for interaction = 0.9) nor by HRT history (ever vs. never) among postmenopausal women (*p*-value for interaction = 0.5).

Cancer subtype

In a multinomial logistic regression model, we explored the association of both nighttime fasting duration and time of breakfast with the RR of each breast cancer subtype. Among all women, we did not observe significant differences in nighttime fasting duration or time of breakfast and different cancer subtypes (Table 3). Among premenopausal women, later time of breakfast (per hour increase) was associated with a higher risk of HER2 + tumors compared with positive hormonal receptors and triple-negative (Table 3, RR = 1.38, 95% CI: 1.03–1.85, RR = 1.19, 95% CI: 0.99–1.42, and RR = 1.11, 95% CI: 0.69–1.78, *p*-value from Wald test = 0.03). No differences were observed for postmenopausal women by subtype.

TABLE 3 Multinomial logistic regression model investigating the association between nighttime fasting and time of breakfast with breast cancer risk subtype.

	Controls	HER2 +		+ Hormonal receptors		Triple-negative	
	Mean (*SD*) or *N* (%)	Mean (*SD*) or *N* (%)	RR (95% CI)[a]	Mean (*SD*) or *N* (%)	RR (95% CI)[a]	Mean (*SD*) or *N* (%)	RR (95% CI)[a]
All women							
Nighttime fasting (hours)	11.0 (1.6)	11.5 (1.6)	1.13 (0.96–1.34)	11.0 (1.6)	0.98 (0.89–1.08)	10.9 (1.6)	0.96 (0.75–1.22)
Time of breakfast	8.4 (1.4)	8.8 (1.6)	1.07 (0.89–1.28)	8.5 (1.3)	1.04 (0.93–1.17)	8.4 (1.4)	1.01 (0.75–1.35)
Total	1,232 (54.8)	200 (8.9)		740 (32.9)		75 (3.3)	
Premenopausal women							
Nighttime fasting (hours)	10.6 (1.5)	11.5 (1.8)	1.09 (0.84–1.41)	10.8 (1.7)	0.94 (0.81–1.10)	10.7 (1.6)	0.93 (0.63–1.37)
Time of breakfast	8.2 (1.3)	9.1 (1.8)	1.38 (1.03–1.85)	8.5 (1.4)	1.19 (0.99–1.42)	8.5 (1.5)	1.11 (0.69–1.78)
Total	380 (49.5)	71 (9.3)		290 (37.8)		26 (3.4)	
Postmenopausal women							
Nighttime fasting (hours)	11.2 (1.7)	11.4 (1.5)	1.19 (0.95–1.48)	11.2 (1.5)	1.02 (0.89–1.16)	11.0 (1.6)	1.02 (0.74–1.41)
Time of breakfast	8.5 (1.5)	8.7 (1.5)	0.92 (0.72–1.18)	8.5 (1.2)	0.98 (0.84–1.14)	8.4 (1.4)	0.91 (0.62–1.32)
Total	852 (57.6)	129 (8.7)		450 (30.4)		49 (3.3)	

[a]Models were adjusted for age, center, educational status, family history of breast cancer, menarche, number of children, BMI, contraceptive use, hormonal replacement therapy, menopausal status, breastfeeding, and age of the first child. Models were mutually adjusted for both exposures. Controls were considered as the reference group.
N, sample size; RR, relative Risk; SD, standard deviation.

Sensitivity analyses

Adjustment for lifestyle factors and other potential breast cancer risk factors did not importantly change our estimates (Supplementary Tables 3, 4). We explored further adjustment of our models with other circadian behaviors (Supplementary Table 5). In all women, the additional adjustment of time of breakfast with the time of last meal (without nighttime fasting) strengthened the association between time of breakfast and breast cancer risk (OR = 1.05, 95% CI: 0.94–1.16 to 1.06 and 95% CI: 1.00–1.14). None of the other estimates in these models importantly changed after adjustment (Supplementary Table 5).

We also investigated the associations of interest using the behaviors reported as corresponding to the year prior to baseline. We observed associations between a later time of breakfast (per hour increase) and breast cancer in all, premenopausal and postmenopausal women (Supplementary Table 6, OR = 1.19, 95% CI: 1.08–1.31, OR = 1.21, 95% CI: 1.03–1.43, and OR = 1.18, 95% CI: 1.04–1.33, respectively). In none of the strata, nighttime fasting duration was associated with breast cancer risk after considering the time of breakfast (Supplementary Table 6).

We observed some differences in the correlation between data reported as corresponding to 40 years of age and to the previous year. For premenopausal women, the correlation was high for the time of breakfast (rho = 0.83), time of last meal (rho = 0.86), and nighttime fasting duration (rho = 0.82). For postmenopausal women, we found a low correlation for the time of breakfast (rho = 0.44), a moderate correlation for the time of last meal (rho = 0.52), and a low correlation for nighttime fasting duration (rho = 0.44).

Discussion

This is one of the first epidemiological studies to investigate the association between circadian nutritional behaviors and breast cancer risk. We found that having a late breakfast was associated with an increased risk of breast cancer compared with an earlier breakfast. This pattern was stronger among premenopausal women and was observed across all chronotypes. We observed an association between nighttime fasting and breast cancer, especially among premenopausal women, which disappeared after adjusting for the time of breakfast.

There is few evidence available on circadian timing of diet and cancer risk. To the best of our knowledge, only one other epidemiological study examined the association between the time of breakfast and breast cancer risk (10). The results of this prospective study showed that each hour later in breakfast was associated with a 13% increase in the hazards of developing breast cancer risk (HR = 1.13, 95% 0.99–1.29, *p*-value 0.07) (10). In this French cohort, non-cases were younger (aged 45 years, 14.5 *SD*) compared with controls in this analysis (aged 58 years, 12.5 *SD*). Results were not stratified for menopausal status, which according to our results might be an effect modifier in this association. These and other factors such as the prospective study design in the NutriNet-Santé cohort or the exposure assessment could explain the differences between

the results from the NutriNet-Santé study and the results presented in this study.

Two cross-sectional studies have suggested that prolonged nighttime fasting could reduce systemic inflammation and improve glycemic control, both potential breast cancer risk factors (12, 21). A prospective cohort study showed that elongating the nighttime fasting period could reduce breast cancer recurrence (11). In this study, the mean nighttime fasting duration was 12.5 (1.7, SD) h. Differences between our results and the results by Marinac et al. (11) could be also explained by the mean nighttime fasting of the study population. Although models were adjusted for a binary variable of eating after 8 PM, the time of breakfast was not considered. As suggested in our study and in a previous analysis from the MCC-Spain study, the time of breakfast was confounding the association between nighttime fasting duration and risk of cancer (29). In line with our results, in the NutriNet-Santé study nighttime fasting duration was not associated with breast cancer after adjusting for the time of the first meal. In the French cohort, the mean nighttime fasting duration was 11.9 (1.2, *SD*).

Several hypotheses could explain the association between the time of breakfast and breast cancer risk. Breakfast skipping might be compensated with a higher intake later on the day (6). This could be also the case for a later time of breakfast, but even after adjusting for daily caloric intake, no changes were observed. Similarly, regular breakfast consumption has been linked with healthier lifestyle behaviors (6). It could be that the observations for early breakfast are also indicative of a "healthy user bias." We explored adjustment for alcohol intake, vegetable and fruit intake, and physical activity, and we did not observe important changes.

The association is biologically plausible. Delaying breakfast could be associated with worse glycemic control (15), lipid profile (17), inflammation (16), and alterations in the cortisol rhythm (30), which may then lead to breast cancer risk (11, 12, 31, 32). In animal models, it has been shown that skipping the analogous breakfast, delaying the first active-phase meal by 4 h, can be associated with increased visceral fat (33), increased hepatic lipid accumulation (34), and with a phase delay in the expression of circadian genes in the liver and fat tissue (35, 36). A randomized clinical trial showed that skipping breakfast acutely altered the regulation of clock and clock-controlled genes (37). Supporting this hypothesis, the downregulation of clock genes has been correlated with breast cancer (38).

The differences in menopausal status could be explained by an increased susceptibility of breast tissue to circadian disruption in earlier life stages (28). It could also be that the potential for recall bias is greater among postmenopausal women (older women) with a long time elapsed since exposure. In fact, the correlation between mid-age behaviors and the previous year to the inclusion in this study was lower in postmenopausal women. The strongest association between the time of breakfast and breast cancer risk in premenopausal women was observed in HER2 + cases. However, there is less evidence on differences in circadian parameters and breast cancer subtypes (3). Further studies are needed to confirm and understand these differences.

The main strengths of this study are the large sample size of the study population, which enabled the stratification of our results by menopausal status, and the detailed information on circadian nutritional behaviors. This investigation gives new insights and future questions on the impact of chrononutrition in cancer, an emerging field of study that deserves more attention. The main limitation of this study is the potential for recall bias since women were asked for behaviors at 40 years of age. Circadian nutritional behaviors were assessed at one single time point, which may affect the validity of these exposures. Finally, given the observational nature of the study and its design, residual confounding cannot be completely ruled out, and causal interpretation of these findings should be taken with caution.

Our results suggest that delaying circadian nutritional behaviors, specifically having a late breakfast, is associated with increased breast cancer risk, especially among premenopausal women. Together with our previous study on other circadian timing aspects, this study suggests that when to eat may also be an important aspect of healthy nutritional behaviors, influencing cancer risk. If these results are confirmed by prospective studies and clinical trials, public nutritional recommendations may consider including timing aspects aside from the quality and quantity components of the diet.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by CEIC 2008/3123/I. The patients/participants provided their written informed consent to participate in this study.

Author contributions

BP-G, EA, TF, PA, IG-A, VM, JA, GF-T, AM-B, RM-G, NA, GC-V, MG, AM, MP, and MK performed the data acquisition. AP-C and AE performed the data curation. MP and MK carried funding acquisition. DR and MK performed the supervision of the study. AP-C wrote the first draft of the manuscript. All authors contributed to the study conception and design,

commented on previous versions of the manuscript and read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.941477/full#supplementary-material

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Consumption of fermented dairy products is associated with lower anxiety levels in Azorean university students

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A growing number of studies have found that the gut microbiota is involved in a variety of psychological processes and neuropsychiatric disorders, which include mood and anxiety disorders. Consumption of dairy products may contain bioactive compounds and probiotic bacteria with various therapeutic benefits. The aim of the study was to investigate possible associations between the frequency of consumption of different types of dairy products and the state of anxiety in university students. The subjects were 311 Azorean university students, 231 women and 80 men, with an average age of 20.5 years. Subjects completed a quantitative questionnaire on the frequency of dairy product consumption and a short version of the Spielberger State-Trait Anxiety Inventory (STAI) test. Among dairy products, semi-skimmed milk was the most commonly consumed, followed by cheese (ripened), drinking yogurt, skim milk, and set yogurt, while fresh cheese, whole milk, and dairy ice cream were the least common. Discriminant analysis showed that consumption of fermented products (yogurt and cheese) was significantly higher ($P < 0.05$) in the group with low anxiety level (score < 40 in STAI test) than in the group with higher anxiety level (score ≥ 40). In this analysis, 62.4% of the initially grouped cases were correctly classified according to the frequency of fermented products consumption. No correlations were found between anxiety and unfermented dairy products. The results indicate that the consumption of fermented dairy products has a positive effect on reducing anxiety in young Azorean university students.

KEYWORDS

fermented food, anxiety, dietary intake, health, dairy, yogurt, STAI test

Introduction

Several studies in animals have shown that the commensal microbiota is crucial for the development of the hypothalamic-pituitary stress response (1–3). It has been shown that the absence of gut microbiota in germ-free animals is associated with an increase in risk behaviors and anxiety, which could normalize after gut colonization with bacteria from normal animals (4). In addition, observational studies in humans reported an altered gut microbiome in individuals with depression and depressive symptoms (5).

Components naturally present in the diet may play an important role in the viability, composition, and functionality of the gut microbiota (6). Recent evidence suggests that many aspects of the diet, such as its composition, consumption patterns, and cultural habits, have the potential to influence the interaction between the gut microbiome and the brain through multiple neuroendocrine pathways (7). In addition, there is growing evidence that the gut microbiome plays an active role in depression symptoms, anxiety, cognitive function, sleep, and brain function (8). Although there is still a need to fully understand these mechanisms, some evidence suggests that the gut microbiome may influence brain function through the excretion of metabolites, regulation of the host immune system, and ultimately the ability to secrete neuropeptides and active neurotransmitters (9). Due to the potential effect of probiotics on improving mental health, the term "psychobiotics" has been proposed (10). Psychobiotics refer to a group of probiotics that are able to produce and release neuroactive substances such as dopamine, norepinephrine, serotonin, and γ-aminobutyric acid (GABA), which act through the brain-gut axis and may exert antidepressant effects (11, 12). Current scientific evidence also suggests that lactic acid bacteria (LAB), particularly *Lactobacillus* (Firmicutes) and *Bifidobacterium* (Actinobacteria), help the host correct imbalances in the gut microbiota and consequently maintain and regulate mental health (9, 13). These bacteria are traditionally associated with fermented foods and are the most studied probiotic organisms (14). In this regard, consumption of fermented foods and probiotics have received considerable attention as potential treatments for depression and anxiety (15, 16).

Because fermented dairy products are an important source of probiotic microorganisms, we hypothesized in the current study that the state of anxiety may be influenced by the consumption of dairy products. A cross-sectional study was conducted to assess the association between dairy product consumption and the prevalence of anxiety in young university students.

Materials and methods

Participants

Participants were undergraduate students at the University of the Azores, Portugal. Participation was voluntary and informed consent was obtained before the start of the study. Of the 317 students who voluntarily participated in the survey, a total of 311 healthy students were included who did not meet the exclusion criteria of being over 40 years of age. Thus, the sample consisted of 311 young students of both sexes, 231 females and 80 males, with a mean age of 20.5 years (SD = 3.35). This study was approved by the Ethics Committee of the University of the Azores.

Dairy food frequency questionnaire

The dairy intake survey was based on the Food Frequency Questionnaire (FFQ), adapted for the Portuguese population and validated by Lopes et al. (17). The questionnaire included 10 most common dairy foods and a frequency section with nine response options ranging from never to six or more times per day. Dairy product categories included milk (whole, semi-skimmed, and skimmed), yogurt (set and drinking yogurts), cheese (ripened cheese), fresh cheese (Latin-style cheese and whey-based cheese - requeijão, made without starter cultures), dairy desserts, and dairy ice cream.

Spielberger state-trait anxiety inventory test

The short version of the Spielberger State-Trait Anxiety Inventory (STAI) was used to assess anxiety in university students. This test consists of six items, three of which are formed by questions about the presence of anxiety and three of which are formed by questions about the absence of anxiety (18). The short version (6-item version) of the STAI test was chosen instead of the 20-item STAI test because it is faster to use and the two tests give equivalent results (19). The scale for this test ranges from 20 to 80 points, with a higher score indicating greater anxiety. A cut-off of 39–40 has been proposed to detect clinically significant symptoms of anxiety for the STAI scale (20).

Statistical analysis

The frequency of dairy products consumption was calculated using the validated questionnaires. Validation of the questionnaires was done by calculating Cronbach's alpha to determine the degree of reliability of the data obtained (21). A *t*-test for independent samples was performed to compare the consumption of dairy products between men and women. Discriminant analysis using Wilks' method was used to determine which dairy products provided significant discrimination between individuals exhibiting different levels of anxiety. Participants in the STAI test were divided into two groups according to the cutoff point at score 40 (20): group 1 - STAI score < 40, corresponding to a normal/low anxiety state and group 2 - STAI score ≥ 40, corresponding to a high anxiety state. Dairy products were categorized as follows: milk consumption corresponded to all types of whole milk, semi-skimmed milk and skimmed milk; yogurts included set and drinking yogurts, Latin-style fresh cheese and whey cheese (locally called requeijão) were included in the "fresh cheese" group (non-fermented cheeses) and the last group included dairy ice cream and desserts. The pooled results from the consumption of yogurts (set and drinking) and ripened cheeses

TABLE 1 Frequency of consumption of dairy products among Azorean university students [$n = 311$, female = 231, male = 80].

Item		Frequency of dairy food consumption (%)								
		Never or < 1 × month	1–3 × month	1 × week	2–4 × week	5–6 × week	1 × day	2–3 × day	4–5 × day	≥ 6 × day
Full-fat milk	Female	96.1	1.7	0.0	0.4	0.0	1.3	0.4	0.0	0.0
	Male	95.0	1.3	0.0	1.3	2.5	0.0	0.0	0.0	0.0
	Total	95.8	1.6	0.0	0.6	0.6	1.0	0.3	0.0	0.0
Semi-skimmed milk	Female	29.9	8.2	5.2	18.2	6.9	17.7	10.4	1.3	2.2
	Male	22.5	7.5	3.8	23.8	7.5	18.8	13.8	0.0	2.5
	Total	28.0	8.0	4.8	19.6	7.1	18.0	11.3	1.0	2.3
Skimmed-milk	Female	71.4	7.8	1.7	5.6	3.9	6.1	2.2	0.9	0.4
	Male	81.3	3.8	2.5	1.3	2.5	3.8	3.8	1.3	0.0
	Total	74.0	6.8	1.9	4.5	3.5	5.5	2.6	1.0	0.3
Yogurt (set)	Female	27.7	19.9	18.2	20.3	6.5	5.6	1.7	0.0	0.0
	Male	31.3	21.3	13.8	18.8	3.8	10.0	1.3	0.0	0.0
	Total	28.6	20.3	17.0	19.9	5.8	6.8	1.6	0.0	0.0
Yogurt (drinking)	Female	28.1	19.0	14.7	16.9	9.5	10.0	1.3	0.0	0.4
	Male	28.7	18.8	7.5	23.8	5.0	11.3	3.8	0.0	1.3
	Total	28.3	19.0	12.9	18.6	8.4	10.3	1.9	0.0	0.6
Fresh cheese(Latin-Style)	Female	32.9	34.2	18.2	9.1	2.6	2.6	0.4	0.0	0.0
	Male	41.3	27.5	18.8	8.8	1.3	0.0	1.3	0.0	1.3
	Total	35.0	32.5	18.3	9.0	2.3	1.9	0.6	0.0	0.3
Wey cheese (Requeijão)	Female	85.3	10.4	2.6	1.7	0.0	0.0	0.0	0.0	0.0
	Male	88.8	8.8	1.3	0.0	1.3	0.0	0.0	0.0	0.0
	Total	86.2	10.0	2.3	1.3	0.3	0.0	0.0	0.0	0.0
Matured cheeses	Female	12.1	17.7	14.3	33.8	8.7	10.0	3.0	0.4	0.0
	Male	10.0	15.0	8.8	36.3	10.0	10.0	6.3	2.5	1.3
	Total	11.6	17.0	12.9	34.4	9.0	10.0	3.9	1.0	0.3
Dairy desserts	Female	24.7	46.3	15.6	10.4	1.7	1.3	0.0	0.0	0.0
	Male	22.5	51.2	16.3	3.8	2.5	1.3	1.3	0.0	1.3
	Total	24.1	47.6	15.8	8.7	1.9	1.3	0.3	0.0	0.3
Dairy ice-cream	Female	21.2	52.4	16.9	7.4	2.2	0.0	0.0	0.0	0.0
	Male	18.8	62.5	13.8	2.5	0.0	1.3	0.0	0.0	1.3
	Total	20.6	55.0	16.1	6.1	1.6	0.3	0.0	0.0	0.3

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TABLE 2 Self-reported anxiety scores of the STAI-6 test of Azorean university students ($n = 311$).

	Anxiety	Sex		Total
		Female	Male	
Score	Average	41.3	38.5	40.6
	Standard error	12.3	10.7	11.9
Number of individuals	Normal (<40)	103	45	148
	High (≥40)	128	35	163
%	Normal (<40)	45.9	56.3	47.6
	High (<40)	55.4	43.7	52.4

A score < 40 was considered normal and a score ≥40 corresponds to an elevated anxiety level.

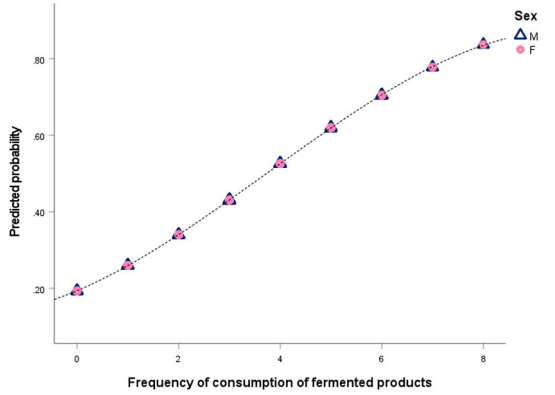

FIGURE 1
Predicted likelihood of having a normal anxiety state (STAI score < 40) and consuming fermented foods (yogurt and cheese). Frequency of consumption of fermented products ranged from 0 (never or < 1 × month) to 8 (≥ 6 × day), as indicated in Table 1. Males are represented by a blue triangle (M) and females (F) by a pink circle.

were classified as "fermented foods." The assumptions of normality and homogeneity of the variance-covariance matrices of each group were tested using the Kolmogorov-Smirnov and M the Box tests, respectively. The association between anxiety according to the score group (low/normal and high anxiety) and consumption frequency of dairy foods, age, and sex was assessed using logistic regression with the forward stepwise method. P values < 0.05 were considered statistically significant. Data were processed using the Statistical Package for the Social Sciences (SPSS IBM, USA, version 27).

Results and discussion

Assessment of ingestion of dairy products

The application of the dairy Food Frequency Questionnaire (FFQ) resulted in a total of 311 validated questionnaires. The average age for both sexes was 20.5 years, with a range between 17 and 37 years. Of the 311 respondents, 231 correspond to the female gender with a mean age of 20.5 years (SD = 3.6) and 80 correspond to the male gender with a mean age of 20.5 years (SD = 2.6). When analyzing the reliability of the questions constructed to evaluate the consumption of dairy products and dairy desserts, a Cronbach's α value of 0.7 was obtained, showing the correction of the internal consistency of the questionnaire according to Landis and Koch (21).

Table 1 shows the frequency of consumption of dairy products by the students. There was no significant effect of gender ($P > 0.05$) on the consumption of each dairy product. Almost all students (93.6%; female: 91.8%, male: 98.8%) consumed at least one serving of dairy products 2 to 4 times per week. Semi-skimmed milk was consumed most frequently (59.3%), followed by ripened cheeses (58.6%), drinking and set yogurts (39.8 and 34.1%, respectively), and skim milk (17.4%), while whey cheese, whole milk, and ice cream were consumed least (1.6, 2.5, and 8.3%, respectively). However, only half of the respondents (53.1%; women: 53.2%, men: 52.5%) consumed at least one dairy product per day. Among those who consumed at least one serving per day, semi-skimmed milk was the most consumed (32.6%), followed by ripened cheeses (15.2%), drinking and set yogurts (12.8 and 8.4%, respectively), and skim milk (9.4%).

Relationship of dairy products ingestion and anxiety

To assess possible associations between consumption of dairy products and state of anxiety, the STAI questionnaire was used. This questionnaire is most commonly used to assess anxiety in both psychiatric patients and the general population (22, 23). Marteau and Bekker (19) proposed a shorter STAI test with 6 items (STAI-6) that reliably replaces the longer questionnaire and can be used in behavioral research. The self-reported anxiety scores are shown in Table 2 and Figure 1 (Supplementary material). The STAI score ranges from 20 to

80, with a score of 34–36 considered normal. A higher score indicates severe anxiety, while a cutoff score of 39–40 has been suggested to identify clinically significant symptoms of state anxiety (20). The results showed that a high percentage of the Azorean college population scored above 40 on the STAI test, indicating high levels of anxiety in this population. The mean score was 40.5 ± 11.6, with 41.3 ± 12.2 in females and 38.4 ± 10.6 in males. Higher STAI scores were observed in females, consistent with other studies (23–25). The mean scores were also consistent with other studies conducted with college students (24, 25).

Stepwise discriminant analysis extracted a discriminant function that retained the variables "yogurt" and "cheese" (ripened cheese) with significant discriminant power ($P < 0.001$). Table 3 shows the standardized coefficients of the yogurt and cheese variables in the discriminant function that explains the variability between the groups (group 1 - normal/low anxiety state and group 2 - high anxiety state). This function significantly discriminated the two groups ($\Lambda = 0.930$; $X^2 = 22.378$; $P < 0.001$). Therefore, the consumption of fermented products (yogurt and ripened cheese) was higher in the group of subjects with normal anxiety state (score < 40) than in the subjects with high anxiety state (STAI score ≥40; Table 4). The percentage of subjects correctly classified by discriminant analysis was 62.4%. Therefore, the consumption of fermented products, which include yogurt and ripened cheeses (fresh cheese without starter cultures excluded), may have a significant positive effect ($P < 0.05$) on the anxiety levels of the student respondents.

TABLE 3 Standardized coefficients of variables with discriminatory power (yogurt and aged cheese).

Variables	Co-efficients in the discriminant function
Yogurt	0.768
Cheese	0.571
Eigenvalue	0.077
Explained variance	100.0 %

Logistic regression with all predictors showed that age, sex, and consumption of dairy products (except fermented products) had no significant effect ($P > 0.05$) on the probability of having a normal anxiety level. In contrast, consumption of fermented products increased the probability of having a normal anxiety level (odds ratio, OR = 1.47, $P < 0.05$). Therefore, a new model was fitted with only the variable fermented products. The probability function for a normal/low anxiety state (STAI score < 40) is shown in Figure 1.

There are few studies investigating the relationship between consumption of fermented foods and mental health. The present study confirms some evidence presented by other authors indicating the beneficial effects of fermented food consumption on the central nervous system (26–28). The mechanisms by which fermented foods affect mood can be explained in part by the production of neurotransmitters by certain microorganisms (29). Bacteria (e.g., *Lactobacillus* and *Bifidobacterium* species) associated with fermented foods may influence brain health through modulation of gut microbiota. Some gut microorganisms have been shown to alleviate anxiety and depression and improve cognitive performance (30). Moreover, mental disorders such as depression and anxiety are often associated with gut problems, suggesting a bidirectional relationship between mental health and gut microbiota (31).

Gamma-aminobutyric acid (GABA) production by lactic acid bacteria present in the gut and fermented foods has been proposed as one of the mechanisms involved (27, 28, 32). Fermented dairy products may contain probiotic microorganisms capable of surviving in the gastrointestinal environment and synthesizing GABA (26, 32). In the study by Luo et al. (33), ingestion of a GABA-producing strain of *Lactobacillus* was shown to reduce anxiety and improve cognitive function in animals with anxiety. Ingestion of milk fermented with a GABA-producing *Lactobacillus* strain has also been used to reduce anxiety and induce sleep in animals (28). In addition, several studies have shown that oral administration of GABA can affect the brain neurotransmitter system and improve symptoms of anxiety and depression (6, 32, 34–36). Other studies indicated that oral administration of selected probiotic bacteria may have beneficial effects in the treatment of

TABLE 4 Ranking of discriminant analysis results for fermented foods consumed by subjects divided into two groups: STAI score < 40 and STAI score ≥ 40.

	Group	Predicted group membership[a]		Total
		<40	≥40	
Number of individuals	Normal anxiety	88	67	148
	High anxiety	50	113	163
%	Normal anxiety	54,7	45,3	100,0
	High anxiety	30,7	69,3	100,0

[a]62.4% of the original cases were correctly classified.

gastrointestinal and psychological stress-related disorders (37–39). Recent human studies have shown that taking a dietary supplement containing probiotics, prebiotics, and phytobiotics (phytonutrients with gut-enhancing effects) improves beneficial gut bacteria and psychological well-being (40).

In conclusion, the present study demonstrates the association between the consumption of fermented dairy products such as yogurt and cheese (with the exception of fresh cheese) and lower anxiety levels in young university students. The results of this study should be considered in light of its limitations. Compared with other studies that investigated the eating behaviors of university students, the results of the present study are based on a relatively small sample size (311). Although the analysis was conducted with a small sample size, the homogeneity of the group in terms of age and ethnicity is a strength of the study. However, the use of university students may limit the generalizability of the results to the general population.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the University of the Azores. The patients/participants provided their written informed consent to participate in this study.

Author contributions

RS and CS acquired the data, conducted the data analyses, conceived, and designed the study. All authors contributed to the interpretation of data, drafted the manuscript, and approved the final version for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.930949/full#supplementary-material

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Coffee and caffeine consumption and risk of renal cell carcinoma: A Mendelian randomization study

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Background: The association between coffee and caffeine consumption and the risk of renal cell carcinoma was inconsistent among observational studies, and whether these observed associations were causal remained unclear. Therefore, we performed two-sample Mendelian randomization (MR) study to assess the causal nature of the association.

Materials and methods: In this study, 12 and two independent single nucleotide polymorphisms (SNPs) related to coffee and caffeine consumption at a genome-wide significance level of $p < 5 \times 10^{-8}$ were used as instrumental variables (IVs), respectively. Summary-level data for renal cell carcinoma were taken from the FinnGen consortium with up to 174,977 individuals, and the International Agency for Research on Cancer (IARC) with 13,230 individuals. We used inverse-variance weighted (IVW) as the main method, followed by the weighted median method, the MR-Egger regression method, and the MR robust adjusted profile score method. Outlier and pleiotropic variants were assessed by the MR Pleiotropy RESidual Sum and Outlier test and MR-Egger regression. We used meta-analysis methods in fixed-effects to combine the estimates from the two sources.

Results: The genetically predicted coffee consumption was not associated with the risk of renal cell carcinoma in the FinnGen consortium, and the relationship was consistent in the IARC consortium. The pooled odds ratio (*OR*) per 50% increase of coffee consumption was 0.752 [95% confidence interval (*CI*), 0.512–1.105; $p = 0.147$]. In addition, complementary analyses that separated the coffee-related SNPs according to their relationship with blood levels of caffeine metabolites (higher, lower, or unrelated) found no relationship with renal cell carcinoma. The results were consistent after excluding eight SNPs due to potential risk factors at genome-wide significance ($p < 5 \times 10^{-8}$). Moreover, genetically predicted per 80-mg increase in caffeine consumption was not associated with the risk of renal cell carcinoma (pooled *OR* = 0.872, 95% *CI*: 0.676–1.125, $p = 0.292$).

Conclusion: Our MR study provided no convincing evidence for a causal effect between coffee and caffeine consumption and the risk of renal cell carcinoma. The associations for renal cell carcinoma need to be verified in well-powered studies.

KEYWORDS

renal cell carcinoma, coffee consumption, caffeine consumption, Mendelian randomization, causal effect

Introduction

The incidence rate of renal cell carcinoma has increased significantly by approximately 1.1% every year in the past few years (1). Compared with 1990, the global incident cases in 2019 were higher by 154.78% for renal cell carcinoma (2). During the 30-year study period, there was an upward trend in the age-standardized mortality rate and the age-standardized disability-adjusted life-years rate for renal cell carcinoma (estimated annual percentage change = 0.35 and 0.12, respectively) (2). Previous studies demonstrated several risk factors for renal cell carcinoma, but the accurate pathogenesis remains unclear (3, 4). Given that renal cell carcinoma is a fatal disease, it is crucial to identify interventions that can reduce the risk of this disease.

Coffee is one of the most popular beverages worldwide. A traditional cup of coffee could contain up to 1,000 bioactive compounds, such as a wide variety of aromatic compounds, antioxidants, and most importantly, caffeine (5). Several researchers have been interested in the relationship between coffee and caffeine consumption and tumor risk, given the anti-inflammatory and antioxidant properties of the beneficial ingredients (6–8). However, the relationship between coffee and caffeine consumption and renal cell carcinoma remains controversial. For instance, certain observational studies suggested that the consumption of coffee and caffeine is a protective factor for renal cell carcinoma (9, 10). Other studies, however, suggested otherwise (11).

As the kidney is an excretory organ, the role of fluid consumption could also be important in the development of renal cell carcinoma. However, observational studies on coffee and caffeine consumption and renal cell carcinoma risk may have several potential limitations. First, the observed acute effects of coffee and caffeine may not reflect their long-term effects, because the body can develop a tolerance to caffeine (12). Second, traditional epidemiological studies on coffee and caffeine intake and renal cell carcinoma risk may be affected by confounding factors (such as smoking or other unhealthy lifestyles) and reverse causation, and early studies that did not fully consider these biases may have produced misleading results (11, 13–16). Even in recent studies that have conducted a more comprehensive correction for potential confounding factors, residual confounding is still a worrying issue (10). Since the available evidence on the association between coffee and caffeine consumption and renal cell carcinoma risk came from traditional observational studies, the conclusions might be biased by reverse causation and residual confounding factors. Therefore, it is still indistinct whether coffee and caffeine intake plays a causal role in the renal cell carcinoma risk.

Randomized controlled trials (RCTs) cannot be conducted due to practice, cost considerations, and ethics issues. Mendelian randomization (MR) analysis could enhance casual inference on the association of exposure and an outcome of interest by employing genetic instrumental variants as instrumental variables (IVs) of exposure factors. The IVs are unlikely to be correlated with confounders associated with exposure and outcome of interest, as they are randomly allocated at the time of pregnancy. In addition, MR analysis reduces reverse causation because allele randomization is occurred before the development of disease.

We performed an MR analysis to evaluate the causal relationship between coffee and caffeine consumption and the risk of renal cell carcinoma.

Materials and methods

Study design

We performed an MR analysis based on three core assumptions: (1) the IVs are robustly related to the exposure of interest, (2) the IVs are not affected by any confounding factors, (3) and the IVs do not influence the outcome *via* any variable other than the exposure (17). The flowchart of this MR study design is displayed in **Figure 1**.

Genetic instrument selection for coffee and caffeine consumption

This study was on the strength of publicly retrievable summary-level data from large-scale genome-wide association studies (GWASs) and consortium that previously obtained

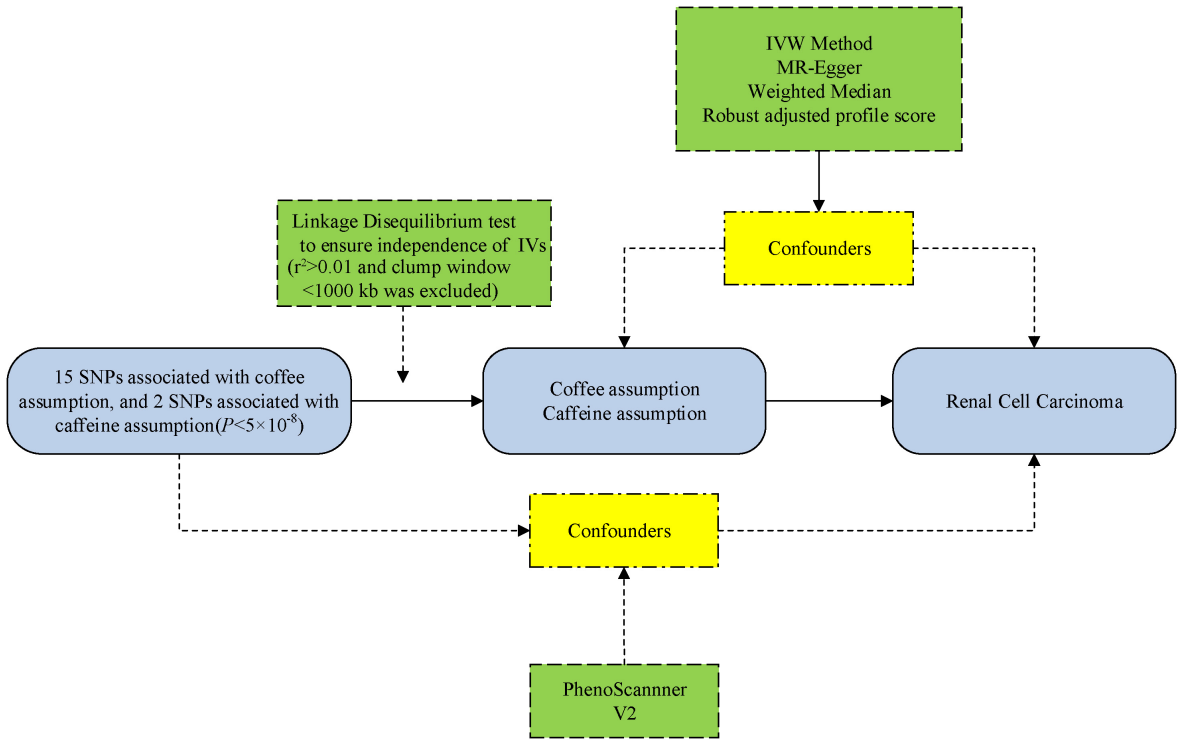

FIGURE 1
The flowchart of the Mendelian randomization (MR) study. IVs, instrumental variables; SNP, single nucleotide polymorphism; IVW, inverse-variance-weighted.

informed consent and ethics review board approvals. The single-nucleotide polymorphisms (SNPs) closely related to coffee intake were derived from a meta-analysis of four GWASs (the United Kingdom Biobank and three United States cohorts) (18). The GWASs adjusted for sex, age, total energy, body mass index, and top 20 principal components. In the United Kingdom Biobank (discovery phase), a touch screen questionnaire was applied to collect coffee consumption from all participants at baseline: "How many cups of coffee do you drink each day (including decaffeinated coffee)?" In the United States cohorts (replication phase), a semi-quantitative food frequency questionnaire was used to collect the regular and decaffeinated coffee consumption. The GWASs contained 375,833 individuals of European descent, and the median coffee consumption ranged from 1.1 to 2.5 cups per day. The effect sizes for the SNP-coffee associations were expressed per 1% of increase in coffee consumption in the GWASs. The odds ratio (*OR*) estimates of renal cell carcinoma were scaled to a per 50% of increase in coffee consumption in our study. To fulfill the first MR assumption that IVs are robustly related to the exposure of interest, 15 SNPs that were related to coffee consumption at the level of genome-wide significance ($p < 5 \times 10^{-8}$) (18) were identified as IVs (**Supplementary Table 1**). Selected SNPs explained approximately 0.48% phenotypic variance in coffee consumption (18). We calculated the linkage disequilibrium (LD) between the 15 SNPs based on an LD reference panel from 1,000 Genomes of European populations. We excluded SNPs in LD ($r^2 > 0.01$ and clump window < 1,000 kb) and retained the SNP with the lowest *p*-value. To fulfill the second MR condition that the IVs are not affected by any confounding factors, we evaluated the pleiotropic relationships of the SNPs with potential confounding factors by searching the PhenoScanner V2 website.[1] The LD of these 15 SNPs was computed utilizing the 1,000 genomes LD European individual panel as a reference population (19). There were 3 of the 15 SNPs excluded due to LD (rs117692895, rs4719497, and rs12699844 in chromosome 7). Therefore, 12 SNPs were implemented as IVs for coffee consumption in the primary analysis. Among the selected 12 SNPs in the primary analysis, the coffee-raising allele has been found to be correlated with higher (one SNP, rs2330783), lower (four SNPs, rs1260326, rs1057868, rs4410790, and rs2472297), and irrelevant to blood levels of caffeine metabolites (five SNPs, rs574367, rs10865548, rs597045, rs1956218, and rs66723169); and for two SNPs (rs34060476 and rs73073176), the relationship was unclear (20, 21). We then excluded eight SNPs due to potential risk factors at genome-wide significance ($p < 5 \times 10^{-8}$) (**Supplementary Tables 2, 3**).

1 http://www.phenoscanner.medschl.cam.ac.uk/

The rest of the four SNPs were employed as IVs for coffee consumption in the sensitivity analysis.

A total of two SNPs correlated with caffeine consumption ($p < 5 \times 10^{-8}$) were applied as IVs for caffeine from a meta-analysis of six GWAS (a total of 9,876 people of European descent) (21). A self-reported questionnaire was used to measure caffeine consumption from the consumption of coffee, tea, and cola (22). The summary-level data for SNPs related to caffeine intake (i.e., beta coefficient and standard error [SE]) were obtained. The effect size for the SNP-caffeine association was scaled to an 80-mg raise, which was equal to the caffeine dose of one cup of coffee (22). These two SNPs approximately explained a 1.31% variance for caffeine consumption (**Supplementary Table 5**).

Data source for renal cell carcinoma

We acquired summary-level data for genetic association with renal cell carcinoma from the FinnGen consortium and the International Agency for Research on Cancer (IARC) (**Supplementary Tables 4, 5**). The fifth release of the FinnGen consortium data included a total of 174,977 men and women of Finnish ancestry after the removal of individuals with excess heterozygosity (±4 SD), high genotype missingness (>5%), ambiguous gender, and non-Finnish ancestry (23). All genetic association effect sizes were computed by multivariable logistic regression and adjusted for age, sex, and genetic principal components. In IARC, the dataset of renal cell carcinoma was a gender-specific GWAS meta-analysis comprising two kidney cancer genome-wide scans for women (1,992 cases and 3,095 controls) and men (3,227 cases and 4,916 controls) of European descent (24). The quality control, imputation, and sex-specific association analysis protocols were described in one study (24).

Statistical analysis

We used the inverse-variance weighted (IVW) method with random effects to assess the relationships for genetically predicted coffee consumption and the IVW method with fixed effects (for analysis with less than three SNPs) to assess the relationships for genetically predicted caffeine consumption (25). Several other analyses were carried out, including the weighted median (WM) method (26), the MR-Egger regression method (27), and the MR robust adjusted profile score (MR-RAPS) method (28). The IVW method uses coefficients and standard errors uniting with risk factors and regresses the results of each genetic variation in turn (25). The WM combines data from multiple genetic instruments for the consistency analysis by computing a single weighted median estimator (26). The MR-Egger method allows each IV to exhibit pleiotropy effects and is consistent if the instrument strength is independent of these pleiotropic effects (27). The MR-RAPS is more robust to weak instrument bias (28). The MR pleiotropy residual sum and outlier (MR-PRESSO) method was applied to identify potential outlier SNPs (29). We used Cochran's *Q* statistic to test the heterogeneity of the IVW and MR-Egger methods for the causal estimates of individual SNPs. To examine the third MR assumption that the IVs do not influence the outcome *via* any variable other than the exposure, we calculated the intercept and 95% confidence interval (*CI*) of the MR-Egger regression line for testing horizontal pleiotropy (27). In the complementary analysis, we grouped the coffee-related SNPs according to their relationship with blood levels of caffeine metabolites (20, 21) and separately conducted MR analyses for each group of SNPs by the IVW method. We computed the F-statistic to evaluate the strength of the instruments (30). The statistical analysis was implemented using R (version 4.0.2), through MR-PRESSO and TwoSample MR packages. All statistical tests were two-sided, and the evidence of association was cutoff at a prespecified *p*-value below 0.05 in the final MR analyses.

Results

F-statistic and outlier detection

The F-statistic for coffee and caffeine consumption was 159 and 67, respectively, suggesting that the selected SNPs were adequate in strength as IVs for both coffee and caffeine consumption. In addition, no outlier SNPs were found in the MR-PRESSO in all primary and sensitivity analyses.

Causal relationships between coffee and caffeine consumption and renal cell carcinoma

The MR-Egger analysis identified no directional pleiotropy (all $p > 0.05$) (**Figure 2**). Additionally, we detected no heterogeneity by the IVW and MR-Egger regression. In the primary analysis, genetically predicted coffee consumption was not correlated with renal cell carcinoma in the FinnGen consortium study (*OR* = 0.623, 95% *CI*: 0.313–1.242) by the IVW method in the random-effects model (**Figure 2**). In addition, there was no effect of coffee on renal cell carcinoma in IARC consortium [men (*OR* = 0.768, 95% *CI*: 0.399–1.478, $p = 0.430$) and women (*OR* = 0.874, 95% *CI*: 0.454–1.683, $p = 0.688$)]. The combined *OR* of per genetically predicted 50% increase of coffee consumption was 0.752 (95% *CI*: 0.512–1.105, $p = 0.147$). Moreover, there was no obvious difference between the results of the sensitivity analysis and the primary analysis (**Figure 3**).

Complementary analyses that distinguished the genetically predicted coffee-related SNPs according to their correlation with

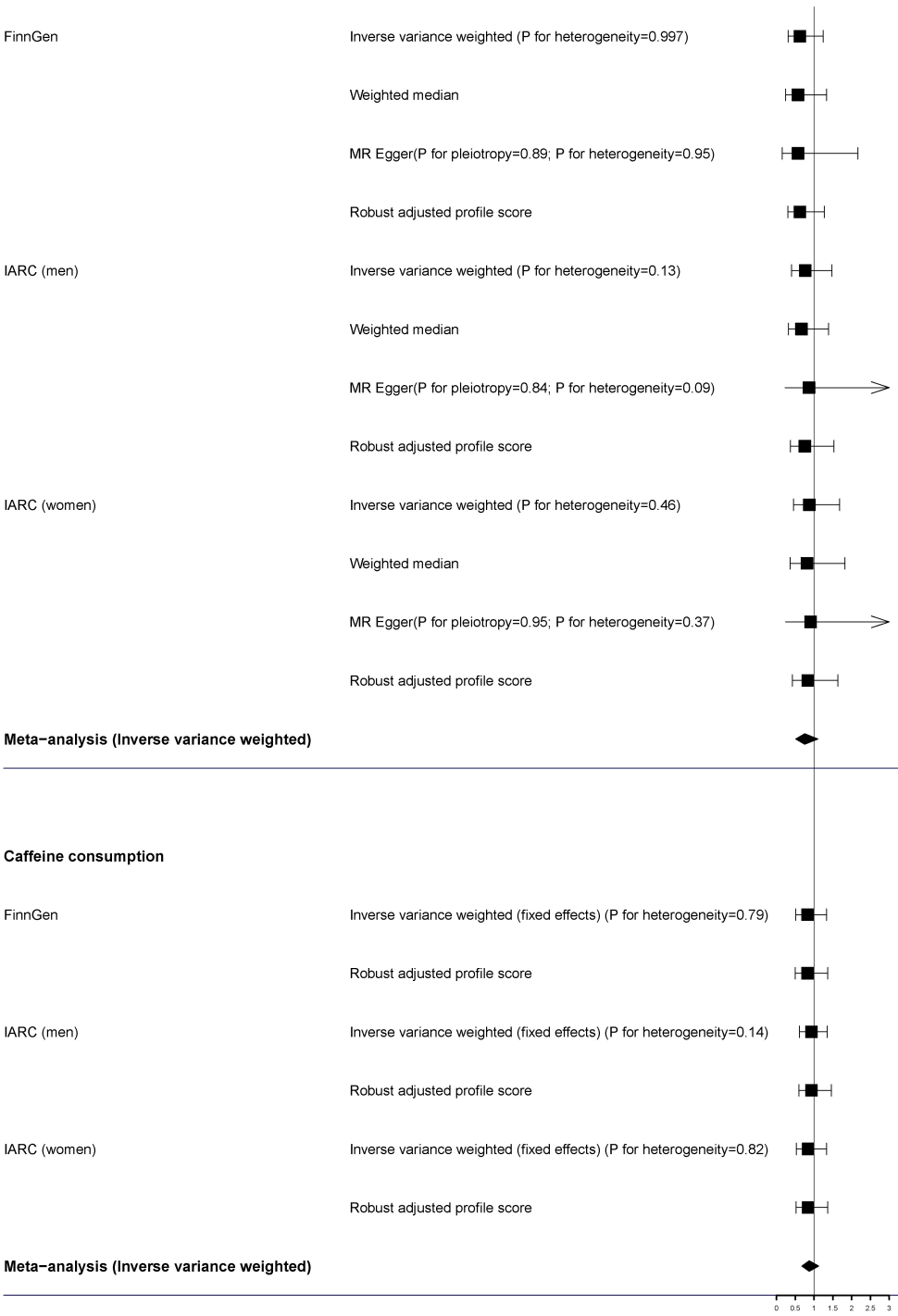

FIGURE 2
The association of genetically predicted coffee and caffeine consumption with renal cell carcinoma. Pooled estimates were combined using the fixed-effects meta-analysis methods. *ORs* for renal cell carcinoma were scaled to a genetically predicted 50% of increase in coffee consumption and an 80-mg increase in caffeine consumption. FinnGen, FinnGen Consortium; IACC, the International Agency for Research on Cancer; *OR*, odds ratio; CI, confidence interval.

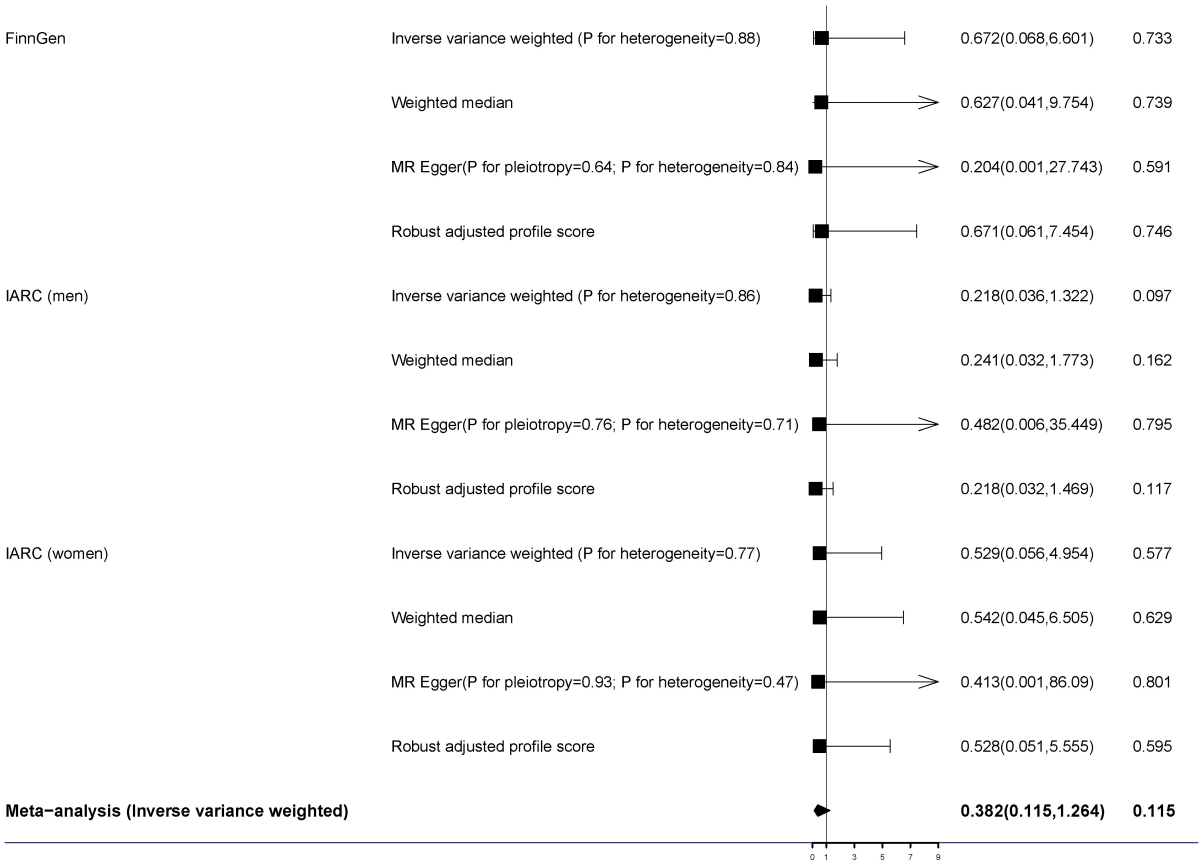

FIGURE 3
Results of sensitivity analyses association of genetically predicted coffee consumption with renal cell carcinoma. Estimates were obtained from the inverse-variance weighted methods and combined using the fixed-effects meta-analysis methods. *ORs* for renal cell carcinoma were scaled to a genetically predicted 50% of increase in coffee consumption. FinnGen, FinnGen Consortium; IACC, the International Agency for Research on Cancer; *OR*, odds ratio; CI, confidence interval.

caffeine metabolites blood levels (higher, lower, or unrelated) showed no significant difference (**Supplementary Table 6**).

The per 80-mg increase in caffeine consumption was also not associated with a risk of renal cell carcinoma (combined *OR* = 0.872, 95% *CI*: 0.676–1.125, $p = 0.292$) (**Figure 2**).

Discussion

Our study did not find a relationship between genetically predicted coffee and caffeine consumption and the risk of renal cell carcinoma. No outlier SNPs or pleiotropy were detected. Sensitivity analyses showed consistent results. In addition, coffee related genetically predicted SNPs that are correlated with higher or lower levels of caffeine metabolites in the blood, reflecting slower or faster caffeine metabolism, respectively, showed no significant difference.

Previous studies have reported inconsistent results on the association between coffee or caffeine consumption and the risk of renal cell carcinoma. A recent meta-analysis including 16 case-control and 6 cohort studies identified no significant relationship between coffee consumption and the risk of renal cell carcinoma in men and women, with a relative risk of 0.87 (95% *CI*: 0.72–1.04) and 1.15 (95% *CI*: 0.85–1.55), respectively (13). In addition, a meta-analysis including 13 cohorts found insignificant association for coffee consumption and kidney cancer in a dose-response analysis (14). However, a meta-analysis of 10 cohort studies detected a pooled relative risk of 0.85 (95% *CI*: 0.76–0.96) comparing the risk of renal cancer between the highest and the lowest category of coffee consumption, and this inverse relationship still remained among

studies adjusting for body mass index (BMI) and smoking (16). Meanwhile, a case-control study with 669 renal cell carcinoma cases and 1,001 matched controls found an inverse correlation between caffeinated coffee intake and renal cell carcinoma risk (*OR* = 0.74, 95% *CI*: 0.57–0.99) in comparison with no coffee consumption, whereas there was a trend toward increased risk of renal cell carcinoma for decaffeinated coffee consumption (*OR* = 1.47, 95% *CI*: 0.98–2.19) (15). Generally, the cancer-coffee relationship is strongly modified by smoking, and this phenomenon was observed not only for renal cell carcinoma only but generally for cancer (31). A recently published large prospective cohort study with a total of 420,118 participants found an inverse relationship between coffee consumption and renal cell carcinoma after adjustment for several risk factors, with a 20% reduction in the risk of developing renal cell carcinoma during a 16-year follow-up for those who drank $\geq$ 2 cups of coffee per day over 16 years of follow-up (10). The inverse relationship was detected among non-smokers but not ever-smokers.

Whereas, those observational studies might be limited by their research design and could not rule out residual confounding (such as healthy lifestyle factors and dietary components) and reverse causality that influenced the results. Besides, the measurement of long-term coffee and caffeine consumption in observational epidemiological studies may be inexact.

This is the first two-sample MR method to assess the potential relationship between coffee and caffeine consumption and the risk of renal cell carcinoma. The two-sample MR method is not subject to interferences by confounding factors, such as lifestyle and social environment and reverse causation by using IVs as a proxy for lifetime coffee and caffeine consumption. In this study, LD analysis was used to exclude three SNPs of coffee IV, and the MR-Egger intercept test identified that the selected 12 SNPs did not have pleiotropic properties, which also increased the credibility of our study. In our study, the IVs for coffee consumption may be related to other risk factors for renal cell carcinoma, and thus the relationship between the genetic variants and renal cell carcinoma may be susceptible to confounding by these factors. The four SNPs in the sensitivity analysis were not related to other traits, indicating that this association was not affected by confounding factors between coffee-related SNPs and renal cell carcinoma. Moreover, we assessed these relationships in two independent populations, and the consistent results ensured the stability of the research findings.

There were several limitations. One restriction is that the current MR analysis assumes a linear relationship between exposure and the outcome of interest. If there is a non-linear relationship or threshold effect between habitual coffee and caffeine consumption and renal cell carcinoma, we cannot detect this association. Therefore, our null detections may not rule out a possible beneficial effect of moderate but not massive coffee and caffeine consumption on renal cell carcinoma. In addition, in our complementary analyses, we might not have enough power to detect the weak relationships between various levels of caffeine metabolites and the risk of renal cell carcinoma. Therefore, further research on the causal relationship between caffeine metabolite exposure and the risk of renal cell carcinoma is needed to verify our findings. Beyond that, the GWAS for coffee consumption was based on regular and decaffeinated coffee consumption. However, we added the effects of caffeine consumption on the risk of renal cell carcinoma, and the results were consistent with coffee consumption. Moreover, it is worth mentioning that the population of our study is limited to European individuals; this conclusion may not be directly applicable to other populations. Furthermore, there is an urgent need for more studies with larger sample sizes to explore the relationship among different coffee bean types, roasting procedures, brewing methods, and renal cell carcinoma.

Conclusion

Our MR study provided no convincing evidence for a causal effect between coffee and caffeine consumption and the risk of renal cell carcinoma. Further longitudinal and experimental studies are still demanded to authenticate our results.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Author contributions

Y-BW, Y-HJ, and B-HL contributed to the protocol development, data collection, and statistical analysis. B-HL, S-YY, X-HL, and QH drafted the manuscript. L-SL, Y-YW, and JH supervised the method and visualized the results. All authors contributed to manuscript revision, read, and approved the submitted version.

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Genetic instruments for coffee and caffeine consumption were obtained from two published meta-analyses of genome-wide association studies. Genetic association estimates for renal cell carcinoma were obtained from the FinnGen consortium and the International Agency for Research study. The authors acknowledge the participants and investigators of the FinnGen and International Agency for Research on Cancer studies.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.898279/full#supplementary-material

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Beef intake and risk of rheumatoid arthritis: Insights from a cross-sectional study and two-sample Mendelian randomization

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Background: Beef is common in daily diet, but its association with the risk of rheumatoid arthritis (RA) remains uncertain. The objective of this study is to explore the relationship between beef intake and the risk of RA.

Materials and methods: We investigated the association between beef intake and risk of RA by multivariate logistic regression, based on the National Health and Nutrition Examination Survey (NHANES) 1999–2016 involving 9,618 participants. The dose–response relationship between beef intake and RA was explored as well. Furthermore, we performed Mendelian randomization (MR) analysis to examine the causal effect of beef intake on RA. Genetic instruments for beef intake were selected from a genome-wide association study (GWAS) including 335,576 individuals from the UK Biobank study, and summary statistics relating to RA were obtained from a GWAS meta-analysis of 14,361 RA patients and 43,923 controls. The inverse-variance weighted (IVW) approach was used to estimate the causal association, and MR-Egger regression and Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test were applied to evaluate the pleiotropy and outliers.

Results: Compared with the lowest quintile (0 to ≤33.50 g/d), beef intake was found to be significantly associated with the risk of RA [odds ratio (OR): 1.94; 95% confidence interval (CI): 1.20–3.12] in the third quintile (50.26 to ≤76.50 g/d). Moreover, a reversed "U" dose–response relationship between beef and RA ($P_{non-linearity}$ = 0.023) was found. In the MR analysis, beef intake was associated with an increased risk of RA (OR: 3.05; 95% CI: 1.11–8.35; P = 0.030) by the IVW method. The results from MR-Egger regression and MR-PRESSO test showed that there were no pleiotropic variations and outliers.

Conclusion: This study indicated that there is suggestive evidence to support the causal effect of beef intake on the risk of RA, while further studies are warranted to elucidate the exact association.

KEYWORDS

beef, rheumatoid arthritis, NHANES, cross-sectional, Mendelian randomization

Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease that corrodes arthrosis and causes progressive articular damage (1). The annual incidence rate of RA was 14.9% in 2017, which had increased by 8.2% compared with that in 1990 around the world (2). It was estimated that 20–30% of RA patients would be invalidity for work permanently without any medical treatment within 2–3 years of diagnosis (3).

Accumulating risk factors have been found to play an important role in RA, such as smoking, breastfeeding, silica exposure, and educational level (4–7). Recently, it has received growing attention that dietary patterns and nutrients are potentially modifiable factors affecting the occurrence and development of RA (8–10). A Mediterranean diet is recommended to prevent the occurrence and complications of RA, due to the abundance of antioxidants and anti-inflammatory foods (11). In contrast, a western diet that contains high consumption of red meat and saturated fat may be associated with a high risk of RA by directly causing inflammation or indirectly raising insulin resistance and body mass index (BMI) (12–14). However, the role of red meat in the risk of RA remains controversy. For example, a large cohort study consisting of 80,551 post-menopausal women in the United States suggested that high red meat intake was associated with an elevated risk of RA (15). However, a 22-year Nurses' Health Study (NHS) cohort study (16), which collected diet information from 82,063 participants by semi-quantitative food frequency questionnaires (FFQ), showed that there was no significant association between red meat intake and RA risk. One reason that could explain the discrepancy in findings may be due to the differences in the composition of red meat in previous studies, since different types of red meat have different nutritional contents, which may lead to different health outcomes and risk of diseases (17, 18). Beef, is a major source of red meat, although it provides various nutrients that are essential to humans (19, 20), its high protein and fat content hold the potential to increase the risk of RA (21). Nevertheless, limited epidemiological studies have explored the association between beef consumption and the risk of RA.

To be noted, diets are associated with a variety of clinical and social factors, and it is difficult to assess the causal effects of diets on multiple outcomes. Mendelian randomization (MR) is an analytical method that assesses the causal association between exposure and outcome by introducing genetic instrumental variables (IVs), such as single nucleotide polymorphisms (SNPs) (22). Since IVs are independent of other traits and are inherited randomly, MR analysis can largely reduce the interference of confounding factors and reduce the possibility of reverse causality (23, 24). This approach is increasingly applied in assessing and screening potentially causal associations (25–27), which would be useful to detect the causal effect of beef intake on the risk of RA.

In this study, we first conducted a cross-sectional study based on the National Health and Nutrition Examination Survey (NHANES) to determine the observational association between beef intake and the risk of RA. Then, we further implemented MR analysis to assess the causal relationship between beef intake and the risk of RA.

Abbreviations: CI, confidence interval; FFQ, food frequency questionnaires; FNDDS, Food and Nutrient Database for Dietary Studies; GWAS, genome-wide association study; IVs, instrumental variables; IVW, inverse-variance weighted; MAF, minor allele frequency; MR, Mendelian randomization; MR-PRESSO, Mendelian randomization pleiotropy residual sum and outlier; NHANES, National Health and Nutrition Examination Survey; NHS, Nurses' Health Study; OR, odds ratio; RA, rheumatoid arthritis; SNPs, single nucleotide polymorphisms.

Materials and methods

Cross-sectional study

Study population in National Health and Nutrition Examination Survey

The NHANES is a cross-sectional survey designed to assess the health and nutritional status of Americans, and it has been a continuous program since 1990 and is updated every 2 years (28). In this current study, we combined data from 1999 to 2016 to increase the sample size. We included non-Hispanic whites aged more than 20 years and excluded the participants with missing information of covariates. All study participants supplied the written informed consent and the study was approved by National Center for Health Statistics Research Ethics Review Board.

Beef consumption and rheumatoid arthritis assessment

Participants were asked to complete two 24-h dietary recalls for each cycle except only once in the 1999–2000 wave. Each food consumption was assigned an 8-digit Food and Nutrient Database for Dietary Studies (FNDDS) code and the code for beef products was 21000000–21800000 (29). We assessed the beef intake by calculating the sum of the weight of all beef products consumed by participants over a 24-h dietary recall. For RA assessment, the participants were asked two questions about RA: (1) Have doctors ever said they had arthritis? (2) Which type of arthritis? If participants answered yes to the first question and answered "rheumatoid arthritis" to the second question, then he/she would be considered RA patients. Otherwise, he/she was considered a non-RA individual.

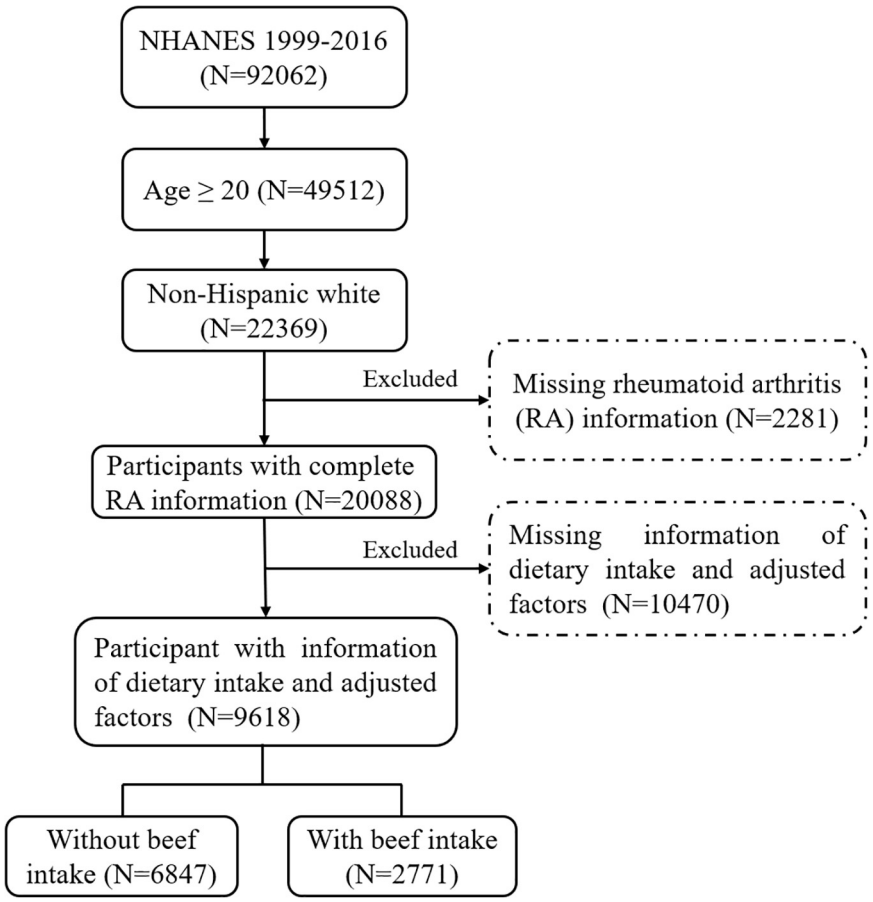

FIGURE 1
The flow diagram of participant selection.

Statistical analysis

Weighted analysis was conducted using the sample weights, stratification, and clustering variables to account for the complex sampling design in NHANES. In this study, we rebuilt a new 18-year dietary weight because of combining nine 2-year survey cycles of NHANES.[1]

The multivariate logistic regression was applied to estimate the odds ratio (OR) and 95% confidence interval (CI) for the association between beef intake and risk of RA. First, we explored the effect of beef intake (none and yes) on RA independently. Second, we investigated the association of beef consumption with RA by categorizing beef consumption into quintiles (Q1, 0 to ≤33.50 g/d; Q2, 33.50 to ≤50.26 g/d; Q3, 50.26 to ≤76.50 g/d; Q4, 76.50 to ≤118.00 g/d; Q5, >118.00 g/d). Two sets of adjusting covariates were constructed in the logistic regression model. Model 1 was assembled by adjusting for age, sex, education, poverty-income rate, and marriage. In addition to the factors adjusted in model 1, smoking, alcohol drinking, history of diabetes, and BMI (kg/m^2) were considered in model 2. Furthermore, the method described by Greenland and Longnecker (30) was used to estimate the dose–response relationship. For the highest dose group, the lower limit plus the width of the previous group was supposed as the corresponding beef consumption. The other dose groups were assigned the midpoint of the lower and upper bound.

Statistical analyses were performed by SAS version 9.4 and $P < 0.05$ was regarded as statistically significant.

Mendelian randomization study

Summary dataset of rheumatoid arthritis

The genetic association data of RA was obtained from a genome-wide association study (GWAS) meta-analysis of 14,361 RA cases and 43,923 controls of European ancestry (31). A total of 42 loci were identified to be significantly associated with RA at the genomic level ($P < 5 \times 10^{-8}$). More information and details about this study have been reported in the previous article (31). The written informed consent was provided by

1 https://wwwn.cdc.gov/nchs/nhanes/tutorials

TABLE 1 Baseline characteristics of selected participants from National Health and Nutrition Examination Survey (NHANES) 1999–2016.

Characteristics	RA ($N = 906$)	Non-RA ($N = 8712$)
Age		
20∼65 years	430 (47.46%)	6510 (74.72%)
≥65 years	476 (52.54%)	2202 (25.28%)
Sex		
Female	480 (52.98%)	4027 (46.22%)
Male	426 (47.02%)	4685 (53.78%)
BMI (kg/m^2)	29.73 ± 7.31	27.84 ± 6.21
Poverty-income ratio	2.75 ± 1.66	3.09 ± 1.65
Education		
Less than high school	243 (26.82%)	1717 (19.71%)
High school graduate	348 (38.41%)	3122 (35.84%)
More than high school	315 (34.77%)	3873 (44.46%)
Married		
Yes	527 (58.17%)	5189 (59.56%)
No	379 (41.83%)	3523 (40.44%)
Diabetes		
Yes	154 (17.00%)	636 (7.30%)
No	752 (83.00%)	8076 (92.70%)
Smoked at least 100 cigarettes in life		
Yes	585 (64.57%)	4816 (55.28%)
No	321 (35.43%)	3896 (44.72%)
Frequency of alcohol drinks in the past 12 months, median (IQR)	1 (0, 3)	2 (1, 3)

IQR, interquartile range; RA, rheumatoid arthritis.

all study participants and the study was allowed by each local agency review board.

Selection of beef intake associated single nucleotide polymorphisms

Beef intake-associated SNPs were selected from a large-scale GWAS based on 335,576 individuals of white European descent from the UK Biobank study (32). Beef consumption was assessed according to a diet questionnaire by asking "How often do you eat beef?" and a competitive analysis was used to test the association between genotype and phenotype (32). **Supplementary Table 1** lists the details of the GWAS studies and datasets used in the MR study. A total of seven loci were associated with beef intake at the genome-wide significant threshold ($P < 5 \times 10^{-8}$). All of them were not in linkage disequilibrium ($r^2 < 0.1$) and not overlapped with the known risk of RA (33). However, one SNP (rs66495454) was eliminated because it was not found in the outcome GWAS, thus six SNPs were used as IVs. The details of instrumental SNPs in this study are shown in **Supplementary Table 2**.

Furthermore, to assess the strength of the IVs, the F-statistics were calculated by the formula of $F = R^2 \times (N-k-1)/k \times (1-R^2)$ (34), where R^2 is the total variance explained by the IVs, N represents the sample size and k indicates the number of included IVs. The variance of each IV was computed by minor allele frequency (MAF) and β value (35). In addition, the statistical power of MR analysis to detect causal association was calculated (36).

Statistical analysis

The inverse-variance weighted (IVW) method was used to evaluate the causal association between beef intake and the risk of RA. The IVW method performs a meta-analysis of Wald values (i.e., the beta coefficient of the SNP for outcome divided by the beta coefficient of the SNP for exposure) to estimate the overall causal association between exposure and outcome (37). In addition, the maximum-likelihood method was used to validate the result from the IVW method, which is assessed by assuming that there was a linear relationship between beef intake and risk of RA (38).

Then we used MR-Egger regression to assess potential directional pleiotropy by checking the intercept term. It indicates that directional pleiotropy may not exist when the intercept term was close to zero (39). Moreover, to evaluate the horizontal pleiotropy level of the IVs, Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) were employed, which is comprised of three parts [(a) detection of horizontal pleiotropy, (b) correction by removal of offending IVs, and (c) test of significant differences in the causal estimates before and after removal of outlier] (40). Furthermore, we used Cochran's Q test to estimate the consistency of the association between beef intake and the risk of RA across each IV.

Furthermore, the GWAS Catalog[2] was searched to find whether the instrumental SNPs were related to other traits. We also conducted sensitivity analysis by the "leave-one-out" method to assess the reliability of causality. We eliminated each SNP one by one and combined the effect value of the remaining. The fluctuation of the results before and after removing the SNP reflects the stability of the association.

Statistical analyses were performed by using R version 4.0.5 and $P < 0.05$ was regarded as statistically significant.

Results

Cross-sectional study

The details of the inclusion and exclusion criteria of subjects are shown in **Figure 1**. Consequently, a total of 9,618 participants were eventually included in this cross-sectional

2 https://www.ebi.ac.uk/gwas/

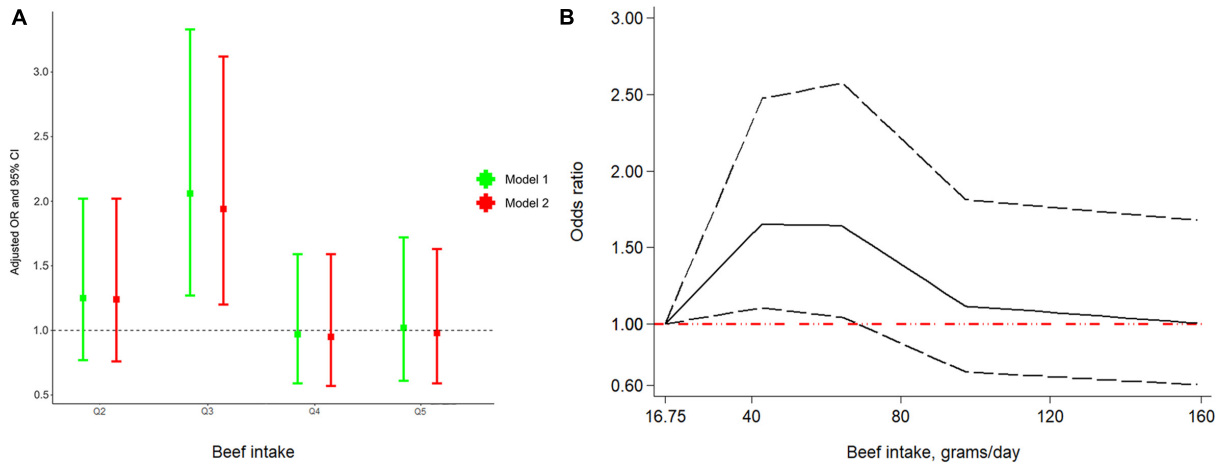

FIGURE 2
Odds ratio (OR) between quintiles of beef intake and RA **(A)** and dose-response relationship between beef intake per day and RA **(B)**. Abbreviation: RA, rheumatoid arthritis.

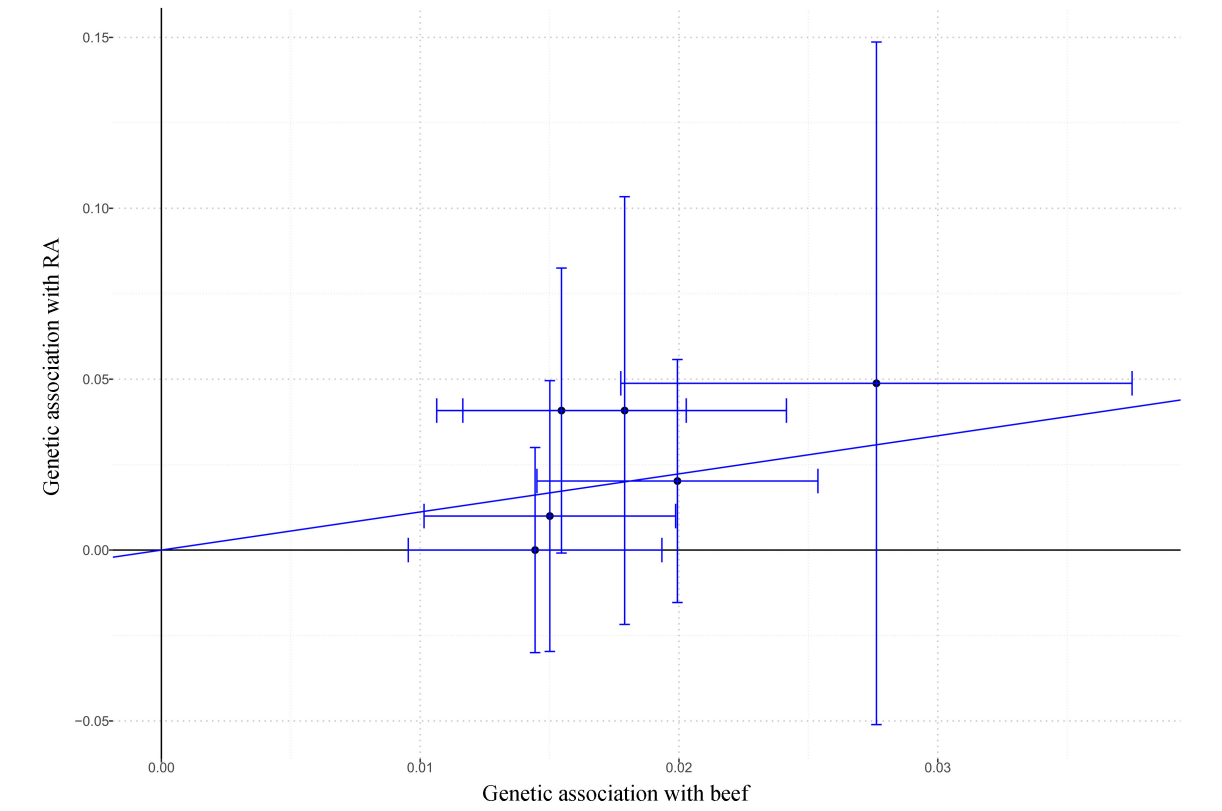

FIGURE 3
The effect size and 95% CI of each SNP on the association between beef intake and RA risk by IVW. Abbreviations: CI, confidence interval; IVW, inverse-variance weighted; RA, rheumatoid arthritis; SNP, single nucleotide polymorphism.

study. Compared with non-RA individuals, patients with RA seemed to have higher BMI and lower poverty-income ratio. The detailed characteristics of the included participants are presented in Table 1.

Even though ever beef intake was not significantly associated with the risk of RA (Supplementary Table 3), we found that the risk of RA in the third quintile was 2.06 times than in the first quintile (OR: 2.06; 95% CI 1.27–3.33) in

TABLE 2 A causal association between intake of beef and risk of rheumatoid arthritis (RA).

Outcomes and methods	Number of SNPs	OR	95% CI	*P* for association	*P* for Cochran's *Q* test	*P* for MR-PRESSO global test
IVW (fixed)	6	3.05	1.11–8.35	0.030	0.698	
MR-Egger	6	/	/	0.547[a]		
Maximum-likelihood	6	3.12	1.10–8.79	0.032		
MR-PRESSO (0 outliers)	6	3.05	1.40–6.66	0.038		0.708

[a] *P*-value for the intercept of MR-Egger regression analysis.
CI, confidence interval; IVW, inverse-variance weighted; MR-PRESSO, Mendelian randomization pleiotropy residual sum and outlier; OR, odds ratio; SNP, single nucleotide polymorphism.

model 1 (Figure 2A). Similarly, in model 2, the association between beef and RA remained robust (OR: 1.94; 95% CI: 1.20–3.12). Additionally, as depicted in Figure 2B, there was an interesting non-linear relationship between RA and beef ($P_{non-linearity}$ = 0.023). In particular, an increased risk of RA was observed when beef intake ranged from 16.75 to 68.67 g/day.

Mendelian randomization study

As shown in Figure 3, beef intake was associated with an increased risk of RA (OR: 3.05; 95% CI: 1.11–8.35; P = 0.030) by the IVW method. Similarly, genetically predicted beef intake was positively associated with the risk of RA by the maximum-likelihood method (OR: 3.12; 95% CI: 1.10–8.79; P = 0.032). There was no indication for directional pleiotropy effects (P = 0.547) as assessed by the MR-Egger intercept (Table 2). Also, there was no evidence for heterogeneity (P = 0.698) in the association of any IV with the risk of RA as measured by the Cochran's Q test, and no outlier SNPs (P = 0.708) were detected with the MR-PRESSO test (Table 2). The F-statistics ranged from 30.05 to 51.82, suggesting the IVs were unlikely to be affected by weak instruments (Supplementary Table 2). Statistical power was calculated to be 89.77% to detect an effect size of 3.05 at a significance level of 0.05.

Of the six IVs used in MR analysis, there were four SNPs statistically correlated with different traits, such as rs4676964 was associated with biological sex ($P = 7 \times 10^{-14}$), smoking status measurement ($P = 1 \times 10^{-9}$), and risk-taking tendency ($P = 8 \times 10^{-18}$) (Supplementary Table 4). In the "leave-one-out" method, we found that the causal association between beef intake and RA fluctuated slightly after removing three SNPs (rs9379833, rs61853274, and rs7873152) stepwise (Supplementary Figure 1).

Discussion

In this current study, the stepwise analysis of a cross-sectional study from NHANES 1999–2016 and a two-sample MR study were combined to explore the association between beef intake and the risk of RA. We found a reversed "U" relevance between beef consumption and RA based on NHANES, and a positive association between beef intake and risk of RA by MR. Therefore, the findings indicated that beef intake is suggestively associated with an increased risk of RA.

Previously, a large number of investigators have explored the relationship between different types of meat and the risk of RA. For example, Nguyen et al. conducted a large-scale cohort study including 62,639 participants and suggested that moderate fish consumption was negatively associated with RA risk (41). In addition, a cohort study performed by Sundström et al. in Sweden showed that there was no statistically significant association between poultry intake and risk of RA (42). However, beef is a staple of the American diet, but there was no specific observational study to explore the association between beef intake and RA to date. In our cross-sectional study based on a serial NHANES survey (1999–2016), we found individuals who consumed 50.26–76.50 g of beef per day had a higher risk of RA than those who consumed less than 33.50 g of beef per day. However, except for the third quintile, the risk of RA kept uncertain in other quintiles due to the poor statistical power. Moreover, a reversed "U" relevance between beef consumption and RA was found in dose–response relationship analysis. The non-significant increased risk of more beef consumption might derive from a relatively small sample size but not real effect. Large-sample and well-designed studies should be developed in the future to demonstrate this turning point. Furthermore, the observational studies are easily biased by potential confounding factors and reverse causation (43–45), though we have adjusted age, sex, education level, diabetes, etc. in our analysis. Hence, to further determine the causal association between beef intake and the risk of RA, we conducted a two-sample MR study.

In the MR analysis, we interestingly found that beef consumption is positively associated with RA risk. For MR analysis, it should satisfy three assumptions, which are the premises of causal inference (46). First, there is a robust and strong correlation between IVs and exposure. To ensure this, the loci strongly

associated with beef intake reaching the genome-wide significant threshold ($P = 5 \times 10^{-8}$) were selected as IVs from a genome-wide association study of 335,576 participants. Second, the IVs must be independent of confounding factors affecting the exposure-outcome relationship. Because genetic alleles are randomly assigned at conception, they could rule out the possibility of the association with confounding factors such as socioeconomic and behavioral factors (47). Third, IVs do not influence the outcome through pathways other than exposure. In the MR-Egger and MR-PRESSO analysis, we found no evidence of directional pleiotropy. For "leave-one-out" analysis, we found the association between beef intake and RA risk was enhanced after excluding rs9972653 and rs4676964, which have the most potential pleiotropic effects. However, the results fluctuated after the exclusion of rs7873152 (P = 0.117), rs61853274 (P = 0.060), or rs9379833 (P = 0.060). These three SNPs were not associated with other traits except beef intake among the European population by searching GWAS Catalog.

A potentially positive association between beef intake and the risk of RA is biologically plausible (14, 48). One explanation is that beef is rich in iron (49), which has been found to be abundant in the rheumatoid synovium mainly in the form of ferritin, contributing to the inflammatory reaction damage (50, 51), such as the promotion of inflammatory mediators including IL-6, IL-8, and IL-1β (52). Another possible explanation is that high collagen in beef increased collagen sensitivity and produced anti-collagen antibodies (21). Besides, the saturated fatty in beef could translocate endotoxin such as lipopolysaccharide toxins and release them into the bloodstream, thus stimulating the immune system and enhancing inflammation (53). High ingestion of fat also promotes the production of endogenous antioxidants, uric acid, and mercaptan, which obviously affects dietary-induced inflammation (54).

There were some limitations that should be noted. First, the imprecise measurement of beef intake along with recall bias and the retrospective diagnosis of RA based on questionnaires might affect the estimation of the association between beef intake and risk of RA in the cross-sectional study. Thus, we conducted an MR study to further clarify the causal relationship. Second, the poor power limited the exploration of a possible non-linear relationships between beef and RA. Third, in view of the data from NHANES and two-sample MR analysis that came from the participants of non-Hispanic white and European descent, it is unknown whether the same results can be applied to other ethnic groups. Forth, the "leave-one-out method" of MR analysis showed an unstable association between beef and RA, which needs to be careful to interpreted this connection. In addition, the limitation of public summary data of other subtypes of red meat, prevented multivariate MR analysis to assess the independent influence of beef intake on RA. Therefore, the role of beef in the development of RA needs further prospective and mechanistic studies to verify.

Conclusion

Our study suggested a possible causal association between beef intake and risk of RA, while further epidemiologic studies are needed to clarify this suggestive association and the possible dose-response relationship.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZH and DY conceived and designed the study. WC and KL conducted data analysis and interpreted the results. WC drafted the manuscript. LH, YM, and CW revised the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.923472/full#supplementary-material

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Prevalence of vegan/vegetarian diet and eating behavior among Saudi adults and its correlation with body mass index: A cross-sectional study

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Background: Globalization has steered the spread of vegetarianism around the world. Vegetarianism has achieved increased acceptance by different populations.

Objective: The present study aims to assess vegetarian diet, and eating behavior prevalence among Saudi adults and their association with demographics and body mass index.

Method: A cross-sectional study conducted on 1,143 Saudi adults [418 (36.6%) males and 725 (63.4%) females]. An online survey questionnaire containing questions on demographics, type of diet, eating behavior and physical activity was provided to participants for self-administration. Statistical analysis was performed to associate demographic and eating behavior variables with the type of diet using Pearson's Chi-square test and Spearman's partial correlation test was used to correlate BMI and eating behavior.

Results: Prevalence of veganism was 4.7% ($n = 54/1,143$) and vegetarianism was 7.8% (89/1,143). A significantly higher prevalence of vegan diet was observed in females than males (79.6% vs. 20.4%, $p < 0.0001$). A significantly higher proportion of participants on vegetarian diet selected "Always" as response for eating breakfast, vegetables and fruits as well as for eating or drinking dairy foods, and for eating canned food than participants on non-vegetarian diet ($p < 0.0001$). A significantly higher proportion of participants on vegan diet selected 'Never' for eating fast-food and fried food as well as for drinking fizzy or soft drinks ($p < 0.0001$). A positive moderate correlation was found between BMI and eating fast-food and fried food [$r_{(1,140)} = 0.529$, $p < 0.0001$], drinking fizzy or soft drinks with meals [$r_{(1,140)} = 0.495$, $p = 0.001$], and eating canned food [$r_{(1,140)} = 0.510$, $p < 0.0001$].

Conclusion: Our study shows that vegan and vegetarian diet have gained access into the lifestyle of Saudi adults with a prevalence of 4.7 and 7.8%, respectively. Participants on vegetarian diet showed better lifestyle like higher physical activity level, higher consumption of fruits, vegetables, dairy products and low intake of fast-foods and fizzy beverages.

KEYWORDS

BMI, demographics, physical activity, questionnaire, survey

Introduction

The Global Burden of Diseases report (2015) highlighted an increased prevalence of obesity in Eastern Mediterranean Region (EMR) 21%, much higher than the global obesity average 12% (1). A number of studies were conducted in EMR during 2016-2017 and reported lowest obesity rates 17.0% in Yemen and highest 32.3% in United Arab Emirates (2, 3). A nation-wide survey 2020 from Saudi Arabia reported the weighted prevalence of obesity as 24.7% and unweighted prevalence 21.7% (4). The main reason of high obesity rates in EMR includes nutrition transition, inactivity, and urbanization (5).

Vegetarian diet has gained popularity in western countries and had shown variation in the prevalence rate from 4.3 to 9% (6–9) among the general population, with the highest prevalence from India (30%) (10). A recent nutrition report from Germany stated that the number of vegetarians and vegans have doubled from pre-COVID (5%) to post-COVID (10%) (11). Vegetarians refer to those who do not eat any meat, poultry or fish and may or may not consume egg or dairy products (12), while vegan refers to those who refrain from eating any animal product including dairy, eggs and other animal-derived food (12).

Scientific research findings reported vegan diet is associated with various health benefits (13–15). Studies conducted in western countries have consistently shown that vegetarian diet is associated with low BMI and lower blood pressure (16–18). Further, a vegetarian diet has presented positive effect on preventing and treating non-communicable diseases (17, 18). Despite the health benefits of vegetarian diet, adopting a vegan/vegetarian diet may lead to micro-nutrient deficiencies and can result in negative consequences like impaired cognition, muscular pain, neural tube defect, reduced physical performance and endurance (19, 20). However studies have reported a well-planned vegan and vegetarian diet is capable to fulfill nutrients needs of an individual and may be beneficial to their health (14, 21).

The major transitional changes in lifestyle usually occur in youths, as parental supervision is reduced and new friend relationships are established. The feeling of independence leads to the establishment of personal principles and choices, including diet and eating habits. The change in principles (ethical, environmental, and psychological) is reflected by the adoption of certain dietary patterns (22).

As the overweight and obesity prevalence are high in Saudi Arabia, vegan and vegetarian diet can help to mitigate the problem, information regarding prevalence of vegetarianism among adult Saudi nationals is crucial. Therefore, this study aims to assess vegetarian diet, and eating behavior prevalence among Saudi adults and their association/correlation with demographics and body mass index.

Materials and methods

Study design

The present cross-sectional study was conducted in February-March, 2022 after receiving approval from the Research Ethics Committee, Saudi Electronic University, Saudi Arabia. The study was carried out among Saudi adults from 4 cities of Saudi Arabia (Riyadh, Dammam, Jeddah and Abha). Malls were selected as the place to recruit the participants as it is one of the common visiting places of Saudi nationals during the weekends. Randomly 2–4 malls were selected from each city to visit on weekends (Fridays and Saturdays) between 1 pm to midnight.

Participants

A total of 1,282 Saudi nationals in the age group 18–45 years, who agreed and gave their informed consent to participate in study, were recruited. All the eligible participants height and weight was measured using a portable stadiometer weighing scale machine (Model HW-700z, LEKA) which was capable of measuring height range (60–200 cm with 0.5 cm minimum division) and weight range (8–200 kg with 0.1 kg minimum division). After the measurements the participants completed the online survey questionnaire. Among 1,282 participants, 139 (10.8%) participants reported to be ignorant about the vegetarian diet, so were excluded. After data cleaning a total 1,143 participants' complete and correct data was available for the analysis.

Measure

BMI was calculated using World Health Organization (WHO) standard formula (23). Participants were categorized as "Under-weight" for BMI <18.5 kg/m^2, "Normal weight" for 18.5–24.9 kg/m^2, "Over-weight" for BMI 25–29.9 kg/m^2, and "Obese" for BMI 30 kg/m^2 and above according the WHO classification (23).

Questionnaire

A closed-ended multiple-choice questionnaire was used to assess the diet type, eating behavior and physical activity level of the participants. The questionnaire had 3 sections; the first section included demographic questions (age, sex, education level, BMI). The second section had questions on the awareness of a vegetarian diet, types of vegetarian diet and additional questions for the vegetarian participants included reason for adapting vegan/vegetarian diet, duration of practicing

the diet and favorite substitute for meat. The participants were classified as vegan, vegetarian, and non-vegetarian. Vegan refers to those who refrain from eating any animal product including dairy, eggs and other animal-derived food; vegetarians refers to those who do not eat any meat, poultry or fish; while non-vegetarians refers to those who consume meat, poultry, fish and their products (12). The third section had questions related to the eating behavior of the participants (like eating breakfast daily, having three meals a day, fruit & vegetable and dairy product intake, fast-food consumption, sugar-sweetened beverages consumption). The eating behavior questions are presented in a 5-Likert scale with a score "0" for "Never", "1" for "Rarely", "2" for "Sometimes", "3" for "Often" and "4" for "Always". The physical activity level of participants was assessed by using International Physical Activity Questionnaire-SF questions (24, 25). The criteria used to classify the physical activity level are shown in Table 1.

Ethical statement

The study was approved by the Saudi Electronic University Research Ethics Committee (SEUREC-−22,008, 13th February 2022).

Statistical Analysis

IBM SPSS Version 24 (IBM Corp, Armonk, NY, United States) was used for the statistical analysis. The demographic characteristics of the participants and their responses to the type of diet, physical activity and eating behavior are reported as numbers and percentages. The comparison of the demographic characteristics, and eating behavior were performed between the types of diet using Pearson's chi-square test. Spearman's partial correlation analysis was used to correlate BMI with eating behavior by controlling for physical activity. A p-value of < 0.05 was considered significant.

Results

A total of 1,143 Saudi adults (35.5% males and 64.5% females) were included in the study for statistical analysis. The socio-demographic characteristic of the participants is presented in Table 2. Among the study participants 64.5% ($n = 737$) participants were in the 18–30 age group, and 83.2% ($n = 951$) participants were graduates. The participants recruited from Riyadh were 45.5% ($n = 520$) followed by Dammam 32.5% ($n = 371$). The BMI (mean ± SD) of the participants was 25.5 ± 5.3 kg/m^2. The classification of BMI into categories revealed that 41.0% ($n = 469$) participants had normal weight, while prevalence of under-weight, over-weight and obesity was 8.8, 31.2, and 19.0%, respectively. The high level of physical activity was reported by 41.3% ($n = 472$) participants. The eating behavior reported by the participants is shown in Figure 1. "Always" was selected as response by 38.2% ($n = 437$) participants for eating breakfast daily, and by 22.2% ($n = 254$) participants for eating three meals a day, while <10% participants selected "always" as a response for eating fast food and fried foods, drinking fizzy or soft drinks with meals as well as eating canned food.

The prevalence of vegan and vegetarian diet was 4.7% ($n = 54/1,143$) and 7.8% (89/1,143), respectively. The socio-demographic characteristic of the participants based on type of diet is shown in Table 2. A significantly higher prevalence of vegan diet was observed in age group 18–30 years and among females compared to their counter parts ($p < 0.05$). A significant difference was observed for BMI between vegan, vegetarians, and non-vegetarian participants [$X^2_{(6)} = 77.56$, $p < 0.0001$], the prevalence of underweight was higher in vegan (25.9%) followed by vegetarian participants (16.1%) than non-vegetarians participants (7.7%) and inversely, obesity prevalence was higher among non-vegetarian participants (21.0%) than vegetarian participants (7.9%). Comparison of physical activity with type of diet showed a significant difference between vegan, vegetarian, and non-vegetarian participants [$X^2_{(6)} = 123.46$, $p < 0.0001$] (Table 2). The distribution of eating behavior reported by the participants based on the type of diet is shown in Figure 2. A significantly higher proportion of participants on vegetarian diet selected "Always" as response for eating breakfast daily [$X^2_{(8)} = 17.24$, $p = 0.028$], eating vegetables and fruits [$X^2_{(8)} = 132.28$, $p < 0.0001$] as well as for eating or drinking dairy foods [$X^2_{(8)} = 524.10$, $p < 0.0001$], for eating three meals daily [$X^2_{(8)} = 72.07$, $p < 0.0001$], for eating snacks between meals [$X^2_{(8)} = 114.85$, $p < 0.0001$] and for eating canned food [$X^2_{(8)} = 171.85$, $p < 0.0001$] than participants on non-vegetarian diet ($p < 0.0001$), while significantly higher proportion of participants on vegan diet selected "never" as a response for eating fast food and fried foods [$X^2_{(8)} = 247.11$, $p < 0.0001$], and drinking fizzy or soft drinks with meals [$X^2_{(8)} = 73.97$, $p < 0.0001$] (Figure 2).

The participants who selected vegan and vegetarian diet were provided with additional questions regarding reasons for adapting vegetarian diet, duration on vegetarian diet and favorite substitute for meat (Table 3). Among the 143 participants who reported to be on vegetarian diet, 37.8% ($n = 54$) were on vegan diet, 25.2% ($n = 36$) were on semi-vegetarian diet and 19.6% ($n = 28$) were on lacto-ova diet. The primary reason for adapting vegan diet was animal ethics (37.0%, $n = 20$), while vegetarian diet adapting mainly for better health/nutrition 59.5% ($n = 33$). 72.9% ($n = 41$) participants had adapted vegan diet within a year of the survey, while 14.6%

TABLE 1 Criteria used to classify physical activity level.

	Low intensity (Walking)	Moderate intensity (Cycling, swimming)	Vigorous intensity (Gardening, climbing stairs)
"High" activity Graded "3"	Every day (>60 min)	At least 5 days (>60 min)	At least 3 days (>60 min)
	7 or more days of any combination of walking, moderate intensity or vigorous intensity activities.		
"Moderate" activity Graded '2'	Every day (>30 min)	5 or more days (>30 min)	3 or more days (>30 min)
	5 or more days of any combination of walking, moderate intensity or vigorous intensity activities.		
"Low" activity Grade "1"	3–5 days	3–5 days	1–3 days
	< 5 days of any combination of walking, moderate intensity or vigorous intensity activities.		
Sedentary Graded "0"	0–2 days	0–2 days	0 days

TABLE 2 Socio-demographic characteristics of participants ($n = 1,143$) based on type of diet.

	Total $n = 1,143$	Vegan $n = 54$	Vegetarian $n = 89$	Non-vegetarian $n = 1,000$	Statistics
Age (years)					$X^2_{(2)} = 7.15, p = 0.028$
18–30	64.5% (737)	77.8% (42)	71.9%	63.1% (631)	
31–45	35.5% (406)	22.2% (12)	28.1% (25)	36.9% (369)	
Sex					$X^2_{(2)} = 8.15, p = 0.017$
Male	36.6% (418)	20.4% (11)	43.8% (39)	36.8% (368)	
Female	63.4% (725)	79.6% (43)	56.2% (50)	63.2% (632)	
Education					$X^2_{(4)} = 4.96, p = 0.29$
High school	13.0% (149)	13.0% (7)	15.7% (14)	12.8% (128)	
Graduate	83.2% (951)	85.2% (46)	84.3% (75)	83.0% (830)	
Postgraduate	3.8% (43)	1.9% (1)	0.0% (0)	4.2% (42)	
City					
Riyadh	45.5% (520)	35.2% (19)	38.2% (34)	46.7% (467)	
Dammam	32.5% (371)	51.9% (28)	37.1% (33)	31.0% (310)	
Jeddah	12.3% (141)	5.6% (3)	16.9% (15)	12.3% (123)	
Abha	9.7% (111)	7.4% (4)	7.9% (7)	10.0% (100)	
BMI (kg/m^2)					$X^2_{(6)} = 77.56, p < 0.0001$
<18.5	8.8% (100)	25.9% (14)	16.1% (9)	7.7% (77)	
18.5–24.9	41.0% (469)	74.1% (40)	49.7% (31)	39.8% (398)	
25–29.9	31.2% (357)	0.0% (0)	47.2% (42)	31.5% (315)	
≥30	19.0% (217)	0.0% (0)	7.9% (7)	21.0% (210)	
Physical activity					$X^2_{(6)} = 123.46, p < 0.0001$
Sedentary	20.2% (231)	11.1% (6)	1.1% (1)	22.4% (224)	
Low	16.3% (186)	7.4% (4)	2.2% (2)	18.0% (180)	
Moderate	22.2% (254)	42.6% (23)	3.4% (3)	22.8% (228)	
High	41.3% (472)	38.9% (21)	93.3% (83)	36.8% (368)	

Data are presented as prevalence (along with number of participants). BMI, body mass index; χ^2, chi-square. P, p-value for difference between the groups. Non-vegetarian—those who consume fruits, vegetables, pulses or beans, animal products (chicken or meat, fish, eggs, milk or curd) either daily, weekly or occasionally. Vegetarians—those who avoid all flesh foods but consume egg and/or dairy products. Vegan—those who avoid all foods and ingredients from animal sources.

($n = 13$) were following a vegetarian diet for more than 10 years. The favorite meat substitute was soya in both groups (Table 3).

The Spearman's partial correlation analysis controlling for physical activity showed a negative weak correlation between BMI and eating vegetables and fruits [$r_{(1,140)} =$

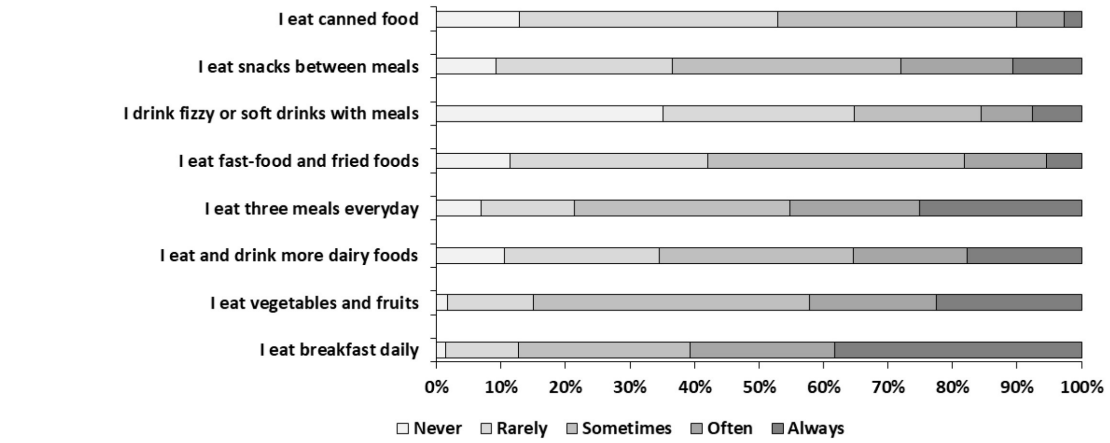

FIGURE 1
Distribution of eating behavior in the study population.

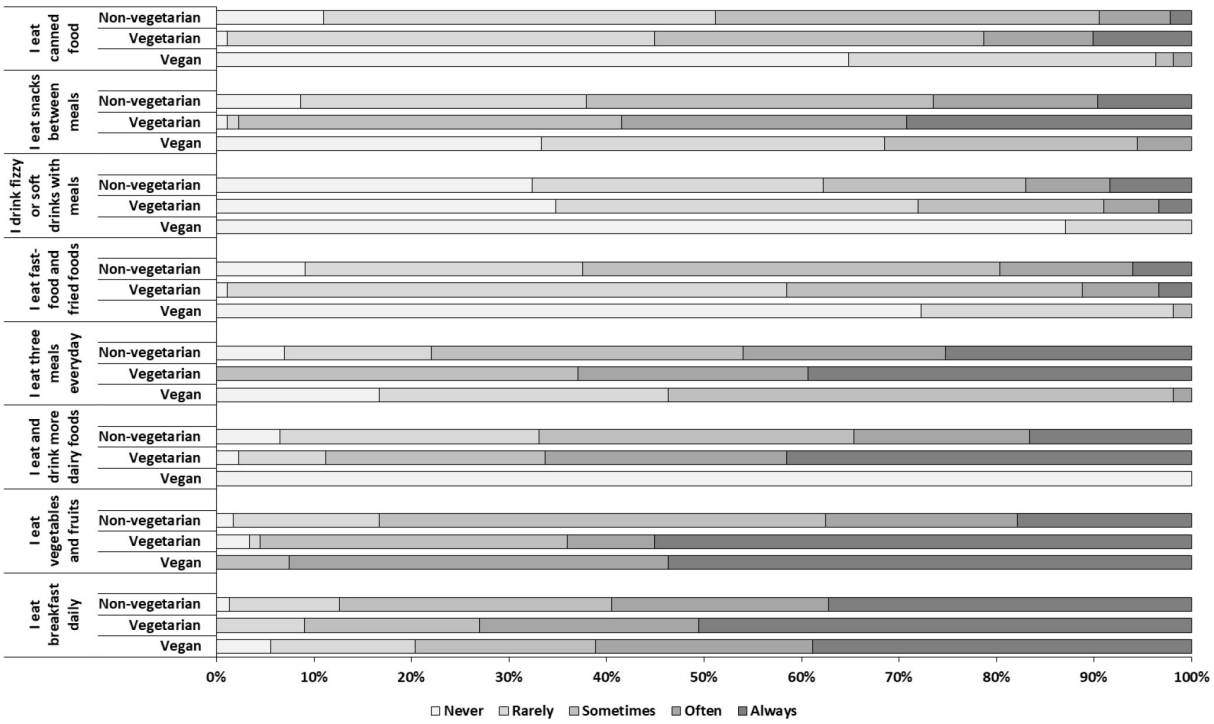

FIGURE 2
Distribution of eating behavior based on type of diet in the study population.

-0.269, $p < 0.0001$], while a positive weak correlation was observed between BMI and eating and/or drinking dairy foods [$r_{(1,140)} = 0.347$, $p < 0.0001$], eating three meals a day [$r_{(1,140)} = 0.125$, $p < 0.0001$] and eating snacks between meals [$r_{(1,140)} = 0.218$, $p < 0.0001$]. A positive moderate correlation was found between BMI and eating fast-food and fried food [$r_{(1,140)} = 0.529$, $p < 0.0001$], drinking fizzy or soft drinks with meals [$r_{(1,140)} = 0.495$, $p = 0.001$], and eating canned food [$r_{(1,140)} = 0.510$, $p < 0.0001$] (Figure 3).

Discussion

Since there are limited research studies on the prevalence of vegetarianism from Saudi Arabia, the study was designed

TABLE 3 Distribution of reason for adopting vegan/vegetarian diet, duration and favorite meat substitute among vegan and vegetarian participants ($n = 54$).

	Vegan $n = 54$	Vegetarian $n = 89$
What is the primary reason you decided to become vegetarian?		
For better health/nutrition	18.5% (10)	59.5% (53)
Weight control	31.5% (17)	7.9% (7)
Animal ethics	37.0% (20)	3.4% (3)
I don't like animal products	13.0% (7)	14.8% (8)
I have a health condition	0.0% (0)	14.8% (8)
My friends or family are vegetarian	0.0% (0)	1.1% (1)
Other	0.0% (0)	10.1% (9)
How long have you been a vegetarian		
<1 year	72.9% (41)	38.2% (34)
1–2 years	11.1% (6)	5.6% (5)
2–4 years	13.0% (7)	24.1% (13)
4–10 years	0.0% (0)	27.0% (24)
>10 years	0.0% (0)	14.6% (13)
What is your favorite meat substitute		
Soya	59.3% (32)	23.6% (21)
Tofu	33.3% (18)	15.7% (14)
Egg	0.0% (0)	16.8% (15)
Quorn	7.4% (4)	1.1% (1)
Other	0.0% (0)	42.7% (38)

Data are presented as prevalence (along with number of participants).

to assess prevalence of vegetarian diet, eating behavior and to correlate them with demographics and BMI. The present study is the first and only study presenting the prevalence of vegetarianism and eating behavior of Saudi nationals.

The most important findings of the study are (i) vegan prevalence 4.7% and vegetarianism 7.8%; (ii) vegan diet more prevalent in youth (77.8%) and females (79.6%); (iii) eating breakfast is common behavior of Saudi nationals (iv) eating behavior and physical activity level is more favorable among participants on vegetarian diet; and (v) consumption of fast-food and fizzy beverages are positively correlated with BMI.

The prevalence of vegetarian diet is much higher among Saudi nationals as compared to the vegetarian prevalence reported in Americans (5%) (7) and lower than Germans (10%) (11) and Indians (30%) (10). Moreover, the present results are similar to vegetarian prevalence among Canadians (8%) (8). However, differences in the social and cultural perspective between Saudi Arabia and other western nations may also affect the vegan/vegetarian diet adoption prevalence. Only one study is available from Saudi Arabia which reported a higher prevalence of veganism (8%) than vegetarianism (5%), contrary to our findings which might be due to the difference in the selection of the study population. Our study participants were only Saudi nationals, while study by AlHusseini et al. (26), included both Saudi nationals and residents (42 and 58%, respectively). Traditionally, the Saudi nationals follow a non-vegetarian diet, but changes in the perception of youth regarding nutrition, ethics, and health are reflected in increased adoption of vegan and vegetarian diet in the recent year. This change will lead to increased fiber intake and decreased consumption of unhealthy fat leading to positive impact on BMI (27).

Similar to our study results, a study from Germany also reported a significantly higher proportion female ($p < 0.001$) among vegetarians/vegans and were significantly younger than omnivores ($p < 0.001$) (28). These results are in line with most studies that show the same trend for more females being vegetarian than males, regardless of nation/culture (6–10, 21, 26). Moreover, there are studies that showed a strong association between red meat consumption and masculinity (29–32). Our study shows that participants on vegetarian diet have a lower BMI and a high physical activity level compared to non-vegetarian participants. These results are in accordance with other studies conducted in different parts of the world (7, 28). Moreover, the reason for adopting a vegetarian diet was to improve their health and control body weight, which supports the higher physical activity level in our study population.

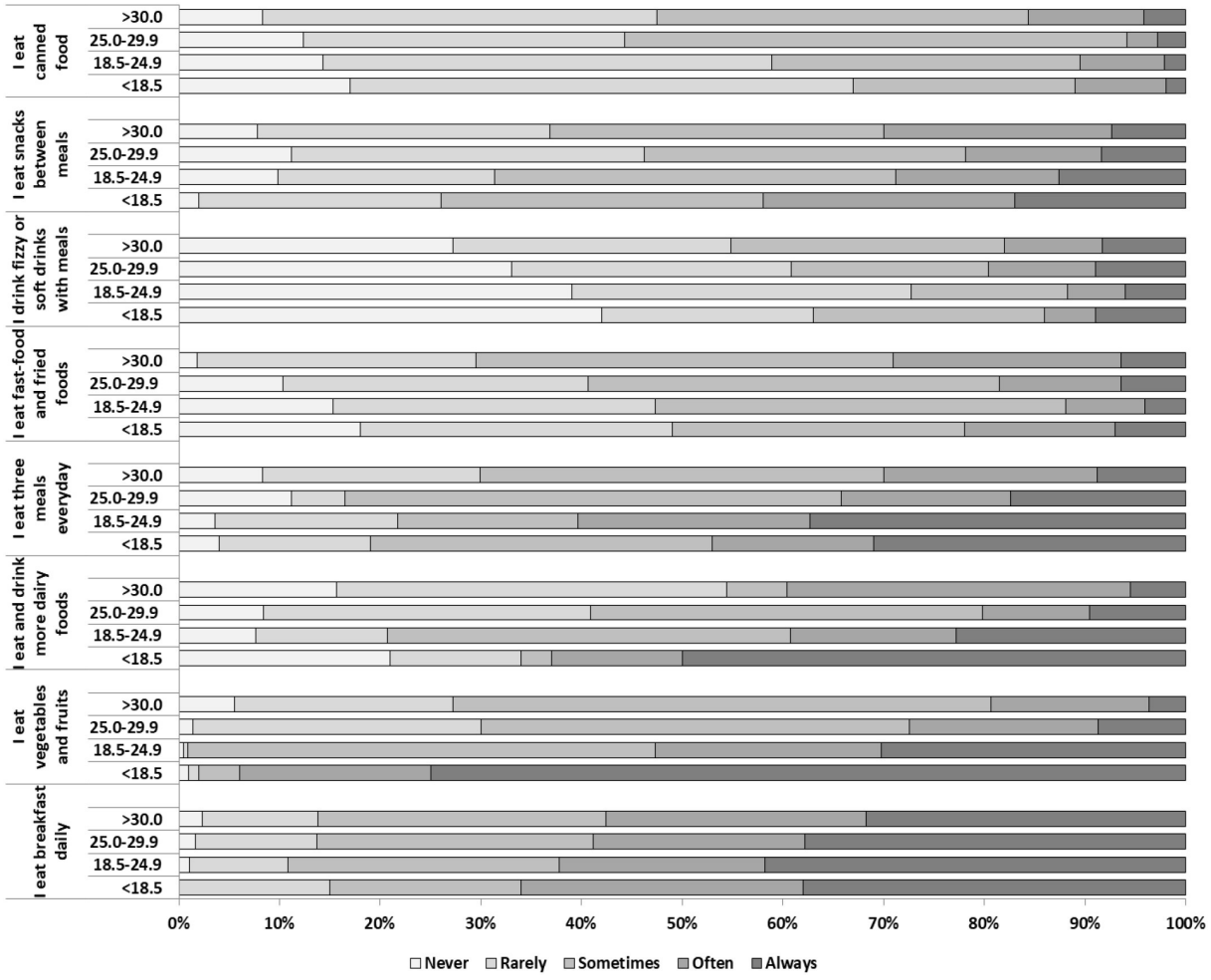

FIGURE 3
Distribution of eating behavior based on BMI category in the study population..

However, a study from Brazil reported no significant difference in the BMI and physical activity level between the vegetarian and omnivorous group (33). In our study, all underweight vegan participants and most of the vegetarian participants were young females. It is possible that these participants might have poorly planned their diet due to lack of knowledge about a healthy balanced diet. There are studies which have reported that a well-planned vegan/vegetarian diets can provide nutritional requirements to the individuals who are involved in endurance activities (14, 15, 21). The prevalence of underweight among vegan and vegetarian diet participants is high and need to be addressed through awareness programs on healthy balanced diet especially in colleges and universities as vegan and vegetarian diet adoption is higher among the youth. Moreover, students should be encouraged to consult dietitian when adopting vegan/vegetarian diet to achieve a healthy weight and to avoid micronutrient deficiency.

Our study results of eating behavior vary from a previous study, which reported a higher prevalence for eating breakfast (88.6%) at least three times per week, rarely eating vegetables and fruits (32.2 and 36.1%) and eating snacks (31.7%), while a lower prevalence 31.4% for eating three meals per day (34). This deviation in eating behavior may be due to the difference in study population which comprised of only 18–24 years male students. Further, there is a duration of more than 10-years between the two surveys, which might be another reason for a better eating behavior witnessed in our study. The eating behavior from nationally representative study of Saudi population revealed a low intake of vegetables and fruits, which are source of high-fiber content along with other nutrient and a higher consumption of protein and carbohydrate through meat products and rice (35, 36). An encouragement toward a well-planned vegan and vegetarian diet among Saudi population may help in controlling the obesity.

In the present study a significantly higher intake of vegetables and fruits was observed in participants on a vegetarian diet than on a non-vegetarian diet, while eating fast-foods and drinking fizzy drinks was more popular among participants on a non-vegetarian diet. Similar results have been reported in studies conducted in western countries (32, 37, 38). Our study findings indicate that the population following a vegan and vegetarian diet shows an overall healthier lifestyle with better eating behavior, and high level of physical activity than non-vegetarians. This finding is supported by a previous study conducted among the members of the Seventh-day Adventist Church which reports, irrespective of their religion-based lifestyles, that people following a vegetarian diet are usually less likely to smoke, drink less alcohol, and are more physically active (39).

There was significant decrease in the BMI of the participants who consumed more vegetable and fruit. However, a study from Saudi Arabia (34) reported no significant correlation between BMI and eating three meals a day and the consumption of vegetables and fruits, which may be due to the small sample size, and the age and gender of the participants. In our study, an increased consumption of fast food, canned food and fizzy drinks showed a significant association with body weight and subsequently BMI. Our study findings were similar to the results reported by other studies (40–42).

Though the present study provides important findings which were not reported earlier for Saudi nationals, the study has some limitations. The level of awareness of good eating behavior and importance of physical activity might have resulted in social desirability bias with higher responses to the good eating behavior and high level of physical activity. However, this study results are reliable firstly for prevalence of BMI categories as the BMI was calculated after taking the measurements and secondly for correlation of eating behavior with BMI as the correlation was performed by controlling for the confounder physical activity level.

Conclusion

Our study shows that vegan and vegetarian diet have gained access into the lifestyle of Saudi adults with a prevalence of 4.7 and 7.8%, respectively, especially vegan diet was more prevalent amongst youth (77.8%) and females (79.6%). Participants on vegetarian diet showed better lifestyle like higher physical activity level, higher consumption of fruits, vegetables, dairy products and low intake of fast-foods and fizzy beverages.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Saudi Electronic University Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MA-M made substantial contributions to conception and design, in charge of data collection and curation, writing of the manuscript, performed the statistical analysis, and approved the submitted version of the manuscript.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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The relationship between processed meat, red meat, and risk of types of cancer: A Mendelian randomization study

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Background: Observational studies have suggested processed and red meat may increase the risk of cancer. However, the causal effects and direction between them were still unclear. We conducted two-sample Mendelian randomization (MR) analysis to evaluate the causal effect of processed meat and red meat on the risk of nine common types of cancer, namely, lung, ovarian, endometrial, breast, kidney, gastric, prostate, skin, and oropharyngeal cancer.

Methods: Genome-wide association studies (GWAS) for processed meat and red meat (pork, beef, and mutton) were obtained from the UK Biobank. GWAS of types of cancer in this study were extracted from the genetic consortia and the FinnGen consortium. The inverse variance weighted (IVW) was carried out as the main method for two-sample MR analysis. Sensitivity analyses were used to assess the robustness of the results.

Results: Genetically predicted processed meat intake was causally associated with increased risk of lung cancer (OR [odds ratio] = 1.923, 95% CI = 1.084–3.409, $P = 0.025$). There is no convincing evidence for the associations between genetically determined processed meat, red meat, and the risk of other cancers we studied.

Conclusion: Our results suggested that intake of processed meat may increase the risk of lung cancer. These findings provided no evidence to support that consumption of processed and red meat has a large effect on the risk of other cancers we studied. Further research is needed to clarify the results.

KEYWORDS

processed meat, red meat, cancer, Mendelian randomization, genome-wide association studies

Background

Cancer is the main cause of morbidity and mortality in the world. According to the research of the International Agency for Research on Cancer (IARC), it was estimated that there were approximately 20 million new cancer cases and nearly 10 million cancer deaths globally in 2020, which had become the main health burden of all countries (1). Previous studies found that dietary factors were associated with cancer risk, especially red and processed meat intake may be an important risk factor for many types of cancer (2).

Red meat (pork, beef, mutton, etc.) is an important source of protein, vitamins, amino acids, minerals, and other nutrients (3). Processed meat refers to improving the taste of meat or increasing the shelf life through several processes such as salting, curing, fermentation, and smoking (4). In recent decades, the consumption of meat has been increasing all over the world. However, it has been reported that high consumption of red and processed meat may increase the risk of cancer (5).

Myoglobin, hemoglobin, and cytochrome which have high levels in red meat were transformed into denatured protein hemes, hemichromes, and hemochromes during cooking and other processing. Oxidative reactions catalyzed by hemoglobin and iron can damage various components of biological systems, such as lipids, proteins, nucleic acids, and other substances. Free radical damage caused by oxidative stress can lead to cancer (6). The IARC working group has shown that consumption of red meat may increase the chance of colorectal cancer, pancreatic cancer, and prostate cancer, while consuming processed meat may increase the possibility of colorectal cancer and gastric cancer (7).

According to previously published systematic reviews and meta-analyses, red and processed meat consumption may lead to an increased risk of cancer (8–11). However, there are still many studies showing that the consumption of processed and red meat may not be linked to higher cancer risk (12–16). Observational studies evaluating the relationship between processed meat, red meat, and the risk of cancers have reported inconsistent results, most likely due to sampling size limitations. Furthermore, observational epidemiological studies are susceptible to confounding and reverse causation (17). Whether there is a causal relationship between the intake of processed or red meat and cancer remains unclear (18). Compared with the observational studies, randomized controlled trials (RCTs) on the consumption of red meat and processed meat could potentially help establish the causal relationship (19). A recent RCT on this topic showed that processed meat intake was not associated with the risk of cancers, and red meat intake could increase the risk of breast cancer (20). However, it is worth noting that volunteers included in the study had more health-conscious behaviors and higher educational levels compared to the general population, which will inevitably bias the results. In addition, the number of cases of cancer at specific sites is relatively small. Therefore, the extrapolation of these results still needs to be cautious.

Mendelian randomization (MR) is a research method used in epidemiology in recent years, mainly through genetic variation to infer the causal relationship between exposure and disease outcomes based on single nucleotide polymorphisms (SNPs) (21). In MR, causal inference of exposure-outcome associations can be improved by using phenotype-related genetic variants as instrumental variables for exposure. Genetic variation follows the rules that alleles segregate randomly from parent to offspring and are determined at conception by genetic variation, so it is not easy to be disturbed by population confounding factors in traditional observational research (22). In addition, the genotypes are not affected by disease phenotypes, so inverse correlation bias can also be avoided (23). Currently, MR has been applied to studies on the causal relationship between dietary habits such as vegetable intake and cancer (24, 25). For example, Chen Jin et al. conducted a two-sample MR analysis to explore the relationship between the causal relationship between dried fruit intake and the risk of cancers. Studies have shown that the consumption of dried fruit may have a protective effect against cancer. It is suggested that health education and reasonable adjustment of dietary ratios may contribute to the primary prevention of cancer (26). There is also a high-quality MR study on the association between processed meat and the risk of cancer. Qi Feng et al. performed both observational analyses with UK Biobank and genetic analysis with MR to explore the effect of processed meat intake on the risk of colorectal cancer. The results showed that heavy consumption of processed meat independently increases the risk of colorectal cancer, and processed meat intake reduction may be an effective strategy for preventing colorectal cancer (27).

In our study, we performed a two-sample MR analysis to assess the potential causal relationship between processed red meat intake and the risk of cancers from the GWASs and UK Biobank that were publicly available.

Methods

We used data from published studies or GWAS summaries that were openly available. Since no primary data were used in this study, ethical approval was not required. All the studies included were permitted by their academic ethics review committees, and each participant signed written informed consent.

Exposure and outcome measures

Dietary exposures (processed meat, pork, beef, and mutton) were obtained from the UK BioBank cohort with 461,981, 460,162, 461,053, and 460,006 individuals of European ancestry, respectively. To minimize the effects of linkage disequilibrium (LD), single nucleotide polymorphisms (SNP) that passed the generally accepted genome-wide significance threshold ($P < 5 \times 10^{-8}$, R^2>0.001 within a 10,000 kb window) for exposures were chosen as instrumental variables (Figure 1A). The detailed information on these independent, genome-wide SNPs was shown in Supplementary material 1. F statistics and proportion of variance explained (PVE) were computed to test whether a weak instrument bias was present.

We use large-scale GWAS data for nine types of cancer as outcome factors. Breast cancer data were obtained from GWAS

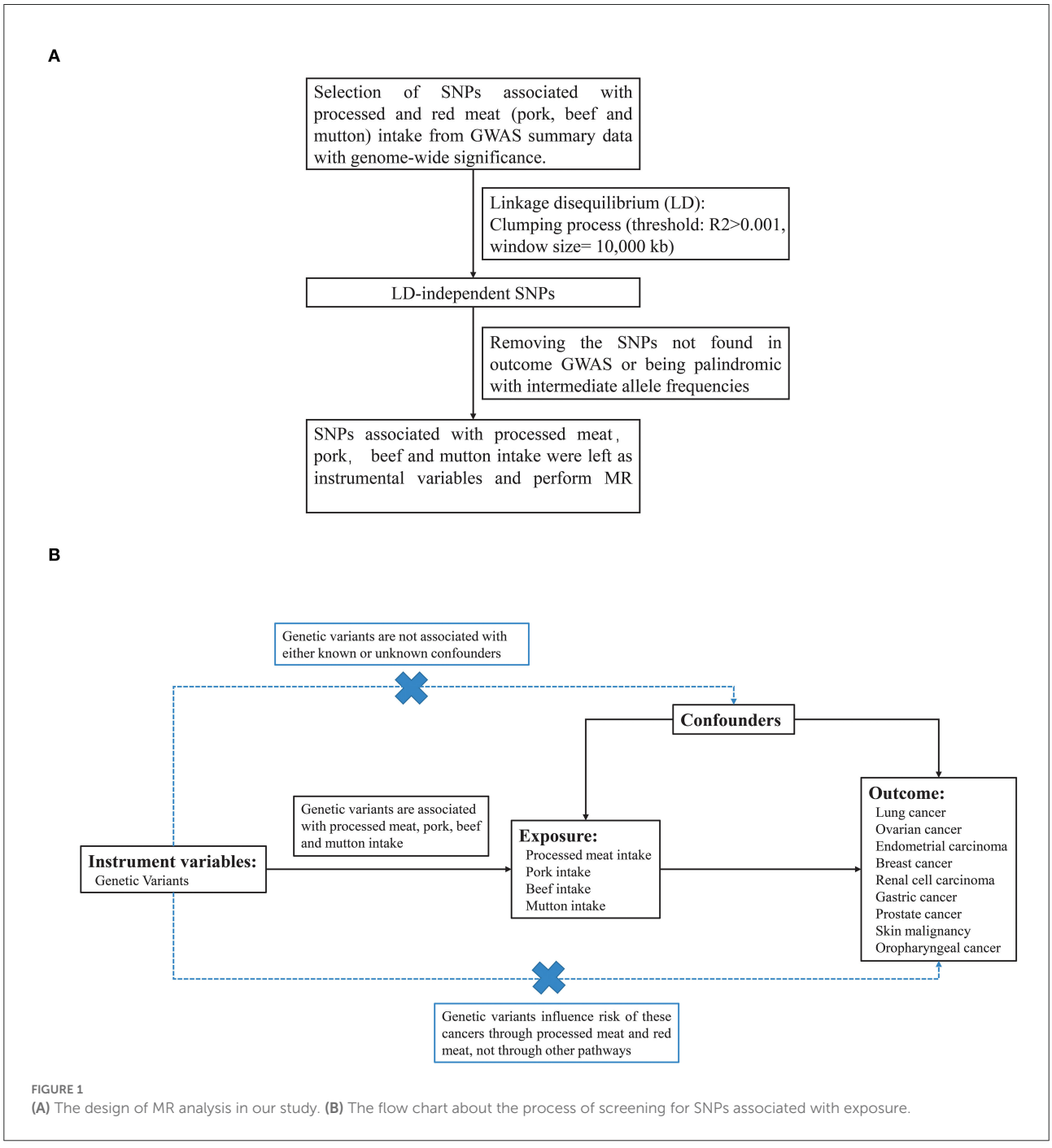

FIGURE 1
(A) The design of MR analysis in our study. **(B)** The flow chart about the process of screening for SNPs associated with exposure.

meta-analysis from Breast Cancer Association Consortium (BCAC) studies involving people of European ancestry (46,785 cases and 42,892 controls). Data for prostate cancer was derived from a genome-wide association analysis of 79,148 patients and 61,106 controls of European ancestry by the Prostate Cancer Association Group to Investigate Cancer-Associated Alterations (PRACTICAL) in the Genome Consortium. For lung cancer, we used data from the International Lung Cancer Consortium, consisting of 11,348 cases and 15,861 controls of European descent. GWAS data for ovarian cancer were acquired from the Ovarian Cancer Alliance Consortium, which included 25,509 patients of European ancestry. Genome-wide association analysis results for gastric cancer, renal cell carcinoma, and skin malignancy were all derived from European ancestry data in FinnGen Biobank analysis (Table 1). Our study only utilized the results of published GWAS and did not involve individual-level data. All exposure and outcome summary data were downloaded from the IEU OpenGWAS project (https://gwas.mrcieu.ac.uk/).

Mendelian randomization

The MR analysis was carried out using the TwoSampleMR R package and the "MR-PRESSO" R package (version 0.4.13, http://github.com/MRCIEU/TwoSampleMR). All of our studies were based on a two-sample MR framework, which obtained SNP-exposure (processed meat intake, pork intake, beef intake, and mutton intake) associations and SNP-outcome (lung cancer, ovarian cancer, endometrial carcinoma, breast cancer, renal cell carcinoma, gastric cancer, prostate cancer, skin malignancy, and oropharyngeal cancer) associations from different cohorts to estimate the causal effects of exposure on the outcome. In total, six MR methods were used to estimate the effect of genetically predicted exposure on cancers namely the main analysis method inverse variance weighted (IVW), and other five additional analysis methods, Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO), maximum likelihood, MR Egger, weighted median, and penalized weighted media. For the IVW method, we used a random-effects model when the results were heterogeneous, and a fixed-effects model was used when there was no heterogeneity. The maximum likelihood method was performed by estimating the causal effects of the effect of SNPs on exposure and outcome by direct maximization of the likelihood (28). The MR-PRESSO method was used to detect outlier variables in IVW analysis by comparing the actual distance of the genetic variants to the expected distance from the regression, assuming the absence of horizontal pleiotropy and evaluating the causal estimates after removing outliers (29). The MR–Egger approach utilizes InSIDE to perform a weighted linear regression of exposure results but is susceptible to IVs (30). In addition, the weighted median method can significantly improve the detection ability of causal effects and reduce type I errors (31).

Pleiotropy and sensitivity analyses

To test for heterogeneity, MR Egger and IVW were carried out. The SNP-exposure association and the SNP-outcome association estimates were involved in MR Egger. Using the slope of the weighted regression line, we estimated the causal effect of exposure on the outcome, independent of horizontal pleiotropy. An estimate of the causal effect of exposure on outcome was provided by the slope of the weighted regression line and was not affected by horizontal pleiotropy. In the MR-Egger test, the intercept assesses the mean pleiotropy of genetic variation, with values greater or less than zero indicating possible bias in IVW estimates. The sensitivity of the results was analyzed using the leave-one-out method. The SNPs were sequentially removed one at a time to examine whether the individual SNPs with potentially large horizontal pleiotropic effects could affect MR estimate.

TABLE 1 Number of cancer cases and controls in the Mendelian randomization study on the association of processed meat and red meat intake with risk of site-specific cancer.

Outcome	Data source	Cases (n)	Controls (n)	Population
Lung cancer	ILCCO	11,348	15,861	European
Ovarian cancer	OCAC	25,509	40,941	European
Endometrial carcinoma	Consortium (Tracy et al.)	12,906	108,979	European
Breast cancer	BCAC	46,785	42,892	European
Kidney cancer	The FinnGen consortium	971	174,006	European
Gastric cancer	The FinnGen consortium	633	174,006	European
Prostate cancer	PRACTICAL	79,148	61,106	European
Skin malignancy	The FinnGen consortium	10,384	208,408	European
Oropharyngeal cancer	Consortium (Corina et al.)	2,497	2,928	European

ILCCO, International Lung Cancer Consortium; OCAC, Ovarian Cancer Alliance Consortium; BCAC, Breast Cancer Association Consortium; PRACTICAL, Prostate Cancer Association Group to Investigate Cancer-Associated Alterations in the Genome Consortium.

Result

Selection of instrumental variables

We used the summary GWAS data from UK Biobank for each processed meat, pork, beef, and mutton as exposures and risk for 9 types of cancer as the outcome in different studies (Figure 1B). Two-sample MR analysis was performed to explore the causal relationship between processed/red meat and cancer. Supplementary material 1 showed the SNP information of four exposures (intake of processed meat, pork, beef and mutton), consisting of the name, chromosome location, genes, function, effect allele (EA), other alleles, and effect allele frequency (EAF). We calculated the F statistic for instrumental variable selection and F statistics were >10, which indicated that we have effectively avoided the bias caused by weak instrumental variables (Supplementary material 1) (32).

The causal effect of processed red meat and cancer

The inverse variance weighted (random effect and fixed effect), maximum likelihood, MR Egger, weighted median, and penalized weighted media were used to estimate causal associations between genetically predicted processed/red meat and the risk of 9 types of cancer. It showed that processed meat was associated with an increased risk odds of lung cancer (IVW: OR = 1.923, 95% CI = 1.084–3.409, $P = 0.025$) (Figure 2). A higher processed meat intake was not associated with the risk of ovarian cancer, endometrial carcinoma, breast cancer, renal cell carcinoma, gastric cancer, prostate cancer, skin malignancy, and oropharyngeal cancer. Consumption of red meat did not significantly increase the risk of cancer (Supplementary material 2).

Sensitivity analyses

The horizontal pleiotropy between SNPs and outcomes was assessed by MR-Egger regression, which showed no evidence of horizontal pleiotropy (Supplementary material 4). The funnel plots showed a symmetric pattern of effect size variation around the point estimates, indicating no apparent horizontal pleiotropy (Supplementary material 3). The results of the leave-one-out sensitivity analyses demonstrated that no potentially influential SNPs drive the causal link and the stability of our conclusion (Supplementary material 3).

Discussion

In this study, a two-sample MR analysis was performed using the instrumental variables of large-scale GWAS to assess the causal relationship between processed/red meat and cancers using genetic data from populations of European descent. In our MR analysis, genetic predisposition to processed meat consumption was associated with a higher risk of lung cancer, with an OR of 1.923 [95% CI, 1.084–3.409; $P = 0.025$]. Results from a two-sample MR analysis suggested that processed meat consumption was not associated with the risk of ovarian cancer, endometrial carcinoma, breast cancer, renal cell carcinoma, gastric cancer, prostate cancer, skin malignancy, and oropharyngeal cancer. In this study, no strong evidence was found to support associations between red meat intake and the risk of types of cancer.

There was growing evidence that high levels of red meat intake, and processed meat consumption were linked to an increased risk of types of cancer (33, 34). A large observational study involving more than 470,000 people with a follow-up of 11.4 years showed a reduced risk of colorectal cancer and breast cancer in people who consumed less red meat (35). World Cancer Research Fund/American Institute for Cancer

MR methods	OR	95% CI	P value
IVW (random effect)	1.923	1.084-3.409	0.025
IVW(fixed effects)	1.923	1.084-3.409	0.025
MR-PRESSO	1.923	1.087-3.401	0.035
Maximum likelihood	1.977	1.106-3.534	0.022
MR Egger	3.044	1.737-5.336	0.225
Weighted median	2.865	1.947-4.217	0.008
Penalised weighted media	2.892	2.308-3.624	0.015

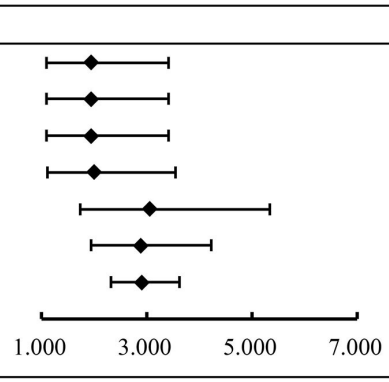

FIGURE 2
Associations of genetically predicted processed meat intake with risk of lung cancer. OR, odds ratio; CI, confidence interval.

Research (WCRF/AICR) also advised that limiting red meat intake and avoiding consumption of processed meat may modestly reduce the risk of cancer (36). Giuseppe et al. (37) compiled 24 meta-analyses of the association of red meat and 39 processed meat consumption with the risk of cancer published between 2005 and 2015. The results indicated an increased risk of cancer in subjects consuming large amounts of red and processed meat. It is possible that high-temperature cooked meats can produce N-nitroso compounds (NOCs). Heterocyclic amines formed in meat smoking can become carcinogens after metabolic activation (38). Heterocyclic aromatic amines (HAAs) can be derived from high-temperature cooked red meat (39). The rich heme in red meat can catalyze the production of NOC and lipid peroxidation products (LPO). These carcinogens combine with DNA to form DNA adducts, which interfere with DNA replication and repair, and cause gene mutations during cell division, inducing the occurrence of cancer (40). The 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP), a heterocyclic amine widely present in processed and red meat, induced cancer through cytochrome P450-mediated DNA damage and metabolic activation of mutagens (41, 42).

In MR analysis, it failed to detect significant associations of genetic predisposition to processed/ red meat with most of the cancers studied ($P > 0.05$). This was consistent with the conclusions of some guidelines and observational studies (43–47). A meta-analysis of 6 million participants in 56 cohort studies also found evidence of low quality that with the reduction of unprocessed red meat, the total cancer mortality would decrease. An intake reduction of three servings of processed meat per week was not associated with a lower incidence of cancer of the mouth, stomach, small bowel, liver, pancreas, endometrial, or prostate. Although studies have shown that reducing the intake of processed meat can reduce the risk of esophageal, colorectal, and breast cancer, the certainty of the evidence was very low due to the observation design and inaccuracy. In addition, there was low-certainty evidence that reducing the intake of red meat was associated with a very small overall reduction in cancer mortality (48). According to the Nutritional Recommendations (NutriRECS) Consortium's report, there was a low and very low quality that meat consumption could lead to potential adverse health outcomes. The probability of esophageal cancer, colorectal cancer, and breast cancer caused by high consumption of processed meat was not significantly different from that of low consumption (49). A definitive causal relationship requires more in-depth mechanism studies and RCT studies in the future.

The Mendelian randomization can avoid bias from unmeasured confounding and avoid bias from reverse causation and offer some protection against biases that can be conceptualized as reverse causation (50, 51). Our study tried to avoid some problems of confounding factors and reverse causality, but there were still some limitations. First, this is a pooled analysis of individual studies, due to the lack of original data, we could not conduct a patient-level analysis. Second, like all MR studies, horizontal pleiotropy, as a common issue, is difficult to avoid. Although some MR methods such as the leave-one-out method and MR-Egger were used to test, which indicated our results were not affected by pleiotropy, the possibility of bias could not be ruled out. Third, our results suggested a potential causal relationship between processed/red meat and types of cancer, the analysis presented here does not provide evidence for specific mechanisms of tumorigenesis. Fourth, wide CIs was observed under the MR-Egger method in MR analyses, which may hint at low potency. However, the MR-Egger method is often underpowered in studies and other Mendelian analyses were qualitatively consistent with the primary analysis of the inverse-variance weighted method. The last but not least, although using a single European population to investigate the causal relationship between processed/red meat and cancer can minimize population stratification bias, it might not be generalizable to other populations.

Conclusion

In conclusion, there is an obvious positive causal relationship between the genetically predicted processed meat and lung cancer. We did not find a causal relationship between processed, red meat, and other studied cancers. Observational studies had previously suggested an association between processed/red meat and cancer. Although traditional epidemiological studies can help us preliminarily understand the correlation between meat consumption and cancer, traditional epidemiological studies are influenced by confounding factors, such as social and demographic components. In addition, unrecognized bias may lead to inaccurate results. Further MR studies may be needed to assess the relationship between meat consumption and important risk factors for cancer.

Data availability statement

All summary statistics based on association data are available free of charge. The data of processed meat intake (ID: ukb-b-6324), pork intake (ID: ukb-b-5640), beef intake (ID: ukb-b-2862), mutton intake (ID: ukb-b-14179), lung cancer (ID: ieu-a-966), prostate cancer (ID: ieu-b-85), oral cavity and pharyngeal cancer (ID: ieu-b-89), breast cancer (ID: ieu-a-1130), ovarian cancer (ID: ieu-a-1120), endometrial cancer (ID: ebi-a-GCST006464), skin malignancy (ID: finn-b-C3_SKIN), kidney cancer (ID: finn-b-C3_KIDNEY_NOTRENALPELVIS) and gastric cancer (ID:finn-b-C3_STOMACH_EXALLC) can be obtained from https://gwas.mrcieu.ac.uk/.

Author contributions

XS and DX contributed to the study's conception and design. Material preparation, data collection, and analysis were

performed by KW, TS, and AL. The first draft of the manuscript was written by KW and LL. All authors commented on previous versions of the manuscript and read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.942155/full#supplementary-material

SUPPLEMENTARY MATERIAL 1
Instrumental SNPs from processed meat and red meat (pork, beef, and mutton) GWASs.

SUPPLEMENTARY MATERIAL 2
MR estimates from each method of the causal effect of exposure on cancers.

SUPPLEMENTARY MATERIAL 3
Leave-one-out analysis, funnel plot, and MR effect size for processed meat, pork, beef, and mutton intake on cancer.

SUPPLEMENTARY MATERIAL 4
Heterogeneity and level pleiotropy test in the Mendelian randomization analyses.

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Association of dietary pattern and Tibetan featured foods with high-altitude polycythemia in Naqu, Tibet: A 1:2 individual-matched case-control study

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This study focused on the association of dietary patterns and Tibetan featured foods with high-altitude polycythemia (HAPC) in Naqu, Tibet, to explore the risk factors of HAPC in Naqu, Tibet, to raise awareness of the disease among the population and provide evidence for the development of prevention and treatment interventions. A 1:2 individual-matched case-control study design was used to select residents of three villages in the Naqu region of Tibet as the study population. During the health examination and questionnaire survey conducted from December 2020 to December 2021, a sample of 1,171 cases was collected. And after inclusion and exclusion criteria and energy intake correction, 100 patients diagnosed with HAPC using the "Qinghai criteria" were identified as the case group, while 1,059 patients without HAPC or HAPC -related diseases were identified as the control group. Individuals were matched by a 1:2 propensity score matching according to gender, age, body mass index (BMI), length of residence, working altitude, smoking status, and alcohol status. Dietary patterns were determined by a principal component analysis, and the scores of study subjects for each dietary pattern were calculated. The effect of dietary pattern scores and mean daily intake (g/day) of foods in the Tibetan specialty diet on the prevalence of HAPC was analyzed using conditional logistic regression. After propensity score matching, we found three main dietary patterns among residents in Naqu through principal component analysis, which were a "high protein pattern," "snack food pattern," and "vegetarian food pattern." All three dietary patterns showed a high linear association with HAPC ($p < 0.05$) and were risk factors for HAPC. In the analysis of the relationship between Tibetan featured foods and the prevalence of HAPC, the results of the multifactorial

analysis following adjustment for other featured foods showed that there was a positive correlation between the average daily intake of tsampa and the presence of HAPC, which was a risk factor. Additionally, there was an inverse correlation between the average daily intake of ghee tea and the presence of HAPC, which was a protective factor.

KEYWORDS

Tibet, dietary pattern, featured foods, high altitude polycythemia, 1:2 individual matched case-control study

Introduction

High-altitude polycythemia (HAPC) is a clinical syndrome that occurs among natives or long-term residents living ≥ 2,500 m above sea level. It is characterized by excessive erythrocytosis (in women, hemoglobin > 19 g/dL; in men, hemoglobin > 21 g/dL), severe hypoxemia, and (in some cases) moderate or severe pulmonary hypertension, which may evolve to cor pulmonale, leading to congestive heart failure (1). The 2004 definition of the disease considers HAPC and chronic plateau disease (CMS) to be the same thing. However, in naming, typing, and diagnosing altitude sickness in China (2), HAPC is considered to be a separate clinical subtype, whereas CMS refers to the clinical manifestations of both HAPC and high-altitude heart disease (3). In our study, HAPC was considered to be a separate clinical subtype to study its associated risk factors.

Excessive erythrocytosis is accompanied by excessive blood volume and extensive vasodilatation. Due to excessive red blood cells and hypoxemia, patients present with profound cyanosis. Characteristic pestle-like fingers and toes can usually be observed, with deep cyanosis of the oral and throat mucosae (4–6). Undoubtedly, the clinical features described above impose a serious disease burden and economic burden on patients with HAPC.

In the Tibetan Plateau, HAPC occurs in 5–18% of the total population (5, 6). HAPC affects people in the plateau region, but not all people in the plateau region have a high probability of developing HAPC. Studies have shown that local high-altitude populations have a unique set of heritable traits that can allow them to tolerate hypoxia, though between 1.2 and 33% of high-altitude populations globally are not adapted to this low-oxygen environment. Other research have shown that the prevalence of high-altitude erythrocytosis is not only related to genetic factors but also to the altitude, length of residence, age, and gender of the population (7, 8). Naqu, Tibet, is located in northern Tibet, with an average altitude of > 4,500 m. Its residents live in a harsh environment of low-pressure oxygen deprivation, high altitude, low temperature, and high ultraviolet light exposure for a long time (9). Therefore, the diet of Tibetans is very characteristic (10). Their featured foods are mainly ghee, tsampa, sweet tea, ghee tea, milk dregs, Tibetan cheese, and barley wine.

Currently, there is no study on the association of dietary patterns and Tibetan featured foods with HAPC. In this study, answers to the Health Status and Health Needs of People in Highland Areas Questionnaire and the 70-item Food Frequency Questionnaire (FFQ), which were modified according to the characteristics of the Tibetan diet, were collected from patients attending a tertiary care hospital in Naqu, Tibet, from December 2020 to December 2021, from which case and control groups were selected for analysis. Our study focused on the relationship between dietary patterns and Tibetan featured foods with HAPC in Naqu, Tibet, to explore the risk factors of HAPC in Naqu, Tibet, to raise awareness of the disease among the population, provide evidence for the development of prevention and treatment interventions, and guide people to eat scientifically.

Materials and methods

Study subjects

Residents of three villages in the Naqu region of Tibet were selected as the study population. A face-to-face questionnaire was administered to the study population from December 2020 to December 2021. A sample of 1,171 cases was collected, including 100 cases in the case group and 1,071 cases in the control group.

We enrolled patients (1) with HAPC who were diagnosed by a tertiary care hospital in Naqu, Tibet, between December 2020 and December 2021; (2) who completed all anthropobiological measurements and questionnaires, including lifestyle characteristics as well as dietary assessments; (3) without missing data for the required variables; and (4) with hemoglobin > 19 g/dL (women) or hemoglobin > 21 g/dL (men) (1). Those with true erythrocytosis, other types of secondary erythrocytosis, or other HAPC complications (2) were excluded.

Enrollees in the control group met the same inclusion and exclusion criteria as the case group, with no abnormal

blood test results and no true erythrocytosis, other secondary erythrocytosis, or other HAPC complications.

Data collection

The data of cases and controls were collected through the Questionnaire on Health Status and Health Needs of People in Highland Areas and a reliability-validated FFQ (11) (**Supplementary Data 1**), which were modified according to the characteristics of the Tibetan diet, by the investigator in face-to-face interviews with patients. At least two investigators were Tibetan and could communicate in both the native Tibetan language and Mandarin. Subjects filled in the FFQ according to their average intake per year, month, week, or day, which was converted to the average daily intake for statistical purposes. A physical examination, including the measurement of height and weight, was performed, and we calculated BMI as weight (kg) divided by height (m) squared. The data-collection process ensured that all participants completed questionnaires, and the final factors included in the study were (**Table 1**) gender, age, BMI, smoking status, alcohol status, length of residence, working altitude, and average daily frequency of intake of each food item (g/day). We excluded foods with an intake of less than 0.5 g/d, finally leaving 70 kinds of food. In the meantime, we calculated the average daily total energy intake, protein intake, carbohydrate intake, and fat intake for each person according to the Chinese Food Composition Tables (12). And corrected the intake of each food according to the mean value of total energy by the residual method. After combining previous studies, we excluded data from samples with a daily energy intake of less than 450 kcal or more than 5,000 kcal (13–15). Therefore, 12 participants were excluded for reporting implausible energy intake. Finally, we determined 1,159 samples, including 100 cases in the case group and 1,059 cases in the control group. This study was approved by the Ethics Review Committee of Naqu People's Hospital, and all participants signed a consent form before completing the interview.

Propensity score matching

After referring to previous literature, we performed 1:2 PSM using R 4.1.2 to exclude the influence of confounding factors on dietary patterns and featured foods. The case and control groups were individually matched 1:2 according to gender, age, body mass index (BMI), length of residence, working altitude, smoking status, and alcohol consumption according to the propensity score matching (PSM) method to reduce confounding bias. The 1:2 match of case and control groups was completed, involving 1,059 controls, based on gender, age, BMI, length of residence, working altitude, smoking status, and alcohol status for 100 case groups. To ensure that the 100 cases in the case group were matched to 200 controls, the caliper distance was selected to be as small as possible. Propensity scores were calculated using a logistic regression model, and to try to match all cases and ensure a low caliper distance (16), the final matching was done at a caliper distance of 1.5. The standardized mean difference (SMD) was used to assess the equilibrium of baseline information between the case and control groups after PSM. A SMD of 0.1 usually indicates a good balance and can be considered a tiny difference between the case and control groups.

Identification of dietary patterns

Based on the intake frequency of each food item in the dietary survey questionnaire, the average daily food intake (g/day) was calculated, energy correction based on total energy intake, and 70 food items were divided into 14 food groups according to the similarity of types and nutrient composition, including "staple foods," "meat," "milk and milk products," "eggs," "beans," " dishes," "snacks and nuts," "fungi and mushrooms," "all vegetables," "fruits," "tea and beverages," "sugars," "oils," and "salt" (**Table 2**). Then, the dietary intake frequencies of each food group were calculated, and the data were standardized to facilitate principal component analysis for dimensionality reduction (16–18). Principal component analysis was done using R 4.1.2 and GraphPad Prism 9. The dietary patterns were selected according to the parallel analysis scree plot, and the factor loadings accounted for by the food groups were also calculated. Dietary patterns were defined according to the common characteristics of the top three factors, accounting for the factor loadings of each dietary pattern. The score for each dietary pattern was calculated for each study subject, with a high score representing greater adherence to the dietary pattern and a low score representing less adherence to the dietary pattern. On this basis, we analyzed the association between the prevalence of HAPC and dietary pattern scores in

TABLE 1 PSM of the name of each variable and the meaning of the assigned value.

Variable	Variable meaning	Variable assignment
Age	The age of the research subjects	
Gender	Gender of study subjects	1: Male, 2: Female
Body mass index	Subject's height (m) divided by weight squared (kg)	
Smoke	Smoking status	1: Yes, 0: No
Alcohol	Alcohol status	1: Yes, 0: No
Length of residence	Years of residence in the Naqu region of Tibet	1: From birth, 2: Migrate
Working altitude	Usually performs work at the altitude	1: < 4,500 m, 2: > 4,500 m

TABLE 2 FFQ groups and foods contained and the average intake after energy correction of the two groups (before PSM).

Group	Foods contained and average intake of the two groups*
Staple foods	Rice (41.85, 49.49), tsampa (64.83, 35.75), wheat flour (19.92, 8.40), rice flour (17.34, 19.78), ginseng fruit (0.03, 2.30), hanging noodles (2.21, 9.72), corn (2.92, 8.67), sweet potato (0.92, 0.47)
Meat	Pork (muscle) (−3.41, 11.06), pork (fat and muscle) (−5.19, 2.91), beef (−6.93, 67.12), lamb (31.26, 7.94), chicken (2.04, 11.48), sausage (−1.44 3.13), pork legs, and feet (0.01, 0.22)
Milk and milk products	Whole fresh milk (30.87, 22.83), low-fat fresh milk (5.95, 6.33), fresh goat's milk (6.61, 2.16), yogurt (12.81, 10.39), ice cream (5.06, 4.65), cheese (4.69, 10.61), milk dregs (5.47, 5.19), ghee (−17.48, 19.81)
Eggs	Eggs (−11.37, 12.97), duck eggs (1.17, 0.42)
Beans	Tofu (5.74, 7.27), dry bean-curd (0.00, 2.29), soy milk (1.35, 2.29)
Savory dishes	Salted radish (4.29, 1.58), salty cucumber (4.35, 0.72), mustard (2.65, 0.55), pickle (6.76, 1.21)
Snacks and nuts	Cakes (−2.88, 4.01), bread (4.32, 7.68), cookies (3.05, 4.01), instant noodles (13.14, 5.52), potato chips (0.07, 1.35), peanuts (21.31, 4.17), walnuts (1.60, 1.09), chestnuts (−0.13, 0.57)
Fungi and mushrooms	Dried mushrooms (0.46, 4.08), fresh mushrooms (2.97, 1.37), kelp (4.86, 0.75), nori (7.29, 0.78)
All vegetables	Cabbage, carrot, potato, cabbage, green pepper, bean sprout, lettuce, cauliflower, bell pepper, winter squash, pumpkin (Due to the wide variety of vegetables, only the average daily intakes of the major vegetable categories were collected in the questionnaire design and survey, and these vegetables mentioned in the table were only for the respondents' reference recall to obtain the most realistic data. Vegetables were also counted as a category only in the process of counting FFQ food types and quantities.) (115.01, 48.36)
Fruits	Watermelon (24.50, 11.00), grapes (5.16, 8.21), apples (59.43, 23.09), bananas (76.25, 9.85), pears (53.36, 5.95), oranges (33.13, 13.07), raisins (0.68, 2.08), peaches (2.36, 1.40), strawberries (−0.13, 1.43), lychees (26.59, 0.21), mangoes (0.03, 1.15), hawthorn (0.59, 4.40), persimmons (8.15, 0.22), dates (5.30, 0.45)
Tea and beverages	Ghee tea (40.01, 99.73), sweet tea (12.50, 13.11), green tea (62.90, 69.94), cola (34.33, 22.93), juice (26.69, 9.04), coffee (2.22, 1.97), black tea (−1.16, 3.00), milk tea (3.32, 3.91)
Sugars	Sugar (26.44, 6.24) (The sugar in this case is refined sugar made from molasses extracted from sucrose and beets.)
Oils	Peanut oil (15.25, 16.09)
Salt	Edible salt (7.47, 10.17)

*The numbers in parentheses represent the average intake of each food in the case and control groups, respectively, after energy correction. Intake units in g/day. Example: food (mean intake for the case group, mean intake for the control group).

Naqu, Tibet. In addition, we selected six kinds of Tibetan food items, namely "tsampa," "ghee," "milk dregs," "cheese," "sweet tea," and "ghee tea," and analyzed the relationship between Tibetan featured foods and HAPC.

Statistical analysis

Data were entered using Epidata 3.1, and all data were collated and analyzed using R 4.1.2. Energy-corrected food group intakes were computed using the residual method. A *t*-test was used for continuous variables, and the chi-squared test was used for categorical variables to describe the differences between groups. Univariate and multivariate conditional logistic regression analyses were performed to analyze the association of dietary pattern scores and Tibetan specialty foods with HAPC. We calculated the odds ratio (OR) with 95% confidence interval (CI) values, and $p < 0.05$ was considered to be statistically significant (19, 20).

Results

Baseline characteristics and propensity score matching

A total of 1,171 Tibetan herdsmen were collected before matching in this study, and 1,159 cases were retained. About 12 participants were excluded for reporting implausible energy intake of less than 450 kcal or more than 5,000 kcal. The average total energy intake of the study subjects was 1,689.00 ± 839.25 kcal/d, the average daily intake of protein was 48.11 ± 34.16 g/d, the average daily intake of carbohydrates was 203.94 ± 120.32 g/d, and the average daily intake of fat was 76.14 ± 37.30 g/d. Therefore, we included 100 cases in the case group and 1,059 cases in the control group. And all cases were matched according to the PSM method to achieve 1:2 matching based on gender, age, BMI, length of residence, working altitude, smoking status, and alcohol status.

The baseline characteristics of the study subjects before and after matching are shown in Tables 3, 4. A *t*-test was used for continuous variables, and the chi-squared test was used for categorical variables to describe the differences between groups. The calculated *p*-values for all factors after matching were > 0.05 and the SMD values were < 0.1, which were considered indicative of good equilibrium between the case and control groups after matching.

In the case group, the average age was 43.01 ± 17.43 years, the average BMI was 26.80 ± 5.04 kg/m^2, there were 40 (40.0%) men and 60 (60.0%) women, and 99 (99.0%) participants had lived in the Naqu region of Tibet since birth. The number of people who usually worked at an altitude of > 4,500 m was 94 (94.0%). The number of smokers was 5 (5.0%) and the number of drinkers was 10 (10.0%).

TABLE 3 Baseline characteristics of study subjects before PSM.

		Control (n = 1,059)	Case (n = 100)	p	SMD
Age (years), mean (*SD*)		32.17 (13.50)	43.01 (17.43)	<0.001	0.695
BMI (kg/m^2), mean (*SD*)		24.05 (5.77)	26.80 (5.04)	<0.001	0.508
Gender (%)	Male	603 (56.9)	40 (40.0)	0.002	0.344
	Female	456 (43.1)	60 (60.0)		
Length of residence (%)	From birth	834 (78.8)	99 (99.0)	<0.001	0.680
	Immigrated	225 (21.2)	1 (1.0)		
Working altitude (%)	< 4,500 m	360 (34.0)	6 (6.0)	<0.001	0.747
	> 4,500 m	699 (66.0)	94 (94.0)		
Smoking (%)	Yes	144 (13.6)	5 (5.0)	0.021	0.299
	No	915 (86.4)	95 (95.0)		
Alcohol (%)	Yes	129 (12.2)	10 (10.0)	0.631	0.070
	No	930 (87.8)	90 (90.0)		

BMI, body mass index, *SD*, standard deviation; SMD, standardized mean difference.

TABLE 4 Baseline characteristics of study subjects after PSM.

		Control (n = 200)	Case (n = 100)	p	SMD
Age (years), mean (*SD*)		41.65 (16.09)	43.01 (17.43)	0.501	0.081
BMI (kg/m^2), mean (*SD*)		26.49 (6.51)	26.80 (5.04)	0.672	0.054
Gender (%)	Male	81 (40.5)	40 (40.0)	1.000	0.010
	Female	119 (59.5)	60 (60.0)		
Length of residence (%)	From birth	197 (98.5)	99 (99.0)	1.000	0.045
	Immigrated	3 (1.5)	1 (1.0)		
Working altitude (%)	< 4,500 m	12 (6.0)	6 (6.0)	1.000	<0.001
	> 4,500 m	188 (94.0)	94 (94.0)		
Smoking (%)	Yes	10 (5.0)	5 (5.0)	1.000	<0.001
	No	190 (95.0)	95 (95.0)		
Alcohol (%)	Yes	21 (10.5)	10 (10.0)	1.000	0.016
	No	179 (89.5)	90 (90.0)		

BMI, body mass index; *SD*, standard deviation; SMD, standardized mean difference.

In the control group, the mean age before matching was 32.17 ± 13.50 years, the mean BMI was 24.05 ± 5.77 kg/m^2, there were 603 (56.9%) men and 456 (43.1%) women, and 834 (78.8%) participants had lived in the Naqu area of Tibet since birth. The number of people who usually worked at an altitude of > 4,500 m was 699 (66.0%). The number of smokers was 144 (13.6%) and the number of alcohol drinkers was 129 (12.2%).

After matching, the mean age of the control group was 41.65 ± 16.09 years, the mean BMI was 26.49 ± 6.51 kg/m^2, there were 81 (40.5%) men and 119 (59.5%) women, and 197 (98.5%) participants had lived in the Naqu area of Tibet since birth and 3 (1.5%) had migrated here. The number of people who usually worked at an altitude of > 4,500 m was 188 (94.0%). The number of smokers was 10 (5.0%) and the number of drinkers was 21 (10.5%).

Identification of dietary patterns

According to the recommendations of the parallel analysis scree plot (Figure 1), 14 food groups were grouped into three dietary patterns. According to the scores derived from the factor loadings (Table 5), the top three food groups in the factor loadings of the first dietary pattern were eggs (0.93), meats (0.92), and milk and milk products (0.90), which were significantly characterized as being rich in protein, thus defining such dietary patterns as a "high protein pattern." The top three factor loadings of the second dietary pattern were snacks and nuts (0.81), fruits (0.76), and beans (0.64), which are not the main source of energy intake in the general sense (not staple foods, meat, or vegetables), so we defined them in general as a "snack food pattern." The top three factor loadings of the third dietary pattern were fungi and mushrooms (0.80), tea and beverages (0.58), and all vegetables (0.45), which

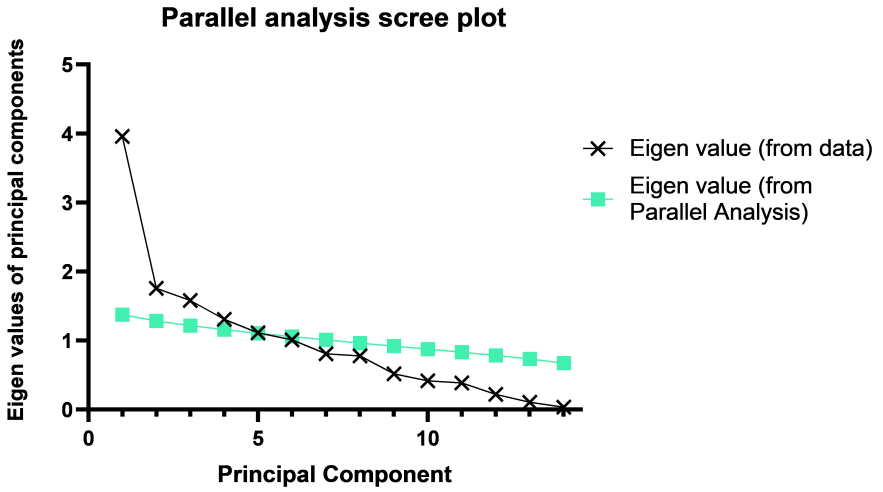

FIGURE 1
Parallel analysis scree plot. The principal component analysis of the 14 food groups and the component folds of the actual data and their slopes indicate that we should choose three principal components.

TABLE 5 Dietary pattern factor loadings.

Factor	High protein pattern	Factor	Snack food pattern	Factor	Vegetarian food pattern
Eggs	0.93	Snacks and nuts	0.81	Fungi and mushrooms	0.80
Meat	0.92	Fruits	0.76	Tea and beverages	0.58
Milk and milk products	0.90	Beans	0.64	All vegetables	0.45
Savory dishes	0.80	Savory dishes	0.12	Savory dishes	0.34
Staple foods	0.52	Fungi and mushrooms	0.09	Sugars	0.21
Salt	0.23	All vegetables	0.06	Fruits	0.14
All vegetables	0.01	Sugars	0.05	Beans	0.03
Fungi and mushrooms	0.00	Salt	0.01	Milk and milk products	0.00
Snacks and nuts	−0.07	Eggs	−0.15	Snacks and nuts	−0.12
Tea and beverages	−0.08	Milk and milk products	−0.16	Eggs	−0.14
Beans	−0.16	Meat	−0.23	Meat	−0.14
Fruits	−0.17	Staple foods	−0.23	Salt	−0.22
Sugars	−0.20	Oils	−0.26	Oils	−0.25
Oils	−0.25	Tea and beverages	−0.36	Staple foods	−0.32

were characterized by the absence of animal-based foods and belong to the vegetarian mode and therefore indicative of a "vegetarian food pattern." The variance contributions of these three dietary patterns were 0.26, 0.14, and 0.12, respectively, and their cumulative variance contribution was 0.52.

Association between dietary patterns and high-altitude polycythemia

The results from before and after adjusting for the scores of the other two dietary patterns showed (Table 6) that greater adherence to the snack food pattern and vegetarian food pattern had a significant linear correlation with the prevalence of HAPC ($p < 0.001$). In the analysis of the high protein pattern, the univariate analysis did not show a statistically significant difference, but after adjusting for the other two dietary patterns, the high protein pattern showed an association with the prevalence of HAPC. Univariate conditional logistic regression analysis showed that, for each 1-unit increase in adherence to the snack food pattern (OR, 1.84; 95% CI, 1.34–2.53), the risk of developing HAPC increased by 1.84 times; whereas the vegetarian food pattern (OR, 2.47; 95% CI, 1.73–3.54) increased the risk of HAPC by 2.47 times for each additional 1 unit of compliance.

The multifactorial conditional logistic regression analysis showed that, after adjusting for the other two dietary patterns,

TABLE 6 Conditional logistic regression analysis of dietary pattern scores.

	Control (n = 200)	Case (n = 100)	Crude		Adjusted†	
	Mean (*SD*)	Mean (*SD*)	OR (95% CI)	*p*	OR (95% CI)	*p*
High protein pattern	−0.05 (1.05)	0.11 (0.89)	1.18 (0.92–1.51)	0.202	1.52 (1.13–2.04)	0.005**
Snack food pattern	−0.18 (1.13)	0.35 (0.51)	1.84 (1.34–2.53)	<0.001***	2.11 (1.51–2.94)	<0.001***
Vegetarian food pattern	−0.24 (1.06)	0.48 (0.64)	2.47 (1.73–3.54)	<0.001***	2.80 (1.94–4.06)	<0.001***

CI, confidence interval; SD, standard deviation; OR, odds ratio. †Multifactorial conditional logistic regression, adjusted for scores of the other two dietary patterns, *$p < 0.05$, **$P < 0.1$, ***$p < 0.001$.

the OR of the high protein pattern score was 1.52 (1.13–2.04), the OR of the snack food pattern score was 2.11 (1.51–2.94), and the OR of the vegetarian food pattern score was 2.80 (1.94–4.06). All three dietary patterns were risk factors affecting the prevalence of HAPC.

Association between Tibetan featured foods and high-altitude polycythemia

The results of the univariate conditional logistic regression analysis showed (Table 7) that tsampa (OR, 1.05; 95% CI, 1.03–1.06) increased the risk of HAPC by 1.05 times for each 1-unit increase in daily intake. In addition, cheese (OR, 0.95; 95% CI, 0.93–0.98) was associated with a 0.95-fold reduction in the risk of HAPC for each additional 1 unit of daily intake, and ghee tea (OR, 0.99; 95% CI, 0.98–0.99) was associated with a 0.99-fold reduction in the risk of HAPC for each additional 1 unit of daily intake. Ghee (OR, 1.00; 95% CI, 0.99–1.00) had no effect on this disease. No correlation was found between milk dregs or sweet tea and the prevalence of HAPC.

In the multifactorial conditional logistic regression analysis, after adjusting for the average daily intake of several other specialty foods, tsampa (OR, 1.04; 95% CI, 1.03–1.05) was associated with a 1.04-fold increased risk of HAPC for each 1-unit increase in daily intake, while ghee tea (OR, 0.99; 95% CI, 0.98–1.00) was associated with a 0.99-fold reduction in the risk of HAPC for each 1-unit increase in daily intake.

The combined results of the univariate and multifactorial analyses concluded that tsampa intake is a risk factor for the development of HAPC, while ghee tea intake is a protective factor for HAPC.

Discussion

Our study focuses on the association of dietary patterns and Tibetan featured foods with HAPC in Naqu, Tibet, to explore the risk factors of HAPC in Naqu, Tibet, to raise awareness of the disease among the population and provide evidence for the development of prevention and treatment interventions. In this study, the 70 food items in the food frequency questionnaire were grouped into 14 food groups according to the similarity of food types and nutrient contents, and downscaled into three dietary patterns—namely, a "high protein pattern," "snack food pattern," and "vegetarian food pattern." According to the principal components analysis of each dietary pattern of the study subjects, univariate and multivariate conditional logistic regression analyses were conducted, respectively. Then, the average daily intakes of six foods with Tibetan characteristics (tsampa, ghee, cheese, milk dregs, sweet tea, and ghee tea) were analyzed to determine their effect on HAPC among Tibetan residents in the Naqu area.

Selection of matching variables in propensity score matching

Previous research have indicated that high-altitude adaptation occurs at the expense of being more prone to CMS (21). Lorenzo et al. (22) demonstrated that the *EGLN1* haplotype in Tibetans is associated with protection from polycythemia (22). Simonson et al. (7) suggested that Tibetan high-altitude adaptation is not determined by a single gene but instead by multiple evolved genetic adaptations acting in concert with each other. Three of these genes, *EPAS1*, *EGLN1*, and *PPARA*, regulate or are regulated by the hypoxia-inducible factor, a principal controller of erythropoiesis and other organismal functions (7). Hurtado et al. (23) demonstrated that variants in *EPAS1* correlate with lower hemoglobin concentrations, supporting their roles in maintaining a blunted erythropoietic response to lower oxygen saturation values, which is a hallmark of altitude adaptation in Tibetans (23). Julian et al. (24) demonstrated that perinatal hypoxia increases susceptibility to HAPC (24). These demonstrations of adaptation to high altitude suggested to us that genetics plays an important role in the development of HAPC among Tibetans due to their adaptation to altitude, and those individuals who newly migrate to the area are more likely to develop HAPC. Zhang et al. (25) demonstrated that an elevation of around 4,500 m represented a turning point for Tibetans, with a dramatic increase in both hemoglobin concentration and polycythemia prevalence (25). In the Naqu region of Tibet, the average altitude is > 4,500 m, and our grouping for working altitude was also bounded by

TABLE 7 Conditional logistic regression analysis of Tibetan featured foods.

	Control (n = 200)	Case (n = 100)	Crude		Adjust†	
	Mean (*SD*)	Mean (*SD*)	OR (95% CI)	*p*	OR (95%)	*p*
Tsampa	34.80 (26.35)	64.83 (22.09)	1.05(1.03–1.06)	<0.001***	1.04 (1.03–1.05)	<0.001***
Cheese	11.08 (15.07)	4.69 (5.55)	0.95 (0.93–0.98)	<0.001***	0.99 (0.95–1.03)	0.495
Milk dregs	5.07 (13.05)	5.47 (4.07)	1.00 (0.98–1.03)	0.768	1.01 (0.97–1.04)	0.776
Ghee	18.26 (107.84)	−17.48 (89.46)	1.00 (0.99–1.00)	0.009**	1.00 (0.99–1.00)	0.292
Ghee tea	98.93 (65.83)	40.01 (55.65)	0.99 (0.98–0.99)	<0.001***	0.99 (0.98–1.00)	0.002**
Tibetan sweet tea	12.63 (28.83)	12.50 (15.69)	1.00 (0.99–1.01)	0.962	1.00 (0.99–1.01)	0.947

CI, confidence interval; *SD*, standard deviation; OR, odds ratio. †Multifactorial conditional logistic regression, adjusted for mean daily intake of other featured foods, *$p < 0.05$, **$p < 0.1$, ***$p < 0.001$.

4,500 m to eliminate the confounding bias brought by altitude. In addition, the influencing factors of HAPC recognized by the medical community are gender, age, smoking status, and alcohol consumption: men are more likely to have the disease than women, older people are more likely to have the disease, and smoking and alcohol consumption could also lead to the development of HAPC.

In summary, we selected confounding factors, such as gender, age, BMI, length of residence, work altitude, smoking status, and alcohol status, which may have influenced the prevalence of HAPC in previous studies, as PSM variables to achieve a 1:2 match for all cases.

Association between dietary patterns and high-altitude polycythemia

The results of the principal component analysis showed that the 14 food groups were suggested to be interpreted in three patterns which were termed a "high protein pattern," "snack food pattern," and "vegetarian food pattern," respectively, according to their top three highest factor loadings. In previous studies, the Tibetan diet has been characterized by high meat and low vegetable intake, and Tibetan residents follow a high grain and meat intake and high fat and sodium diet pattern (10). The contribution of variance of the "high protein pattern" was 0.26, which was the highest among the three dietary patterns—that is, it was the most important among the three dietary patterns. Among them, the factor loadings of eggs, meat, and milk and milk products were all as high as 0.9 or more. This dietary pattern was not only the most dominant but also has distinctive features and was very characteristic of Tibet. In the snack food pattern, snack foods, fruits, and beans all had factor loadings greater than 0.6, and they were not universally satiating foods. They were eaten mostly between meals or with meals. In the last "vegetarian food pattern," the top three factor loadings were fungi and mushrooms, tea and beverages, and vegetables. This is a dietary pattern without animal food, which may be related to religious beliefs.

Our study showed that the mean protein and carbohydrate intakes of Tibetans were lower than the recommended intakes (26), while the total energy intake of Tibetans was also low. This is quite different from the results of a 1992 study of Naqu pastoralists (27). However, we also found that in the 1992 study, the intake of vegetables among the Naqu pastoralists was only 1.8 g/d. In our study, the intake of vegetables was 115.01 g/d and 48.36 g/d. And the intake of staple foods such as tsampa, flour, and rice all differed significantly from the 1992 findings. The intake of vegetables by the population of Nagqu has greatly increased and the intake of staple foods has decreased, and the change in dietary structure may be the main reason for the decrease in total energy intake in our study. However, there has been a decreasing trend in energy intake among residents in Tibet in recent years (28). Our results of lower energy intake and protein intake are similar to some previous studies (17, 29). However, their fat intake was higher than the recommended intake and the findings in 1992. This may be related to a variety of factors such as the vast and sparsely populated Tibet, lack of resources, religious beliefs, long, cold winters, and a low-oxygen environment. In addition to that, Tibetans may reduce the intake and consumption of energy and protein, and increase the intake of fat to keep warm at the same time in recent year. And from these three dietary patterns and energy intake, it was easy to see that the residents in this area still follow the traditional high-protein and high-fat dietary pattern of the Tibetan region. With both total energy intakes below the recommended values, Tibetans still followed a dietary pattern based on eggs, meats, and milk. The dietary variety of residents has become richer by analyzing dietary patterns, which is different from the previously focused intake of Tibetan specialty foods (17). In our results, all three dietary patterns were risk factors for the prevalence of HAPC (**Table 6**). A forest plot (**Figure 2**) shows the ORs and 95% CIs for the dietary pattern scores for the prevalence of HAPC, and a nomogram plot (**Figure 3**) shows the hazard predictions for the prevalence of HAPC for the three dietary model scores.

The cumulative variance contribution of these three dietary patterns reached 0.52, explaining the dietary patterns of more

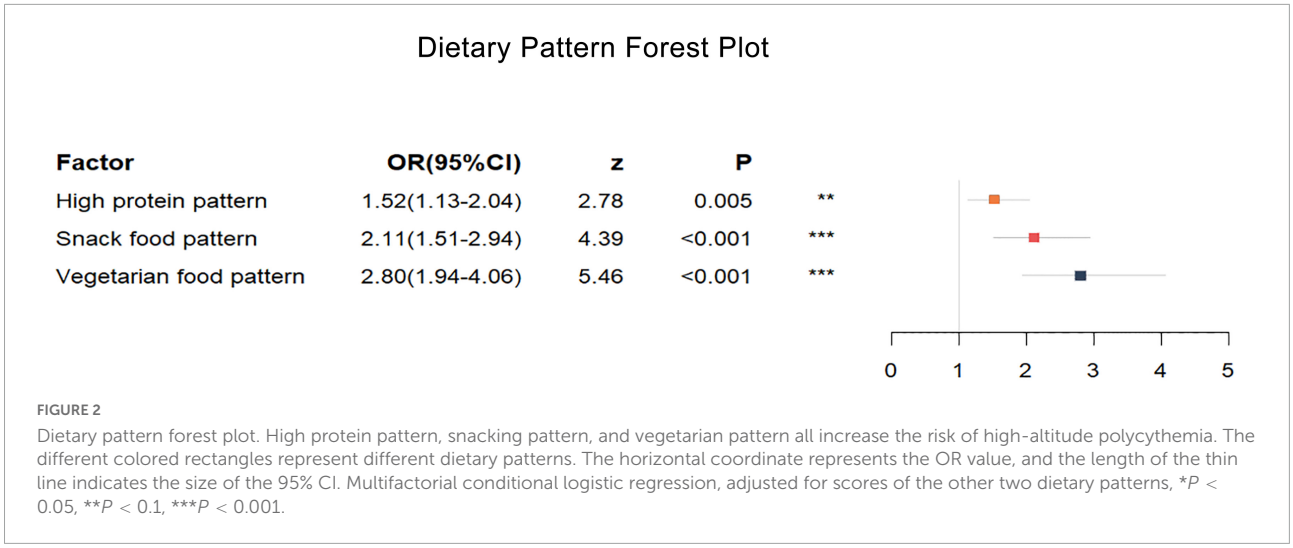

FIGURE 2
Dietary pattern forest plot. High protein pattern, snacking pattern, and vegetarian pattern all increase the risk of high-altitude polycythemia. The different colored rectangles represent different dietary patterns. The horizontal coordinate represents the OR value, and the length of the thin line indicates the size of the 95% CI. Multifactorial conditional logistic regression, adjusted for scores of the other two dietary patterns, $*P < 0.05$, $**P < 0.1$, $***P < 0.001$.

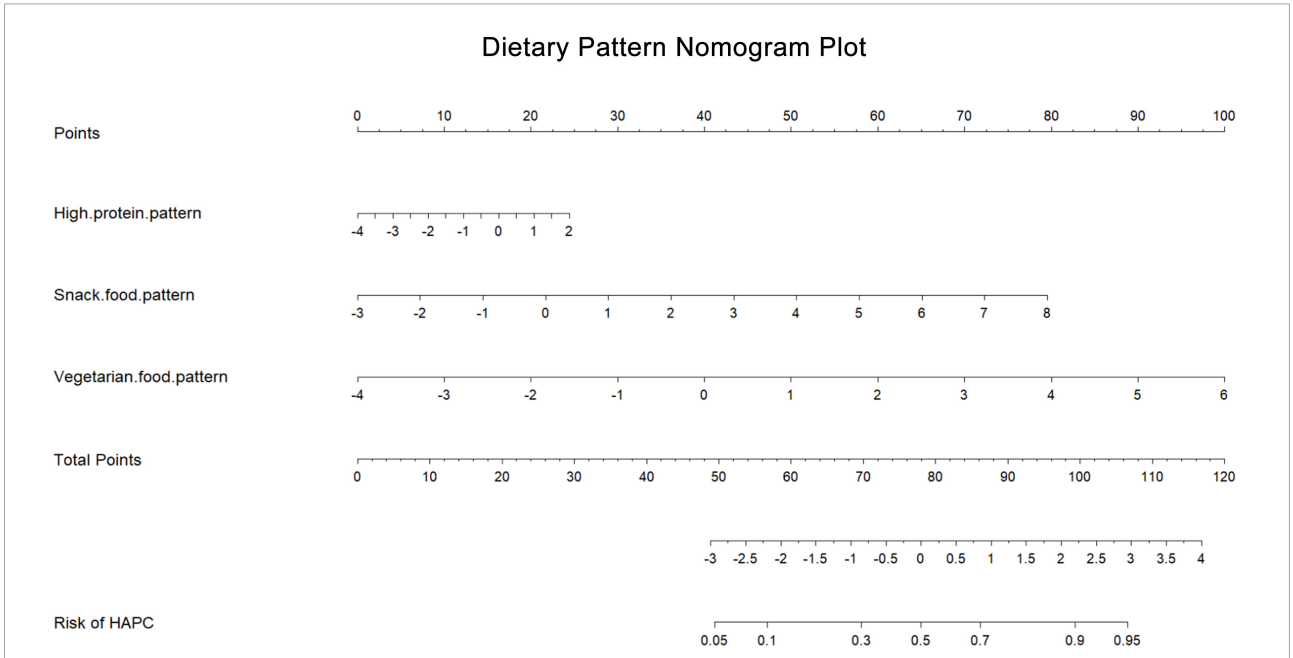

FIGURE 3
Dietary pattern nomogram plot. Based on the contribution of each dietary pattern to the prevalence of HAPC in the conditional logistic regression analysis, a score was assigned to each intake level of each dietary pattern. The individual scores were then summed to obtain the total score, which resulted in the predicted value of different dietary pattern intake levels in relation to the prevalence of HAPC.

than half of the population, but we believed that the dietary characteristics represented by these three dietary patterns have certain limitations and do not facilitate a balanced diet, that is, the currently advocated for the Mediterranean dietary pattern (30, 31). With the development of the Tibetan economy and infrastructure, the dietary characteristics of the Tibetan region have gradually changed, and residents' dietary patterns now tend to be more balanced, but an unhealthy dietary pattern is still a risk factor for HAPC. In addition, only the frequency of food intake was counted in our questionnaire; the analysis did not incorporate the specific processing of these foods by residents, and it was shown in both our study and previous ones that people in Tibetan areas have the cooking characteristics of grilling and high levels of salt addition (32). This may also affect the relationship between dietary patterns and the prevalence of HAPC. In addition, Lotti et al. demonstrated that the morning chronotype is associated with higher adherence to the Mediterranean diet (33), while Gokhale and Rao suggested that income affects dietary diversity and dietary patterns (34). These studies offer us some hints that both diet timing and

income may impact dietary patterns, which may contribute to the risk of dietary patterns causing HAPC.

Association between Tibetan featured foods and high-altitude polycythemia

The Tibetan region of China has a special alpine climate and lack of oxygen, and to adapt to this climate, Tibetans have a unique lifestyle and unique regional dietary characteristics (35). The unique regional diet of Tibetans is strongly influenced by the biogeography of the region, indigenous traditions, popular religious beliefs, and dietary taboos (17). Studies had shown that about 25% of the foods regularly consumed by Tibetans are traditional Tibetan foods, such as tsampa, yak meat, and ghee tea (11). In our study, six foods with Tibetan characteristics, namely tsampa, ghee, sweet tea, ghee tea, milk dregs, and cheese, were analyzed separately to correlate each with the prevalence of HAPC.

The results of a univariate logistic regression analysis of Tibetan specialty foods and HAPC showed that tsampa, cheese, ghee, and ghee tea were associated with the prevalence of HAPC, and cheese and ghee tea had protective effects. After adjusting for other featured foods, multifactorial logistic regression analysis showed that tsampa and ghee tea still affected the prevalence of HAPC: tsampa (OR, 1.04; 95% CI, 1.03–1.05) was a risk factor and ghee tea (OR, 0.99; 95% CI, 0.98–1.00) was a protective factor. A forest plot (**Figure 4**) shows the ORs and 95% CIs for the mean daily intake of the Tibetan featured foods according to the prevalence of HAPC, and a nomogram plot (**Figure 5**) shows the predicted risk of the mean daily intake of the featured foods relative to the prevalence of HAPC.

These Tibetan-featured foods each have their own special nutritional value. The nutrients in tsampa are mainly crude protein, crude fat, crude cellulose, total sugar, amino acids, and minerals (36). Ghee is also a typical yak dairy product, similar to butter, that is extracted from yak milk, and Tibetans consume yak and its dairy products to supplement their protein and energy intake. Compared to common butter, ghee has greater functional and nutritional values, mainly because of its higher unsaturated fatty acid content and richer functional lipids than common butter (37, 38). There are many cows and sheep in Tibetan pastoral areas, and there are also many dairy products. Among them, the representative ones are milk dregs and cheese. Milk dregs are substances left over after milk is refined from ghee; after boiling and water evaporation, what remains are milk dregs (39). The types of cheese consumed by Tibetan residents are mostly yak milk cheese, which is rich in nutritional value due to its special geographical location and climatic conditions and contains large amounts of protein, amino acids, lactose, and minerals (40). Ghee tea and sweet tea both have the function of tea in Tibet. Sweet tea is made by adding fresh milk or milk powder, white enamel, and a little salt to black tea. Ghee tea is made with ghee and brick tea and a certain amount of salt. Ghee tea and sweet tea can keep the drinker warm, replenish their energy, fight plateau reactions, and mitigate the lack of vitamins due to the low intake of fruits and vegetables (41). Tea is rich in tea polyphenols and catechins, and it is generally believed that these nutrients have a positive effect on the body by reducing blood lipids and blood pressure. Studies have shown that ghee has a certain effect on blood lipids. However, the effect of ghee tea on blood lipids is not obvious, and some studies have also shown that ghee can increase the concentration of bound catechins by decreasing the concentration of free catechins in plasma, thus altering the metabolism of catechins in the body (42, 43). These studies suggest that the effects of ghee and ghee tea on the prevalence of some diseases are not uniform among Tibetan residents in highland areas. Therefore, in this study, ghee and ghee tea were analyzed separately.

When combining our findings with previous literature, it is clear that these foods have a special protective role against many diseases. This point leads us to speculate that these Tibetan-featured foods have special nutrients that are protective against many diseases. The statistical results showed that the effect of these foods on the prevalence of HAPC was not significant, with OR values close to 1. We believe the reason for this is that the prevalence of HAPC is not influenced by the intake of featured foods alone. The intake of featured foods is common throughout the Tibetan region, but the dietary pattern is not the same for each family or individual. To explore the relationship between dietary factors and HAPC, we should not only consider it in terms of specialty foods but also return to the macroscopic dietary pattern.

In this study, we did not investigate factors such as food preparation methods and daily meal duration. The effects of food preparation methods, daily meal duration, and income on dietary patterns and thus the causes of HAPC need to be investigated. Based on the results of this study, to reduce the prevalence of HAPC, we advocate that Tibetan residents should increase their dietary diversity and balance, not eat only vegetables and fruits, eat fewer snacks, and have three regular meals; process food as simple as possible and use local, seasonal fresh fruits and vegetables as ingredients to avoid loss of trace elements and antioxidant components; use vegetable oils (containing unsaturated fatty acids) instead of animal oils (containing saturated fatty acids) in cooking.

In recent years, the economic level of the Tibetan region has developed rapidly, living conditions have improved, and food variety in the diet has increased, but many living in the Tibetan region still prefer featured foods such as ghee, ghee tea, tsampa, milk dregs, and sweet tea, and the traditional dietary habits of high protein and high-fat intakes have not changed significantly. Our study is the first to focus on the effects of dietary patterns and featured foods on the prevalence of HAPC, and the results of our analysis also show that Tibetan dietary

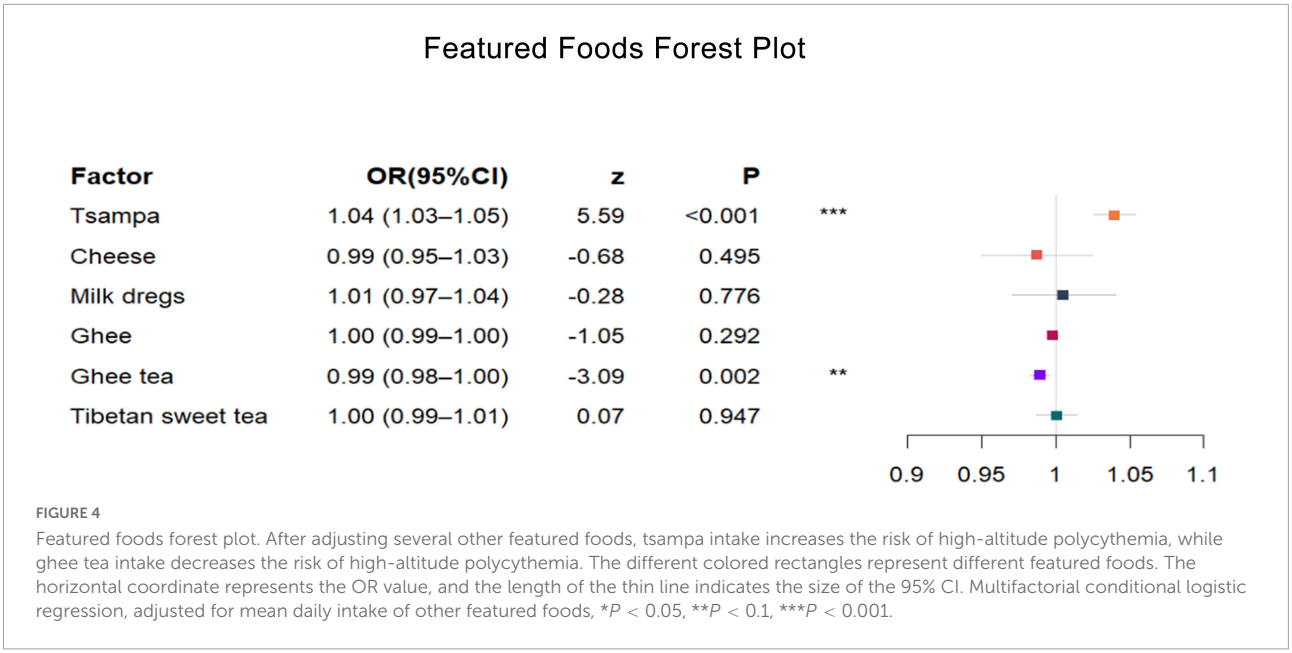

FIGURE 4
Featured foods forest plot. After adjusting several other featured foods, tsampa intake increases the risk of high-altitude polycythemia, while ghee tea intake decreases the risk of high-altitude polycythemia. The different colored rectangles represent different featured foods. The horizontal coordinate represents the OR value, and the length of the thin line indicates the size of the 95% CI. Multifactorial conditional logistic regression, adjusted for mean daily intake of other featured foods, *$P < 0.05$, **$P < 0.1$, ***$P < 0.001$.

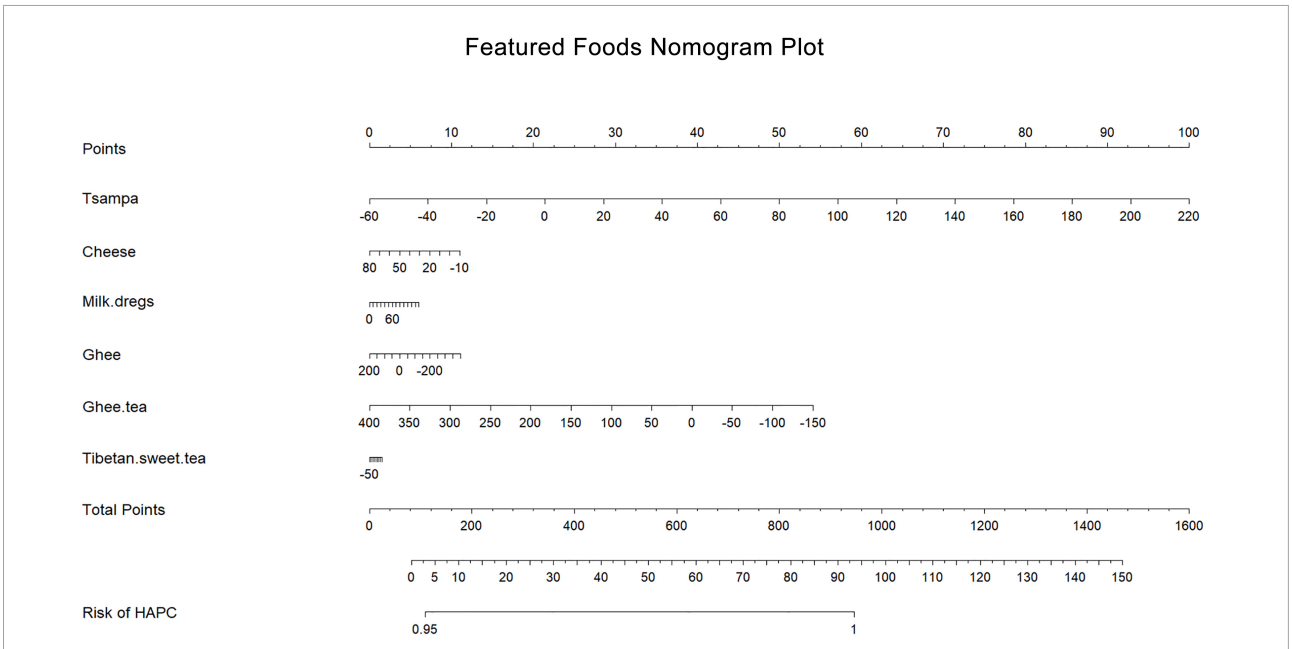

FIGURE 5
Featured foods nomogram plot. Based on the contribution of each featured food to the prevalence of HAPC in the conditional logistic regression analysis, a score was assigned to each intake level of each featured food. The individual scores were then summed to obtain the total score, which resulted in the predicted value of different featured food intake levels in relation to the prevalence of HAPC.

patterns and specialty diets are independent risk factors for HAPC in the Tibetan region.

Strengths and limitations

Our study is the first to focus on the association of dietary patterns and Tibetan-featured foods with HAPC in the Naqu region of Tibet. We included Tibetan featured foods in the FFQ to make it more regional, and the conduct of a 1:2 individual-matched case-control study makes the analysis results more convincing.

The limitations in this study are as follows: in the analysis of dietary patterns, because of the structure of the questionnaire and the excessive variety of foods, each food could only be included in large food groups and then identified in the dietary

models, making the analysis not detailed enough to accurately locate the foods that play a major influence on each dietary pattern. And we may not have included many foods in this study, thus resulting in low energy intake levels. Additionally, the cooking methods of foods and the daily meal duration of the population were not investigated. Finally, alcohol intake is an important factor affecting energy intake, and our FFQ did not address the investigation and analysis of alcohol intake. We have used PSM in our study to individually match for alcohol consumption, eliminating the effect of whether to drink alcohol on the prevalence of HAPC, and we were unable to determine the effect of alcohol intake on prevalence among drinkers.

Conclusion

Unbalanced dietary intake of the high protein pattern, snacking pattern, and vegetarian pattern all increase the risk of HAPC. Tsampa intake increases the risk of HAPC, while ghee tea intake decreases the risk of HAPC.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Naqu People's Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

JC: original draft preparation, methodology, software analysis, formal analysis, and data curation. XL and XX: investigation, resources, manuscript review, editing, and supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.946259/full#supplementary-material

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Childhood socioeconomic status and adulthood dietary diversity among Indonesian adults

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Food insecurity problems still exist among people in low-to-middle income countries. The long-term disadvantages of socioeconomic status may contribute to chronic food insecurity. However, whether childhood socioeconomic status factors are related to food insecurity in adulthood remains unclear. Thus, the aim of this study was to test the association between childhood socioeconomic status factors and one of the proxies for adulthood food security, dietary diversity. This study used the 2014 RAND Indonesia Family Life Survey dataset with 22,559 adult participants as study samples. The childhood socioeconomic status factors consisted of 16 questions about the participants' conditions when they were 12 years old. Adult dietary diversity was assessed using the United Nations World Food Programme's food consumption score. A linear regression model was used to analyze the association between variables. This study found that the number of owned books (β coef.: 3.713–7.846, $p < 0.001$), the use of safe drinking-water sources (β coef.: 0.707–5.447, $p < 0.001$–0.009) and standard toilets (β coef.: 1.263–4.955, $p < 0.001$–0.002), parents with the habit of alcohol consumption (β coef.: 2.983, $p = 0.044$) or the combination with smoking habits (β coef.: 1.878, $p < 0.001$), self-employed with the permanent worker (β coef.: 2.904, $p = 0.001$), still married biological parents (β coef.: 1.379, $p < 0.001$), the number of rooms (β coef.: 0.968, $p < 0.001$), people (β coef.: 0.231, $p < 0.001$), and younger siblings (β coef.: 0.209–0.368, $p < 0.001$–0.039) in the same house were positively and significantly associated with the outcome variable. Furthermore, in the order of childhood socioeconomic status factors, self-employment without permanent workers and casual work types (β coef.: −9.661 to −2.094, $p < 0.001$–0.001), houses with electricity facilities (β coef.: −4.007, $p < 0.001$), and parents with smoking habits (β

coef.: −0.578, p = 0.006) were negatively and significantly associated with the food security proxy. In conclusion, childhood and early socioeconomic disadvantage is related to adult food security status and may lead to poor health.

KEYWORDS

dietary diversity, adult, childhood, socioeconomic status, Indonesian

Introduction

The Indonesian population who lived below the national poverty line in 2021 was around 10% (1, 2). The proportion of the employed population earning less than one dollar ninety cents purchasing power parity per day decreased from 10.4% in 2013 to 3.5% in 2019 (1). Besides the income level used to assess poverty, a previous researcher suggested child malnutrition as one of the poverty indicators (3). Meanwhile, Indonesia faces a child malnutrition problem called the "double burden of malnutrition" (4). For example, 24 children die for every 1,000 babies born in Indonesia in 2020 before they reach their fifth birthday (2).

Furthermore, socioeconomic status is related to people's health status. However, economic situations such as poverty are related to a public health problem called food insecurity (5, 6). Thus, Indonesia is still dealing with food insecurity problems (6–8). Food insecurity is defined as an individual's hardship in maintaining a healthy and active life with the consumption of a nutritious and balanced diet (9, 10). Another definition of food insecurity is a person's complex situation that maintains the sustainability of food availability, food accessibility, and utilization of food for them to live an active and healthy lifestyle (11). Therefore, people who live below the poverty line or have food insecurity are more likely to have difficulty providing a nutritious and balanced diet for themselves and their families.

Food insecurity has three types based on its duration: chronic, transitory, and seasonal (12, 13). Chronic food insecurity is mainly due to persistent and long-term causes such as poverty. The impact of food insecurity on a person's life includes the potential for adverse physical or mental health outcomes (7, 14–16). Furthermore, the long-term disadvantage in socioeconomic status may also contribute to chronic food insecurity (17, 18). Parents' long-term income volatility and economic deprivation contribute to their children's socioeconomic and food security status. Moreover, socioeconomic status during childhood is associated with adult health status and behavior (19–22). However, it is still unclear which socioeconomic status factors during childhood are related to adulthood food insecurity.

Conversely, food insecure people who live in urban areas and low-income people are considered vulnerable targets of the food insecurity intervention program. Food security assessment includes the following three pillars: availability, accessibility, and utilization of food (23). Food-secure people are more likely to consume more diverse foods because they have access to many available food types and their bodies can utilize foods well (24, 25). Dietary diversity is positively associated with food security pillars (24, 26). Dietary diversity is a qualitative measurement of food consumption that reflects the variety of foods accessed and is a proxy for nutrient adequacy (27). Meanwhile, one of the food security measurements, the food consumption score, considers dietary diversity and food frequency in composite score analysis (28, 29). Thus, we aimed to test the association between childhood socioeconomic status factors and food consumption scores.

Materials and methods

Dataset

This study used data from the Indonesia Family Life Survey (IFLS) by the RAND Corporation. The first IFLS collected samples representing approximately 80% of Indonesia's population in 1993 (30). We used the fifth wave of the RAND-IFLS 2014 datasets. We included the basic information of the participants, such as anthropometric measurements, dietary information based on the 7 days before the survey, and information related to their health status. Our sample participants were those with complete data, were not breastfed or pregnant, did not have any disabilities, and were never diagnosed with cancer to minimize the analysis bias. This study included 22,559 adult participants aged 18–64 years. Trained nurses collected anthropometric (e.g., height, body weight, and waist circumference for the participants $\geq$ 40 years) and health-related data (i.e., blood pressure). The body mass index calculation uses body weight and height and is categorized into three groups based on the Indonesian BMI cutoff points (31). Abdominal obesity was defined as having a waist circumference cutoff point larger than 90 and 80 cm for men and women, respectively. Furthermore, we calculated physical activity assessment using the frequency, duration, and metabolic equivalent

of task (MET) scores for each type of physical activity intensity (32). The data selection process is illustrated in **Supplementary Figure 1**.

Childhood socioeconomic status factors and the outcome variable

The 2014 Indonesian Family Life Survey questionnaire included questions on childhood socioeconomic status. The assessment of childhood socioeconomic status uses subset questions from the China Health and Retirement Longitudinal Study (CHARLS) (30, 33). The childhood socioeconomic status questionnaire consists of information about the parents' behavior, family, and housing situation when participants were 12 years old. The family situation section consisted of three questions about who lived with the participant, whether they lived with their biological mother or father, or whether their parents were still married. The housing situation questions include electricity and a standard toilet facility, drinking water source type, number of rooms, and people and siblings who lived in the same dwelling (30). A list of questions is presented in **Supplementary Table 1**. Childhood socioeconomic variables use categorical data except for the variables of the number of rooms, people, and siblings.

The aim of this study was to examine the association between socioeconomic status during childhood and food security proxy dietary diversity during adulthood. Furthermore, our study outcome variable is the food consumption score because the use of the food frequency questionnaire is relevant for food security assessment if defined by dietary diversity and food frequency (24, 29). The food consumption score used continuous data for statistical analysis. The United Nations World Food Programme (WFP) has developed a way to analyze food security using the dietary diversity proxy approach, called food score analysis, which results in a food consumption score (29). This study's analysis of food scores used the number of days of ten eaten food types listed in the IFLS Food Frequency Questionnaire (FFQ). The steps to analyze food scores were to group the 10 food types into five groups (i.e., staples, protein, dairy products, fruits, and vegetables) and multiply the days of the food types with the score weighted for each food group. The weighted score is based on the food group's nutrient density. The next step is to summarize the food group scores into scores for food consumption, which are further categorized into three levels of food consumption (i.e., poor, borderline, and acceptable) and two classes of food security (15, 29). The food security classes are food secure if the score falls within the acceptable level and food insecure if the score falls within the poor and borderline score.

The ethical matter and statistical analysis

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the RAND Corporation in the United States and the University of Gajah Mada in Indonesia. The RAND's Human Subjects Protection Committee (RAND IRB) gave IFLS5 s0064-06-01-CR01. Informed consent was obtained from all participants involved in the study prior to data collection from the Indonesian Family Life Survey. Secondary data from the RAND-IFLS 2014 were used, and adult participants' characteristics were reported as numbers (percentages) for categorical data and mean ± standard deviation for continuous data. Chi-square tests and *t*-tests were used to report the participants' characteristics. We used a linear regression model to assess the association between food consumption and childhood socioeconomic status variables. The results of the linear regression are presented as coefficients and confidence intervals. Adjustments for the regression model included age and sex variables. Furthermore, *a* p-value < 0.05 was set as statistically significant. We used the STATA statistical software (v17.1; Stata Corp. LP, College Station, TX, USA).

Results

Study participants' characteristics

The aim of this study was to assess the association between childhood socioeconomic status and adulthood food security proxy using RAND-IFLS 2014 secondary data. The total number of participants was 22,559, including 11,594 men and 10,965 women. The mean age of the participants was 38 ± 12 years, while the mean age of the men and women was 37 ± 12 and 38 ± 13 years, respectively. As shown in **Table 1**, most participants attended academic education for less than 12 years, with 11,888 people (52.70% of the total participants). Furthermore, 18,883 participants (83.70% of the total participants) were currently or ever married.

Meanwhile, 13,411 participants (59.45% of the total participants) had never smoked. The physical activity volumes of moderate and vigorous exercise among women were lower than in men ($p < 0.001$). Moreover, the mean food consumption score among women (34.34 ± 14.79) was lower than that of men (35.10 ± 14.76), with a p-value less than 0.001. However, women had a higher mean body mass index, body shape index, and waist circumference than men. The number of women with abdominal obesity, classified as overweight or obese, and diagnosed with cardiovascular diseases was significantly higher than that of men (**Table 1**).

TABLE 1 Participants characteristics.

Variable	All	Men	Women	*P*-value
N	22,559 (100.00)	11,594 (51.39)	10,965 (48.61)	
Age (years), mean ± SD	38 ± 12	37 ± 12	38 ± 13	<0.001
Academic attainment, *n* (%)				<0.001
Less than 12 years	11,888 (52.70)	5,654 (48.77)	6,234 (56.85)	
More than equal to 12 years	10,671 (47.30)	5,940 (51.23)	4,731 (43.15)	
Matrimonial situation, *n* (%)				<0.001
Never married	3,676 (16.30)	2,266 (19.54)	1,410 (12.86)	
Currently or ever married	18,883 (83.70)	9,328 (80.46)	9,555 (87.14)	
Housing areas, *n* (%)				0.240
Rural	9,125 (40.45)	4,733 (40.82)	4,392 (40.05)	
Urban	13,434 (59.55)	6,861 (59.18)	6,573 (59.95)	
Smoking habit, *n* (%)				<0.001
Non-smoker	13,411 (59.45)	2,780 (23.98)	10,631 (96.95)	
Ex-smoker	1,006 (4.46)	936 (8.07)	70 (0.64)	
Smoker	8,142 (36.09)	7,878 (67.95)	264 (2.41)	
Food consumption score, mean ± SD	34.73 ± 14.78)	35.10 ± 14.76	34.34 ± 14.79	<0.001
Moderate PA volume (METs min/w), mean ± SD	1951.02 ± 2077.35)	2010.93 ± 2160.63	1894.32 ± 1993.84	<0.001
Vigorous PA volume (METs min/w), mean ± SD	4755.87 ± 4689.75)	5117.28 ± 4814.12	3483.47 ± 3970.80	<0.001
Waist circumference (cm), mean ± SD	84.64 ± 11.67)	83.16 ± 11.16	86.07 ± 11.98	< 0.001
Abdominal obesity[a], *n* (%)				<0.001
No	4,748 (51.10)	3,322 (72.74)	1,426 (30.19)	
Yes	4,543 (48.90)	1,245 (27.26)	3,298 (69.81)	
Body shape index ($m^{11/6}\ kg^{-2/3}$), mean ± SD	0.0812 ± 0.0058	0.0806 ± 0.0050	0.0819 ± 0.0065	<0.001
Body mass index (kg/m^2), mean ± SD	23.56 ± 4.43	22.58 ± 3.90	24.59 ± 4.71	<0.001
Body mass index classification[b], *n* (%)				<0.001
<18.5	2,279 (10.10)	1,424 (12.28)	855 (7.80)	
18.5–25.0	12,792 (56.70)	7,359 (63.47)	5,433 (49.55)	
25.1–27.0	2,873 (12.74)	1,260 (10.87)	1,613 (14.71)	
>27.0	4,615 (20.46)	1,551 (13.38)	3,064 (27.94)	
Systolic blood pressures (mmHg), mean ± SD	128.82 ± 19.87	130.38 ± 17.51	127.17 ± 21.97	<0.001
Diastolic blood pressures (mmHg), mean ± SD	79.36 ± 12.05	79.46 ± 11.82	79.24 ± 12.30	0.171
Hypertension, *n* (%)				0.308
No	15,534 (68.86)	8,019 (69.17)	7,515 (68.54)	
Yes	7,025 (31.14)	3,575 (30.83)	3,450 (31.46)	
Cardiovascular diseases[c], *n* (%)				<0.001
No	22,112 (98.02)	11,406 (98.38)	10,706 (97.64)	
Yes	447 (1.98)	188 (1.62)	259 (2.36)	

METs min/w, metabolic equivalent of tasks for minutes per week; PA, physical activity; SD, standard deviation. The categorical data were presented using *n* (%), and the continuous data were presented using mean ± SD.

[a]The definition of abdominal obesity for women and men was based on waist circumference with cutoff points > 80 cm or > 90 cm, respectively.

[b]The body mass index was calculated using the adult categorization of body mass index for the Indonesian population.

[c]Cardiovascular diseases are defined as the event of any stroke or cardiac heart disease that is diagnosed by the doctor. A significant *p*-value was set to <0.05.

Childhood socioeconomic status

Table 2 shows the distribution of childhood economic status by gender. Childhood socioeconomic status was measured when the participants were 12 years old. Childhood socioeconomic status was divided into three parts to simplify the table. First, the family situation consists of the participants' family conditions, such as the parents' marriage life, where and with whom the participants lived at that time and the job type of the breadwinner in the house. Second, parental behavior consisted of smoking habits, heavy alcoholic beverage consumption, and whether parents had mental problems. The last part was the participants' housing situations, such as electricity and toilet ownership, drinking water source type, the number of books they owned, and the number of people and siblings who lived in the same dwelling.

Furthermore, in the family situation, most of the participants' parents were still married ($p < 0.001$). The number of participants who lived with their biological mothers was significantly higher among men (n = 10,809) than among women (n = 10,146), with a p-value of 0.041. The number of participants who lived with their biological fathers was also significantly higher among men than among women ($p = 0.008$). Regarding parental behaviors, the percentage of parents with smoking habits, mental health problems, or alcoholic beverage consumption was higher among male participants ($p < 0.001$)

TABLE 2 Distribution of childhood socioeconomic status.

Variable	All	Men	Women	*P*-value
Family situation				
Married biological parents				<0.001
No	2,133 (9.46)	1,006 (8.68)	1,127 (10.28)	
Yes	20,426 (90.54)	10,588 (91.32)	9,838 (89.72)	
Live with biological mother				0.041
No	1,604 (7.11)	785 (6.77)	819 (7.47)	
Yes	20,955 (92.89)	10,809 (93.23)	10,146 (92.53)	
Live with biological father				0.008
No	2,818 (12.49)	1,382 (11.92)	1,436 (13.10)	
Yes	19,741 (87.51)	10,212 (88.08)	9,529 (86.90)	
Live at the born place				0.284
No	18,171 (80.55)	9,307 (80.27)	8,864 (80.84)	
Yes	4,388 (19.45)	2,287 (19.73)	2,101 (19.16)	
Employment type of the breadwinner				0.100
Government/Private worker	6,056 (26.85)	3,169 (27.33)	2,887 (26.33)	
Self-employed	5,052 (22.39)	2,514 (21.68)	2,538 (23.15)	
Self-employed with temporary worker	8,012 (35.32)	4,173 (35.99)	3,839 (35.01)	
Self-employed with permanent worker	288 (1.28)	150 (1.29)	138 (1.26)	
Unpaid family worker	27 (0.12)	12 (0.10)	15 (0.14)	
Casual worker in agriculture	1,477 (6.55)	726 (6.26)	751 (6.85)	
Casual worker not in agriculture	1,387 (6.15)	722 (6.23)	665 (6.06)	
Transfer	144 (0.64)	70 (0.60)	74 (0.67)	
Pension	116 (0.51)	58 (0.50)	58 (0.53)	
Parental behaviors				
Smoke, *n* (%)				<0.001
No	7,096 (31.46)	3,417 (29.47)	3,679 (33.55)	
Yes	15,463 (68.54)	8,177 (70.53)	7,286 (66.45)	
Heavily alcohol beverages consumption, *n* (%)				0.011
No	22,459 (99.56)	11,530 (99.45)	10,929 (99.67)	
Yes	100 (0.44)	64 (0.55)	36 (0.33)	
Have mental problems, *n* (%)				0.043
No	22,548 (99.95)	11,585 (99.92)	10,963 (99.98)	
Yes	11 (0.05)	9 (0.08)	2 (0.02)	
Parental behaviors combination, *n* (%)				
Smoke + Heavily alcohol beverages consumption				<0.001
No	21,760 (96.46)	11,077 (95.54)	10,683 (97.43)	
Yes	799 (3.54)	517 (4.46)	282 (2.57)	
Smoke + Have mental problems				0.104
No	22,515 (99.80)	11,566 (99.76)	10,949 (99.85)	
Yes	44 (0.20)	28 (0.24)	16 (0.15)	
Smoke + Alcohol + Mental problems				0.198
No	22,546 (99.94)	11,585 (99.92)	10,961 (99.96)	
Yes	13 (0.06)	9 (0.08)	4 (0.04)	
Housing situation				
Having electricity, *n* (%)				0.158
No	10,000 (44.33)	5,192 (44.78)	4,808 (43.85)	
Yes	12,559 (55.67)	6,402 (55.22)	6,157 (56.15)	
Toilet ownership, n (%)				0.031
Yes, with septic tank	9,023 (40.00)	4,595 (39.63)	4,428 (40.38)	
Yes, without septic tank	3,443 (15.26)	1,784 (15.39)	1,659 (15.13)	
Shared toilet	894 (3.96)	428 (3.69)	466 (4.25)	
Public toilet	1,840 (8.16)	994 (8.57)	846 (7.72)	
Others	7,359 (32.62)	3,793 (32.72)	3,566 (32.52)	
Drinking-water source type, *n* (%)				0.030
Piped water	2,911 (12.90)	1,484 (12.80)	1,427 (13.01)	
Closed-well/Pump (Electric, Hand)	3,386 (15.01)	1,699 (14.65)	1,687 (15.39)	
Opened-well water	11,719 (51.95)	6,061 (52.28)	5,658 (51.60)	
Mineral water	615 (2.73)	286 (2.47)	329 (3.00)	
Others	3,928 (17.41)	2,064 (17.80)	1,864 (17.00)	

(Continued)

TABLE 2 (Continued)

Variable	All	Men	Women	*P*-value
Number of book in the house, *n* (%)				0.068
None or very few (0–10 books)	18,344 (81.32)	9,382 (80.92)	8,962 (81.73)	
Enough to fill 1 shelf (11–25 books)	2,814 (12.47)	1,459 (??)	1,355 (12.36)	
Enough to fill 1 bookcase (26–100 books)	1,129 (5.00)	594 (5.12)	535 (4.88)	
Enough to fill 2 bookcases (101–200 books)	172 (0.76)	95 (0.82)	77 (0.70)	
Enough to fill 2 or more bookcases (more than 200 books)	100 (0.44)	64 (0.55)	36 (0.33)	
Number of people live in the same house, mean ± SD	6.80 ± 2.90	6.16 ± 2.32	6.33 ± 2.63	<0.001
Number of room in the house, mean ± SD	3.49 ± 1.75	3.61 ± 1.67	3.65 ± 1.64	0.070
Number of older brother, mean ± SD	0.82 ± 1.22	0.78 ± 1.06	0.75 ± 1.04	0.032
Number of older sister, mean ± SD	0.74 ± 0.99	0.72 ± 0.99	0.71 ± 1.02	0.455
Number of younger brother, mean ± SD	1.06 ± 1.23	0.75 ± 0.99	0.82 ± 1.02	<0.001
Number of younger sister, mean ± SD	1.02 ± 1.29	0.72 ± 0.95	0.78 ± 1.03	<0.001

SD, standard deviation. The categorical data were presented using number (%), and the continuous data were presented using mean ± SD. Significant *p*-value was set to < 0.05.

and (p = 0.011–0.043), respectively. The number of parents with alcoholic beverage consumption and mental health problems was low. The percentage of a combination of parental behavior (smoking habit and heavily alcoholic beverage consumption) was significantly higher among men (4.46%) than women (2.57%), with a p-value less than 0.001.

Furthermore, in the housing situation part, most participants had toilets with a septic tank in the house, while the drinking water source type was open-well water (p = 0.030–0.031). A toilet with a septic tank in a house is one of the standards of living in Indonesia. The number of people, books, older brothers, younger sisters, and younger brothers who live in the same dwelling as the participants was significantly different between male and female participants (p < 0.001–0.032).

Association between childhood socioeconomic status and food security proxy

Table 3 shows the association between childhood socioeconomic status and food consumption scores. In this study, the food consumption score represents the food security proxy, named dietary diversity. In the family situation of childhood socioeconomic factors, a variable of biological parents who were still married was positively and significantly associated with the food consumption score in both crude and adjusted models (p < 0.001). The employment types of the breadwinners in the house were significantly associated with the food consumption score compared to the government/private worker type in both crude and adjusted models (p < 0.001–0.001), except for transfer and pension. The self-employed with permanent workers were positively associated (p = 0.001) with the outcome variable compared to the government/private workers, with exponentiated β-coefficients of 2.920 (95% CI: 1.188–4.652) in the crude model to 2.904 (95% CI: 1.172–4.636) in the adjusted model. Meanwhile, other employment types of breadwinners were negatively associated with the outcome variable (p < 0.001). Conversely, parents' smoking habits in the parent's behavior part were negative and significantly associated with the food consumption score (exponentiated β-coefficients of –0.540 (95% CI: –0.956 to –0.125, p = 0.011) in the crude model to –0.578 (95% CI: –0.993 to –0.162, p = 0.006) in the adjusted model). In contrast, the consumption of heavy alcoholic beverages and the combination of smoking and heavy alcoholic beverage consumption in parents' behavior was positively and significantly associated with the food consumption score (p < 0.001–0.044).

Regarding the housing situation of the childhood socioeconomic factors, owning an electricity facility in the house during childhood was significantly negatively associated with adulthood's food consumption score in both regression models (p < 0.001). Toilet ownership (i.e., toilet ownership with a septic tank, public toilet, and shared toilet) was positively and significantly associated with food consumption scores (p < 0.001–0.015). The drinking water source type variable was positively and significantly associated with the food consumption score (p < 0.001–0.012). Furthermore, the number of books owned in the house was positively and significantly associated with the outcomes [exponentiated β-coefficients of 3.624–7.267 (95% CI: 3.043–8.147, p < 0.001)] in the crude model and the adjusted model (exponentiated β-coefficients of 3.713–7.333 (95% CI: 3.129–8.214, p < 0.001). Moreover, in the housing situation, the number of people who lived in the same dwelling was significantly positively associated with the food consumption score in both the crude and adjusted models, with exponentiated β-coefficients of 0.224 (95% CI: 0.146–0.301) to 0.231 (95% CI: 0.152–0.309), with p < 0.001. The food consumption score will increase by 0.224–0.231 units for every one-unit increase in the number of people who live in the same house as participants during childhood. The food consumption score increased by 0.939–0.968 units for every one-unit increase in the number of owned rooms in the participants' houses when they were 12 years old (p < 0.001). Finally, the food consumption score increased by 0.198–0.209

TABLE 3 Regression model of the association between childhood socioeconomic status and outcome.

Variables	Crude				Adjusted			
	Coef.	95% CI		*p* value	Coef.	95% CI		*P*-value
Family situation								
Married biological parents (ref.: no)	1.402	0.743	2.061	<0.001	1.379	0.719	2.039	<0.001
Live with biological mother (ref.: no)	0.365	−0.385	1.116	0.340	0.348	−0.403	1.098	0.364
Live with biological father (ref.: no)	0.531	−0.052	1.115	0.074	0.515	−0.069	1.098	0.084
Live at the born place (ref.: yes)	−0.206	−0.694	0.281	0.406	−0.208	−0.696	0.280	0.404
Employment status of the breadwinner								
Government/Private worker	**Ref.**				**Ref.**			
Self-employed	−2.088	−2.635	−1.540	<0.001	−2.094	−2.642	−1.546	<0.001
Self-employed with temporary worker	−2.394	−2.883	−1.905	<0.001	−2.432	−2.923	−1.941	<0.001
Self-employed with permanent worker	2.920	1.188	4.652	0.001	2.904	1.172	4.636	0.001
Unpaid family worker	−9.650	−15.189	−4.110	0.001	−9.661	−15.200	−4.123	0.001
Casual worker in agriculture	−7.549	−8.382	−6.715	<0.001	−7.588	−8.424	−6.752	<0.001
Casual worker not in agriculture	−3.836	−4.691	−2.981	<0.001	−3.817	−4.672	−2.962	<0.001
Transfer	−1.559	−3.981	0.862	0.207	−1.469	−3.891	0.952	0.234
Pension	−0.287	−2.979	2.406	0.835	−0.299	−2.990	2.393	0.828
Parental behaviors								
Smoke (ref.: no)	−0.540	−0.956	−0.125	0.011	−0.578	−0.993	−0.162	0.006
Heavily alcohol beverages consumption (ref.: no)	3.066	0.164	5.969	0.038	2.983	0.080	5.886	0.044
Having mental problems (ref.: no)	−2.638	−11.373	6.098	0.554	−2.872	−11.606	5.862	0.519
Parental behaviors combination								
Smoke + Heavily alcohol beverages consumption (ref.: no)	1.964	0.921	3.007	<0.001	1.878	0.833	2.923	< 0.001
Smoke + Having mental problems (ref.: no)	−1.298	−5.669	3.073	0.561	−1.398	−5.768	2.972	0.531
Smoke + Alcohol + Mental problems (ref.: no)	3.506	−4.531	11.542	0.393	3.363	−4.671	11.397	0.412
Housing situation								
Having electricity (ref.: yes)	−2.791	−3.177	−2.404	<0.001	−4.007	−4.465	−3.550	< 0.001
Toilet ownership								
Yes, with septic tank	4.351	3.901	4.802	<0.001	4.955	4.479	5.430	< 0.001
Yes, without septic tank	0.137	−4.790	−3.640	0.651	0.318	−0.275	0.911	0.293
Shared toilet	1.257	−4.101	−2.089	0.015	1.576	0.558	2.593	0.002
Public toilet	1.103	−3.982	−2.514	0.004	1.263	0.515	2.011	0.001
Others	**Ref.**				**Ref.**			
Drinking-water source type								
Piped water	4.084	3.379	4.788	<0.001	4.421	3.707	5.134	< 0.001
Closed-well/Pump (Electric, Hand)	3.171	2.495	3.847	<0.001	3.586	2.897	4.276	< 0.001
Opened-well water	0.684	0.153	1.216	0.012	0.707	0.176	1.238	0.009
Mineral water	4.639	3.389	5.889	<0.001	5.447	4.170	6.724	< 0.001
Other	**Ref.**				**Ref.**			
Number of book in the house								
None or very few (0–10 books)	**Ref.**				**Ref.**			
Enough to fill 1 shelf (11–25 books)	3.624	3.043	4.205	<0.001	3.713	3.129	4.298	< 0.001
Enough to fill 1 bookcase (26–100 books)	7.267	6.387	8.147	<0.001	7.333	6.452	8.214	< 0.001
Enough to fill 2 bookcases (101–200 books)	7.676	5.478	9.874	< 0.001	7.701	5.504	9.898	< 0.001
Enough to fill 2 or more bookcases (more than 200 books)	7.912	5.035	10.789	< 0.001	7.846	4.970	10.722	< 0.001
Number of people live in the same house	0.224	0.146	0.301	< 0.001	0.231	0.152	0.309	< 0.001
Number of room in the house	0.939	0.823	1.055	< 0.001	0.968	0.850	1.085	< 0.001
Number of older brother	−0.012	−0.196	0.172	0.900	−0.018	−0.202	0.166	0.848
Number of older sister	0.129	−0.063	0.321	0.189	0.125	−0.068	0.317	0.204
Number of younger brother	0.350	0.158	0.541	< 0.001	0.368	0.173	0.563	< 0.001
Number of younger sister	0.198	0.003	0.392	0.046	0.209	0.011	0.407	0.039

The adjustment variables are age and gender. Significant *p*-value was set to < 0.05.

units and by 0.350–0.368 units for every one-unit increase in the number of younger sisters and younger brothers who lived in the same house as the participants during childhood, respectively ($p < 0.001$–0.046).

Discussion

The aim of this study was to assess the association between childhood socioeconomic status and food consumption scores.

Most of the participants in this study were men aged <40 years, had low educational attainment, were married, or had ever had a marriage experience, and reported that they never had smoking habits. Furthermore, the food consumption score and physical activity volume were low among the female participants. Meanwhile, the mean body mass index, waist circumference, body shape index, and percentage of participants with abdominal obesity classified as overweight and diagnosed with CVD were high among women. Based on our study results, female participants are more likely to be food insecure, have less than 12 years of educational attainment, and have a disadvantaged health status (e.g., obesity and CVD). Previous researchers have suggested that vulnerable targets of food insecurity include women, people with low education levels, and people with low socioeconomic status, which leads to their poor health status (34–39). Low education levels lead to poor employment and low income, which leads to poverty and food insecurity among women, particularly those who live alone or are single parents (40–42). Among all the vulnerable targets, we still need to identify the determinant factors and see a prospective solution using multidisciplinary integrated approaches to solve food insecurity problems. Identifying determinant factors may start with socioeconomic status factors during childhood, which contributes to the experience of chronic food insecurity. This study assessed how socioeconomic status during childhood is associated with the food insecurity proxy or dietary diversity in adulthood.

Furthermore, childhood socioeconomic status was assessed using 16 questions and presented in three parts to simplify the reading results table and better understand the concept. Part one was the family part, which was about the situation of parental marriage-life during the participant's life in childhood. Our study results showed that parents who were still married were positively and significantly associated with the food consumption score, which means that parental marital status change (i.e., becoming divorced, widowed, or separated) will decrease the food consumption score by 1.379 units. Children who live with married parents have a better chance of accessing various available foods. Parental socioeconomic status, including education, job, and financial factors, is related to parenting style, which may explain the association between parents' marital status and the outcomes. The parents' situation affects how they treat and feed their children. The healthy human body absorbs and utilizes adequate quality nutrients, which results in good health status. In addition, healthcare needs are related to individual health status, particularly for children, because they also need good nutrients to grow. Meanwhile, children with two parents are more likely to have met their healthcare needs than children with single mothers (43–45). Healthcare needs are important for all household members because they are related to food utilization (46), which is one of the pillars of food security.

Furthermore, parents' socioeconomic status depends on their job type and income level. The job types in Indonesia commonly have stable income government/private workers. Another job type with a stable income is self-employed permanent workers, which shows a positive and significant association with the food consumption score. Our study participants' parents with casual workers or other types of self-employed jobs were negatively and significantly associated with the food consumption score, with government/private workers as a reference in the parents' job type regression analysis. The regression analysis showed that any changes in the parents' job type, particularly in job types with an unstable income, will decrease the score of food consumption by 2.094–9.661 units. Financial resources and environmental food factors may affect the individual's food security status (e.g., from the food access or food availability pillar) differently based on their geographical areas (47). Financial resources may be the key to food access. For example, although food is available, if the individual has difficulty buying or reaching the nearest food market, there will be a food insecurity problem. The difficulty in buying food may be affected by the type of breadwinner's job and low or unstable income. A family with a stable income is more likely to have sustainable access to the available food in the market (48).

Moreover, self-employed workers or those self-employed with temporary workers may be more likely to receive unstable incomes because they do not guarantee their specific income. Although the size of the self-employed company may affect the income amount, when there is a fluctuation in the economic situation because of some issues in the political or social environment, these people may be more impacted than those with a stable income (48). However, the self-employed with permanent workers may provide a more stable income due to their ability to pay salaries regularly or monthly for their workers, which means they have enough benefits for themselves.

Meanwhile, casual workers (in or not in the agriculture sector) are associated with the climate, geography, and environmental situation. Some changes in climate or natural disasters affect agricultural results or harvest times (49, 50). The harvest amount and quality affect the worker's income, which relates to the food access of food insecurity pillars if they sell the crops and will affect their food stock (related to food access and availability) if they consume it for themselves. An individual's access to the variety of available foods in the market eventually affects their dietary diversity and food frequency.

Besides the parental situation, part two of the childhood socioeconomic factors was about the parents' behavior. This study found that in the parental behavior part, smoking and alcoholic beverage consumption habits, in particular, were associated with the food security proxy. Parents' smoking habits were negatively and significantly associated with their food consumption scores. In contrast, alcoholic beverage consumption habits and combination variables (smoking and alcohol consumption) were positively and significantly

associated with the outcome variable. Smoking and alcoholic beverage consumption habits lead to chronic conditions such as hypertension and respiratory or cardiovascular diseases (51, 52). However, food insecurity is more prevalent among people with smoking habits (53–56). Some people may develop a smoking habit as a coping strategy in stressful situations, but buying tobacco products may also account for some proportion of the food expenditure, which leads to difficulty accessing a more diverse variety of available food for the family (57, 58). However, based on the majority religion of the Indonesian population, the consumption of alcoholic beverages is not a popular culture (59). The price of alcoholic beverages is high because of the additional tax that comes with it for both national and imported products. A family with financial ability can provide a greater proportion of non-food expenditure than food expenditure (60), which is less likely to happen among food insecure people, which may explain the positive association between alcoholic beverage consumption and the food security proxy in this study.

The third part of the childhood socioeconomic status factor was the participants' housing situation during childhood. The house situation included any facility owned and the number of occupants in the same dwelling as the participants. One of the food insecurity indicators in the food security vulnerability atlas is the percentage of households with access to electricity (61). A person without access to electricity in their house was negatively associated with the food security proxy in our study results. Furthermore, electricity is essential for people to maintain their food in a refrigerator or room at a controllable temperature. Another benefit of electricity is street lighting, in-house lighting, or other electronic devices to support food supply, preparation, production, and distribution systems. A family with electricity needs to spare some of its income to pay for the electricity bill. Thus, families with better or stable incomes may pay this bill without participating in food expenditure. Based on the electricity benefit of providing the power for refrigerators to prolong the shelf life of various food types, which leads to a more diverse diet, people with food security are more likely to receive benefits from it than food insecure ones.

Furthermore, one of the food security pillars is food utilization, which is about how a healthy person's body utilizes nutrients from food, which results in good health. To maintain an optimal health status related to food security, we must prevent food utilization problems, such as use of safe and good quality water for drinking and preparing food, or hygiene sanitation for all household members. Poor water quality and hygienic sanitation can be sources of infectious diseases that lead to food utilization problems. The infection disrupts food utilization in the body and lowers the diversity of the consumed diet due to the lack of appetite, which eventually results in malnutrition in children, such as stunting (62, 63), which has long-term effects on children's lives. Water quality (i.e., physical, chemical, and microbiological) and water safety, which may have immediate health consequences, play an important role in infectious disease prevention (64). The quality of the water in the house for food preparation leads to good food utilization with a more diverse consumed diet, which may explain the positive association between safe drinking water source types and the outcome variable in our study.

Moreover, in hygiene sanitation management, toilet ownership must meet the standard to prevent fecal contamination through soil water, which is dangerous to the drinking water source of the house. The standards for toilet ownership must have a septic tank. Meanwhile, this study results showed that house situations with toilets, septic tanks, and shared or public toilets were positively and significantly associated with the food consumption score. However, public or shared toilets may help them access the toilet facility together because not all people can afford to have proper toilets in their houses or those who live under the poverty level. Public or shared toilets usually meet the standard and have hygiene sanitation facilities that prevent infectious diseases, which may explain the positive association between toilet-type ownership and the outcome variable.

A positive association was also found in the housing situation between the number of books owned and the outcomes of this study. The number of books owned in the house may relate to better education and literacy level of the household members or a higher non-food expenditure that is more likely to happen among food-secure people with no problem fulfilling a diverse diet. In contrast, although the study result showed a positive association between the number of owned rooms in the house, the number of people, younger sister, younger brother, and food consumption scores, the exponentiated beta value was less than one, which means that the association is weaker than the beta value that is larger than one. The increased number of rooms in the house may be related to the presence of additional family members. A family can increase the number of rooms in their house if they have sufficient money, which means that their food expenditure is not affected by house renovation fees. A family with five or more people in a house is considered large. One of the determining factors of urban household food insecurity is the large size of the family (65). The number of people or younger siblings living in the same dwelling represents the size of the family. Family size grows when younger siblings are born, and parents already prepare savings for the new family member, which means they have more than enough money for food and non-food expenditure. A stable financial situation leads to better access to a more diverse diet that can be purchased in the market.

Our study has some limitations. The childhood socioeconomic status questionnaire was prone to recall bias, which may potentially lead to underestimation of the association between variables. However, the questionnaire has been widely used in previous research (33, 66–68). Another limitation was that the food consumption score did not represent all food insecurity proxies. However, food consumption score analyses are widely used for food security

assessment as a composite score of dietary diversity and food frequency in developing countries (15, 16, 29, 69). Further research should consider additional information on food recall to provide complete information on dietary diversity related to nutrient adequacy. In addition, more variables that become possible proof of childhood socioeconomic status, such as the ownership of tertiary products (i.e., a refrigerator and a vehicle that is not used for work), should be included.

Conclusion

In respective order among the childhood socioeconomic status factors, the number of owned books, the use of safe drinking-water sources and standard toilets, parents with alcohol consumption habits or a combination of smoking habits, self-employed permanent workers, still married biological parents, the number of rooms, people, and younger siblings were positively and significantly associated with the food security proxy. Contrarily, in respective order among the childhood socioeconomic status factors, self-employment without permanent workers and casual work types, houses with electricity facilities, and parents with smoking habits were negatively and significantly associated with the outcomes. Therefore, children with early socioeconomic disadvantages may experience chronic food insecurity, which affects their adult food security status and leads to poor health status. Thus, integrated work with all sectors may consider food insecure adults with the possibility of chronic food insecurity as the priority of vulnerable targets of food and nutrition security improvement programs.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: http://www.rand.org/labor/FLS/IFLS/download.html#updates.

Ethics statement

The studies involving human participants were reviewed and approved by RAND's Human Subjects Protection Committee, RAND Corporation. The patients/participants provided their written informed consent to participate in this study.

Author contributions

EI: conceptualization, methodology, and writing—original draft preparation. EI, Y-CC, and S-HY: writing—review and editing. S-HY: supervision. All authors read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Fear of COVID-19, healthy eating behaviors, and health-related behavior changes as associated with anxiety and depression among medical students: An online survey

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Background: Medical students' health and wellbeing are highly concerned during the COVID-19 pandemic. This study examined the impacts of fear of COVID-19 (FCoV-19S), healthy eating behavior, and health-related behavior changes on anxiety and depression.

Methods: We conducted an online survey at 8 medical universities in Vietnam from 7th April to 31st May 2020. Data of 5,765 medical students were collected regarding demographic characteristics, FCoV-19S, health-related behaviors, healthy eating score (HES), anxiety, and depression. Logistic regression analyses were used to explore associations.

Results: A lower likelihood of anxiety and depression were found in students with a higher HES score (OR = 0.98; 95%CI = 0.96, 0.99; $p = 0.042$; OR = 0.98;

95%CI = 0.96, 0.99; $p = 0.021$), and in those unchanged or more physical activities during the pandemic (OR = 0.54; 95%CI = 0.44, 0.66; $p < 0.001$; OR = 0.44; 95%CI = 0.37, 0.52; $p < 0.001$) as compared to those with none/less physical activity, respectively. A higher likelihood of anxiety and depression were reported in students with a higher FCoV-19S score (OR = 1.09; 95%CI = 1.07, 1.12; $p < 0.001$; OR = 1.06; 95%CI = 1.04, 1.08; $p < 0.001$), and those smoked unchanged/more during the pandemic (OR = 6.67; 95%CI = 4.71, 9.43; $p < 0.001$; OR = 6.77; 95%CI = 4.89, 9.38; $p < 0.001$) as compared to those stopped/less smoke, respectively. In addition, male students had a lower likelihood of anxiety (OR = 0.79; 95%CI = 0.65, 0.98; $p = 0.029$) compared to female ones.

Conclusions: During the pandemic, FCoV-19S and cigarette smoking had adverse impacts on medical students' psychological health. Conversely, staying physically active and having healthy eating behaviors could potentially prevent medical students from anxiety and depressive symptoms.

KEYWORDS

fear, COVID-19, smoking, physical activity, healthy eating behavior, anxiety, depression, medical students

Introduction

The COVID-19 pandemic has been causing the ever-increasing number of confirmed cases and deaths worldwide (1), placing a huge burden on the healthcare system (2–4). Amidst the pandemic, healthcare workers (HCWs) have directly involved in providing care and treatment for COVID-19 patients. The HCWs have faced stressful challenges, including lack of experience, overwhelming workload, shortages of personal protective equipment, and fear of contagion for their loved ones (5–7). Besides, long working hours and under high-pressure situations make HCWs physically and mentally exhausted which may also increase the risk of infection. In addition to frontline HCWs, most medical staff, regardless of their profession, have experienced remarkable changes in their working conditions and time, and a lack of interaction with colleagues and patients (8). These factors may significantly contribute to the development of psychological burdens among healthcare staff. Recent scientific literature also showed that HCWs were at increased risk of developing psychological illnesses during the COVID-19 crisis (7, 9, 10). Medical students are the future health workforce for the medical system, they had significant roles in containing the COVID-19 pandemic (11–15), their mental health requires more attention and additional support.

Unlike students studying in other disciplines, medical students have been reported to have a greater risk of developing psychological disorders during the pandemic (16–18). Before the pandemic, factors influencing their mental health were documented, including heavy academic programs, rigorous exams, work-life imbalance, the difficulty in adapting to clinical environments and exposing to critically ill and dying patients (19, 20). During the pandemic, Vietnam has adopted various preventive measures to control the spread of COVID-19, including a nationwide lockdown from April 1–20, 2020 (21, 22). Since educational institutions had to be closed for a long time (23), education programs transitioned from the classroom learning to the online learning (24). Meanwhile, medical students have to perform practical training, online studying may affect their academic progress. Besides, prevention measures (e.g., lockdown, and home quarantine) could cause feelings of isolation, leading to harmful lifestyles (25–27). These factors may have negative impacts on their psychological health. Therefore, it is essential to understand the impact of COVID-19 on the mental health of medical students, thereby promoting appropriate strategies to help them reduce the risk of developing mental disorders during the pandemic.

The uncertainty of the COVID-19 pandemic can cause increased fear in the community. Previous studies indicated that there were high rates of anxiety, depression, and psychological distress in the general population during the COVID-19 pandemic (28–30). In addition, COVID-19-related fear was found to be positively associated with mental disorders (31, 32). Particularly, medical students have a better knowledge of the disease and its severity, making them more fearful and worried during the pandemic (33). Therefore, anxiety and depression in relation to the fear of COVID-19 should be investigated in medical students to assess their mental health.

Engaging in positive lifestyles (e.g., healthy diet, staying physically active, avoiding alcohol and smoking) were documented to have benefits for mental health (34, 35). However, when facing the unfamiliar situations that the

COVID-19 pandemic and preventive measures (e.g., lockdown), people had an increased tendency to consume alcohol and smoke cigarettes as a coping method (36–38). In addition, a recent systematic review of 87 articles indicated that there were increased food and alcohol consumption, and increased sedentary hours during the COVID-19 pandemic (39, 40). Another research also showed that a higher intake of unhealthy foods was documented during the lockdown period (40). The changes of lifestyle may further affect people's psychological health. Therefore, health-related behaviors should be investigated as independent variables for medical student's mental health.

Previous literature indicated that the prevalence of anxiety and depression varied across demographic characteristics, such as age, gender, income, education (41, 42). In the context of the pandemic, several health-related factors were reported to be important predictors of mental disorders, such as underlying health conditions, symptoms like COVID-19, or BMI (41–43). In addition, recent studies also showed that higher digital healthy diet literacy (DDL) and health literacy (HL) were associated with better mental health in different populations (e.g., healthcare workers, and outpatients) (43–45). Therefore, DDL and HL may have potential impacts on anxiety and depression among medical students during the pandemic.

This study was conducted to examine the associated factors of anxiety and depression among undergraduate medical students, in which impacts of fear of COVID-19, health-related behavior changes and healthy eating behaviors were emphasized.

Methods

Study design and sample

We conducted a cross-sectional study among medical students at 8 medical universities across Vietnam, including 5 universities in the Northern area, 1 university in the Central area, and 2 universities in the Southern area. An online questionnaire survey was carried out to collect data from 7th April to 31st May 2020. A sample of 5,765 students (out of 28,737 possible students) completed the online survey (46).

This study received ethical approval from the Institutional Ethical Review Committee of Hanoi University of Public Health, Vietnam (IRB No. 133/2020/YTCC-HD3).

We used a convenience sampling method to recruit medical students. Researchers (university lecturers) informed and invited their students to participate in the survey. Next, the online survey link was sent to student leaders, who were responsible for sharing the link with other students in their class *via* email, Facebook messenger, or Zalo. Study purposes were informed to students before they signed the electronic consent forms. Students then completed all the survey questions. There was no missing data as marking required fields for all the questions. Students' data is kept confidential and used for research purposes only.

Measurements

Outcome variables

Anxiety and depression were assessed using the 7-item Generalized Anxiety Disorder (GAD-7) questionnaire and the 9-item Patient Health Questionnaire (PHQ-9), respectively. The original translated versions of GAD-7 and PHQ-9 were used in this study and in the Vietnamese context (47–49). In the current study, the Cronbach's alpha values for the GAD-7 and PHQ-9 questionnaires were 0.94 and 0.91, respectively. These questionnaires investigate participants about the extent to which different symptoms of anxiety and depression bothered them in the last 2 weeks with four possible responses from "0 = not at all" to "3 = almost every day". A sum GAD-7 score (a range of 0–21) of $\geq$ 8 was classified as anxiety (50). Similarly, a sum PHQ-9 score (a range of 0–27) of $\geq$ 10 was classified as depression (51).

Fear of COVID-19

We used the 7-item fear of COVID-19 scale (FCoV-19S) to evaluate fear. This instrument was validated and used in Vietnamese medical students in a previous study (52). Students were asked to rate their agreements with varying degrees of the COVID-19 related fear. The possible answers range from "1 = strongly disagree" to "5 = strongly agree". The sum scores varied between 7 and 35, in which students with higher scores have greater degrees of FCoV-19S.

Healthy eating behavior

We used the healthy eating score (HES-5) questionnaire to evaluate healthy eating behavior (53, 54). This instrument consists of 5 food items, which investigate how often students consumed these foods in the last 30 days, including vegetables, fruits, fish, whole grains, and dairy products. The frequency of food consumption ranges from 0 (rarely or never) to 5 (three or more times every day). The sum score of HES is between 0 and 25, in which students with higher scores have healthier eating habits. This tool was validated and used for assessing a healthy diet in different Vietnamese populations (44, 47). The Cronbach's alpha for HES-5 in the current study was 0.73.

Health-related behavior changes

Students reported their health behaviors amidst the pandemic compared to the pre-pandemic, including cigarette smoking, alcohol drinking, physical activity with five choices (never, quitted/stopped, less, unchanged, and more); and eating habits with 3 choices (less healthy, unchanged, and healthier).

It is recommended that people should maintain or make better positive behaviors (e.g., physical activity, healthy diet) to stay healthy during the pandemic. Inversely, harmful behaviors (e.g., smoking, drinking) should be abandoned or reduced gradually (55). Therefore, we classified health-related behavior changes into two categories as follows: cigarette smoking ("none or smoke less" vs. "unchanged or smoke more"), alcohol drinking ("none or drink less" vs. "unchanged or drink more"), physical activity ("none or less active" vs. "unchanged or more active") and eating habits ("unchanged or less healthy" vs. "healthier").

Digital healthy diet literacy and health literacy

We used the 4-item digital healthy diet literacy questionnaire (DDL-4) and 12-item short-form health literacy questionnaire (HLS-SF12) to evaluate student's digital healthy diet literacy (DDL) and health literacy (HL). The DDL-4 was developed, validated, and used in previous studies during the pandemic (44, 46, 56), while the HLS-SF12 was commonly utilized for research in Asian nations (57) and Vietnam (58–62). The Cronbach's alpha for the DDL-4 and HLS-SF12 in our study were 0.87 and 0.89, respectively. Students rated their performance difficulty for each questionnaire item on four-level responses ranging from "1 = very difficult" to "4 = very easy". We standardized the DDL and HL scores into unified metrics ranging from 0 to 50, in which students with higher DDL scores or higher HL scores have better DDL or HL. The standardized formula was represented in previous research (46, 57).

Socio-demographic and clinical characteristics

Data related to student's characteristics were also collected, including age, sex (female vs. male), ability to pay for medical care (easy vs. difficult), and academic year (1–2 vs. 3–6). Bodyweight (kg) and height (cm) were self-reported by students, which were used to calculate body mass index (BMI, kg/m^2). We used the Charlson Comorbidity Index items (63) to evaluate underlying health conditions (none vs. one or more). Students also reported Suspected COVID-19 symptoms (S-COVID-19-S) that they had at the time of the survey. These symptoms include fever, cough, difficult breathing, myalgia, fatigue, sputum production, confusion, headache, sore throat, rhinorrhea, chest pain, hemoptysis, diarrhea, and nausea (64). Students had S-COVID-19-S if they had at least one of S-COVID-19-S.

Data analysis

First, descriptive analyses were used to summarize the features of independent variables (IVs), including frequency, percentage, mean, and standard deviation. Second, we used the Chi-squared test to compare the distribution of anxiety and depression according to different groups of IVs. Third, simple and multiple logistic regression models were conducted to explore the associated predictors of anxiety and depression in medical students. We chose variables related to outcomes at $p < 0.1$ in bivariate models to perform multivariate models. The Spearman correlation test was utilized to test relationships between IVs to deal with multicollinearity. We found that age highly correlates with the academic year ($rho = 0.82$); cigarette smoking moderately correlates with alcohol drinking ($rho = 0.49$); health literacy moderately correlates with the DDL ($rho = 0.63$) (Supplementary Table S1). Thus, age, gender, cigarette smoking, health literacy, and other independent factors were included in the multiple logistic regression models. We set $p\text{-}value < 0.05$ as a significant level. All analyses were performed using the IBM SPSS Version 26.0 (IBM Corp, Armonk, NY, United States).

Results

Student's characteristics

The average age of the sample was 21.7 ± 1.9. Out of all participants, 47.3% were female, 46.3% responded to difficult payment for medical care, and 35.3% were first-year or second-year students. The mean score of FCoV-19S was 16.6 ± 5.2. The prevalence of anxiety and depression was 8.1 and 12.2%, respectively. The proportion of anxiety varied by different groups of gender, ability to pay for medical care, S-COVID-19-S, underlying health conditions, cigarette smoking, alcohol drinking, physical activity, and eating habits. The proportion of depression varied by different groups of ability to pay for medical care, S-COVID-19-S, underlying health conditions, cigarette smoking, alcohol drinking, physical activity, and eating habits (Table 1).

Associated factors of anxiety among medical students

The multiple logistic regression models show that medical students had a lower likelihood of anxiety were male (odds ratio, OR: 0.79; 95% confidence interval, 95% CI: 0.65, 0.98; $p = 0.029$), those with higher healthy eating scores (OR: 0.98; 95% CI: 0.96, 0.99; $p = 0.042$) and those with unchanged or more physical activity (OR: 0.54; 95%CI: 0.44, 0.66; $p < 0.001$) as compared to those with none or less physical activity during the pandemic.

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TABLE 1 Characteristics of medical students by anxiety and depression ($n = 5765$).

Variables	Total ($n = 5765$)	Anxiety disorders			Depressive symptoms		
		GAD < 8 ($n = 5298$)	GAD ≥ 8 ($n = 467$)		PHQ < 10 ($n = 5061$)	PHQ ≥ 10 ($n = 704$)	
	n (%)	*n* (%)	*n* (%)	p^*	*n* (%)	*n* (%)	p^*
Age, year (mean ± SD)	21.7 ± 1.9	-	-	-	-	-	-
Gender				0.001			0.089
Female	2,726 (47.3)	2,470 (90.6)	256 (9.4)		2,372 (87.0)	354 (13.0)	
Male	3,039 (52.7)	2,828 (93.1)	211 (6.9)		2,689 (88.5)	350 (11.5)	
Ability to pay for medical care				<0.001			<0.001
Very or fairly easy	3,096 (53.7)	2,397 (89.8)	272 (10.2)		2,257 (84.6)	412 (15.4)	
Very or fairly difficult	2,669 (46.3)	2,901 (93.7)	195 (6.3)		2,804 (90.6)	292 (9.4)	
Academic year				0.834			0.825
1–2	2,036 (35.3)	1,869 (91.8)	167 (8.2)		1,790 (87.9)	246 (12.1)	
3–6	3,729 (64.7)	3,429 (92.0)	300 (8.0)		3,271 (87.7)	458 (12.3)	
Suspected COVID-19 symptoms				<0.001			<0.001
No	4,695 (81.4)	4,376 (93.2)	319 (6.8)		4,205 (89.6)	490 (10.4)	
Yes	1,070 (18.6)	922 (86.2)	148 (13.8)		856 (80.0)	214 (20.0)	
Underlying health conditions				<0.001			<0.001
None	5,517 (95.7)	5,096 (92.4)	421 (7.6)		4,878 (88.4)	639 (11.6)	
One or more	248 (4.3)	202 (81.5)	46 (18.5)		183 (73.8)	65 (26.2)	
BMI, kg/m^2				0.228			0.066
Normal weight (BMI <25.0)	5,313 (92.2)	4,889 (92.0)	424 (8.0)		4,676 (88.0)	637 (12.0)	
Overweight/obese (BMI ≥25.0)	448 (7.8)	405 (90.4)	43 (9.6)		381 (85.0)	67 (15.0)	
Cigarette smoking				<0.001			<0.001
None or smoke less	5,577 (96.7)	5,180 (92.9)	397 (7.1)		4,957 (88.9)	620 (11.1)	
Unchanged or smoke more	188 (3.3)	118 (62.8)	70 (37.2)		104 (55.3)	84 (44.7)	
Alcohol drinking				<0.001			<0.001
None or drink less	5,346 (92.7)	4,967 (92.9)	379 (7.1)		4,749 (88.8)	597 (11.2)	
Unchanged or drink more	419 (7.3)	331 (79.0)	88 (21.0)		312 (74.5)	107 (25.5)	
Physical activity				<0.001			<0.001
None or less active	1,810 (31.4)	1,607 (88.8)	203 (11.2)		1,478 (81.7)	332 (18.3)	
Unchanged or more active	3,955 (68.6)	3691 (93.3)	264 (6.7)		3583 (90.6)	372 (9.4)	
Eating habits				0.003			0.002
Unchanged or less healthy	3,429 (59.5)	3,121 (91.0)	308 (9.0)		2,973 (86.7)	456 (13.3)	
Healthier	2,336 (40.5)	2,177 (93.2)	159 (6.8)		2,088 (89.4)	248 (10.6)	
Healthy eating score, mean ± SD	14.5 ± 4.7	-	-	-	-	-	-
Fear of COVID-19 score, mean ± SD	16.6 ± 5.2	-	-	-	-	-	-
Health literacy index, mean ± SD	34.6 ± 7.0	-	-	-	-	-	-
Digital healthy diet literacy, mean ± SD	34.0 ± 8.7	-	-	-	-	-	-

PHQ, patient health questionnaire; GAD, Generalized Anxiety Disorder; SD, standard deviation; BMI, Body Mass Index.
*Results of the Chi-square test.

Whereas, medical students had a higher likelihood of anxiety were those who found it difficult to pay for medical care (OR: 1.55; 95% CI: 1.27, 1.90; $p < 0.001$), those with symptoms like COVID-19 (OR: 2.11; 95% CI: 1.69, 2.63; $p < 0.001$), those with one one more underlying health conditions (OR: 2.45; 95% CI: 1.71, 3.53; $p < 0.001$), those with unchanged or more smoking during the pandemic (OR: 6.67; 95% CI: 4.71, 9.43; $p < 0.001$), and those with higher fear of COVID-19 scores (OR: 1.09; 95% CI: 1.07, 1.12; $p < 0.001$), as compared to their counterparts (Table 2).

TABLE 2 Factors associated with anxiety disorders among medical students ($n = 5765$).

Variables	Anxiety disorders			
	Simple regression		Multiple regression	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Age	0.98 (0.93, 1.03)	0.464	0.99 (0.94, 1.05)	0.758
Gender				
Female	Ref.		Ref.	
Male	0.72 (0.59, 0.87)	0.001	0.79 (0.65, 0.98)	0.029
Ability to pay for medical care				
Very or fairly easy	Ref.		Ref.	
Very or fairly difficult	1.69 (1.39, 2.04)	<0.001	1.55 (1.27, 1.90)	<0.001
Academic year				
1–2	Ref.		-	-
3–6	0.98 (0.80, 1.19)	0.834	-	-
Suspected COVID-19 symptoms				
No	Ref.		Ref.	
Yes	2.20 (1.79, 2.71)	<0.001	2.11 (1.69, 2.63)	<0.001
Underlying health conditions				
None	Ref.		Ref.	
One or more	2.76 (1.97, 3.85)	<0.001	2.45 (1.71, 3.53)	<0.001
BMI, kg/m^2				
Normal weight (BMI <25.0)	Ref.		-	-
Overweight/obese (BMI ≥25.0)	1.22 (0.88, 1.70)	0.229	-	-
Cigarette smoking				
None or smoke less	Ref.		Ref.	
Unchanged or smoke more	7.74 (5.66, 10.58)	<0.001	6.67 (4.71, 9.43)	<0.001
Alcohol drinking				
None or drink less	Ref.		-	-
Unchanged or drink more	3.48 (2.69, 4.51)	<0.001	-	-
Physical activity				
None or less active	Ref.		Ref.	
Unchanged or more active	0.57 (0.47, 0.69)	<0.001	0.54 (0.44, 0.66)	<0.001
Eating habits				
Unchanged or less healthy	Ref.		Ref.	
Healthier	0.74 (0.61, 0.90)	0.003	0.87 (0.70, 1.08)	0.200
Healthy Eating Score, 1 score increment	0.97 (0.95, 0.99)	0.004	0.98 (0.96, 0.99)	0.042
Fear of COVID-19 score, 1 score increment	1.11 (1.09, 1.14)	<0.001	1.09 (1.07, 1.12)	<0.001
Health literacy index, 1 score increment	0.98 (0.97, 1.00)	0.092	0.99 (0.98, 1.01)	0.681
Digital healthy diet literacy, 1 score increment	0.99 (0.98, 1.00)	0.165	-	-

GAD, Generalized Anxiety Disorder; OR, odds ratio; CI, confidence interval; BMI, Body Mass Index.

Associated factors of depression among medical students

The multiple logistic regression models indicate that medical students had lower odds of depression were those with higher healthy eating scores (OR: 0.98; 95% CI: 0.96, 0.99; $p = 0.021$), those with unchanged or more physical activity (OR: 0.44; 95% CI: 0.37, 0.52; $p < 0.001$) as compared to those with none or less physical activity during the pandemic. Whereas, medical students had higher odds of depression were those who found it difficult to pay for medical care (OR: 1.62; 95% CI: 1.37, 1.92; $p < 0.001$), those with COVID-19-like symptoms (OR: 2.00; 95% CI: 1.66, 2.42; $p < 0.001$), those with one one more underlying health conditions (OR: 2.50; 95% CI: 1.82, 3.43; $p < 0.001$), those with unchanged or more smoking during the pandemic (OR: 6.77; 95% CI: 4.89, 9.38; $p < 0.001$), those with higher fear of

TABLE 3 Factors associated with depression among medical students ($n = 5765$).

Variables	Depressive symptoms			
	Simple regression		Multiple regression	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Age	1.00 (0.96, 1.04)	0.967	1.01 (0.96, 1.05)	0.755
Gender				
Female	Ref.		Ref.	
Male	0.87 (0.74, 1.02)	0.089	0.96 (0.81, 1.15)	0.684
Ability to pay for medical care				
Very or fairly easy	Ref.		Ref.	
Very or fairly difficult	1.75 (1.49, 2.06)	<0.001	1.62 (1.37, 1.92)	<0.001
Academic year				
1–2	Ref.		-	-
3–6	1.02 (0.86, 1.20)	0.825	-	-
Suspected COVID-19 symptoms				
No	Ref.		Ref.	
Yes	2.14 (1.79, 2.56)	<0.001	2.00 (1.66, 2.42)	<0.001
Underlying health conditions				
None	Ref.		Ref.	
One or more	2.71 (2.02, 3.64)	<0.001	2.50 (1.82, 3.43)	<0.001
BMI, kg/m^2				
Normal weight (BMI <25.0)	Ref.		Ref.	
Overweight/obese (BMI ≥25.0)	1.29 (0.98, 1.69)	0.066	1.17 (0.87, 1.57)	0.302
Cigarette smoking				
None or smoke less	Ref.		Ref.	
Unchanged or smoke more	6.46 (4.79, 8.71)	<0.001	6.77 (4.89, 9.38)	<0.001
Alcohol drinking				
None or drink less	Ref.		-	-
Unchanged or drink more	2.73 (2.15, 3.45)	<0.001	-	-
Physical activity				
None or less active	Ref.		Ref.	
Unchanged or more active	0.46 (0.39, 0.54)	<0.001	0.44 (0.37, 0.52)	<0.001
Eating habits				
Unchanged or less healthy	Ref.		Ref.	
Healthier	0.77 (0.66, 0.91)	0.002	0.95 (0.79, 1.14)	0.563
Healthy Eating Score, 1 score increment	0.97 (0.95, 0.98)	<0.001	0.98 (0.96, 0.99)	0.021
Fear of COVID-19 score, 1 score increment	1.07 (1.05, 1.09)	<0.001	1.06 (1.04, 1.08)	<0.001
Health literacy score, 1 score increment	0.98 (0.97, 0.99)	<0.001	0.99 (0.98, 1.01)	0.105
Digital healthy diet literacy, 1 score increment	0.98 (0.97, 0.99)	0.002	-	-

PHQ, Patient Health Questionnaire; OR, odds ratio; CI, confidence interval; BMI, Body Mass Index.

COVID-19 scores (OR: 1.06; 95% CI: 1.04, 1.08; $p < 0.001$), as compared to their counterparts (Table 3).

Discussion

In this study, our results indicate that medical students with higher fear of COVID-19 were more likely to have mental disorders. Our finding was consistent with recent literature conducted in China, Turkey, and the United Arab Emirates among university students (65–67). It is understandable that medical students have a better knowledge of the disease, making them more aware of the severity and danger of the virus. Especially, students in clinical training years are required to work in teaching hospitals and emergency units, which are high-risk environments. Therefore, students may feel more anxious and depressed because they are at higher risk of getting infected and may pass the virus on to loved ones. This

explanation also partially elucidates the result of this study that students with symptoms resembling COVID-19 were prone to be anxious and depressed. Previous studies conducted on different populations, such as outpatients, HCWs also reported similar results (41, 43, 48). In addition, we found that underlying health conditions were positively associated with anxiety and depression, which was in line with the findings of recent studies (42, 48, 68). The explanation for this association is that medical students have a better understanding of the disease. Therefore, they know that underlying medical conditions may worsen health outcomes after COVID-19 infections (69), making students with comorbidities more anxious and depressed during the period of the pandemic. Thus, psychological supports that mitigate the fear and enhance mental resilience could be essential to prevent medical students from developing anxiety or depressive symptoms.

The result of this study shows that medical students who smoked unchanged or more during the pandemic were more likely to be anxious and depressed. Our result was in line with previous studies conducted during the pandemic (49, 70). It is reported that smoking is quite common among medical students, especially among male students (71, 72). Although, smoking could help to relieve stress and pressure when facing uncomfortable events, especially in hospital settings. However, the long-term effects of smoking on the development of later anxiety and depression have been demonstrated in various studies (73). In addition, the present study found that maintaining physical activity unchanged or more active during the pandemic could help to prevent medical students from mental disorders. Similar findings were found in other studies carried out in Australia, North America, Brazil, and China among different populations during the pandemic (70, 74–76). Furthermore, physical activity was highly recommended for depression treatment (77). Regular exercise could help to enhance immune function (78–80), which prevents the body from pathogens, improving physical and mental health during the pandemic. This study also highlights the role of healthy eating behaviors in protecting medical students against the development of anxiety and depressive symptoms. The beneficial effect of a healthy diet on psychological health was reported in recent literature (35, 47, 48). Recent research on college students carried out during a home-confinement period in Italy indicated that healthy dietary behaviors were negatively linked with poorer mental states (81). However, restrictions to outdoor activities and daily lives caused by the COVID-19 pandemic may lead to weight gain, and adversely influence eating habits, and sleeping patterns, thereby increasing eating disorder risk and symptoms (82, 83). In addition, due to precaution measures applied during the pandemic, people were more likely to engage in harmful lifestyles (e.g., increased screen time and sedentary behaviors, or increased alcohol consumption) (25, 39, 40). Therefore, our findings related to health-related behaviors provide timely evidence that helps to encourage medical school students to engage in positive lifestyle habits, reducing the likelihood of mental disorders during the pandemic.

Moreover, we found that male medical students had a lower likelihood of developing anxiety than their female colleagues. This result is similar to previous studies conducted in the United States, Bangladeshi among medical students (84, 85). Our study also indicated that medical students who responded to payment difficulty for medical care had higher odds of anxiety and depressive symptoms than their counterparts, which is comparable to the findings of other studies conducted on outpatients and HCWs during the pandemic (47, 49). The difficult affordability of healthcare may lead to delays in examinations and treatments, which negatively affect students' physical and mental health. It may also partially reflex the financial constraints (86). Particularly, the unemployment rate was higher during the pandemic, affecting the household income (87, 88), making medical students more worried and stressed about the cost of living, rent, and education. Therefore, medical universities should consider strategies to assess and support the psychological health of vulnerable demographic groups, including medical students who are female and who find it difficult to pay for healthcare.

Our study was strengthened by its large sample size collected from 8 medical universities across Vietnam. It is also the first study to evaluate the impact of COVID-19 on mental health among medical students in Vietnam. Therefore, this pilot study could provide timely evidence for future research and practices to protect mental health against the adverse impact of the COVID-19 pandemic. However, some limitations need to be acknowledged in this paper. First, the causality of the associations could not be inferred in the study with a cross-sectional design. Second, as students recruited in the survey were not randomly selected, the generalization of these results should be applied cautiously to medical school students. Final, several factors, which may affect outcomes, were not investigated in this study, such as the changes in teaching methods, financial problems, history of mental disorders, relationships with friends and family, and academic workload. Future studies should take these variables into consideration in assessments.

Conclusions

Amidst the COVID-19 pandemic, medical students with higher fear scores were more likely to have anxiety and depression. Students who smoked had a higher likelihood of having anxiety and depression. Fortunately, staying physically active and having healthy eating behavior were found to be protective factors of mental health among medical students. Medical universities should develop strategic programs to encourage students to actively engage in physical activity, healthy diet, and avoid smoking, which could prevent medical students from psychological disorders during the pandemic.

Data availability statement

The raw data supporting the conclusions of this article will be made available on reasonable request to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Ethical Review Committee of Hanoi University of Public Health, Vietnam (IRB No. 133/2020/YTCC-HD3). The patients/participants provided their written informed consent to participate in this study.

Author contributions

MHN, TXD, ThTN, MDP, TTMP, KMP, GBK, BND, HTN, N-MN, HTBD, YHN, KTN, TTPN, TrTN, and TVD: conceptualization, methodology, validation, investigation, data curation, and writing-review and editing draft. MHN, TD, and TD: formal analysis and writing-original draft. MHN, TTMP, and TTPN: project administration. TD: funding acquisition, supervision. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.938769/full#supplementary-material

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Reaching and maintaining higher dietary diversity is associated with decreased risk of all-cause mortality: A longitudinal study from the China Health and Nutrition Survey

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It is generally believed that higher dietary diversity is associated with better health status. The dietary diversity of individuals may change with age; however, evidence on the trajectory of change in the long-term and whether it is related to all-cause mortality is still scant. In this study, we used data from the China Health and Nutrition Survey (CHNS) collected in five follow-ups between 2004 and 2015 to explore the association between changes in dietary diversity scores (DDS) and all-cause mortality, as well as the dynamic change in DDS with age. In total, 6,737 subjects (aged between 30 and 60 at enrollment) were included in the analysis. Latent Class Trajectory Modeling (LCTM) was used to explore the different trajectories of DDS changes among participants. Four classes were identified: class 1 with the lowest average DDS (3.0) that showed a gradual decline during the follow-ups; class 2 with relatively low DDS (4.0) that experienced slight growth; class 3 with medium DDS (5.2) that also demonstrated similar growth rate to class 2; and class 4 with the highest DDS (6.7) maintained at a high level. Cox proportional hazards regression models were applied to investigate the association between the DDS trajectories and the risk of death. Only class 4, which was characterized by the highest and stable DDS, had significant reduced risk of all-cause mortality of 71.0% (hazard ratio *[HR]*: 0.29; 95% confidence interval *[CI]*: 0.10–0.83), 68% (*HR*: 0.32; 95% *CI*: 0.11–0.89), and 66.0% (*HR*: 0.34; 95% *CI*: 0.12–0.94), compared to classes 1, 2, and 3, respectively, while the first three classes showed no significant inter-class differences. When considering the average DDS during the study period, each point of increase in DDS corresponded to a 22% reduced risk of mortality (*HR*: 0.78; 95% *CI*: 0.69–0.89). In summary, reaching and maintaining

a higher DDS was associated with a decreased risk of all-cause mortality. Therefore, promoting diversified eating and increasing the accessibility of varieties of foods should be paid more attention from policymakers and be more emphasized in dietary guidelines.

KEYWORDS

dietary diversity, mortality, trajectory modeling, longitudinal study, China Health and Nutrition Survey

Introduction

Consuming a variety of foods is one of the principles of dietary guidelines in many countries (1–3). The variation in food consumption can be measured by the dietary diversity score (DDS), reflecting nutrient adequacy and dietary quality of individuals (4). Previous studies showed that a higher DDS was related to reduced risks of obesity (5) and many age-related diseases, such as diabetes (6), cognitive, and memory status decline (7–9). It was suggested that higher dietary diversity was also inversely associated with all-cause mortality among elderly people (10) and people who enjoyed greater diversity of diet maintained a longer healthy life expectancy (11). However, another study showed mixed results, indicating that a higher DDS might only decrease mortality among females but not males (12). Therefore, whether DDS is associated with all-cause mortality, and how strong the association is in different populations remain unclear.

Furthermore, food intake and dietary diversity may change over the lifetime due to multiple reasons. With age, the loss of appetite decreased total energy intake, and a worse financial situation may lead to a higher risk of malnutrition (13, 14). Lower chewing ability and eating alone may also decrease the diversity of diet (15, 16). A study showed that dietary diversity significantly declined in women older than 63 years old, and a trend of decrease was also observed in men at the age of 65 years and higher, although not statistically significant (17). However, studies investigating the dynamic change of dietary diversity over time are still scant.

China, the country with one of the largest populations in the world, has experienced a rapid change to an aging society, accompanied by dietary patterns and transitions of its citizens due to the growth of the economy during the past decades (18, 19). Coming along with abundant food options, the prevalence of diet-related non-communicable diseases, such as hypertension, stroke, type-2 diabetes, coronary heart disease, as well as obesity are rising (18). Meanwhile, micronutrient deficiencies also exist, more than half of Chinese adults' micronutrient intake did not meet the Chinese estimated average requirement, and more than 80% of Chinese adults had two or more kinds of micronutrient deficiencies (20). Because of the triple burden of undernutrition, overnutrition, and micronutrient deficiency faced by Chinese adults, more effective dietary guidance that is easy to be followed by the public is urgently needed (21, 22). Increasing dietary diversity and maintaining a high-dietary diversity can be a feasible and effective way to increase nutrient adequacy and dietary quality.

To close the gaps between DDS changes and their associations with mortality, as well as provide valuable scientific evidence to improve the current dietary guidelines, the present study aims to investigate the long-term dynamic change in dietary diversity and its association with all-cause mortality using data from the China Health and Nutrition Survey (CHNS).

Materials and methods

Study design and population

The CHNS is a nationwide prospective cohort study. The initial recruitment of participants was conducted in 1989, and follow-ups were conducted within a 2–3-year interval. Participants were recruited from nine provinces and three autonomous cities. The detailed description can be found elsewhere (23). In this study, we used the data from the CHNS collected in 2004, 2006, 2009, 2011, and 2015. Since the dietary data for 2015 are not yet available, only mortality data were included from that interval. To cover the dietary diversity changes in the aging process, and to avoid significant dietary restriction by diseases, we excluded the participants who were younger than 30 years old or older than 60 years old at baseline (age at baseline was identified as the actual age in the year of entering the cohort during 2004–2015 because the year of participants entering the cohort varied), pregnant or during breastfeeding, and those who only participated in two or fewer follow-ups, had cancers and extreme energy intake (<500 kcal/day or >8,000 kcal/day) throughout the follow-ups period. A total of 6,737 participants were included in the analysis (Figure 1).

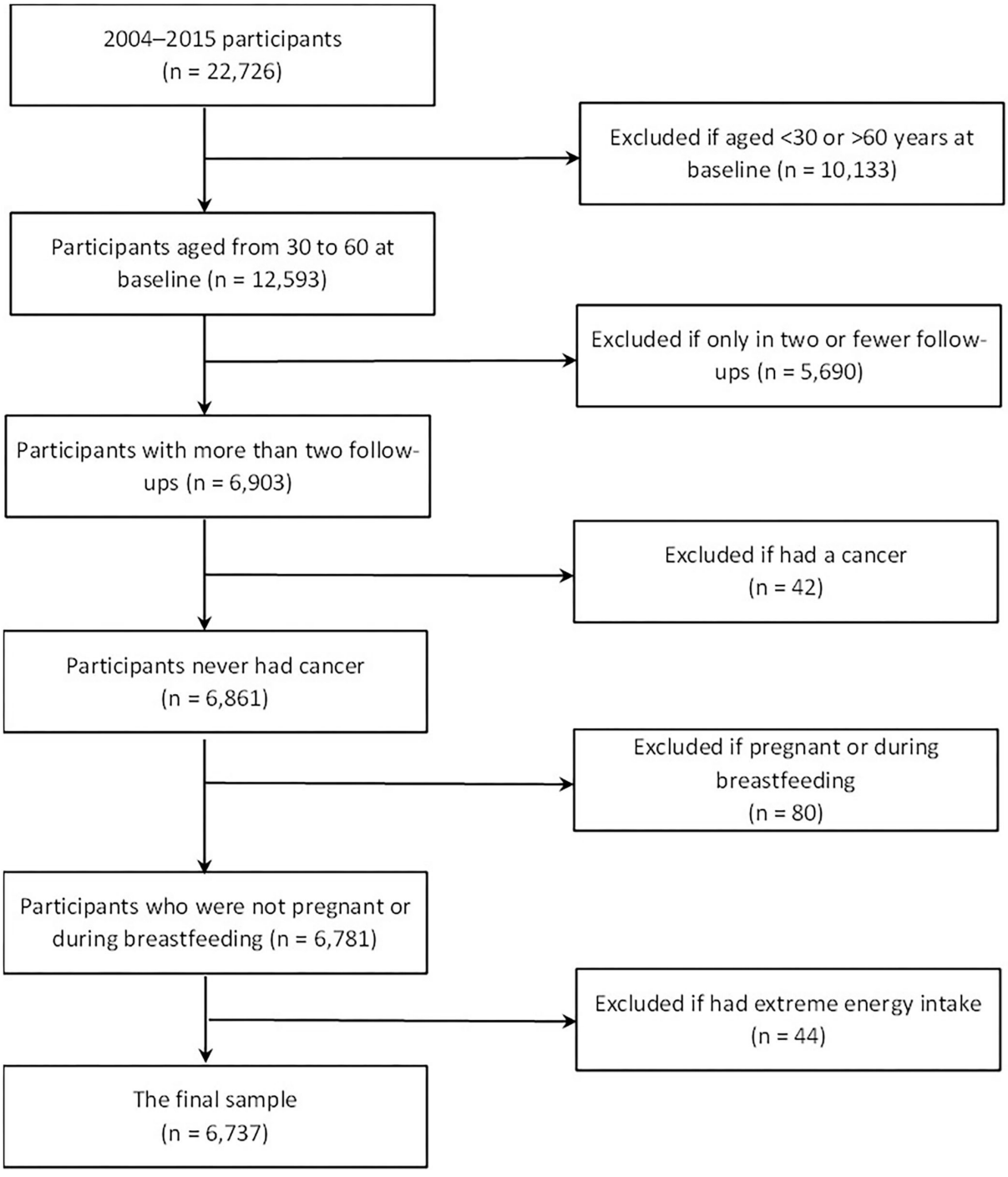

FIGURE 1
Flowchart of sample selection.

The CHNS was approved by institutional review boards at the University of North Carolina (Chapel Hill, NC, the US) and the National Institute of Nutrition and Food Safety (Chinese Center for Disease Control and Prevention). Informed consent was given to all participants before participation. The current study was further approved by the Institution Review Board of Tsinghua University (project identification 20 210072).

Dietary assessment and dietary diversity score

Dietary data at the individual level were collected using 24-h dietary recalls for consecutive 3 days. Participants were required to report all the food consumed at home and away from home over the past 24 h (24). The quality of dietary data was controlled in several ways. First, besides 24-h dietary recalls, changes in the inventory of certain food, such as rice, flour, edible oil, and condiments, were weighed and measured at the household level, which served as a validation of 24-h dietary recall. The significant discrepancies were resolved according to revisiting and investigating their food consumption. Second, all the interviewers were trained nutritionists who were professionally engaged in nutrition work and had participated in other national surveys. In addition, three days of specific training in the collection of dietary data have been provided for this survey (29). In total, 34 kinds of foods and 21 kinds of nutrients were recorded (25). Energy intakes of subjects were calculated by multiplying the consumption of each food and their macronutrients' content acquired from the Chinese Food Composition Tables (26–28). The detailed method of dietary assessment was described elsewhere (29).

DDS was developed based on Chinese dietary guidelines 2022 (CDG-2022) and Chinese Food Composition Tables (26–28, 30). Ten food groups are included in the CDG-2022: cereals and tubers, vegetables, fruits, meat, soybeans and nuts, eggs, aquatic products, milk, and dairy products, as well as salt and oil, in which salt and oil were not included in the DDS assessment (31). Particularly, mixed beans (dried legumes other than soybeans, such as azuki bean and mung bean) and tubers (such as potato and sweet potato) belong to the category of cereals and tubers. Fresh beans and tuber vegetables belong to the category of vegetables. Soybeans, nuts, and seeds belong to the category of soybeans and nuts (32). For each participant, the daily average DDS was calculated during each follow-up.

The trajectory of dietary diversity was analyzed by Latent class trajectory modeling (LCTM), which is a relatively new method in epidemiology studies. Populations with heterogeneous characteristics could be divided into several simplified homogeneous classes by this method. For the modeling process, we referred to the systematic framework introduced by Lennon et al. and adapted it for our study (33). The modeling contained six steps: (1) building a scoping model with five classes; (2) refining the number of classes; (3) refining model structure to a flexible random-effect specification; (4) model adequacy assessment; (5) graphical presentations; and (6) checking clinical characterization and plausibility, that is, the plausibility of DDS scores of each class and the changes over years.

The "lcmm" package (version 1.9.3) in R Statistical Software was used for modeling. The optimal number of classes was chosen based on the lowest Bayes information criterion (BIC) and the criterion that the number of participants in each class should not be less than 5% of the total participants (34). The trajectory model was selected by comparing the BIC and number of participants of models with (1) only fixed effects, (2) fixed effects and random intercepts, and (3) fixed effects and random slopes. The trajectory shapes (whether including cubic and quadratic terms) of the classes were determined according to the significant coefficients of model running results, and linear models were constructed if the polynomial coefficients were not significant (35). For the adequacy assessment, the model with the lowest BIC and the posterior probability of assignments (APPA) of each class ≥ 0.7 was selected as the final model.

Outcome assessment

The primary outcome of the present study is all-cause mortality. For each participant in the CHNS, the household register system would continuously update their status, either alive or deceased, and the year and month of death. The year of follow-up was calculated from enrollment to the date of passed away or loss of follow-up of the participant during 2004–2015.

Statistical analysis

Normality was examined by a combination of histogram and the Kolmogorov-Smirnov test because of the large sample size, and the data with an asymmetrically distributed histogram or $P < 0.05$ in the Kolmogorov–Smirnov test were defined as non-normality. Characteristics for all eligible participants are described as mean and standard deviation (SD; with a normal distribution) or median and interquartile range (P_{25}, P_{75}; without normal distribution) for continuous variables, and frequencies and percentages for categorical variables. Total person-years during the follow-ups were calculated by the sum of all the participants' total person-years participating in the survey during 2004–2015. The differences in sociodemographic characteristics, history of diseases, and diet consumption across classes were examined using the ANOVA (with a normal distribution and homoscedasticity) or the Wilcoxon rank-sum test (without distribution or homoscedasticity) for continuous variables, the chi-square (χ^2) test for categorical variables, and the Bonferroni correction was used to adjust p-value (P) to the pairwise comparison between the classes ($P < 0.007$).

Cox proportional hazard regression was applied to test the association between DDS trajectories and the risk of mortality. An unadjusted model and two adjusted models were established. The confounders were identified from univariate analysis (when factors showed a $P<0.05$). In addition, since the history of chronic disease is usually associated with dietary intake and mortality, it is also considered as a potential confounder in the current analysis. Particularly, in adjusted models, Model

1 adjusted for sociodemographic characteristics, including age at baseline, gender, educational level, region of residence, place of residence, and individual annual income. Model 2 was further adjusted for lifestyle characteristics, including the history of smoking and alcohol consumption, BMI, chronic disease history, physical activity, using hypotensive or hypoglycemic medicine, and energy intake. The proportional hazard assumption of covariates for the Cox regression was tested according to the Schoenfeld residuals test, and the results for DDS trajectory (Supplementary Figure S1) and average DDS (Supplementary Figure S2) showed that all the covariates met the assumption based on a *p*-value threshold of 0.05. The collinearity of the covariates in the adjusted models was examined according to variance inflation factors (VIFs), and the results showed that no collinearity of these covariates existed (all the VIFs were < 5).

Additionally, the Restricted Cubic Spline (RCS) Cox regression was performed to investigate the non-linear relationship between average DDS level and the risk of death, with class 1 (DDS = 3) as the reference and adjusting for the same confounders as Model 2 mentioned above. The regression model with five knots was selected because of the largest coefficient of determination (R^2) and optimism-corrected discrimination index (D_{xy}) in this model.

All statistical analyses were performed using the R Statistical Software (version 4.1.1, R Development Core Team, Vienna, Austria) (36). *P*-value < 0.05 (two-tailed) was considered statistically significant.

Results

Trajectory of DDS changes with age

In total, 6,737 eligible participants were included in the analysis. To explore the trajectory changes of DDS with age, the lowest BIC was reached when the number of classes was four ($BIC_4 = 71920.9$), and the number of participants in each class was higher than 5% of the total participants. When only including fixed effects in the trajectory model, the BIC was the lowest and with a better fit, it was therefore selected. No statistical significance was found when adding cubic and quadratic terms in linear models, therefore only a linear model was used.

Levels and changes in DDS with age in all four classes are shown in Figure 2. Most of the participants belonged to class 2 and class 3, with 40.6% and 42.2% of participants, respectively. In class 1, the initial DDS was relatively low (intercept = 3.663, $P < 0.001$), and a trend of decreasing with age was observed ($\beta = -0.008$, $P = 0.008$). In class 2, the initial DDS was relatively low (intercept = 3.698, $P < 0.001$) and slightly increased with age ($\beta = 0.010$, $P < 0.001$). In class 3, the initial DDS was medium (intercept = 4.718, $P < 0.001$) and also slightly increased during the follow-ups ($\beta = 0.010$, $P < 0.001$); and in class 4, the initial DDS was the highest among all the classes (intercept = 6.106, $P < 0.001$) and maintained stable ($\beta = 0.005$, $P = 0.147$). The mean DDS of classes 1, 2, 3, and 4 during the survey were 3.0, 4.0, 5.2, and 6.7, respectively.

Baseline characteristics among participants across different classes

Of the studied population, 3,293 (48.9%) were male and 3,444 (51.1%) were female. The median (P_{25}, P_{75}) follow-up time was 12.04 (8.94, 13.94) years with 7,6024.36 person-years. During the follow-ups, 326 deaths occurred. Specifically, 64, 151, 106, and 5 participants died in class 1, class 2, class 3, and class 4, respectively.

Table 1 shows the baseline characteristics across classes. The ages of participants at baseline were significantly different between classes, with a decreasing trend from class 1 (median = 48.0) to class 4 (median = 42.0). Compared to class 1, class 4 was more likely to have a higher education level, annual individual income, BMI, and energy intake, and class 4 tended to have less physical activity and live in urban areas.

Association between the trajectory of DDS changes, average DDS, and all-cause mortality

A total of 326 deaths occurred during the follow-ups. The highest and lowest incidences of death were observed in class 1 and class 4, with 8.4 deaths/1,000 person-years and 0.9 deaths/1,000 person-years, respectively. Results of Cox proportional hazard regression showed that significant differences in mortality among all the classes were observed in the unadjusted model. The most substantial difference was found in class 4, with 86% of lower mortality (*HR*: 0.14; 95% *CI*: 0.06–0.35) compared to class 1. When adjusted for sociodemographic characteristics (model 1), only class 4 showed significantly lower mortality compared with other classes. After adjusting for both sociodemographic characteristics and lifestyle characteristics (model 2), the results were similar: class 4 was associated with 71% (*HR*:0.29; 95% *CI*: 0.10–0.83), 68% (*HR*:0.32; 95% *CI*: 0.11–0.89), and 66% (*HR*:0.34; 95% *CI*: 0.12–0.94) of reduced risk of mortality compared to class 1, 2, and 3, respectively. However, no significant inter-class differences were observed between the other three classes (Table 2).

Furthermore, we calculated the average DDS among participants during the follow-ups and found that after adjusting for both sociodemographic characteristics and lifestyle characteristics (model 2), a higher average DDS was significantly associated with a reduced risk of mortality. Each point of

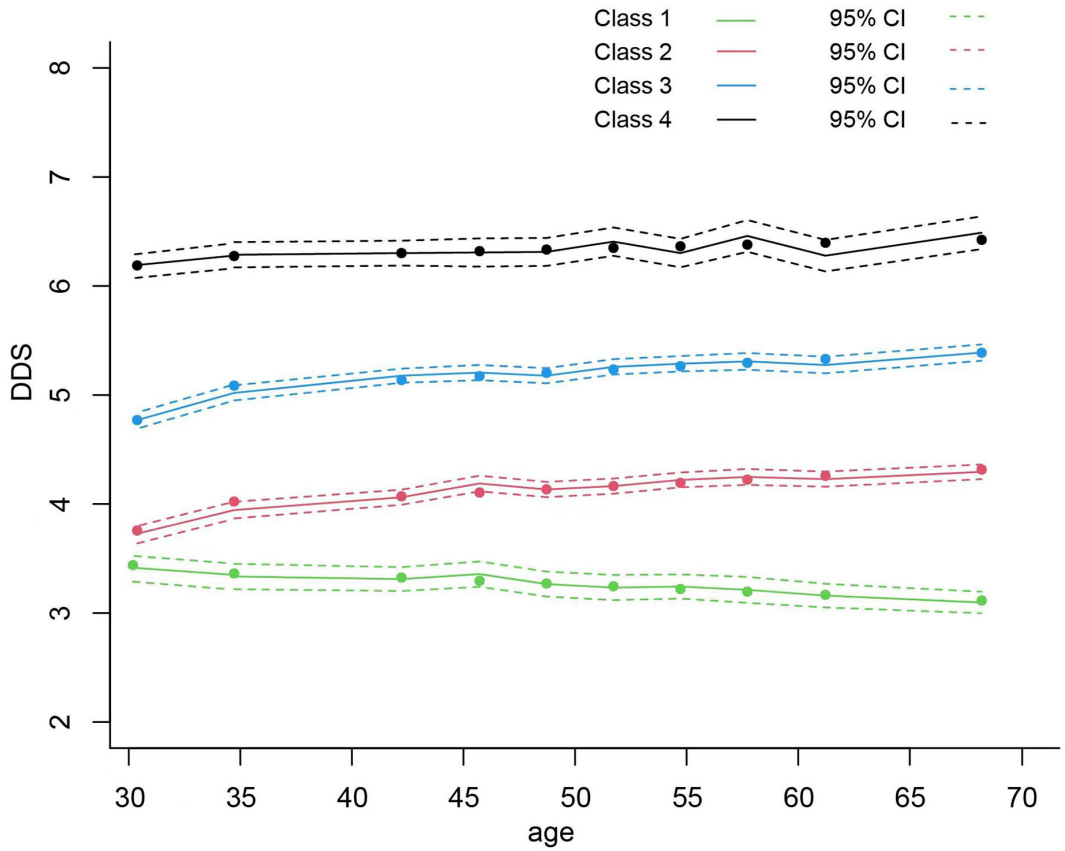

FIGURE 2
The association between average DDS and mortality hazard ratio using restricted cubic spline analysis with five knots. DDS, dietary diversity score; HR, hazard ratio; CI, confidence interval.

increase in DDS corresponded to a 22% reduced risk of mortality in the whole research population (*HR*:0.78; 95% *CI*: 0.69–0.89).

Additionally, the results of the cubic spline curve showed a negatively non-linear association between all-cause mortality and average DDS during the follow-ups. Compared with DDS = 3, DDS < 3 was associated with a higher risk of mortality, and DDS > 3 was associated with an increased risk of mortality, despite a slight fluctuation between DDS = 5 and DDS = 6 (Figure 3).

The hazard ratio was indicated by the solid line, and 95% confidence intervals were represented by the shaded area. The reference point was DDS = 3, adjusted for age at baseline, gender, levels of education, regions of residence, places of residence, and individual annual income, as well as the history of smoking and alcohol consumption, BMI, history of chronic disease, physical activity, hypotensive or hypoglycemic medicine, and energy intake.

Discussion

To the best of our knowledge, this is the first study investigating a long-term dynamic change of DDS and its association with mortality in the Chinese population. Our study revealed that reaching and maintaining a higher dietary diversity was significantly associated with lower mortality in the Chinese population.

Average DDS and mortality

DDSs have been widely accepted as relatively uncomplicated and promising tools to quantify dietary diversity on a large scale (37). The development of DDSs could be based on different measurements, such as the number of individual food items consumed (38), the number of different food groups consumed with the standard weight for each food group (39), as well as the number of food groups consumed that are allocated with different weights according to dietary guidelines (40). Types of dietary assessment also varied. The most commonly used method is 24-h recall, followed by a food frequency questionnaire, food record, and diet history (41). In our study, we used the food-group-based measurement to calculate DDS, which is also the most frequently used method of developing DDSs (41). And food intakes of individuals were calculated

TABLE 1 Characteristics of participants across different classes.

	Class 1 $N = 614$	Class 2 $N = 2735$	Class 3 $N = 2841$	Class 4 $N = 547$	*P*
Age at baseline, years, *M* (P_{25}, P_{75})	48.0 (41.0, 54.0)^{abc}	44.0 (37.0, 50.0)^{ade}	42.0 (35.0, 49.0)^{bd}	42.0 (35.0, 48.0)^{c}	<0.001
Gender, *n* (%)					0.036
Male	285 (46.4)	1341 (49.0)	1426 (50.2)	241 (44.1)	
Female	329 (53.6)	1394 (51.0)	1415 (49.8)	306 (55.9)	
Level of education, *n* (%)					<0.001
Junior high school or below	571 (94.4)^{abc}	2308 (85.1)^{ade}	1991 (70.6)^{bdf}	246 (45.2)^{cef}	
Senior high school or above	34 (5.62)	403 (14.9)	830 (29.4)	298 (54.8)	
Region of residence, *n* (%)					<0.001
Eastern city	100 (16.3)^{abc}	724 (26.5)^{ade}	1122 (39.5)^{bdf}	296 (54.1)^{cef}	
Central city	367 (59.8)	1134 (41.5)	1259 (44.3)	220 (40.2)	
Western city	147 (23.9)	877 (32.1)	460 (16.2)	31 (5.67)	
Place of residence					<0.001
Urban area	91 (14.8)^{ab}	528 (19.3)^{cd}	1141 (40.2)^{ace}	417 (76.2)^{bde}	
Rural area	523 (85.2)	2207 (80.7)	1700 (59.8)	130 (23.8)	
Annual individual income, yuan, *M* (P_{25}, P_{75})	3221 (1250, 5567)^{abc}	5217 (2168, 10737)^{ade}	10317 (4934, 17294)^{bdf}	15789 (9642, 23339)^{cef}	<0.001
Smoking history, *n* (%)					0.004
Never	351 (57.2)^{a}	1569 (57.5)^{b}	1681 (59.3)^{c}	358 (56.5)^{abc}	
Yes	263 (42.8)	1162 (42.5)	1153 (40.7)	188 (34.4)	
Alcohol history, *n* (%)					<0.001
Never	349 (56.8)^{ab}	1435 (52.5)^{c}	1413 (49.8)^{a}	246 (45.0)^{bc}	
Yes	265 (43.2)	1300 (47.5)	1423 (50.2)	301 (55.0)	
BMI, kg/m^2, *M* (P_{25}, P_{75})	22.0 (20.4, 24.8)^{ab}	22.8 (20.9, 25.2)^{cd}	23.6 (21.4, 25.8)^{ac}	23.3 (21.4, 25.6)^{bd}	<0.001
Chronic disease history, *n* (%)					0.069
No	414 (67.4)	1910 (69.8)	1891 (66.6)	369 (67.5)	
Yes	200 (32.6)	825 (30.2)	950 (33.4)	178 (32.5)	
Hypotensive or hypoglycemic medicine taking, *n* (%)					0.009
No	506 (82.4)	2254 (82.4)^{a}	2249 (79.2)^{a}	433 (79.2)	
Yes	108 (17.6)	481 (17.6)	592 (20.8)	114 (20.8)	
Physical activity, MET hour/week, *M* (P_{25}, P_{75})	2691 (664, 4599)^{abc}	2553 (594, 5083)^{ade}	1243 (378, 3275)^{bdf}	1003 (475, 1942)^{cef}	<0.001
Energy intake, kcal/day, *M* (P_{25}, P_{75})	2013 (1663, 2359)^{abc}	2120 (1825, 2474)^{a}	2157 (1870, 2491)^{b}	2177 (1904, 2505)^{c}	<0.001
Dietary diversity score, *M* (P_{25}, P_{75})	3.0 (2.5, 3.2)^{abc}	4.0 (3.8, 4.3)^{ade}	5.2 (5.0, 5.8)^{bdf}	6.7 (6.3, 7.0)^{cef}	<0.001
Number of death	64	151	106	5	<0.001
Incidence (number of deaths/1000 person-years)	8.4^{abc}	4.8^{ade}	3.4^{bdf}	0.9^{cef}	<0.001

The differences in characteristics across classes were examined by the Wilcoxon rank-sum test for continuous variables, and the chi-square (χ^2) test for categorical variables. Superscript letters (abcdef) denoted statistically significant pairwise comparisons (following Bonferroni correction of $P < 0.007$). BMI, body mass index; M, median; P_{25}, 25th percentile; P_{75}, 75th percentile.

using three days of 24-h recall and validated with weighed food inventory per household, which further improved the accuracy of dietary intake data.

Generally, dietary diversity can be an indicator of nutritional adequacy and food accessibility, thus reflecting the potential risk of malnutrition in elderlies, younger adults, and children (21, 42–44). The Chinese dietary guidelines 2022 recommend that people should eat more than 12 kinds of food every day, and more than 25 kinds of food every week (30). Since the DDS used in our study was developed based on the Chinese dietary guidelines, a higher DDS could reflect adherence to the dietary guidelines. However, in some cases, higher DDS may also lead to excessive energy intake, thus leading to overweight or obesity (42). This could be due to an unbalanced diet, such as consuming more varieties of sweets, snacks, and carbohydrates, especially in upper-middle-income economies (21, 45). Nevertheless, a recent systematic scoping review indicated that higher dietary diversity was positively associated with benefits for various health outcomes such as non-communicable diseases, biomarkers of nutritional status,

TABLE 2 Associations of DDS trajectory and average DDS with mortality.

Comparable group vs. Reference	Unadjusted Model	Model 1	Model 2
Class 2 vs. Class 1	0.71 (0.52, 0.96)*	0.91 (0.67, 1.24)	0.90 (0.65, 1.26)
Class 3 vs. Class 1	0.53 (0.39, 0.73)***	0.85 (0.60, 1.20)	0.84 (0.58, 1.23)
Class 3 vs. Class 2	0.75 (0.58, 0.96)*	0.94 (0.72, 1.22)	0.93 (0.71, 1.24)
Class 4 vs. Class 1	0.14 (0.06, 0.35)***	0.33 (0.13, 0.86)*	0.29 (0.10, 0.83)*
Class 4 vs. Class 2	0.20 (0.08, 0.49)***	0.36 (0.15, 0.92)*	0.32 (0.11, 0.89)*
Class 4 vs. Class 3	0.27 (0.11, 0.66)**	0.39 (0.16, 0.97)*	0.34 (0.12, 0.94)*
Hazard ratio of average DDS and mortality	0.69 (0.62, 0.76)***	0.79 (0.70, 0.89)***	0.78 (0.69, 0.89)***

Cox proportional hazard regression was used to test the associations between DDS trajectory and average DDS with mortality. Model 1 adjusted for age at baseline, gender, level of education, region of residence, place of residence, and individual annual income. Model 2 based on Model 1 further adjusted for history of smoking and alcohol consumption, BMI, history of chronic disease, physical activity, hypotensive or hypoglycemic medicine, and energy intake. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. DDS, dietary diversity score.

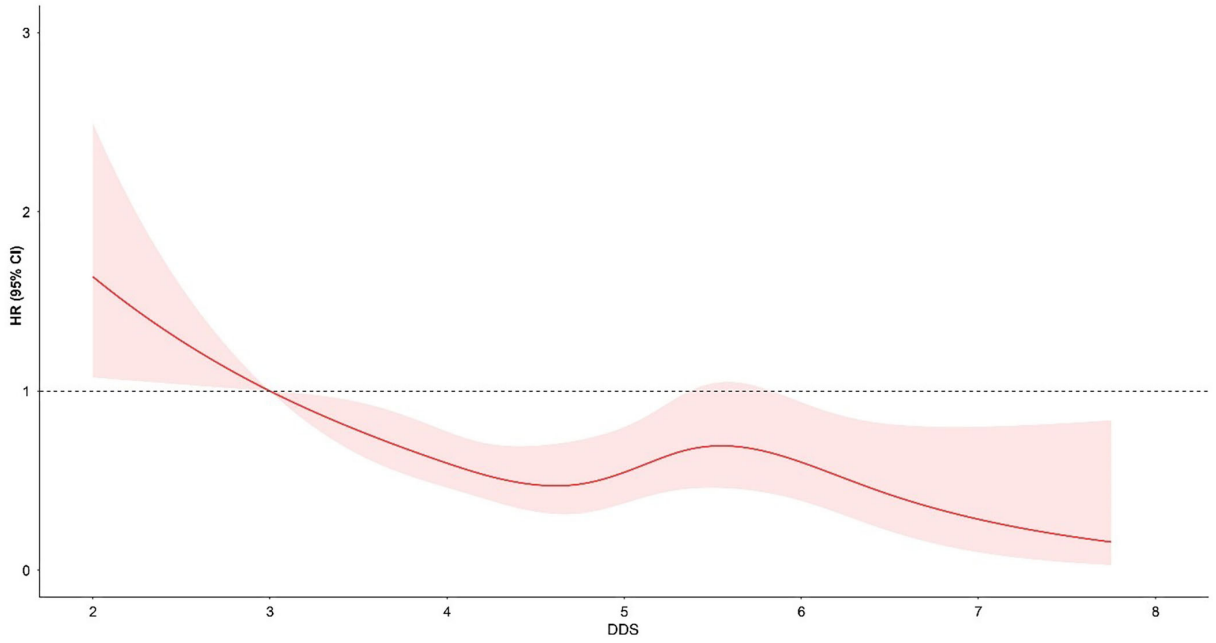

FIGURE 3
Levels and trajectories of DDS change with the age of participants per class. DDS, dietary diversity score.

and mental health as well as cognitive functions among both adolescents and adults (41). However, studies on the relation between dietary diversity and all-cause mortality, especially in developing countries, are still scant. Nevertheless, this review also pointed out that the mixed or null associations between DDS and health outcomes sometimes were shown. Furthermore, the follow-up time of most previous studies was too short to reveal the relation between dietary diversity and health outcomes (41).

In our study, we found that a high level of average DDS related to significantly lower all-cause mortality. Furthermore, each point of increase in average DDS was associated with a 23% reduced risk of mortality among the middle-aged and elderly population. The fitted curve of association between DDS and mortality hazard ratio showed that DDS of lower than 3 was significantly associated with an increased all-cause mortality rate, and DDS of higher than three was associated with a decreased mortality rate. These results are in line with another study, which concluded that among older adults, a higher overall DDS was associated with a 30% decreased risk of mortality (46). The higher decrease in risk of mortality compared with our study might be due to the older age of the subjects (65 or older at baseline). The possible explanation for the inverse association between DDS and mortality could be the fact that DDS could reflect micronutrient adequacy (42). Also, a diverse diet could increase gut microbiota diversity, which was associated with

nutritional status, inflammatory disorders, and co-morbidity (47). In addition, DDS lower than three is also regarded as an indicator of malnutrition in Ethiopian children, which predicted to be underweight (48). These results indicated that DDS of lower than 3 might be an important cut-off value to reflect the poor dietary quality and to predict poor health status among not only children but also middle-aged and elderly populations.

It is also noteworthy that people with a lower DDS were more likely to live in rural areas, have lower income, a lower education level, and a higher physical activity level. This could be due to better accessibility to varieties of food in urban areas than in rural areas (49). In addition, less education of them might lead to a lack of nutrition knowledge, engagement in low-paid physical labor, and significantly lower energy intake. Therefore, increasing and maintaining the nutritional status of this population for the long term is crucial. Policymakers should pay more attention to increasing the diversity of food supply in rural areas, promoting the accessibility of different kinds of foods, and providing financial support to people with low income, thereby improving their wellbeing and decreasing the mortality rate of the vulnerable population in China.

Longitudinal changes of DDS and their associations with mortality

Besides the health influence of DDS, the changes of DDS along with age may also bring a significant impact on health. In the aging process, people usually experience changes in multiple physical, mental, or lifestyle factors that may decrease their dietary diversity. Physically, sensory function decline such as loss of taste and smell may reduce total food intake, thus leading to worsened nutritional state (50). Moreover, the lower chewing ability of elderly people is also associated with food intake insufficiency (15). The lifestyle of elderly people such as eating alone is related to depressive mood and thus decreases their appetite and dietary diversity (16). The financial disadvantage is another hurdle that may limit the dietary diversity of the elderlies, as a study reported that a diverse diet was nearly one-fifth more expensive than an undiversified diet (6, 14).

With the LCTM approach, the dynamic changes of DDS along with aging in the Chinese population were first addressed. Interestingly, DDS did not show a trend of decrease in most populations, although it was prevalent in elderly people (17). In our study, only people in class 1 with the lowest average DDS and the highest median age of baseline showed decreased DDS over time. In classes with relatively low to medium DDS (classes 2 and 3), increases in DDS over the years were observed. However, after adjusting for sociodemographic and lifestyle characteristics, no significant positive effects on mortality were detected compared with class 1. Given that more than 90% of the studied participants were in classes 1, 2, and 3, it can be beneficial for them to increase DDS to a higher level.

A previous longitudinal study showed that elderly people with lower DDS caused by poor appetite had a higher risk of all-cause mortality (51). Participants of this study with a low DDS scantly consumed multiple kinds of food, including vegetables and fruits, fish and other seafood, meat, and eggs, which might result in their lower intakes of total energy, protein, vitamin B, iron, and phosphate. Not only malnutrition is widely accepted as a health concern and significantly shortens the life expectancy of older adults, but also low consumption of varieties of food and nutrients caused by low appetite could be a reason for the higher mortality rate (52). Inadequate protein-rich food consumption, together with a lower DDS, was suggested to be associated with a higher all-cause mortality rate in the elderly (53). Also, because of the diminished sense of taste, together with the monotonous diet of people with low DDS, elderly people tend to eat food with strong flavors, such as pickled vegetables with high salt and sodium content and sweet food containing large amounts of sugar (51, 54). High sodium intake may attribute to additional health risks of chronic diseases, such as hypertension, and excessive sugar consumption is associated with a higher risk of type 2 diabetes and obesity (55). Malnutrition and the higher risk of chronic diseases may both increase the mortality rate of elderly people. Nevertheless, it should be noted that an extreme increase or decrease in DDS was associated with a higher risk of mortality (10). The possible explanation could be that dramatic change in lifestyle could be because of unstable family care, irregular physical exercise, or the diagnosis of chronic diseases.

China is transitioning into an aging society. Since aging is associated with various chronic disorders, more healthcare and medical support for elderly people aggravate the burden on their families and society. Diet is a modifiable factor that is relatively easy and inexpensive to change. Thus, promoting a higher dietary diversity can be a promising way to decrease the mortality rate and prolong health spans in elderly people. The CDG-2022 suggests that elderly people should consume at least 12 kinds of foods every day, and increase their appetite with varieties of methods, such as changing cooking recipes and eating with families and friends (30). Yet, policymakers should place more emphasis on promoting a higher dietary diversity by multiple means such as increasing the accessibility and affordability of a wider range of foods.

Strengths and weaknesses

This study has several strengths. First, the data we used was based on a large sample cohort with a long follow-up time of more than 10 years. Second, we performed a new and reasonable method for trajectory analysis, which could reflect the dynamic changes of DDS over the years.

However, limitations in this study warrant careful consideration. Although we have adjusted for many potential confounders, there could still be unmeasured or residual confounders that could not be completely ruled out. In addition, we have only investigated the association between DDS changes and all-cause mortality, because the cause-specific mortality rates were not available in the CHNS. Therefore, the association between DDS and cause-specific mortality is still needed to be explored to understand the important role that DDS played in human health. Also, specific food groups involved in DDS, such as plant- or animal-based food groups, and their associations with mortality should be further investigated.

Conclusion

Reaching and maintaining a higher DDS among middle-aged and the elderly is significantly associated with a decreased risk of all-cause mortality. These findings demonstrate the significance of promoting and maintaining a higher DDS over middle to late adulthood. Future studies should focus on not only DDS and its influencing factors but also the change in DDS over the life course to further clarify the health implications of maintaining a high dietary diversity, such as malnutrition, metabolic diseases and other non-communicable diseases, and mental health.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: https://www.cpc.unc.edu/projects/china.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Boards at the University of North Carolina (Chapel Hill, NC, the US); the National Institute of Nutrition and Food Safety (Chinese Center for Disease Control and Prevention); Institution Review Board of Tsinghua University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

AZ designed the study, edited, and proofread the final manuscript, has full access to all data used in this study, and has taken responsibility for the integrity of the data and the accuracy of the data analysis. XN performed the statistical analyses. XQ wrote the manuscript, interpreted the results, and composed the discussion. JY, HY, and AC supported literature searching and data analysis. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.947290/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Proportional hazard assumption of covariates for the Cox regression of dietary diversity score trajectory and mortality according to the Schoenfeld residuals test, and the results showed that all the covariates met the assumption based on a *p*-value threshold of 0.05 ($P = 0.08$).

SUPPLEMENTARY FIGURE S2
Proportional hazard assumption of covariates for the Cox regression of average dietary diversity score and mortality according to the Schoenfeld residuals test, and the results showed that all the covariates met the assumption based on a *p*-value threshold of 0.05 ($P = 0.14$).

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Neighborhood effects on dietary behaviors—evidence from older adults in China

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Individual neighborhood environment is an important predictor of dietary behavior. Using data from four waves of the China Health and Nutrition Survey (CHNS, 2004–2011), this study applied a panel data approach to examine the effects of neighborhood diet quality on the eating behaviors of older adults living in the same community. Results of the fixed effects estimation indicated a significant neighborhood effect within the community, and neighborhoods with high-quality diets had a significantly positive effect on the eating behavior of the elderly. The neighborhood effects on elderly eating behaviors were manifested in improved dietary structure, including decreased consumption of cereals and increased consumption of vegetables and fruits, as well as meat, eggs, and dairy products. In terms of nutrient intake, there was a significant increase in protein intake, and hence, a greater percentage of calories from protein. The estimation results were robust when different estimation methods or diet quality measures were used. Future policies for improving diet quality should consider neighborhood-level conditions, especially in rural areas where residents are closely connected and socially interact with one another.

KEYWORDS

dietary behaviors, China, neighborhood effects, nutrient intake, older adults

Introduction

Population aging has become a public health issue in China. According to the National Bureau of Statistics of China, the number of people aged 65 and over is 200.56 million, accounting for approximately 14.19% of the total population of the mainland in 2021. The development of chronic diseases in the elderly population is closely related to dietary structure and health behaviors. A low-quality diet and lack of physical activity are major risk factors for overweight/obesity, diabetes, hypertension, cardiovascular diseases, and other health-related consequences (1–4). Dietary patterns have become a key topic in health research, and many studies have focused on the association between dietary intake and obesity (5–8). For example, Papandreou et al. (7) found that the intake of energy, protein, carbohydrates, and thiamine was positively associated with obesity in children, whereas children with dietary iron deficiency were more likely to be obese after adjusting for energy intake. Through a cross-sectional household-based study, Zou et al. (8) extracted five dietary patterns, including "cereal, animal, and plant foods," "high-protein foods," "plant foods," "poultry" and "beverages" dietary

patterns among Chinese adults and found that the "cereal, animal, and plant foods" and "beverages" dietary patterns increased the intake of protein and fat and the intake of carbohydrates, which ultimately lead to obesity. Moreover, dietary patterns have been shown to be associated with the occurrence of non-communicable diseases [(9–11)]. For instance, the GBD 2017 Diet Collaborators (12) conducted a systematic study and found that high sodium intake and low whole grain and fruit intake were major dietary risk factors for death and disability-adjusted life expectancy in many countries worldwide. Similarly, Wang et al. (9) identified five dietary patterns and found that "protein dietary pattern," "balanced dietary pattern," and "beans dietary pattern" show protective effects on cardio-cerebrovascular disease, whereas "prudent dietary pattern" and "traditional dietary pattern" were positively associated with hypertension.

In terms of factors influencing dietary behaviors, the residential environment is an important predictor, in addition to individual and household characteristics (13–15). Most existing research has focused on home and neighborhood built environments to differentiate between dietary behaviors among adolescents and adults (16–19). For instance, Ho et al. (17) found that fast food shops, restaurants, and convenience stores that are available to adolescents in the neighborhood, especially poor ones, may have a negative effect on their dietary intake. Similarly, Berge et al. (20) found that healthy neighborhoods and home environments were associated with adolescents' healthy dietary intake and low BMI. Additionally, neighborhood socioeconomic conditions and external shocks may lead to changes in individuals' dietary patterns (21). Mayne et al. (22) focused on the associations of neighborhood social environment exposures, including perceived safety, collective efficacy, and crime, with dietary intake and found that high perceptions of the neighborhood environment were associated with high consumption of some healthy foods among preschool-aged children and their mothers.

To better understand the pathway to healthy aging, not only the neighborhood built environment but also the dietary behaviors of neighbors should be considered, especially in areas where residents are closely connected and socially interact with one another. However, few studies have addressed the relationship between dietary behavior and neighbors. Except for Leonard et al. (18), their research found that neighbors' dietary patterns are related to individuals' food consumption (higher fruit and vegetable intake) while controlling for food sources and neighborhood built environment.

This study investigates whether neighborhood diet quality is a predictor of dietary behaviors in older life stages in the context of China. Using data from the China Health and Nutrition Survey (CHNS, 2004–2011), we applied a fixed effects approach to account for unobserved individual heterogeneity. We found significant and positive neighborhood effects on dietary behavior among older adults. Neighborhood diet quality had a significant positive effect on the eating behavior of the elderly. This study provides evidence for a consistent association between neighborhood effects and dietary behaviors by dividing the sample into subgroups. However, no significant gender or regional differences were observed in the heterogeneity analysis.

The remainder of this paper is organized as follows. Section Materials and methods describes the empirical framework, data, and measurements of the study variables. Section Results presents our estimation results. The final section concludes the paper and discusses future policies.

Materials and methods

Data source

The data employed in this study were derived from the China Health and Nutrition Survey (CHNS) conducted by the Carolina Population Center at the University of North Carolina at Chapel Hill and the National Institute for Nutrition and Health at the Chinese Center for Disease Control and Prevention. The CHNS is a longitudinal survey covering detailed information, such as background demographics, work activities and income, health service and disease history, and food consumption. A multistage random cluster method was used to draw samples from nine provinces of China, including Henan, Hubei, Heilongjiang, Liaoning, Shandong, Guizhou, Jiangsu, Guangxi, and Hunan. The first round of CHNS was collected in 1989. Nine additional panels were collected for 1991, 1993, 1997, 2000, 2004, 2006, 2009, 2011, and 2015.

Notably, the CHNS adopted a new food code system after the wave 2004, which is consistent with the coding in the Chinese Food Composition. Hence, four waves of data from 2004, 2006, 2009, and 2011 were used to construct the panel dataset[1]. In this study, we focused on adults aged 55 years or older in urban and rural areas. After excluding samples with missing values and outliers, 11,547 observations were used. Specifically, the sample sizes in 2004 were 2,264, those 2006, 2009, and 2011 in 2,817, 3,387, and 3,079, respectively. The detailed sampling process is illustrated in Figure 1.

Measures

Dietary behaviors

Based on the China Food Composition (23, 24), and detailed food consumption records in the CHNS, we constructed several indicators to describe the dietary behaviors of older adults.

1 The latest wave is not included in this study because data from the dietary questionnaire have not been published in 2015.

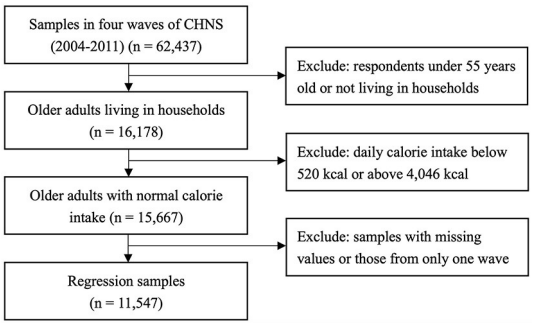

FIGURE 1
Flow chart of study population selection.

Diet quality

In this study, Chinese Healthy Eating Index (CHEI) was used to describe overall diet quality for Chinese people, which is developed on the basis of the Healthy Eating Index (HEI)[2] (25, 26). According to the CHEI, diet quality is assessed from daily food consumption, which can be divided into adequacy components (12 food groups) and limitation components (5 groups) (see Table 1). Recommended amounts were converted into standard portions per 1,000 calories (SP/1,000 kcal) at different caloric levels[3] for each adequacy or limitation component. Scoring for CHEI components is based on energy density, among which cooking oils, sodium, fruits, and dairy are assigned 10 points[4], and other components are assigned 5 points. The sum of an older individual's CHEI score ranges from 0 to 100 points, with higher scores indicating higher food quality. Details of the scoring are described in Yuan et al. (25).

In addition to CHEI, the Chinese Diet Quality Distance (DQD) was constructed with reference to the Chinese Dietary Balance Index-07 (DBI-07) (28, 29). Similarly, DQD contains seven food groups (Table 1). For each food group, if a respondent's daily intake reached the recommended intake level, a score of 0 points was recorded. Negative (ranging from −12 to −1) and positive scores (ranging from 1 to 12) were recorded to evaluate inadequate and excessive food intake. Details of the scoring are described in Xu et al. (29). DQD was calculated by summing the absolute values of the positive and negative scores of each DBI-07 component, ranging from 0 to 84 points. Contrary to CHEI, higher points of DQD indicate a less balanced diet.

TABLE 1 Components of diet quality indicators.

Diet quality indicators	Components
Chinese Healthy Eating Index (CHEI)	***Adequacy components***
	Total grains; Whole grains and mixed beans; Tubers; Total vegetables; Dark vegetables; Fruits; Dairy; Soybeans; Fish and seafood; Poultry; Eggs; Seeds and nuts
	Limitation components
	Red meat; Cooking oils; Sodium; Added sugars; Alcohol
Chinese Diet Quality Distance (DQD)	Cereals; Vegetables and fruits; Dairy products, soybean, and soybean products; Animal food; Condiments and alcoholic beverages; Dietary variety*; Drinking water

*The dietary variety of DQD was based on 12 food subgroups, and −1/0 points were recorded for each subgroup according to daily intake.

Food consumption

In addition to diet quality, we computed the daily food consumption of each participant. The Chinese Food Guide Pagoda (2016) follows the principle of a balanced diet and reflects a nutritionally desirable basic food composition. Based on the structure of the Chinese Food Guide Pagoda (2016), we divided daily food consumption into three food groups: (1) cereals (and cereal products); (2) vegetables and fruits; and (3) meat, eggs, and dairy products. Therefore, the dietary behavior of older adults can be evaluated by observing their daily intake of different food groups.

Nutrient intake

Furthermore, we computed the daily intake of four macronutrients for each older adult: (1) dietary energy, (2) carbohydrate, (3) fat, and (4) protein, among which dietary energy[5] is an overall indicator that measures an older individual's daily energy intake. Carbohydrates, fats, and proteins are the three main macronutrients that an individual needs to maintain their body's structure and systems. Moreover, the distribution of total calories, expressed as a percentage of

2 Healthy Eating Index (HEI) has been widely used in previous studies for assessing diet quality for Americans.

3 Cooking oils and alcohol are measured in grams per 1,000 calories (g/1,000 kcal), whereas sodium is measured in milligrams per 1,000 calories (mg/1,000 kcal).

4 Owing to data availability in China Food Composition, added sugars is not included in this study. Following Liu et al. (27), we reassigned Dairy with 10 points (instead of 5 points) to ensure that the maximum total score of CHEI is 100 points.

5 Following Tian and Yu (30), older adults with an average daily calorie intake below 520 kcal (energy requirement for a newborn baby) and above 4,046 kcal were dropped in this study (exceeding three standard deviations in the sample).

calories obtained from carbohydrates, fats, and proteins, was used to assess the nutrient structure.

Neighborhood dietary behaviors

In this study, we defined the neighborhood effect as the average diet quality of all adults living in the same community, measured by CHEI and DQD. As mentioned above, both indicators depict the overall evaluation of an individual's eating behavior. Hence, a higher average CHEI value represents better dietary behaviors in the neighborhood, whereas a higher DQD value describes worse dietary behaviors in the neighborhood.

Control variables

To investigate neighborhood effects on dietary behaviors among older adults, we controlled for the following variables. (1) Individual characteristics: age, marital status (married/others), education level (in years), working status (yes/no), and number of chronic diseases, which is a proxy for the health endowment of each older adult. (2) Household characteristics: the number of household members, per capita household income (in thousands of yuan), age, gender (male/female), and education level (in years) of the main cook in the household. (3) Community-level characteristics: the number of grocery stores and supermarkets, and the type of road within communities (dirt/stone, gravel, or mixed material/paved road). Following Liu et al. (15), we included year-month dummy variables to eliminate potential seasonal variations in food consumption. In addition, we included interactive fixed effects of each city and survey time to reduce the potential bias caused by time-varying unobservables at the city level, such as external shocks or regional policies.

Method

To estimate the effects of neighborhood diet quality on the dietary behaviors of elderly people in China, we specified a high-dimensional fixed effects model, which can be written as follows:

$$Dietary_{it} = \beta_0 + \beta_1 MDietary_{jt} + \beta_2 X_{it}^{I} + \beta_3 X_{it}^{H} + \beta_3 X_{it}^{V} + u_i + T_t + \delta_c w_t + \varepsilon_{it} \quad (1)$$

where $Dietary_{it}$ represents individual i's dietary behavior in year t, including both diet quality and dietary intake of his/her meals, and $MDietary_{jt}$ represents neighborhood effects, measured by the average value of the dietary indicator for all adults living in the same community in year t. Other control variables included individual characteristics X_{it}^{I}, household characteristics X_{it}^{H}, and community-level characteristics X_{it}^{V}. u_i denotes the individual fixed effects; T_t represents time fixed effects, including year-month dummies; $\delta_c w_t$ is interactive fixed effects, namely, an interactive term of city dummies and year dummies; and ε_{it} is the idiosyncratic error term.

In particular, the current setting of the fixed effects model controls for time-invariant unobservables, such as dietary habits and food preferences, through within-group differencing, which removes omitted variable bias. Moreover, time and interactive fixed effects are included in the regressions, accounting for potential seasonal variations in food consumption and time-varying unobservables at the city level, such as external shocks and regional policies, respectively. Considering the potential correlation in residuals across individuals living in the same community, we reported robust standard errors by clustering at the community level. Singletons were excluded from the regression analysis to avoid incorrect inferences (31, 32). In addition, given the imperfection of fixed effects models for potential endogeneity problems, we attempt to construct instrumental variables and apply two-stage least squares (2SLS) to estimate the neighborhood effects in the robustness check.

Results

Descriptive analysis

Table 2 shows descriptive statistics of the variables, among which older adults are grouped into two sub-samples: (1) urban older adult and (2) rural older adults. The average age of older adults in the sample was about 66 years old, among which around 47% are male and 53% were female. Compared with the rural elderly, the urban elderly are about 1 year older and have better dietary behaviors. Specifically, they had higher quality diet, measured by CHEI and DQD indicators, and better dietary structure, namely, lower consumption of cereals and higher consumption of vegetables and fruits as well as meat, eggs, and dairy products. Hence, urban adults have higher nutrient intake of fat and protein but less intake of carbohydrates. In terms of dietary energy, the rural elderly had a slightly higher daily intake than their urban counterparts. In general, urban elderly belong to higher socioeconomic groups, with more years of education and higher per capita household income. However, in terms of health endowment, urban elderly suffer from more chronic diseases than rural elderly. Urban–rural differences are also evident in terms of community environments. The percentage of having paved road is higher in urban (88%) than in rural areas (75%).

Neighborhood effects and dietary behaviors of older adults

Table 3 presents the fixed effects estimates. As shown in column (1), the coefficient of neighborhood CHEI is positive and statistically significant, which indicates healthier diets among

TABLE 2 Summary statistics.

Variables	Unit	All		Urban		Rural	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Dietary behaviors							
CHEI	point	43.59	10.12	47.33	10.85	41.60	9.11
DQD	point	42.24	9.14	38.47	8.99	44.25	8.57
Cereals	g	417.31	188.01	376.95	168.69	438.75	194.13
Vegetables and fruits	g	337.98	184.38	356.13	194.78	328.34	177.87
Meat, Eggs and Dairy products	g	130.13	113.23	177.02	130.86	105.22	93.54
Dietary energy	kcal	2001.64	630.97	1940.82	611.35	2033.95	638.84
Carbohydrate	g	279.37	104.04	248.28	92.12	295.88	106.22
Fat	g	68.24	34.84	74.91	36.37	64.70	33.46
Protein	g	61.24	22.50	63.45	23.79	60.06	21.69
Share of calorie obtained from carbohydrate	%	56.17	11.86	51.62	11.58	58.58	11.29
Share of calorie obtained from fat	%	30.47	11.42	34.34	11.31	28.41	10.93
Share of calorie obtained from protein	%	12.37	2.89	13.21	3.21	11.92	2.60
Individual characteristics							
Age	year	65.63	7.85	66.13	8.13	65.37	7.68
Male	1 = male, 0 = female	0.47	0.50	0.47	0.50	0.47	0.50
Married	1 = married, 0 = others	0.81	0.39	0.82	0.39	0.80	0.40
Education in years	year	5.24	4.41	6.65	4.88	4.48	3.93
Dummy variable for work	1 = work, 0 = others	0.35	0.48	0.17	0.37	0.44	0.50
Number of chronic diseases	number	0.34	0.60	0.45	0.68	0.28	0.55
Household characteristics							
Number of household members	number	3.37	1.82	3.03	1.50	3.55	1.95
Per capita income	thousand yuan	11.50	13.02	15.98	13.35	9.12	12.20
Age of household cook	year	61.61	10.80	62.13	10.48	61.34	10.95
Male household cook	1 = male, 0 = female	0.20	0.40	0.24	0.43	0.18	0.38
Household cook's education in years	year	5.11	4.36	6.71	4.68	4.26	3.93
Community-level characteristics							
Number of grocery stores and supermarkets	number	17.73	28.36	17.80	25.63	17.69	29.71
Characteristics of the road 1	1 = dirt, 0 = others	0.04	0.20	0.03	0.17	0.05	0.21
Characteristics of the road 2	1 = stone, gravel, or mixed material, 0 = others	0.16	0.37	0.10	0.29	0.20	0.40
Characteristics of the road 3	1 = paved road, 0 = others	0.80	0.40	0.88	0.33	0.75	0.43

older individuals who live in a community of neighborhoods with a high-quality diet. Columns (2)–(4) in Table 3 show that the neighborhood's diet quality is associated with older adults' improved food structure. Specifically, older adults have decreased consumption of cereals and increased consumption of vegetables and fruits, as well as meat, eggs, and dairy products.

In addition to neighborhood effects, individual socioeconomic status is a significant predictor of dietary behavior. Older adults with higher educational levels tend to consume more vegetables and fruits, as well as meat, eggs, and dairy products. Higher household income is associated with improved diet quality among older adults. Elderly people consume more vegetables and fruits, as well as meat, eggs, and dairy products, but consume fewer cereals, indicating an improved dietary structure. The variables that measured access to community food were statistically significant. Communities with more grocery stores and supermarkets are related to improved diet quality and food consumption among older residents, which is consistent with the literature on the influence of community context on dietary behaviors (16–18).

Neighborhood effects and nutrient intake of older adults

We further estimated the fixed effects models with each older adult's nutrient intake as the dependent

TABLE 3 Estimation of neighborhood effects on dietary behaviors of older adults (fixed effects estimators).

Variables	Diet quality	Food consumption		
	CHEI	Cereals	Vegetables and fruits	Meat, eggs and dairy products
	(1)	(2)	(3)	(4)
Neighborhood CHEI	0.633*** (0.047)	−0.089 (1.077)	2.563** (1.202)	1.797*** (0.497)
Square of age	−0.337 (0.259)	−10.375** (5.026)	−2.959 (5.377)	1.569 (3.275)
Married	1.256** (0.559)	15.733* (9.018)	−11.095 (10.211)	−7.430 (6.294)
Education in years	0.014 (0.056)	−1.588 (1.266)	3.522*** (1.214)	1.371** (0.605)
Dummy variable for work	0.759*** (0.289)	11.721* (6.705)	4.974 (5.989)	3.240 (3.262)
Number of chronic diseases	−0.147 (0.217)	−1.223 (3.987)	−9.597** (4.444)	0.326 (2.291)
Number of household members	0.576*** (0.108)	0.092 (2.440)	4.459** (2.222)	0.305 (1.211)
Per capita income	0.028** (0.011)	−0.429** (0.194)	0.656** (0.277)	0.329*** (0.122)
Age of household cook	−0.018 (0.015)	−0.312 (0.342)	−0.201 (0.323)	0.158 (0.168)
Male household cook	−0.401 (0.353)	0.368 (8.055)	−18.954*** (7.209)	2.410 (4.301)
Household cook's education in years	0.032 (0.060)	−0.649 (1.274)	−0.821 (1.266)	0.504 (0.752)
Number of grocery stores and supermarkets	0.006 (0.006)	0.277* (0.155)	0.133 (0.109)	0.056 (0.061)
Characteristics of the road 1	−1.388*** (0.505)	12.040 (15.701)	−4.766 (11.670)	−2.632 (6.485)
Characteristics of the road 2	−0.418 (0.376)	−4.556 (9.318)	−7.627 (9.335)	−7.889* (4.478)
Constant	27.493** (11.554)	888.987*** (216.908)	341.458 (244.950)	−38.462 (145.341)
Individual FE	Yes	Yes	Yes	Yes
Time FE	Yes	Yes	Yes	Yes
Interactive FE	Yes	Yes	Yes	Yes
Observations	11,547	11,547	11,547	11,547

***p < 0.01, **p < 0.05, *p < 0.1. Standard errors in parentheses are clustered at the community level. Interactive fixed effects are interactive terms for city fixed effects and year fixed effects.

TABLE 4 Estimation of neighborhood effects on nutrient intakes of older adults (fixed effects estimators).

Variables	Nutrients intake				Share of calories obtained from		
	Dietary energy	Carbohydrate	Fat	Protein	Carbohydrate	Fat	Protein
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Neighborhood CHEI	−0.212 (4.043)	0.074 (0.652)	−0.348* (0.191)	0.435*** (0.142)	−0.017 (0.060)	−0.097 (0.060)	0.084*** (0.014)
Control variables	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Individual FE	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Time FE	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Interactive FE	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Observations	11,547	11,547	11,547	11,547	11,547	11,547	11,547

***p < 0.01, **p < 0.05, *p < 0.1. Standard errors in parentheses are clustered at the community level. Interactive fixed effects are interactive terms for city fixed effects and year fixed effects.

variable. To save space, only the neighborhood CHEI was retained, and the estimation results are presented in Table 4.

No significant effects were observed on the total caloric intake, as measured by the individuals' dietary energy. Similarly, no significant neighborhood effects were found for carbohydrate intake among the older adults. However, neighborhood CHEI was positively associated with protein intake and lower fat intake. Furthermore, neighborhood CHEI was associated with an improved nutrient structure in older individuals. In particular, older adults in advantaged neighborhoods consumed a greater percentage of calories from protein and a lower percentage of calories from carbohydrates and fat, although the coefficient for carbohydrate was not statistically significant.

TABLE 5 Estimation of neighborhood effects on dietary behaviors of older adults (fixed effects estimators, DQD indicator).

Variables	Diet quality	Food consumption		
	DQD	Cereals	Vegetables and fruits	Meat, eggs and dairy products
	(1)	(2)	(3)	(4)
Neighborhood DQD	0.624*** (0.048)	6.391*** (1.226)	−2.299 (1.480)	−1.887*** (0.592)
Control variables	Yes	Yes	Yes	Yes
Individual FE	Yes	Yes	Yes	Yes
Time FE	Yes	Yes	Yes	Yes
Interactive FE	Yes	Yes	Yes	Yes
Observations	11,547	11,547	11,547	11,547

***p < 0.01, **p < 0.05, *p < 0.1. Standard errors in parentheses are clustered at the community level. Interactive fixed effects are interactive terms for city fixed effects and year fixed effects.

Robustness check

In this section, we perform robustness tests. First, we changed the key variable to another diet quality indicator (DQD indicator) and re-estimated neighborhood effects on eating behaviors of older adults. Similarly, only the coefficients of the neighborhood effects are reported for each regression. Table 5 presents the estimates. Generally, we found a significant and positive effect of the neighborhood's diet quality on older adults' eating behaviors, which is consistent with the previous results in Table 3. Specifically, a higher neighborhood DQD indicated poorer diet quality in the community. Hence, the impact of neighborhood DQD on older adults' daily consumption of vegetables and fruits as well as meat, eggs, and dairy products is negative and statistically significant. In turn, older adults in disadvantaged neighborhoods had a significantly higher cereal intake.

Furthermore, the average number of illnesses during the past 4 weeks and awareness of having a healthy diet in the neighborhood were employed as instrumental variables to address potential endogeneity. In the CHNS, respondents were asked whether they had any of these symptoms during the past 4 weeks, including (1) fever, sore throat, cough, (2) diarrhea, (3) stomach ache, (4) asthma, (5) headache/dizziness, (6) joint pain, muscle pain, (7) rash, dermatitis, (8) eye/ear disease, (9) heart disease/chest pain, (10) other infectious diseases, and (11) other non-communicable diseases. We summed the number of these diseases and calculated community-level averages to measure health shocks to neighbors in the past 4 weeks. In addition, respondents were asked about the importance of a healthy diet in their lives. The options included: (1) not important at all (1 point), (2) not very important (2 points), (3) important (3 points), (4) very important (4 points), and (5) the most important (5 points). Similarly, we calculated the mean value of the corresponding indicator at the community level to measure awareness of healthy eating among neighborhood residents. Before presenting the empirical results, we conducted a series of tests on the instrumental variables. Specifically, the instrumental variables for neighborhood effects pass the Sargan–Hansen test for exogeneity. The F-statistic of the first-stage regression of the instruments is well above the rule-of-thumb threshold of 10 for weak instruments (33). Table 6 lists the two-stage least squares estimates. The coefficients of neighborhood CHEI and DQD are both positive, indicating statistically significant neighborhood effects on dietary behaviors among older adults, which is consistent with the fixed effects estimators in Table 3.

TABLE 6 Estimation of neighborhood effects on dietary behaviors of older adults (2SLS estimators).

Variables	CHEI	DQD
	(1)	(2)
Neighborhood CHEI	0.616*** (0.210)	
Neighborhood DQD		0.570** (0.257)
Control variables	Yes	Yes
Community dummies	Yes	Yes
Time dummies	Yes	Yes
Observations	13,449	13,449

***p < 0.01, **p < 0.05, *p < 0.1. Standard errors in parentheses are clustered at the community level.

Heterogeneity analysis

Considering the heterogeneity of older adults in China, we further grouped the entire sample based on their gender and place of residence. Table 7 shows that neighborhood effects have a significant positive effect on diet quality and food consumption in both male and female older adults, and the effects do not differ significantly between groups. Hence, both male and female older adults are influenced by neighborhood diet quality. Similarly, there were no significant regional differences, and the diet quality of both urban and rural older adults was positively affected by neighbors' dietary behaviors. However, in terms of food consumption, we observed differences in neighborhood

TABLE 7 Estimation of neighborhood effects on dietary behaviors of older adults (with subsamples, fixed effects estimators).

	Region		Gender	
	Urban	Rural	Male	Female
	(1)	(2)	(3)	(4)
CHEI	0.512*** (0.075)	0.686*** (0.057)	0.649*** (0.059)	0.624*** (0.050)
Cereals	−3.237* (1.895)	1.449 (1.237)	−0.463 (1.182)	0.281 (1.198)
Vegetables and fruits	5.874*** (1.872)	1.287 (1.494)	2.192* (1.279)	2.921** (1.365)
Meat, eggs and dairy products	2.590** (1.123)	1.421*** (0.506)	2.125*** (0.627)	1.603*** (0.506)

***$p < 0.01$, **$p < 0.05$, *$p < 0.1$. Standard errors in parentheses are clustered at the community level.

effects between the urban and rural elderly. In particular, the neighborhood effect among the urban elderly showed a significant decrease in cereals and an increase in vegetables and fruits, whereas among the rural elderly, the neighborhood effects were mainly reflected in an increase in meat, egg, and dairy product intake. Thus, we found a positive impact of neighborhood diet quality on dietary behaviors among male and female older adults, as well as among urban and rural older adults.

Discussion

In this study, we examined the relationship between neighborhood diet quality and dietary behaviors in older adults, using four waves of data from the CHNS. In contrast to previous studies that have examined the influence of neighborhood built environments on dietary behaviors, this study considers the influence of social interactions between residents within the same community by investigating neighborhood effects on elderly dietary behaviors.

The results showed that neighborhood diet quality has a significant and positive relationship with dietary behaviors among older adults in China. The neighborhood effects on elderly eating behaviors manifested in improved dietary structure, including decreased consumption of cereals and increased consumption of vegetables and fruits, as well as meat, eggs, and dairy products. In terms of nutrient intake, there was a significant increase in protein intake, and hence, a greater percentage of calories from protein.

To demonstrate the heterogeneity of older adults' dietary behavior, we examined subsamples by gender and place of residence. Overall, the results for the subsample are consistent with the baseline estimates. Diet quality and food consumption were significantly and positively influenced by neighborhood effects for both male and female older people. Older people living in urban areas were influenced by the neighborhood effect to reduce cereal and increase fruit and vegetable intake, whereas older people living in rural areas increased their intake of meat, eggs, and dairy products.

Future policies for improving diet quality should consider neighborhood-level conditions. The neighborhood effect implies that dietary interventions have positive externalities; therefore, policy interventions should not only start from individuals but should also consider the interpersonal impact of policy interventions and make full use of the demonstration effect of group behaviors, especially in rural areas where residents are closely connected and socially interact with one another.

Although this study provides direct evidence of neighborhood effects on dietary behaviors, it does not directly examine the mechanisms by which this link works. Future research could further test and explore social interactions within communities as a potential channel to explain the importance of the social environment on individual health and dietary behaviors.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: China Health and Nutrition Survey https://www.cpc.unc.edu/projects/china.

Author contributions

CL: conceptualization, methodology, formal analysis, writing—original draft, writing—review and editing, and funding acquisition. HY: writing—original draft and writing—review and editing. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Psychosocial stress accompanied by an unhealthy eating behavior is associated with abdominal obesity in Korean adults: A community-based prospective cohort study

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Psychosocial stress is recognized as a potential modulator of eating behavior. Psychosocial stress also constitutes an independent risk factor for the development of non-communicable diseases. This study examined the gender-stratified associations between perceived stress, eating behavior, and abdominal obesity in 4,411 adults aged 40–69 years during a 10-year follow-up of the Korean Genome and Epidemiology Study (KoGES). Psychosocial stress was evaluated using the Psychosocial Wellbeing Index Short Form (PWI-SF), and eating behavior was analyzed with a focus on the dietary variety score (DVS). The Cox's proportional hazard model was used to examine the risk of abdominal obesity according to stress levels. Higher stress levels were associated with lower DVS in women. Lower DVS scores were positively associated with the consumption of grains and refined grains but was negatively associated with the consumption of fruits. The DVS was not significantly associated with stress levels among men. Prospectively, the highest tertile of grains and refined grains consumption showed an increased risk of abdominal obesity compared to the lowest tertile in women (HR: 1.36, 95% CI: 1.04–1.78, $p < 0.05$; HR: 1.28, 95% CI: 1.03–1.59, $p < 0.05$, respectively). By contrast, in all participants, the highest tertile of fruits consumption decreased the risk of abdominal obesity compared to the lowest tertile (men, HR: 0.56, 95% CI: 0.45–0.70, $p < 0.01$; women, HR: 0.51, 95% CI: 0.40–0.65, $p < 0.01$). Furthermore, high stress levels showed a borderline significant association with the risk of abdominal obesity only in women (HR: 1.27, 95% CI: 1.00–1.59, $p < 0.05$). These findings suggested that psychosocial stress might contribute to abdominal obesity by interacting with eating behavior represented by a low DVS. The approach to consume a diet with a high DVS might help decrease the risk of abdominal obesity among people in stressful environments.

KEYWORDS

psychological stress, obesity, non-communicable disease, dietary quality, dietary variety score, longitudinal study, gender stratification

Introduction

Psychosocial stress, arising from the workplace or socioeconomic disadvantage and discrimination, is known to affect health outcomes through biological and behavioral changes (1). Stress-induced modification of eating behaviors may be particularly important in understanding various health outcomes. Stress appears to alter overall eating in two contrasting ways (2, 3). When individuals experience chronic stress, they may increase their food intake in response to stress; however, there is also support of either no changes in eating behavior or a reduction of food intake in response to stress (4–6). Moreover, the situational changes in stress, such as any noxious event in one's environment that could be appraised as threatening, risky or harmful, might also evoke change in eating behaviors (3). Little is known on what determines the directional changes in eating behavior following stress, though it has been suggested that the hypothalamic pituitary adrenal (HPA) axis is implicated and the eating-stress behavior relationship in those who experience chronic stress (7). The hyperactivation of the HPA axis, accompanied by increased secretion of cortisol, may entice people to consume energy-dense and hyperpalatable foods, such as those high in sugar and fat, which may then increase the risk of obesity or becoming overweight (8).

It is reported that abdominal obesity accompanied by an increase in intra-abdominal fat and waist circumference (WC) (9) is a primary risk factor for the development of metabolic disorders, such as cardiovascular disease, type 2 diabetes, metabolic syndrome, and some types of cancer (10, 11). The prevalence of abdominal obesity is rapidly rising worldwide. In the United States of America, the estimated prevalence of abdominal obesity increased from 59% in 2003–2004 to 64% in 2013–2014 in men and from 40 to 44% in women (12). In addition, a national survey in Korea reported that the prevalence of abdominal obesity increased from 19.0% in 2009 to 23.8% in 2018 (13). The modifiable lifestyle factors associated with abdominal obesity include stress levels, sedentary patterns, and unhealthy eating behavior (9, 14).

Eating behavior is a broad term that encompasses food choice and eating motives, feeding practices, dieting, and eating-related problems (15). Healthy eating behaviors have been identified as eating nutrient-balanced meals and a variety of foods (16). Dietary variety is regarded as an integral component of healthy eating behavior (17). The dietary variety score (DVS) may be an indicator for assessing eating behavior by counting the total number of different food items consumed over a period of time (18). A low DVS was intimately related to increased energy ratios of carbohydrates and grains, as well as nutritionally imbalanced meals (19). When chronically stressed, people tend to engage in unhealthy eating behaviors.

The effect of perceived stress on eating behaviors is thought to differ between men and women. Prior research in the general population has reported gender differences in emotional eating, which is occurring in the presence of negative emotions (20). Women are more likely to change their normal eating behaviors when experiencing stress compared to men (21, 22).

According to a 6.5-year follow-up in a Dutch population of middle-aged and older adults, the experience of stressful life events was associated with an increased incidence of abdominal obesity (23). A meta-analysis showed that the risk of adiposity was increased by about 25% due to psychosocial stress (24). However, to our knowledge, no prospective study has investigated whether stress may modify eating behaviors, which then may consequently contribute to the risk of abdominal obesity. Therefore, we aimed to investigate the associations between perceived stress, eating behavior, and abdominal obesity in middle-aged and older adults stratified by gender, using data from the Korean Genome and Epidemiology Study (KoGES), a large community-based cohort study. We hypothesized that stress accompanied by an unhealthy eating behavior may be associated with an increased risk of abdominal obesity. Moreover, the direction and magnitude of this association may differ by gender.

Materials and methods

Study population

We used data from a prospective population-based Ansan-Ansung cohort study, part of the KoGES, to examine the risk and burden of chronic disease among the general Korean population. Detailed information on the study design and aims of the KoGES has been previously reported (25). In brief, 10,030 participants aged 40–69 years were recruited from the Ansan (urban) and Ansung (rural) areas, and follow-up examinations were conducted biennially. The second follow-up examination provided information on stress levels, so our analysis used this data as the baseline. Data from the baseline (2005–2006) to the seventh examination (2015–2016) were used for the current study. Among the 7,515 participants, we excluded participants who reported implausible total daily energy intake (<500 or >4,000 kcal/day, $n = 96$), those who did not respond to the stress assessment ($n = 256$), and those with missing information on covariates ($n = 184$). An additional 2,568 participants with abdominal obesity at baseline were excluded. Finally, 4,411 participants (2,439 men and 1,972 women) were analyzed (Figure 1). The study was approved by the Institutional Review Board of Ewha Womans University (2021-0316, October 2021).

Definition of abdominal obesity

WC was measured at each follow-up examination. Abdominal obesity was defined as a WC $\geq$ 90 cm in men and $\geq$ 85 cm in women, in accordance with the definition of the Korean Society for the Study of Obesity (KSSO) (26).

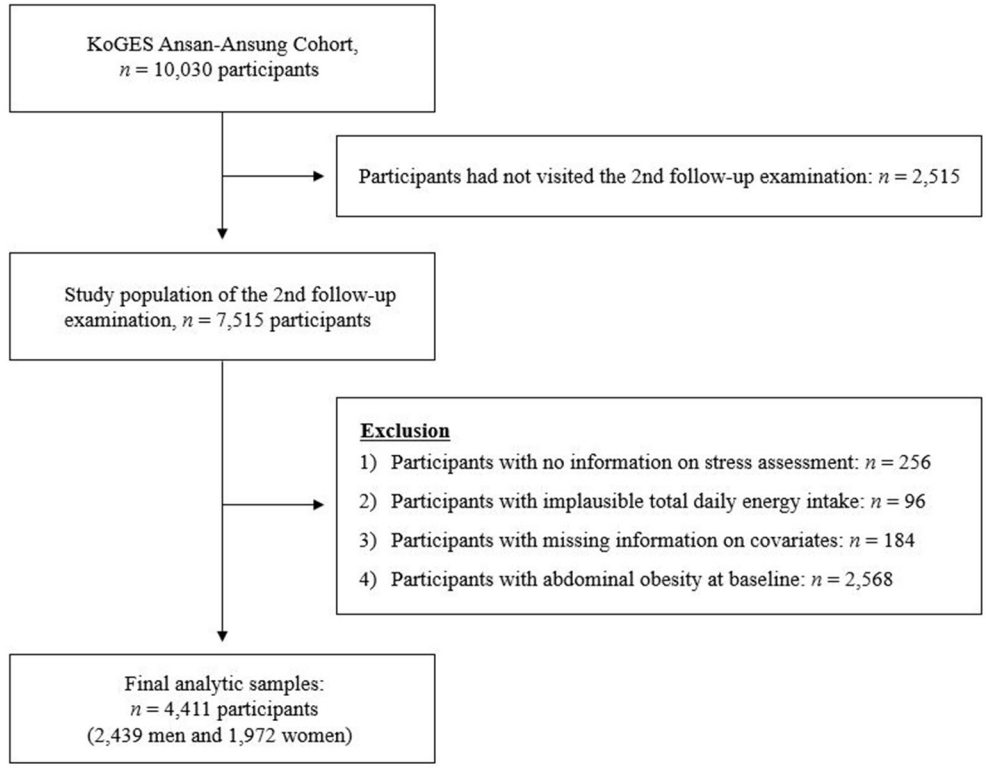

FIGURE 1
Flow diagram of the study population.

Assessment of psychosocial stress

At baseline, the participants' stress levels were assessed using the Psychosocial Wellbeing Index Short Form (PWI-SF) developed by Chang (27), which was based on the general health questionnaire devised by Goldberg (28). The validity of the PWI-SF has been previously demonstrated (27). The PWI-SF consists of 18 items: social performance and self-confidence (eight items), depression (three items), sleep disturbances and anxiety (three items), and overall wellbeing and vitality (four items). Each item ranges from "strongly disagree" (0) to "strongly agree" (3) based on a 4-point Likert scale, and total PWI score is the sum of each subscale. A higher PWI-SF score reflects a higher level of psychosocial stress.

Assessment of food consumption and eating behavior

The dietary intake information was collected using the semi-quantitative food frequency questionnaire (FFQ) developed for the KoGES (29). This FFQ consisted of 106 food items. Food items were classified into 8 groups based on the previous study (30). We modified Leila Azadbakht's method adding highly palatable foods category: grains, refined grains, vegetables, fruits, dairy, meat, fast foods, and highly palatable foods (Supplementary Table 1).

Food consumption was measured once, at baseline of the study, concerning the individual's dietary intake over the past year. Participants were asked to report their average food frequency (on a 9-point scale of "almost none," "once a month," "twice or three times a month," "once or twice a week," "twice or three times a week," "five or six times a week," "once a day," "twice a day," and "three times a day") and the average portion size (on a 3-point scale of "0.5 times the reference," "reference," and "1.5–2.0 times the reference") for each food item for 1 year. The duration of the seasonal variety of fruit consumption was divided into four categories (3, 6, 9, and 12 months). The validation and reproducibility of the FFQ are described in detail (29).

Eating behavior was evaluated based on the DVS, originally devised by Elizabeth Randall et al. (31). In this study, we measured DVS modified by Choi et al. (18), counting the food items consumed at least once per month. Specifically, the food items consumed were counted as 1 point except "almost none," based on the reported frequency from the FFQ. Foods consumed multiple times during the period were counted only once. In

addition, the foods containing the same ingredients, such as pork roast and steamed pork, were considered as one food. Each time another food item was consumed, the DVS increased by 1 point.

Measurements

Anthropometric measurements were obtained by trained research staff at each follow-up visit. Height and body weight were measured with the participants wearing a patient gown and no shoes, and the body mass index (BMI) was calculated as body weight (kg) divided by the square of height (m^2). The WC (cm) was measured at the thinnest point between the lower rib and the iliac crest, and the average of three repeated measurements was used in this study. Blood pressure (BP) was measured in both arms using a mercury sphygmomanometer (W.A Baum Co. Inc., Copiague, NY, USA) after resting for at least 5 min. This study used the average value of repeated measurements to define systolic BP and diastolic BP.

Covariates

The demographic characteristics, socioeconomic status, and lifestyle factors of the participants were surveyed at baseline. Covariates included age, BMI, marital status (others, married), monthly household income (<3 million KRW, ≥3 million KRW), education level (others, ≥college), alcohol consumption (never, former, current), smoking status (never, former, current), and physical activity (<30 min/day, ≥30 min/day).

Statistical analysis

Continuous variables are expressed as mean and standard error (SE), and categorical variables are expressed as numbers and percentages. The generalized linear model and the Chi-square test were used to determine the differences in means and distribution of general characteristics and to test the linear trends according to stress levels. For adjustment in the multivariable model, potential confounders from the previously published scientific literature were taken into account (14, 32, 33) with stepwise regression procedures, such as age, BMI, marital status, monthly household income, education level, alcohol consumption, smoking status, physical activity and total energy intake. The multivariable Cox proportional hazard model was used to assess the hazard ratios (HRs) and 95% confidence intervals (CIs) for the risk of abdominal obesity according to stress levels during the follow-up. Data analyses were performed with SAS software, version 9.4 (SAS Institute, Cary, NC, USA). Statistical significance was considered at $P < 0.05$. We stratified the analysis according to gender, as previous research reported that gender influences the relation between stress and eating behaviors (20–22).

Results

Baseline characteristics

Table 1 describes the characteristics of the study population according to tertiles of stress level at baseline. Compared to those with lower stress levels, men with higher stress levels were more likely to have lower waist circumference (WC) ($p < 0.01$). However, women did not show any difference in WC among groups. In men, participants with higher levels of stress were younger ($p < 0.01$), consumed alcohol currently ($p < 0.01$), and were less physically active ($p < 0.01$), whereas women with higher levels of stress were older ($p < 0.05$). Alcohol consumption and physical activity were not significantly different with stress levels among women. In both men and women, participants with higher levels of stress had lower BMI, had lower household income, were less educated, and were more likely to be current smokers (all $p < 0.05$).

Associations between stress levels and food consumption

The associations of stress levels with food consumption (g/1,000 kcal) are presented in Table 2. Among men, the DVS did not differ significantly according to stress levels, whereas women with higher levels of stress showed a lower DVS ($p < 0.01$). In men, the higher stress levels were associated with a higher consumption of refined grains ($p < 0.05$) and highly palatable foods ($p < 0.05$), but lower consumption of fruits ($p < 0.01$). In women, the higher stress levels were associated with a higher consumption of grains ($p < 0.01$), especially refined grains ($p < 0.01$), but lower consumption of fruits ($p < 0.01$), dairy ($p < 0.05$), and meat ($p < 0.01$).

Associations between DVS and food consumption

The associations of DVS with food consumption (g/1,000 kcal) are shown in Table 3. In all participants, as the DVS decreased, the consumption of grains and refined grains increased (all $p < 0.05$). By contrast, as the DVS decreased, the consumption of fruits, dairy, meat, fast foods, and highly palatable foods decreased (all $p < 0.01$). Consumption of vegetables was not significantly associated with DVS.

TABLE 1 Baseline characteristics of the study population.

	Men				Women			
	T1 (Lowest) [§] (*n* = 976)	T2 (Intermediate) (*n* = 790)	T3 (Highest) (*n* = 673)	*P*-value	T1 (Lowest) (*n* = 495)	T2 (Intermediate) (*n* = 720)	T3 (Highest) (*n* = 757)	*P*-value
PWI-SF score (median)	9.0	17.0	26.0		9.0	17.0	27.0	
Age (years)	55.9 ± 0.3	54.5 ± 0.3	54.8 ± 0.3	0.0013	53.8 ± 0.4	53.2 ± 0.3	54.4 ± 0.3	0.0269
Height (cm)	166.4 ± 0.2	166.0 ± 0.2	166.5 ± 0.2	0.1294	154.1 ± 0.3	154.6 ± 0.2	153.6 ± 0.2	0.0015
Weight (kg)	64.9 ± 0.2	63.9 ± 0.3	63.0 ± 0.3	<0.0001	55.1 ± 0.3	55.2 ± 0.2	53.9 ± 0.3	0.0002
Waist circumference (cm)	82.2 ± 0.2	81.5 ± 0.2	80.8 ± 0.2	<0.0001	76.7 ± 0.2	76.8 ± 0.2	76.7 ± 0.2	0.9102
Body mass index (kg/m^2)	23.4 ± 0.07	23.2 ± 0.08	22.7 ± 0.10	<0.0001	23.2 ± 0.10	23.1 ± 0.08	22.8 ± 0.09	0.0146
Systolic blood pressure (mmHg)	116.0 ± 0.5	115.2 ± 0.5	115.2 ± 0.6	0.4768	112.6 ± 0.8	111.4 ± 0.6	111.9 ± 0.6	0.4665
Diastolic blood pressure (mmHg)	77.8 ± 0.3	77.7 ± 0.4	77.7 ± 0.4	0.9632	74.0 ± 0.5	73.3 ± 0.4	74.0 ± 0.4	0.3297
Marital status (%)				0.3715				0.4612
Others	31 (3.2)	29 (3.7)	34 (5.0)		62 (15.5)	90 (15.5)	118 (15.6)	
Married	945 (96.8)	761 (96.3)	639 (95.0)		433 (87.5)	630 (87.5)	639 (84.4)	
Monthly household Income (≥3 million KRW, %)	350 (35.9)	290 (36.7)	202 (30.0)	0.0143	174 (35.2)	232 (32.2)	174 (23.0)	<0.0001
Education level (≥College, %)	208 (21.3)	177 (22.4)	111 (16.5)	0.0123	56 (11.3)	75 (10.4)	39 (5.2)	<0.0001
Alcohol consumption (%)				0.0067				0.4934
Never	200 (20.5)	171 (21.7)	103 (15.3)		353 (71.3)	520 (72.2)	525 (69.4)	
Past	97 (9.9)	57 (7.2)	64 (9.5)		5 (1.0)	14 (1.9)	14 (1.9)	
Current	679 (69.6)	562 (71.1)	506 (75.2)		137 (27.7)	186 (25.8)	218 (28.8)	
Smoking status (%)				<0.0001				0.0356
Never	295 (30.2)	183 (23.2)	125 (18.6)		476 (96.2)	706 (98.1)	724 (95.6)	
Past	372 (38.1)	308 (39.0)	228 (33.9)		6 (1.2)	5 (0.7)	5 (0.7)	
Current	309 (31.7)	299 (37.9)	320 (47.6)		13 (2.6)	9 (1.3)	28 (3.7)	
Physical activity (%)				<0.0001				0.1298
<30 min	269 (27.6)	261 (33.0)	262 (38.9)		173 (35.0)	264 (36.7)	305 (40.3)	
≥30 min	707 (72.4)	529 (67.0)	411 (61.1)		322 (65.1)	456 (63.3)	452 (59.7)	

KRW, Korean won. Values are expressed as mean (SE) or numbers (percentages). [§]Stress levels were assessed using the Psychosocial Wellbeing Index-Short Form (PWI-SF). The P-value was calculated from the ANOVA test for continuous variables and the Chi-square test for categorical variables.

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TABLE 2 Food consumption according to stress levels.

Food consumption (g/1,000 kcal)	Men				Women			
	T1 (Lowest) [§] (*n* = 976)	T2 (Intermediate) (*n* = 790)	T3 (Highest) (*n* = 673)	*P*-trend	T1 (Lowest) (*n* = 495)	T2 (Intermediate) (*n* = 720)	T3 (Highest) (*n* = 757)	*P*-trend
DVS	53.8 ± 0.43	53.6 ± 0.49	52.8 ± 0.54	0.6432	53.9 ± 0.55	54.5 ± 0.44	51.4 ± 0.48	0.0074
Grains	416.7 ± 2.47	423.4 ± 2.86	421.5 ± 3.07	0.2439	385.6 ± 4.07	399.9 ± 3.22	416.5 ± 3.32	<0.0001
Refined grains	136.8 ± 5.76	153.6 ± 6.84	163.1 ± 7.37	0.0431	68.0 ± 5.88	78.7 ± 5.25	101.4 ± 6.00	0.0006
Vegetables	144.7 ± 2.77	144.3 ± 3.19	141.8 ± 3.14	0.5551	148.7 ± 4.00	146.3 ± 3.35	145.1 ± 3.24	0.3219
Fruits	97.9 ± 2.71	89.7 ± 2.55	82.1 ± 2.66	0.0087	163.0 ± 5.17	154.4 ± 4.07	129.0 ± 3.63	<0.0001
Dairy	56.1 ± 2.07	53.2 ± 2.07	54.4 ± 2.44	0.8738	84.6 ± 3.67	77.6 ± 2.98	72.6 ± 2.68	0.0395
Meat	44.8 ± 0.89	42.9 ± 0.94	42.8 ± 1.02	0.1586	40.0 ± 1.22	37.1 ± 0.89	34.9 ± 0.93	0.0069
Fast foods	1.35 ± 0.15	1.28 ± 0.12	1.60 ± 0.20	0.2951	2.30 ± 0.29	2.06 ± 0.19	1.86 ± 0.18	0.4829
Highly palatable foods	17.1 ± 0.86	18.6 ± 1.02	21.1 ± 1.24	0.0206	15.1 ± 1.00	15.1 ± 1.05	15.2 ± 1.23	0.8612

DVS, dietary variety score. Values are expressed as mean (SE). [§]Stress levels were assessed using the Psychosocial Wellbeing Index-Short Form (PWI-SF). The P-trend was obtained through generalized linear models after adjusting for age, BMI, marital status, monthly household income, education level, alcohol consumption, smoking status and physical activity.

TABLE 3 Food consumption according to dietary variety score.

Food consumption (g/1,000 kcal)	Men				Women			
	T1 (Lowest) (*n* = 792)	T2 (Intermediate) (*n* = 851)	T3 (Highest) (*n* = 796)	*P*-trend	T1 (Lowest) (*n* = 653)	T2 (Intermediate) (*n* = 639)	T3 (Highest) (*n* = 680)	*P*-trend
DVS (median)	40	55	67		41	54	65	
Grains	461.7 ± 2.71	414.5 ± 2.44	385.0 ± 2.47	<0.0001	452.9 ± 3.31	396.0 ± 3.20	360.8 ± 3.06	<0.0001
Refined grains	179.7 ± 7.65	133.9 ± 6.06	136.0 ± 5.80	<0.0001	102.4 ± 7.21	74.3 ± 5.34	77.6 ± 4.56	0.0338
Vegetables	151.6 ± 3.48	143.9 ± 2.92	135.8 ± 2.58	0.6870	157.0 ± 4.05	139.5 ± 3.28	142.8 ± 3.04	0.6130
Fruits	65.8 ± 2.35	101.2 ± 2.80	104.9 ± 2.65	<0.0001	117.2 ± 4.17	164.4 ± 4.33	158.7 ± 3.93	0.0029
Dairy	45.3 ± 2.42	57.5 ± 2.14	60.9 ± 1.91	<0.0001	64.9 ± 3.24	82.8 ± 3.26	84.5 ± 2.58	0.0050
Meat	32.6 ± 0.96	44.2 ± 0.86	53.9 ± 0.88	<0.0001	24.9 ± 0.83	37.2 ± 0.92	48.3 ± 0.10	<0.0001
Fast foods	0.57 ± 0.15	1.06 ± 0.14	2.57 ± 0.17	<0.0001	0.79 ± 0.18	1.97 ± 0.21	3.31 ± 0.22	<0.0001
Highly palatable foods	13.7 ± 1.05	17.8 ± 0.90	24.6 ± 1.07	<0.0001	12.2 ± 1.45	13.1 ± 0.81	20.0 ± 1.04	<0.0001

DVS, dietary variety score. Values are expressed as mean (SE). The P-trend was obtained through generalized linear models after adjusting for age, BMI, marital status, monthly household income, education level, alcohol consumption, smoking status and physical activity.

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Associations between stress levels and nutrients intake

The associations of stress levels with nutrients intake per 1,000 kcal are presented in Table 4. Participants with high stress showed low total energy intake ($p < 0.05$ in men and $p < 0.01$ in women). Women with higher levels of stress showed a higher carbohydrate intake despite a lower total energy intake ($p < 0.05$). In women, there was a negative association between stress levels and most of nutrients intake (all $p < 0.05$). The intake of vitamin A, sodium, zinc, retinol, carotene, and cholesterol was not significantly differed with stress levels among women. In men, only vitamin B_1 intake differed significantly in relation to stress levels ($p < 0.05$).

Longitudinal association of food consumption with the risk of abdominal obesity

Prospectively, the highest tertile of grains and refined grains consumption showed an increased risk of abdominal obesity compared to the lowest tertile (HR: 1.36, 95% CI: 1.04–1.78, $p < 0.05$; HR: 1.28, 95% CI: 1.03–1.59, $p < 0.05$, respectively) after adjusting for all confounding factors in women (Table 5). In men, the highest tertile of refined grains consumption was associated with a higher risk of abdominal obesity compared to the lowest tertile (HR: 1.36, 95% CI: 1.11–1.66, $p < 0.01$) after adjusting for all confounding factors. By contrast, among women, the highest tertile of dairy consumption decreased the risk of abdominal obesity compared to the lowest tertile (HR: 0.79, 95% CI: 0.63–0.99, $p < 0.05$) after adjusting for all confounding factors. In all participants, the highest tertile of fruits consumption decreased the risk of abdominal obesity (men, HR: 0.56, 95% CI: 0.45–0.70, $p < 0.01$; women, HR: 0.51, 95% CI: 0.40–0.65, $p < 0.01$) after adjusting for all confounding factors.

Longitudinal association of stress with the risk of abdominal obesity

High stress showed a borderline significant association with the risk of abdominal obesity (HR: 1.27, 95% CI: 1.00–1.59, $p < 0.05$) after adjusting for age, BMI, marital status, monthly household income, education level, alcohol consumption, smoking status, and physical activity only in women (Table 6).

Discussion

In this prospective cohort study, we found that higher levels of stress affected eating behavior represented by a low DVS, characterized by higher consumption of grains and refined grains, and a lower consumption of fruits only in women. High consumption of grains, especially refined grains, was longitudinally associated with an increased risk of abdominal obesity. In addition, stress levels were positively associated with the risk of abdominal obesity. To the best of our knowledge, this is the first study to examine the associations of perceived stress, eating behavior, and abdominal obesity in Korean adults.

Stress can be defined as the generalized, non-specific response of the body to a real or perceived threat beyond the ability to cope (9). The PWI-SF, a survey used in our study, has been widely adopted to assess the levels of psychosocial stress, including physical and psychological symptoms (27, 34) in different populations (35–37). Chronic psychosocial stress is known to increase the risk of developing numerous diseases, such as metabolic syndrome (38), diabetes mellitus (39), and obesity (8).

In this study, participants who were less educated, had a lower income, and were current smokers reported higher levels of stress. Previous studies have reported that the responses to stress may influence lifestyle behaviors, such as smoking, physical activity, and alcohol use (14, 40). Cohort studies in Finland found that work stress was positively associated with both smoking status and intensity (41). Our findings are consistent with previous studies that linked lower incomes and education levels with higher levels of stress (42, 43).

Several studies have found associations between stress and unhealthy eating behavior (3, 44, 45). Eating behavior was commonly assessed based on food preferences, dietary intake, dietary variety, and eating traits (46). It is known that stressful conditions lead to a decreased dietary variety as people tend to show an increased preference for comfort foods from the same food category under stressful conditions (47, 48). In our study, women with higher levels of stress showed a lower DVS, suggesting that stress might be related to unhealthy eating behavior. Exposure to chronic stress activates the hypothalamic–pituitary–adrenal axis, with the release of cortisol (9). Increased levels of cortisol in response to stress may affect appetite (47) and promote abnormal eating behaviors (49), including preferentially selecting highly palatable foods and energy-dense foods (50, 51). The consumption of energy-dense foods has been associated with high intakes of refined grains, processed foods, and added sugars and fats, but low intakes of fruits, vegetables, and whole grains (52, 53). We found that higher levels of stress were associated with higher consumption of grains, especially refined grains in women, but a lower consumption of fruits in both men and women. Moreover, a low DVS was positively associated with the consumption of grains and refined grains

TABLE 4 Nutrient intake according to stress levels.

	Men				Women			
	T1 (Lowest) [§] (*n* = 976)	T2 (Intermediate) (*n* = 790)	T3 (Highest) (*n* = 673)	*P*-trend	T1 (Lowest) (*n* = 495)	T2 (Intermediate) (*n* = 720)	T3 (Highest) (*n* = 757)	*P*-trend
Energy (kcal)	1,922.7 ± 17.15	1,869.0 ± 18.38	1,839.8 ± 19.39	0.0134	1,732.5 ± 23.09	1,674.4 ± 17.51	1,611.9 ± 17.78	0.0004
Protein (g)	32.7 ± 0.17	32.3 ± 0.19	32.1 ± 0.21	0.0828	33.2 ± 0.28	32.5 ± 0.20	32.1 ± 0.22	0.0161
Fat (g)	15.9 ± 0.18	15.4 ± 0.19	15.9 ± 0.22	0.8258	15.0 ± 0.26	14.4 ± 0.20	13.8 ± 0.20	0.0109
Carbohydrate (g)	178.5 ± 0.51	179.9 ± 0.53	178.7 ± 0.61	0.5906	181.3 ± 0.75	182.8 ± 0.56	184.0 ± 0.57	0.0406
Calcium (mg)	223.4 ± 2.80	218.0 ± 3.17	216.8 ± 3.54	0.2740	274.5 ± 5.28	256.2 ± 3.98	250.0 ± 3.98	0.0026
Phosphorus (mg)	494.3 ± 2.63	487.5 ± 2.90	485.4 ± 3.26	0.0872	526.8 ± 4.69	510.7 ± 3.51	504.3 ± 3.56	0.0040
Iron (mg)	5.23 ± 0.04	5.17 ± 0.05	5.07 ± 0.05	0.1125	5.82 ± 0.07	5.64 ± 0.06	5.47 ± 0.06	0.0023
Potassium (mg)	1,239.3 ± 11.65	1,226.1 ± 13.29	1,204.8 ± 13.71	0.1772	1,424.5 ± 21.77	1,366.1 ± 16.15	1,301.0 ± 16.12	0.0002
Vitamin A (R.E.)	252.8 ± 4.62	245.3 ± 5.27	246.8 ± 5.75	0.4643	278.0 ± 6.94	268.5 ± 6.03	263.6 ± 6.09	0.1737
Sodium (mg)	1,528.0 ± 24.08	1,554.1 ± 27.90	1,539.5 ± 27.85	0.9934	1,527.9 ± 35.79	1,491.7 ± 28.56	1,506.8 ± 28.22	0.5378
Vitamin B1 (mg)	0.58 ± 0.004	0.56 ± 0.004	0.56 ± 0.005	0.0197	0.56 ± 0.005	0.56 ± 0.004	0.55 ± 0.004	0.0191
Vitamin B_2 (mg)	0.49 ± 0.004	0.48 ± 0.005	0.48 ± 0.005	0.1718	0.54 ± 0.008	0.52 ± 0.006	0.50 ± 0.006	0.0008
Niacin (mg)	7.83 ± 0.05	7.81 ± 0.06	7.75 ± 0.06	0.1049	7.90 ± 0.08	7.67 ± 0.06	7.55 ± 0.06	0.0124
Vitamin C (mg)	53.0 ± 0.81	51.2 ± 0.86	49.4 ± 0.87	0.0571	70.3 ± 1.52	67.3 ± 1.22	61.1 ± 1.11	<0.0001
Zinc (μg)	4.32 ± 0.03	4.37 ± 0.06	4.23 ± 0.03	0.4342	4.36 ± 0.04	4.28 ± 0.03	4.24 ± 0.03	0.0656
Vitamin B_6 (mg)	0.86 ± 0.006	0.86 ± 0.007	0.85 ± 0.007	0.0926	0.92 ± 0.009	0.90 ± 0.007	0.89 ± 0.008	0.0101
Folate (μg)	114.7 ± 1.43	115.4 ± 1.71	112.4 ± 1.84	0.5526	133.5 ± 2.31	129.1 ± 1.95	126.4 ± 1.90	0.0417
Retinol (μg)	31.6 ± 0.74	29.5 ± 0.73	30.7 ± 0.86	0.5944	37.2 ± 1.19	34.0 ± 0.92	33.2 ± 0.93	0.0825
Carotene (μg)	1,285.8 ± 26.97	1,251.0 ± 30.59	1,251.7 ± 33.14	0.4552	1,408.3 ± 39.44	1,371.0 ± 35.41	1348.4 ± 34.97	0.2719
Fiber (g)	3.21 ± 0.04	3.17 ± 0.04	3.09 ± 0.04	0.1452	3.64 ± 0.05	3.55 ± 0.04	3.43 ± 0.04	0.0046
Vitamin E (mg)	4.29 ± 0.05	4.22 ± 0.05	4.21 ± 0.05	0.7252	4.88 ± 0.09	4.73 ± 0.06	4.51 ± 0.06	0.0092
Cholesterol (mg)	82.0 ± 1.61	77.5 ± 1.56	81.5 ± 2.01	0.9384	86.9 ± 2.33	81.8 ± 1.84	79.7 ± 1.99	0.1277

Values are expressed as mean (SE). Nutrient intakes were expressed per 1,000 kcal. [§]Stress levels were assessed using the Psychosocial Wellbeing Index-Short Form (PWI-SF). The P-trend was obtained through generalized linear models after adjusting for age, BMI, marital status, monthly household income, education level, alcohol consumption, smoking status and physical activity.

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TABLE 5 Hazard ratios (HRs) and 95% confidence intervals (CIs) for the risk of abdominal obesity according to food consumption.

	Men					Women			
	Model 1 [a]		Model 2 [b]			Model 1		Model 2	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Grains (g/day)					Grains (g/day)				
Tertile 1 ($n = 813$)	1 (reference)	-	1 (reference)	-	Tertile 1 ($n = 689$)	1 (reference)	-	1 (reference)	-
Tertile 2 ($n = 813$)	1.154 (0.945–1.408)	0.1593	1.225 (0.991–1.514)	0.0604	Tertile 2 ($n = 626$)	1.096 (0.883–1.361)	0.4069	1.032 (0.820–1.300)	0.7865
Tertile 3 ($n = 813$)	1.094 (0.893–1.341)	0.3855	1.206 (0.919–1.583)	0.1764	Tertile 3 ($n = 657$)	1.166 (0.947–1.435)	0.1472	1.362 (1.043–1.780)	0.0233
Refined grains (g/day)					Refined grains (g/day)				
Tertile 1 ($n = 813$)	1 (reference)	-	1 (reference)	-	Tertile 1 ($n = 657$)	1 (reference)	-	1 (reference)	-
Tertile 2 ($n = 812$)	1.141 (0.932–1.397)	0.2016	1.098 (0.892–1.352)	0.3763	Tertile 2 ($n = 658$)	0.959 (0.773–1.189)	0.7011	1.031 (0.828–1.284)	0.7826
Tertile 3 ($n = 814$)	1.257 (1.031–1.533)	0.0238	1.359 (1.112–1.661)	0.0027	Tertile 3 ($n = 657$)	1.124 (0.912–1.384)	0.2739	1.282 (1.032–1.593)	0.0247
Vegetables (g/day)					Vegetables (g/day)				
Tertile 1 ($n = 813$)	1 (reference)	-	1 (reference)	-	Tertile 1 ($n = 657$)	1 (reference)	-	1 (reference)	-
Tertile 2 ($n = 813$)	0.863 (0.707–1.054)	0.1488	0.827 (0.675–1.012)	0.0656	Tertile 2 ($n = 658$)	1.052 (0.849–1.304)	0.6432	1.105 (0.889–1.373)	0.3686
Tertile 3 ($n = 813$)	0.961 (0.789–1.169)	0.6889	0.971 (0.789–1.194)	0.7774	Tertile 3 ($n = 657$)	1.156 (0.935–1.430)	0.1801	1.270 (1.016–1.589)	0.0361
Fruits (g/day)					Fruits (g/day)				
Tertile 1 ($n = 813$)	1 (reference)	-	1 (reference)	-	Tertile 1 ($n = 657$)	1 (reference)	-	1 (reference)	-
Tertile 2 ($n = 813$)	0.761 (0.626–0.926)	0.0063	0.700 (0.571–0.858)	0.0006	Tertile 2 ($n = 658$)	0.648 (0.529–0.793)	<0.0001	0.655 (0.531–0.807)	<0.0001
Tertile 3 ($n = 813$)	0.748 (0.615–0.911)	0.0038	0.564 (0.450–0.706)	<0.0001	Tertile 3 ($n = 657$)	0.501 (0.404–0.621)	<0.0001	0.513 (0.405–0.651)	<0.0001
Dairy (g/day)					Dairy (g/day)				
Tertile 1 ($n = 816$)	1 (reference)	-	1 (reference)	-	Tertile 1 ($n = 657$)	1 (reference)	-	1 (reference)	-
Tertile 2 ($n = 811$)	1.022 (0.841–1.241)	0.8282	1.007 (0.826–1.228)	0.9451	Tertile 2 ($n = 656$)	0.848 (0.690–1.043)	0.1183	0.920 (0.741–1.141)	0.4471
Tertile 3 ($n = 812$)	0.850 (0.694–1.041)	0.1153	0.817 (0.661–1.010)	0.0620	Tertile 3 ($n = 659$)	0.740 (0.599–0.915)	0.0053	0.793 (0.634–0.992)	0.0419
Meat (g/day)					Meat (g/day)				
Tertile 1 ($n = 813$)	1 (reference)	-	1 (reference)	-	Tertile 1 ($n = 657$)	1 (reference)	-	1 (reference)	-
Tertile 2 ($n = 813$)	1.000 (0.818–1.223)	0.9995	0.864 (0.698–1.070)	0.1803	Tertile 2 ($n = 658$)	0.824 (0.668–1.017)	0.0714	1.029 (0.822–1.288)	0.8030
Tertile 3 ($n = 813$)	1.078 (0.885–1.314)	0.4539	0.890 (0.700–1.132)	0.3437	Tertile 3 ($n = 657$)	0.796 (0.646–0.980)	0.0316	1.068 (0.833–1.370)	0.6040
Fast foods (g/day)					Fast foods (g/day)				
Tertile 1 ($n = 0$)	-	-	-	-	Tertile 1 ($n = 0$)	-	-	-	-
Tertile 2 ($n = 1,739$)	1 (reference)	-	1 (reference)	-	Tertile 2 ($n = 1,318$)	1 (reference)	-	1 (reference)	-
Tertile 3 ($n = 700$)	0.953 (0.797–1.138)	0.5929	0.988 (0.820–1.191)	0.9013	Tertile 3 ($n = 654$)	0.786 (0.651–0.948)	0.0121	0.903 (0.737–1.107)	0.3276
Highly palatable foods (g/day)					Highly palatable foods (g/day)				
Tertile 1 ($n = 811$)	1 (reference)	-	1 (reference)	-	Tertile 1 ($n = 704$)	1 (reference)	-	1 (reference)	-
Tertile 2 ($n = 817$)	0.953 (0.780–1.163)	0.6329	0.934 (0.762–1.143)	0.5062	Tertile 2 ($n = 611$)	1.134 (0.920–1.399)	0.2382	1.149 (0.928–1.422)	0.2017
Tertile 3 ($n = 811$)	0.993 (0.814–1.212)	0.9464	1.128 (0.906–1.404)	0.2811	Tertile 3 ($n = 657$)	0.960 (0.777–1.186)	0.7044	1.092 (0.872–1.369)	0.4421

HR, hazard ratio; CI, confidence interval; Ref., reference category.

[a]Model 1 was unadjusted.

[b]Model 2 was adjusted for age, BMI, marital status, monthly household income, education level, alcohol consumption, and total energy intake.

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TABLE 6 Hazard ratios (HRs) and 95% confidence intervals (CIs) for the risk of abdominal obesity according to stress levels.

	Men						Women					
		Model 1[a]		Model 2[b]				Model 1		Model 2		
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value			HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Stress levels[§]	Number of cases					Stress levels	Number of cases				
Tertile 1 ($n = 976$)	294 (30.1)	1 (reference)	-	1 (reference)	-	Tertile 1 ($n = 495$)	146 (29.4)	1 (reference)	-	1 (reference)	-
Tertile 2 ($n = 790$)	209 (26.4)	0.849 (0.701–1.028)	0.0941	0.977 (0.804–1.186)	0.8109	Tertile 2 ($n = 720$)	230 (31.9)	1.212 (0.966–1.520)	0.0960	1.324 (1.053–1.664)	0.0163
Tertile 3 ($n = 673$)	188 (27.9)	0.876 (0.717–1.071)	0.1979	1.106 (0.898–1.362)	0.3424	Tertile 3 ($n = 757$)	247 (32.6)	1.154 (0.918–1.451)	0.2206	1.265 (1.003–1.594)	0.0470

HR, hazard ratio; CI, confidence interval; Ref., reference category.

[§]Stress levels were assessed using the Psychosocial Wellbeing Index-Short Form (PWI-SF).

[a]Model 1 was unadjusted.

[b]Model 2 was adjusted for age, BMI, marital status, monthly household income, education level, alcohol consumption, smoking status and physical activity.

but was negatively associated with the consumption of fruits. These results concur with prior studies that lower dietary variety is associated with higher consumption of refined grains (30) and lower consumption of fruits and vegetables (54). Chronic stress may modify eating behaviors, specifically the type of foods chosen, resulting in an increased consumption of refined grains.

Increased consumption of grains, especially refined grains, was longitudinally associated with an increased risk of incident abdominal obesity in women, with a mean WC increase of 3.8 ± 0.2 cm. According to the Framingham Offspring cohort study, the frequent consumption of refined grains (≥4 servings/day) was linked to a greater mean increase in WC than infrequent consumption (<2 servings/day) during 4 years (55). A cross-sectional study conducted among Indian adults suggested that higher consumption of refined grains was significantly associated with a higher WC after adjustment for confounding factors, such as age, sex, BMI, metabolic equivalent, total energy intake, and other dietary factors (56). Another cross-sectional study showed that individuals with higher scores in the "Traditional-carbohydrate" dietary pattern, characterized by higher consumption of refined grains, potatoes, sugar, and sweets, had a 55% higher prevalence of abdominal obesity (57).

Several potential mechanisms have been suggested to explain the association between the consumption of refined grains and the risk of abdominal obesity. Refined-grain foods tend to be quickly digested (58) and have a relatively high glycemic index (GI) compared with whole-grain foods, non-starchy vegetables, legumes, and fruits (59). A high-GI diet may increase hunger and lead to overeating, resulting in excess weight gain (60). A previous study of Iranian adults linked a higher dietary GI with an increased risk of abdominal obesity (61). In experimental animals fed a high-refined carbohydrate diet, the serotonin pathway was altered, accompanied by increased expression of the serotonin transporter (*Sert*), which possibly alters satiety and hunger signals, ultimately driving abdominal obesity (62). We found that women with higher levels of stress showed a higher carbohydrate intake but a relatively low intake of other nutrients. It can be suggested that those with higher levels of stress ate more refined grains and carbohydrates, partially contributing to a higher risk of abdominal obesity after 10 years.

In our study, higher levels of stress were longitudinally associated with an increased risk of abdominal obesity in women only, not men. In a prospective cohort study in the United Kingdom, job strain, a form of psychosocial stress in the workplace, was related to an increased risk of abdominal obesity (63). Moreover, a longitudinal study on stress and metabolic syndrome found a significant positive association between the number of stressful life events and WC (23). Cortisol secretion due to stress exposure might contribute to the accumulation of abdominal fat mass (9, 64). An elevated hair cortisol concentration is positively associated with BMI and WC (65). Regarding gender, there is a difference in the stress response exhibited by men and women (66). Women

have more daily stress from performing routine duties (67) and find themselves in stressful circumstances more often than men (68, 69). In addition, the stress coping styles of women are more emotion-focused compared to men, resulting in increased susceptibility to negative health consequences among women (70).

We found that increased consumption of fruits, containing a lot of antioxidant nutrients and fiber, was longitudinally associated with a decreased risk of incident abdominal obesity in both men and women. High intake of vitamin C, abundant in fruits, was reported to decrease the risk of abdominal obesity in Korean women (71). Also, a major antioxidant nutrient, vitamin E supplementation reduced visceral fat deposition in mice fed a high-fat diet through reduction in the fibrotic process, which is related to adipocyte growth and lipid accumulation (72). Dietary fiber intake has been showed reduced prevalence of abdominal obesity and negative association with WC in diabetic patients (73).

This study has several strengths. It is the first to investigate the associations of perceived stress, eating behavior, and abdominal obesity in Korean adults in a prospective study with long follow-up. Furthermore, our analysis is distinct from other prior studies of eating behavior as it applied the DVS, a novel approach, to assess eating behavior. However, there are some limitations to this study. First, we assessed food consumption only at baseline and did not determine whether the dietary patterns of participants had changed throughout the follow-up. Second, blood analysis was not performed, which could reflect changes in hormones associated with stress and appetite control.

Conclusion

In conclusion, perceived psychosocial stress was associated with an unhealthy eating behavior represented by a low DVS, characterized by high consumption of grains, especially refined grains, and relatively low consumption of fruits in women. There was a positive, longitudinal association of stress, as well as grains consumption, with the risk of abdominal obesity. Therefore, it can be suggested that stress-modified eating behavior may be one factor contributing to the risk of abdominal obesity during the follow-up.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The Ansung-Ansan study protocol was reviewed and approved by the Institutional Review Board of the Korea Centers for Disease Control and Prevention, and all study participants submitted written informed consent. The study was approved by the Institutional Review Board of Ewha Womans University (2021-0316, October 2021).

Author contributions

MK and YK contributed to the conceptualization, design of the research, data analysis, writing the manuscript, and editing. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.949012/full#supplementary-material

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Dietary diversity and determinants of young adults in central China: A cross-sectional study from 2015 to 2020

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Background: Early adulthood is a vulnerable period for improved nutrition at all phases of the life cycle. However, there is limited research on diversity information in young adults from middle-income countries undergoing an apparent nutritional transition. The purpose of this study was to explore dietary diversity and determinants among young adults aged 18–35 years in central China.

Methods: From January 2015 to December 2020, a cross-sectional survey of 49,021 young adults in a health management center of central China was conducted through report and phone-assisted self-report for information. The outcome variable was the Dietary Diversity Score. Independent variables included age, sex, race, material status, education, BMI, taste preference, regular meals, midnight snacks, sugared beverage/coffee consumption, and smoking/drinking status. Multivariate logistic regression was performed.

Results: Of 49,021 young adults, 38,374 (78.3%) reported insufficient dietary diversity, and 422 (0.9%) reported sufficient dietary diversity. Light taste preference [adjusted odds ratio (aOR) = 2.325; 95% CI: 1.779, 3.039] and those who had meals regularly (aOR = 1.241; 95% CI: 1.018, 1.513) and consumed coffee (aOR = 2.765; 95% CI: 2.257, 3.387) were more likely to be associated with sufficient dietary diversity. Midnight snacks (aOR = 0.728; 95% CI: 0.588, 0.901) and sugary beverages (aOR = 0.666; 95% CI: 0.535, 0.829) were less likely to be associated with sufficient dietary diversity. Higher BMI (aOR = 1.092; 95% CI: 1.061, 1.125) was associated with higher odds of sufficient dietary diversity. Additionally, participants who were 18–30 years old, with master or above degree and away from cigarette/alcohol were more likely to report better dietary diversity.

Conclusion: Our results painted a less than ideal nutritional condition affecting young adults. High-fat/sugar/salt dietary practices can lead to low dietary diversity, while high dietary diversity might have adverse BMI outcomes in youth. This study highlighted the importance of increasing the

diversity of healthy and selective food items before wide recommendation for dietary diversity.

KEYWORDS

diet survey, dietary diversity, eating habits, factor analysis, China

Introduction

Early adulthood is recognized as a vulnerable period; optimum nutrition is critical at this time because of growth in nutritional demands and important eating behaviors (1). Adequate nutrition in youth plays an important role in both present and long-term health. Hence, this phase is possibly the only opportunity for the catch-up nutrition needed to avoid the vicious intergenerational effect of malnourishment.

A high-quality diet consists of adequate intake of micronutrients; balanced protein, carbohydrate, and fat intake; and abstemious consumption of unhealthy foods (2). Dietary diversity is generally accepted as a key part of a high-quality diet because consuming various foods across and within different diet groups contributes to adequate levels of vital nutrients (3). Diet diversity indicators have been found to be prospective measurement tools, especially in developing countries, due to their simplicity of implementation and their potential for large-scale use, in contrast to other food-consumption indicators that collect complicated quantitative information (4). Low Healthy Diet Index score was found to be associated with obesity and other chronic diseases (5). International studies have consistently reported a lower-quality diet in younger age groups than in older age groups, who often have a higher-quality diet (6, 7). However, dietary intake and diversity information are almost invisible in young age groups.

To date, there is a narrow understanding of determinants of dietary diversity among young adults. Dietary practice has an important determination of many aspects of diet variety and a substantial influence on youth nutrients. Young adults are usually more likely to engage in unhealthy eating habits, such as common meal skipping (8), frequent fast food consumption and dining out (9), high added sugar intake (i.e., sugar added to beverages during production) (10), and inadequate consumption of fruit and vegetables (11), compared to other age groups. Moreover, China is undergoing an apparent nutritional transition related to rapid economic growth (12–14). The diet pattern of citizens has shifted from traditional food (low in fats, mainly composed of carbohydrates, vegetables, and few animal-based foods) to a western diet (high in fats, sugar, and refined foods) (15, 16). As a long-term unhealthy eating behavior, western diet has proved to be such an important factor associated with non-communicable diseases (17). Given this evidence, there is concern that youth, as a vulnerable population, might be placed at higher risk of suffering from micronutrient deficiencies (18). Furthermore, energy-dense foods give rise to annual weight gain (9, 19). Although previous studies had been conducted in China (Qinghai Plateau and Taiwan), the association between body mass index (BMI) and dietary diversity is still unclear, which may be ascribed to differences in the age of the enrolled population (20, 21).

However, little research has examined how current eating patterns influence dietary diversity in youth populations faced with an increasing variety of food choices. Given that dietary patterns shift as a result of urbanization and the easy accessibility of low-cost processed food in developing countries, it is necessary to understand the potential effect of sociodemographic and diet behavior factors on dietary diversity. Therefore, the objective of this study was to explore dietary diversity and determinants among young adults aged 18–35 years in central China across individual-level sociodemographic, dietary practice, and BMI status characteristics.

Methods

Sample and data

Data for this article came from a cross-sectional survey conducted in a health management center of a general tertiary hospital located in central China between 1 January 2015 and 31 December 2020. This survey focused on young adults, so the inclusion criteria was aged from 18 to 35 years and participated in the survey voluntarily, and 49,648 young adults remained enrolled in after the selection process. Before young adults underwent physical examination, trained interviewers from general tertiary hospitals provided general instructions for this study and invited subjects to participate in the investigation. Interviewers then used structured questionnaires (pre-coded) to record the sociodemographic characteristics and dietary practices of the subjects. Physical examination was performed by trained professionals under standard procedures and by standardized instruments for BMI collection. The food consumption information was gathered using 24-h dietary recalls for three straight days (2 and 1 weekend day), collected by interviewers by phone after physical examination. Altogether, 49,021 individuals completed the survey, for a response rate of 98.7%.

Sample size

Our main guideline was the review of Charan and Biswas (22), in which the sample size of cross sectional surveys is considered to be calculated through $Z^2_{1-\alpha/2}p(1-p)/d^2$. In this formula, $Z_{1-\alpha/2}$ presents standard normal variate, p is expected proportion in population, and d is absolute error/precision. Confidence interval is set as 95% in this study, wherein $Z_{1-\alpha/2} = 1.96$. Based on previous large study (23), the estimated proportion of low DDS in Chinese adults is not more than 55%, and the absolute error/precision is assumed to be 0.03. Therefore, this study has to contain 1,057 subjects at least. In order to decrease bias effectively and obtain information profoundly, more young adults were expected to be included.

Ethical clearance

This study was approved by the Institutional Review Board (IRB) of the general tertiary hospital. All procedures followed the Declaration of Helsinki, and all essential permissions were obtained from the government and health commission. All young adults participating in this survey completely understood the purpose and agreed to participate in the investigation.

Outcome variable

The outcome variable was the Dietary Diversity Score (i.e., DDS), which is based on the Chinese Dietary Guidelines. It was defined as the number of food groups consumed over 3 days based on the 24-h dietary recalls. All food items were classified into nine groups: grains (tubers, cereals, and roots), vegetables, fruits, meat (pork, beef, poultry, and organs), beans (beans, nuts, and seeds), eggs, fish (seafood, freshwater fish, and aquatic products), dairy (milk and products), and oil (animal and vegetable oil). If a participant consumed any food from the abovementioned categories, they would receive one point in the corresponding food category. Otherwise, they would be scored zero (such as sugar beverages, coffee, tobacco, and alcohol). Consuming different foods from the same category did not count repeatedly. The total score was the sum of nine food groups, and the maximum score could reach nine points. For this study, DDS was categorized into three degrees [insufficient DDS (1–3 points), moderate DDS (4–6 points), and sufficient DDS (7–9 points)].

Independent variables

The independent variables included sex, age, race, material status, education, taste preference (24), regular meals, midnight snacks, sugary beverages, coffee, smoking/drinking status, and BMI. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Weight was measured to the nearest 0.1 kg (with light clothes on flat ground), and height was measured to the nearest 0.1 cm (without shoes). Supporting details of other independent variables were exhibited in Table 1.

Statistical analysis

Multinomial logistic regressions were conducted to assess the associations between the DDS and the identified independent variables. Unadjusted and adjusted models were conducted. Bivariate association of the outcome variable with any of the independent variables was first examined using Pearson χ^2 tests or ANOVA, and those that were associated with the outcome variable were included in adjusted models. Only the test of difference across all categories was provided, but 95% confidence intervals were also presented to allow consideration of more nuanced differences. Missing data of continuous variables were filled using the variable's mean. The significance level was set at $p = 0.05$ for the analyses. All statistical analyses were carried out using SPSS, version 25.0 for Windows (IBM Corp, Armonk, New York) and accounted for features of the survey design.

Results

Characteristics of the sample

Sociodemographic information is displayed by DDS in Table 2, which represents the population of 18–35 years old among central China. The sample included 49,021 young adults who participated in and responded to questions about dietary diversity, with a response rate of 98.7%. The proportion of respondents in each dietary diversity category was approximately equally distributed by gender. The majority of the participants were over 25 years old (84.6%), Han nationality (95.4%), married (63.8%), with a higher national diploma or bachelor's degree (78.3%), never-smoker (72.2%), or never-drinker (72.9%). The mean BMI of the participants was 22.72 ($\pm$3.48) kg/m^2. Of note, the total percentages of participants who reported insufficient dietary diversity (78.3%) were much higher than any other.

Bivariate analyses of dietary diversity and variables thought to be related were performed using chi-square tests. Table 2 shows that all variables were associated with three categories of dietary diversity ($p < 0.05$). The results of the eating behavior section showed that the percentage of insufficient dietary diversity was higher among individuals who showed a heavy taste preference (81.0%), ate three meals irregularly (79.2%), liked midnight snacks (79.1%) or sugared beverages (78.9%), and disliked coffee (80.0%).

TABLE 1 Specifics of questionnaire.

Variables	Statement
1. Demographics	
Sex	(1) What is your physiologic sex?
	A. Male
	B. Female
Age	(2) Which is your interval of age this year?
	A. 18–25 years old
	B. 26–30 years old
	C. 31–35 years old
Race	(3) What is your race?
	A. Minority (fifty-five kinds of ethnic minorities)
	B. Han nationality
Material status	(4) What is your current marital relationship?
	A. Married or common law marriage
	B. Single
	C. Divorced or widowed
Education	(5) What is your highest level of education?
	A. HS or lower (middle school, elementary school etc.)
	B. Technical school
	C. HND/Bachelor
	D. Master or above (doctor, post doctor etc.)
2. Dietary Behaviors	
Taste preference	(6) In general, which of the following describe most of the foods that you like?
	A. Heavy [salty (halogen/pickled products, the most common local delicacies locally), spicy (fresh peppers, dried chilies, chili sauce, or pepper oil) or fatty foods (fried foods or sweets) etc.]
	B. Light (unsalted foods, fruits, or vegetables etc.)
	C. Arbitrary taste (neither dislike nor like any of the taste above)
Regular meals	(7) Are you usually able to eat three meals on time and not snacks for meals?
	A. Yes
	B. No
Midnight snacks	(8) Do you often intake energy-dense and sugar sweetened snacks between 21:00 and 06:00?
	A. Yes
	B. No
Sugary beverages	(9) Do you drink sugared beverages?
	A. Yes (sugared fruit/milk drinks; sugared or "no-cal" cola; other soft drinks)
	B. No
Coffee	(10) Do you drink coffee, not coffee beverages?
	A. Yes (refers in particular to those without processing or mostly physical processes)
	B. No
3. Life styles	
Smoker	(11) Which of the following best describes your smoking status?
	A. Never
	B. Former (quit smoking for more than 1 year)
	C. Passive (more than 15 min a day and more than 1 day a week)
	D. Current (continued smoking for more than 1 year)
Drinker	(12) Which of the following best describes your drinking status?
	A. Never
	B. Former (quit alcohol for more than 1 year)
	C. Current (have 1 or more alcoholic drinks per week)

HND, Higher National Diploma; HS, high school.

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TABLE 2 Demographic characteristics of samples across the three dimensions of dietary diversity score ($n = 49,021$).

Variables	Overall	Insufficient ($n = 38,374$) N (%)	Moderate ($n = 10,225$) N (%)	Sufficient ($n = 422$) N (%)	χ^2/F	p-value
Sex					31.628	<0.000
Male	22,800 (46.5)	18,101 (79.4)	4,504 (19.7)	195 (0.9)		
Female	26,221 (53.5)	20,273 (77.3)	5,721 (21.8)	227 (0.9)		
Age (years)					50.028	<0.000
18–25	7,548 (15.4)	5,860 (77.7)	1,618 (21.4)	70 (0.9)		
26–30	21,289 (43.4)	16,414 (77.1)	4,664 (21.9)	211 (1.0)		
31–35	20,184 (41.2)	16,100 (79.8)	3,943 (19.5)	141 (0.7)		
Race					11.478	0.003
Minority	2,279 (4.6)	1,719 (75.4)	537 (23.6)	23 (1.0)		
Han nationality	46,742 (95.4)	36,655 (78.4)	9,688 (20.7)	399 (0.9)		
Material status					46.255	<0.000
Married	31,277 (63.8)	24,779 (79.2)	6,241 (20.0)	257 (0.8)		
Single	17,218 (35.1)	13,183 (76.6)	3,874 (22.5)	161 (0.9)		
Divorced/widowed	526 (1.1)	412 (78.3)	110 (20.9)	4 (0.8)		
Education					544.034	<0.000
HS or lower	1577 (3.2)	1,368 (86.7)	206 (13.1)	3 (0.2)		
Technical school	1,389 (2.8)	1,208 (87.0)	175 (12.6)	6 (0.4)		
HND/Bachelor	38,377 (78.3)	30,487 (79.4)	7,593 (19.8)	297 (0.8)		
Master or above	7,678 (15.7)	5,311 (69.2)	2,251 (29.3)	116 (1.5)		
Taste preference					194.938	<0.000
Light	14,738 (30.0)	11,019 (74.8)	3,539 (24.0)	180 (1.2)		
Arbitrary	18,563 (37.9)	14,616 (78.7)	3,794 (20.5)	153 (0.8)		
Heavy	15,720 (32.1)	12,739 (81.0)	2,892 (18.4)	89 (0.6)		
Regular three meals					52.807	<0.000
Yes	17,027 (34.7)	13,017 (76.4)	3,840 (22.5)	170 (1.0)		
No	31,994 (65.3)	25,357 (79.2)	6,385 (20.0)	252 (0.8)		
Midnight snacks					40.280	<0.000
Yes	31,261 (63.8)	24,721 (79.1)	6,308 (20.2)	232 (0.7)		
No	17,760 (36.2)	13,653 (76.9)	3,917 (22.0)	190 (1.1)		
Sugared beverages					29.240	<0.000
Yes	34,238 (69.8)	27,016 (78.9)	6,952 (20.3)	270 (0.8)		
No	14,783 (30.2)	11,358 (76.8)	3,273 (22.2)	152 (1.0)		
Coffee					196.355	<0.000
Yes	17,373 (35.4)	13,064 (75.2)	4,073 (23.4)	236 (1.4)		
No	31,648 (64.6)	25,310 (80.0)	6,152 (19.4)	186 (0.6)		
Smoker					125.297	<0.000
Never	35,400 (72.2)	27,404 (77.4)	7,678 (21.7)	318 (0.9)		
Former	980 (2.0)	733 (74.8)	231 (23.6)	16 (1.6)		
Passive	2,901 (5.9)	2,222 (76.6)	651 (22.4)	28 (1.0)		
Current	9,740 (19.9)	8,015 (82.3)	1,665 (17.1)	60 (0.6)		
Drinker					28.620	<0.000
Never	35,748 (72.9)	27,933 (78.2)	7,517 (21.0)	298 (0.8)		
Former	377 (0.8)	278 (73.7)	87 (23.1)	12 (3.2)		
Current	12,896 (26.3)	10,163 (78.8)	2,621 (20.3)	112 (0.9)		
BMI (Mean ± SD)	22.72 ± 3.48	22.63 ± 3.46	23.02 ± 3.53	23.35 ± 3.55	58.619	<0.000

BMI, body mass index; HND, Higher National Diploma; HS, high school.

Dietary diversity assessment

As shown in Table 3, two categorical variables (race and material status) were not associated (at the $p = 0.05$ level) with DDS in the unadjusted models and were therefore excluded from the adjusted model. Each independent variable was adjusted in cooperation with other independent variables. Table 4 shows the results of the adjusted model examining the association between DDS and all independent variables.

DDS1 results: Demographics

Males had 0.801 times greater odds of moderate DDS [95% confidence interval (CI) = 0.755, 0.849; $p < 0.001$] but not sufficient DDS ($p = 0.303$) compared to their female counterparts. Compared to those aged 31–35 years old, those aged 18–25 had 1.333 times (95% CI = 1.245, 1.428; $p < 0.001$) and 1.969 times (95% CI = 1.458, 2.659; $p < 0.001$) greater odds of moderate and sufficient DDS, respectively. Compared to those whose highest-level education was master's degree or above, participants whose highest level of education was high school or lower had 0.385 times (95% CI = 0.329, 0.450; $p < 0.001$) and 0.117 times (95% CI = 0.037, 0.370; $p < 0.001$) greater odds of moderate and sufficient DDS.

DDS2 results: Dietary behaviors

Participants who preferred light or arbitrary taste had 1.418- (95% CI = 1.337, 1.504; $p < 0.001$) and 1.156-times (95% CI = 1.094, 1.221; $p < 0.001$) greater odds of moderate DDS and 2.325- (95% CI = 1.779, 3.039; $p < 0.001$) and 1.532-times (95% CI = 1.175, 1.996; $p = 0.002$) greater odds of sufficient DDS vs. insufficient DDS, respectively. Those who ate three meals regularly were 1.145 times (95% CI = 1.093, 1.199; $p < 0.001$) more likely to have moderate DDS and 1.241 times (95% CI = 1.018, 1.513; $p = 0.032$) more likely to have sufficient DDS than their counterparts. Those with coffee consumption were 1.307 times (95% CI = 1.247, 1.370; $p < 0.001$) more likely to have moderate DDS and 2.765 times (95% CI = 2.257, 3.387; $p < 0.001$) more likely to have sufficient DDS than those with insufficient DDS. Those who ate midnight snacks were less likely to have sufficient DDS by 0.728 times (95% CI = 0.588, 0.901; $p = 0.004$), but not moderate DDS ($p = 0.131$). Those with sugared beverage consumption were 0.898 (95% CI = 0.853, 0.945; $p < 0.001$) and 0.666 times (95% CI = 0.535, 0.829; $p < 0.001$) less likely to have moderate DDS and sufficient DDS vs. insufficient DDS, respectively. Equally important, high BMI was associated with higher odds of moderate and sufficient DDS by 1.061 (95% CI = 1.053, 1.068; $p < 0.001$) and 1.092 times (95% CI = 1.061, 1.125; $p < 0.001$).

DDS3 results: Life styles

Never smokers had 1.208 times (95% CI = 1.125, 1.297; $p < 0.001$) and 1.443 times (95% CI = 1.047, 1.988; $p = 0.025$) greater odds of moderate and sufficient DDS, respectively, than their smoker counterparts. Former smokers had 1.426 times (95% CI = 1.215, 1.673; $p < 0.001$) and 2.334 times (95% CI = 1.321, 4.123; $p = 0.004$) greater odds of moderate and sufficient DDS, respectively, than their smoker counterparts. Passive smokers had 1.281 times (95% CI = 1.153, 1.425; $p < 0.001$) greater odds of moderate DDS but not sufficient DDS ($p = 0.067$) than their smoker counterparts. Never drinkers had 0.899 times (95% CI = 0.848, 0.954; $p < 0.001$) greater odds of moderate DDS but not sufficient DDS ($p = 0.056$) than their drinker counterparts. Former drinkers had 3.208 times (95% CI = 1.718, 5.991; $p < 0.001$) greater odds of having a sufficient DDS but not a moderate DDS ($p = 0.426$) than their drinker counterparts.

Discussion

To the best of our knowledge, this is the first study to explore determinants of dietary diversity using individual data from a large, representative young adult sample. Insufficient dietary diversity was found to be widespread in central China (78.3%), even far above the oldest old (55.7%) (25). The findings of this study show that participants with light taste preference who had meals regularly and consumed coffee were more likely to report sufficient dietary diversity. Midnight snacks and sugary beverages were found to be inversely associated with sufficient dietary diversity. Importantly, there was a significant positive association between BMI and dietary diversity. In addition, we found that being away from cigarettes/alcohol, 18–30 years old, and with master or above degree were associated with better dietary diversity.

Considering that lower dietary diversity might be a risk factor for health *via* malnutrition (26), identifying dietary determinants are of interest. Overall, we found that differences in dietary practice accounted for diet variety. Regular meals were associated with higher dietary diversity, which is expected. Young people might skip meals, especially breakfast, which may spontaneously increase nutritional vulnerability in young adulthood, thus leading to a "Snacker" dietary pattern (27). Snacker behavior, as a result of not feeling hunger in the morning, can potentially affect feelings of satiety (28), which can lead to worse dietary diversity throughout the day (29). Midnight snackers, who display disturbed regular meal consumption to some extent, were less likely to report sufficient dietary diversity in this study. This finding is similar to previous evidence, which proved that night eating is related to low diet quality in adolescents (30). In particular, late eaters had fewer daily servings of fruit and vegetables and consumed greater weekly servings of fast food/soda (31, 32). Nocturnal ingestion

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TABLE 3 Unadjusted multinomial logistic regressions of dietary diversity on independent variables (n = 49,021).

Variables	Moderate-DDS vs. insufficient-DDS				Sufficient-DDS vs. insufficient-DDS			
	OR	95% CI		*p*-value	OR	95% CI		*p*-value
BMI	**1.061**	**1.054**	**1.069**	**0.000**	**1.092**	**1.060**	**1.125**	**0.000**
Sex								
Male	**0.798**	**0.753**	**0.846**	**0.000**	0.881	0.688	1.128	0.316
Female			Reference				Reference	
Age								
18–25	**1.236**	**1.141**	**1.338**	**0.000**	**2.068**	**1.463**	**2.923**	**0.000**
26–30	**1.184**	**1.124**	**1.247**	**0.000**	**1.705**	**1.356**	**2.143**	**0.000**
31–35			Reference				Reference	
Race								
Minority	1.097	0.991	1.213	0.073	1.070	0.699	1.638	0.756
Han nationality			Reference				Reference	
Material status								
Married	0.864	0.697	1.072	0.184	0.935	0.344	2.541	0.895
Single	0.961	0.771	1.197	0.722	0.875	0.317	2.416	0.797
Divorced/widowed			Reference				Reference	
Education								
HS or lower	**0.391**	**0.334**	**0.458**	**0.000**	**0.116**	**0.037**	**0.368**	**0.000**
Technical school	**0.365**	**0.308**	**0.432**	**0.000**	**0.258**	**0.112**	**0.592**	**0.001**
HND/Bachelor	**0.596**	**0.563**	**0.631**	**0.000**	**0.469**	**0.375**	**0.586**	**0.000**
Master or above			Reference				Reference	
Taste preference								
Light	**1.417**	**1.336**	**1.503**	**0.000**	**2.325**	**1.778**	**3.039**	**0.000**
Arbitrary	**1.155**	**1.094**	**1.221**	**0.000**	**1.532**	**1.175**	**1.996**	**0.002**
Heavy			Reference				Reference	
Regular three meals								
Yes	**1.149**	**1.097**	**1.203**	**0.000**	1.239	1.016	1.511	0.034
No			Reference				Reference	
Midnight snacks								
Yes	0.960	0.913	1.009	0.106	**0.728**	**0.588**	**0.902**	**0.004**
No			Reference				Reference	
Sugared beverages								
Yes	**0.897**	**0.852**	**0.944**	**0.000**	**0.667**	**0.535**	**0.830**	**0.000**
No			Reference				Reference	
Coffee								
Yes	**1.294**	**1.234**	**1.357**	**0.000**	**2.780**	**2.267**	**3.410**	**0.000**
No			Reference				Reference	
Smoker								
Never	**1.206**	**1.124**	**1.295**	**0.000**	**1.444**	**1.048**	**1.990**	**0.025**
Former	**1.427**	**1.216**	**1.674**	**0.000**	**2.330**	**1.319**	**4.117**	**0.004**
Passive	**1.280**	**1.151**	**1.423**	**0.000**	1.541	0.970	2.447	0.067
Current			Reference				Reference	
Drinking								
Never	**0.900**	**0.848**	**0.954**	**0.000**	0.782	0.607	1.007	0.057
Former	1.109	0.865	1.422	0.415	**3.186**	**1.705**	**5.952**	**0.000**
Current			Reference				Reference	

BMI, body mass index; CI, confidence interval; DDS, dietary diversity score; HND, Higher National Diploma; HS, high school; OR, odds ratio. Significant values are displayed in bold.

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TABLE 4 Adjusted multinomial logistic regressions[a] of dietary diversity on independent variables (n = 49,021).

Variables	Moderate-DDS vs. insufficient-DDS				Sufficient-DDS vs. insufficient-DDS			
	aOR	95% CI		*p*-value	aOR	95% CI		*p*-value
BMI	**1.061**	**1.053**	**1.068**	**0.000**	**1.092**	**1.061**	**1.125**	**0.000**
Sex								
Male	**0.801**	**0.755**	**0.849**	**0.000**	0.879	0.687	1.124	0.303
Female			Reference				Reference	
Age								
18–25	**1.333**	**1.245**	**1.428**	**0.000**	**1.969**	**1.458**	**2.659**	**0.000**
26–30	**1.220**	**1.162**	**1.282**	**0.000**	**1.671**	**1.343**	**2.079**	**0.000**
31–35			Reference				Reference	
Education								
HS or lower	**0.385**	**0.329**	**0.450**	**0.000**	**0.117**	**0.037**	**0.370**	**0.000**
Technical school	**0.359**	**0.304**	**0.425**	**0.000**	**0.260**	**0.113**	**0.596**	**0.001**
HND/Bachelor	**0.592**	**0.560**	**0.627**	**0.000**	**0.469**	**0.375**	**0.587**	**0.000**
Master or above			Reference				Reference	
Taste preference								
Light	**1.418**	**1.337**	**1.504**	**0.000**	**2.325**	**1.779**	**3.039**	**0.000**
Arbitrary	**1.156**	**1.094**	**1.221**	**0.000**	**1.532**	**1.175**	**1.996**	**0.002**
Heavy			Reference				Reference	
Regular three meals								
Yes	**1.145**	**1.093**	**1.199**	**0.000**	**1.241**	**1.018**	**1.513**	**0.032**
No			Reference				Reference	
Midnight snacks								
Yes	0.962	0.915	1.012	0.131	**0.728**	**0.588**	**0.901**	**0.004**
No			Reference				Reference	
Sugared beverages								
Yes	**0.898**	**0.853**	**0.945**	**0.000**	**0.666**	**0.535**	**0.829**	**0.000**
No			Reference				Reference	
Coffee								
Yes	**1.307**	**1.247**	**1.370**	**0.000**	**2.765**	**2.257**	**3.387**	**0.000**
No			Reference				Reference	
Smoker								
Never	**1.208**	**1.125**	**1.297**	**0.000**	**1.443**	**1.047**	**1.988**	**0.025**
Former	**1.426**	**1.215**	**1.673**	**0.000**	**2.334**	**1.321**	**4.123**	**0.004**
Passive	**1.281**	**1.153**	**1.425**	**0.000**	1.542	0.970	2.449	0.067
Current			Reference				Reference	
Drinking								
Never	**0.899**	**0.848**	**0.954**	**0.000**	0.782	0.607	1.007	0.056
Former	1.106	0.863	1.419	0.426	**3.208**	**1.718**	**5.991**	**0.000**
Current			Reference				Reference	

aOR, adjusted odds ratio; BMI, body mass index; CI, confidence interval; DDS, dietary diversity score; HND, Higher National Diploma; HS, high school.
[a]Adjusted model: all variables in the table are included in the model and control for each other. Significant values are displayed in bold.

is consistently rich in carbohydrates but limited in dietary food, such as sugary foods/sweets, breads, cereal products and dairy products (33). Understandably, these palatable foods always have high sugar or high fat contents, which may tend to be on the other side of light taste. Supporting this hypothesis is the finding that participants with heavy taste were less likely to have better dietary diversity in our multivariate logistic regression analyses. The heavy taste pattern followed the high-fat/sugar/salt consumption system and had a poorer nutrient profile than other preferences (29). In particular, solid fat and added sugar are recognized sources of empty calories without nutritional value (34); overconsumption can drive energy intake above

caloric requirements as well as also crowd out more nutrient-rich foods (35, 36), which could possibly explain our findings.

Interestingly, higher coffee consumption increased the likelihood of high dietary diversity in our samples, but sugared beverages showed the reverse pattern. Another study (37) found that both coffee and sugared beverages were associated with unsatisfactory dietary quality. According to a food classification based on the extent of industrial processing, coffee belongs to minimally processed foods, while sugared drinks are part of ultra-processed food products (38). The UK National Diet and Nutrition Survey found that fruit/vegetable, fiber and protein intake notably decreased when minimally processed foods decreased or ultra-processed food intake increased (39). This is not a permit to overindulge, but minimally processed coffee does have some unique advantages. That suggests the food processing should also be taken into consideration in response to healthier food choices (40).

Importantly, we found a positive relationship between BMI and dietary diversity, which was supported by prior literatures in various developing countries. A weight disorders survey among Iranian young population under the age of 18 suggested that the total DDS increasing a unit was associated with BMI z-score increasing 0.08 units, meanwhile, related to an increased risk of overweight, obesity or abdominal obesity (41). Sri Lankan researches in groups of adults over 18 years old also showed significant positive associations between dietary diversity and BMI, as well as the level of energy consumption (42, 43). In fact, it has been suggested that higher dietary diversity is associated with higher intake of total energy (particularly from fat and saturated fat) and is linked to obesity (44). A systematic review including 14 studies indicated that the relationship between diversity and adiposity depends on the healthy degree of eatables. Though variety of less healthy intake had a connection with greater adiposity, variety of all kinds of foods and healthy eating related to reduced risk of metabolic-related outcomes (45). For example, the intake of low energy-dense items (fruits and vegetables) contributes to dietary diversity and that higher diet diversity is linked to a lower risk of obesity (46). Another study assessing diversity scores exclusively for fruits/vegetables found that, although energy intake increased across the variety of fruits and vegetables, the mean BMI decreased (47). Briefly, a varied diet should be selective (e.g., fruits and vegetables) rather than absolute, despite the association between BMI and dietary diversity needs to be further researched in developing regions.

Based on findings from this study, never and former smokers had much better dietary diversity than their current-smoker peers, which is consistent with research in Western populations (48). Notably, the diet-smoking association found in diverse populations is not a culture-oriented phenomenon (49). There are reasons to believe that quitting smoking has implications in the implementation of an effective nutrition improvement program regardless of the culture and ethnicity of the youth population. We expected to find never drinkers to be associated with sufficient dietary diversity, but this trend was non-significant; only former drinkers tended to be predictive. Prior literature shows some substitution of alcohol for foods, which may suggest that restrictions on alcohol consumption may help to achieve better dietary diversity (50).

Additionally, the dietary diversity of young adults has been reported to be associated with sociodemographic characteristics. Consistent with prior report in southwest China (51) and Spain (52–54), males in this study were less likely to report sufficient dietary diversity. Although high DDS score of women in developed country has been considered to a whole higher global dietary quality in women, increased risk of general and central obesity has been reported among developing populations with higher DDS, which may suggest the potential strategies for promoting healthy diet in women living in developing countries require more attention. This study also demonstrated that young adults with master or above degree, females or males, were more likely to report better dietary diversity. Graduate and postgraduate groups have been proved to have high nutrition knowledge and healthy diet habits (55). Residents with an increase in dietary knowledge tend to consume more various foods (56), which may reflect the unique presentation of nutrition literacy and probably accounts for our result.

Although older age groups have been linked to better total diet variety (57), we observed that participants aged between 18 and 30 were more likely to have better dietary diversity than those who aged 31 years or over. All of these may be explainable when there existed some extent of home meals, no matter the frequent cookers were themselves (58) or other inhabitants (59). Cooking preparation behaviors has been found to appear in emerging adulthood instead of adolescence, and closely associated with better dietary quality in the 10-year longitudinal study. It is remarkable that quite a number of young participants may be with attribute "Snacker" and worse dietary diversity in our study, which can also be rationalized by home cooking. Snackers were mainly characterized by consuming co-processed foods (29). Evidence from Canada suggested that the decrease of ultra-processed products consumption is necessary, which composes any substantial improvement of diet (60). Adults who aged 18–23 and consumed snacks frequently have been shown to cook meals at home hardly and have limited nutritional targeting (61), which may be the reason for our opposite findings in DDS. This meant that efforts to encourage more home meals among young adults and allow them to make healthier diet choices.

Limitations and strengths

We recognize that our study has several limitations. First, the causal relationships could not be determined, as this

was a cross-sectional study. Second, a generally accepted limitation for all self-reported food intake data is that a proportion of individuals may alter their diet pattern or misreport (commonly underreport) the foods/beverages they consume (62). Third, only a restricted number of covariates were collected and analyzed, which may have excluded other important factors devoted to dietary diversity. Fourth, selection bias might have also impacted the estimation of results because non-responders may have had dissimilar dietary patterns than those who chose to respond. For example, young adults who perceive themselves as having "unhealthy" eating habits may have refused to participate. Lastly, larger sample sizes in different regions in China are needed in future studies.

Our study was based on a population and focused on vulnerable young adult populations, with a nearly 100% response rate. There have been limited previous research with such an extensive sampling about eating behavior relationships with dietary variety of young adults. Our study adds new evidence based on nutrition-related data from this vulnerable period in the context of a middle-income country.

Conclusions

Although the large youth population plays a critical role as the next generation, these results painted a less than ideal nutritional condition affecting young adults. The findings of young adults indicated dietary diversity was lower in those with high-fat/sugar/salt eating behaviors and was higher in high BMI populations. This study highlighted the importance of improving the diversity of healthy and selective food items before wide recommendation for dietary diversity. As a public health issue, further researches are required to assess dietary diversity associations with known disparities experienced by youth with unhealthy eating behaviors.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of the Third Xiangya Hospital of Central South University (No. 2020-S587). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

The study was designed by JW, YLi, YW, and JX. Data were collected by ZW and YLuo. Analyzed by YZ, YD, and XL. Data interpretation and manuscript preparation were undertaken by YZ, JW, and JX. All authors approved the final version of the paper.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Addictive behavior and incident gallstone disease: A dose–response meta-analysis and Mendelian randomization study

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Background: Previous studies have suggested associations between addictive behavior and gallstone disease (GSD) risk, yet conflicting results exist. It also remains unclear whether this association is causal or due to confounding or reverse associations. The present study aims to systematically analyze the epidemiological evidence for these associations, as well as estimate the potential causal relationships using Mendelian randomization (MR).

Methods: We analyzed four common addictive behaviors, including cigarette smoking, alcohol intake, coffee, and tea consumption (N = 126,906–4,584,729 participants) in this meta-analysis based on longitudinal studies. The two-sample MR was conducted using summary data from genome-wide associations with European ancestry (up to 1.2 million individuals).

Results: An observational association of GSD risk was identified for smoking [RR: 1.17 (95% CI: 1.06–1.29)], drinking alcohol [0.84 (0.78–0.91)], consuming coffee [0.86 (0.79–0.93)], and tea [1.08 (1.04–1.12)]. Also, there was a linear relationship between smoking (pack-years), alcohol drinking (days per week), coffee consumption (cups per day), and GSD risk. Our MRs supported a causality of GSD incidence with lifetime smoking [1.008 (1.003–1.013), P = 0.001], current smoking [1.007 (1.002–1.011), P = 0.004], problematic alcohol use (PAU) [1.014 (1.001–1.026), P = 0.029], decaffeinated coffee intake (1.127 [1.043–1.217], P = 0.002), as well as caffeine-metabolism [0.997 (0.995–0.999), P = 0.013], and tea consumption [0.990 (0.982–0.997), P = 0.008], respectively.

Conclusion: Our study suggests cigarette smoking, alcohol abuse, and decaffeinated coffee are causal risk factors for GSD, whereas tea consumption can decrease the risk of gallstones due to the effect of caffeine metabolism or polyphenol intake.

KEYWORDS

addictive behavior, gallstone, cholecystectomy, meta-analyses, Mendelian randomization

Introduction

Addictive behavior causes a major public health concern, and it has a massive, long-term impact on human suffering and societal costs (1). Gallstone disease (GSD) is one of the most common problems in the digestive tract and a major public health issue worldwide. The incidence of GSD continues to rise (around 10–20% of all adults in Europe), and its etiology remains to be understood (2). The pathogenesis of GSD involves environmental triggers, genetic predispositions, and behavioral factors; and the major pathogenetic factors, including abnormal cholesterol metabolism and slow intestinal motility are related to metabolic syndrome (3). Addictive behavior is of increasing interest as it is one of the leading contributors to the global burden of GSD and can be modified to achieve a desired preventive effect (4). Therefore, it is imperative to understand the relationship between common addictive behaviors and incident GSD, including cigarette smoking, alcohol drinking, coffee intake, and tea consumption.

Epidemiological investigations have consistently shown that current smoking, alcohol drinking, and coffee consumption play a key role in the incidence of GSD (5–7). A previous meta-analysis of 10 studies (N = 4,213,482) has provided evidence that smokers have an estimated 11% increased risk of GSD per 10 cigarettes per day compared to non-smokers (8). Another meta-analysis conducted by Wang et al. (9) that involved 14 studies (N = 316,028) has identified a significant non-linear trend of GSD risk reduction associated with the increment of drinking alcohol (up to about 30 g per day). In addition, one meta-analysis based on six studies (N = 227,749) has observed a dose-dependent association of coffee consumption with GSD [0.95 (0.91–1.00), P = 0.049] (10). These meta-analyses, despite their large sample sizes, have several limitations. First, there lack analyses for different types of alcohol (liquor, beer, and wine) and coffee (caffeinated and decaffeinated). Also, not all addictive behaviors have been comprehensively examined (e.g., pack-year smoking and drinking days per week). Second, the majority of evidence is cross-sectional, and the observational nature of conventional epidemiological studies hinders causal inference hampered by confounding or reverse causality (11).

Mendelian randomization (MR) fills the gap of making causal inferences by using single nucleotide polymorphism (SNP) as an instrumental variable (IV) since SNPs are usually established before the development of disease and therefore independent of confounders (12). Indeed, Yuan et al. have found that smoking is causally associated with GSD risk (13). However, tobacco smoking is a highly addictive behavior that contains large amounts of substances, such as nicotine, cannabis, and exposure to tobacco smoke (ETS) the causality of them with GSD has not yet been investigated. As for drinking alcohol, although common alcohol use was not significantly causally associated with the GSD risk, we additionally analyzed the causality between problematic alcohol use (PAU) and the risk of GSD. Moreover, a recent genome-wide association study (GWAS) of caffeine intake has identified additional SNPs associated with coffee or tea consumption, which can be used as IVs for further MR (14). Note that the effect of consuming tea on the GSD risk lacks systematic evaluation.

The current study aims to comprehensively evaluate the relationship between these common addictive behaviors and the GSD risk. We first summarized the evidence in one updated meta-analysis only including a longitudinal study. Data from the meta-analysis was further tested for potential dose–response relationships and by trial sequential analysis (TSA) to check if the present evidence is conclusive. We then explored a putative causal association of tobacco smoking, alcohol use, caffeine intake, and tea consumption with the risk of GSD using a two-sample MR design.

Materials and methods

Search strategy and meta-analysis

Our meta-analysis has been registered at PROSPERO (CRD42020179076) and following PRISMA checklists. We searched PubMed and Embase databases for studies published before January 2021, and references to the retrieved articles were manually searched for additional information (**Supplementary Table 1**). The flow chart is presented in **Supplementary Figure 1**. GSD was defined as gallstones diagnosed by ultrasonography or a history of cholecystectomy; participants without gallstones or cholecystectomy were considered as the

control group (15). Longitudinal studies, including nested case-control, cohort, and randomized controlled trials, provided sufficient data for calculating the effect sizes with 95% confidence intervals (CI) and were eligible for our analysis (see **Supplementary Table 2**). If the person-years of subgroup GSD cases were not reported, we calculated the proportion of new total cases for each group (dividing the exact number of GSD by RR) and multiplied the proportion by total person-years as described previously (16). Two authors (Y.B. and X.W.) extracted data back-to-back from identified articles in current research, and disagreement was solved by consensus.

DerSimonian and Laird's random-effect meta-analysis was applied to summarize the association between addictive behaviors and GSD when I^1 exceeded 50%; otherwise, a fixed-effect meta-analysis was conducted (17, 18). Heterogeneity sources were explored by conducting subgroup analyses. Funnel plots were drawn to demonstrate the possible publication bias if asymmetry were observed, and the bias would be further tested after combining with Egger's and Begg's test results (19). The pooled effect was adjusted by Duval and Tweedie's trim-and-fill method to account for publication bias (20). Sensitivity was evaluated by omitting each estimate at one time to see to what extent a single study could influence the overall risk estimate. Pooled analyses were done using Comprehensive Meta-Analysis version 3.0 (Biostat, Englewood, NJ, USA).

Dose–response analysis

To investigate whether the dose of addictive substances intake was associated with GSD, we conducted Greenland and Longnecker's method using linear and non-linear models (21). The mean amount was used to assign the exposure levels for each risk estimate. For the open-ended lower boundary, the level was assumed to be zero, and non-taken was considered as the reference category. For the open-ended upper boundary, the highest level was assigned to 1.5 or 1.2 times the lower boundary of the category (22). In this study, we further tested a dose-dependent association of GSD with smoking status (cigarettes per day and pack-year smoking), consuming alcohol (drinking grams per day, alcohol intake times per week, and drinking days per week), and intaking caffeine (coffee or tea consumption-cups per day). These statistical analyses were done with the use of STATA 16.0 (StataCorp. College Station, TX, USA).

Trial sequential analysis

TSA was applied to evaluate the sufficiency of the total sample size of a meta-analysis to investigate the associations. A cumulative *Z*-curve exceeds the trial sequential monitoring limit or the required information size, suggesting conclusive evidence (23). TSA was conducted by the program version 0.9 beta.[2] All statistical significance were determined by $P < 0.05$.

Genetic instruments selection and outcome data sources

SNPs showing genome-wide significance ($P < 5.0 \times 10^{-8}$) and with $R^2 < 0.1$ identified by *LDlink*[2] were used as IVs for lifetime smoking (i.e., ever and never smokers, smoking duration, heaviness, and cessation in ever smokers were taken into account) (24). The selection of IVs for smoking initiation (including ever-smoking, current-smoking, and smoking cessation) and common alcohol drinking, for (PAU, considering both alcohol use disorder and measures of problematic drinking), and for caffeine intake (the caffeine content per cup was multiplied by the number of cups of tea or coffee) were retrieved from three GWASs, respectively (14, 25, 26). All study populations were European descendants. The strength of instruments used in this study has been previously described, and an F-statistic larger than 10 was regarded as a strong instrument (27). Details are available in **Supplementary Tables 3**, **4**.

GSD cases and controls were obtained from the UK Biobank, a cohort of about 500,000 adults recruited during 2006–2010 in the United Kingdom (28). Three sources of case-control GWAS were used. First, data containing 337,199 individuals (6,986 cases and 330213 controls, all patients with definite diagnoses, i.e., ICD10: K80_cholelithiasis) with GWAS performed by the Neale Lab (id: ukb-a-559). Second, self-reported gallstones (N_g = 462,933, 7682/455251) obtained from UKB, MRC-IEU (id: ukb-b-18700). Third, the symptomatic GSD with a history of cholecystectomy from the UKB (id: ukb-b-6235, N_c = 462,933, 18319/444614).

Mendelian randomization analysis

For our MR study, the multiplicative random-effect inverse variance weighted (IVW) method was used to estimate the causal associations between addictive behaviors and GSD risk. In sensitivity analysis, the MR-Egger regression was used to identify and correct for the horizontal pleiotropy, the weighted median method provides the estimates when SNPs accounting for more than half of the weight are valid, and the maximum likelihood method maximizes the likelihood of the model based on the causal association (29–31). The *p*-value of the MR-Egger intercept was used to indicate potential horizontal pleiotropy, and Cochrane's *Q*-value was used to evaluate the heterogeneity

1 http://www.ctu.dk/tsa

2 https://analysistools.cancer.gov

among those SNPs for each addictive behavior (32). In this study, the large sample size allowed us to gain sufficient power (all were greater than 80%) for conclusive estimation of the associations between addictive behaviors and incident GSD. The analyses were performed *via* the MR-Base[3] using the R package "TwoSampleMR" (version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria). Here, the causal association would be considered statistically significant when a Bonferroni corrected *P*-value was less than 0.013 (correcting for four exposures, including tobacco smoking, alcohol drinking, coffee, and tea consumption). A p-value < 0.05 was regarded as the marginal significance.

Results

Meta-analysis

In our meta-analysis, a total of 27 longitudinal studies with 43 datasets were included in the pooled analysis, incorporating cigarette smoking (N = 4,584,729), alcohol intake (N = 1,819,052), coffee consumption (N = 333,773), and tea consumption (N = 126,906). A positive significant association of incident GSD was observed with smoking [RR: 1.17 (95% CI: 1.06–1.29)] and tea consumption [1.08 (1.04–1.12)]; while a negative significant association of intaking alcohol [0.84 (0.78–0.91)] or coffee [0.86 (0.79–0.93)] with GSD was found (**Table 1** and **Supplementary Figure 2**). The associations were directionally consistent when stratified by sex, ethnicity, underwent cholecystectomy, or according to different types of addictive behaviors (**Supplementary Figures 3–7**). Of note, a significant increment in GSD risk was associated with smoking only in males (1.15 [1.11–1.20]), and current smokers increased about 7% risk of GSD compared to former smokers. Consuming coffee was significantly associated with a decrement of GSD risk [0.87 (0.79–0.96)] in females only. Although an association with GSD [0.84 (0.82–0.87)] was found in caffeinated coffee, it was not statistically significant in decaffeinated coffee (**Table 1**). We also assessed the potential publication bias, and the adjusted funnel plot is shown in **Supplementary Figure 8**. Then a sensitivity analysis suggested that one of each included study did not influence the overall estimate of the meta-analysis (**Supplementary Figure 9**). Moreover, there were significant differences across all dose levels of cigarette smoking and alcohol intake with the risk of GSD.

Here, our dose–response meta-analysis showed a non-linear relationship between GSD risk with daily smoking per 10 cigarettes [1.10 (1.08–1.12), $P_{\text{nonlinearity}} \leq 0.01$]. We further detected a linear association that an increment of pack-years of smoking increased the risk of GSD [1.01 (1.01–1.01), $P = 0.08$] (**Figure 1**). However, there was no significant association between alcohol consumed grams per day and GSD, despite a non-linear relationship being found. A significant non-linear association of alcohol intake times per week was observed for GSD risk reduction with an RR of [0.81 (0.67–0.99), $P \leq 0.01$)] per 5 units. We further found an increment of days per week of alcohol drinking decreased the GSD risk with a linear inverse association [0.96 (0.94–0.97), $P = 0.89$]. As for caffeine consumption, a potential linear association was detected between coffee cups per day and GSD risk [0.95 (0.94–0.96), per 1 cup]. Despite a non-linear relationship between GSD risk with consuming tea-cups per day ($P = 0.01$), we found no significant association. In addition, the risk of GSD increased by 4% and 8% with every 5 and 10 pack-years increments in cigarettes-smoking; while the risk was reduced by 20% and 23% per five units increment in alcohol-drinking days per week and coffee-cups per day (**Supplementary Table 5**).

Trial sequential analysis

In the TSA of our meta-analysis, the cumulative Z-curve crossed trial sequential monitoring and/or conventional boundary and penalized tests adjusted Z-curves also presented similar results, denoting that this evidence was robust and conclusive (**Supplementary Figure 10**). Compared with the control, the adjusted RR of GSD was 1.13 (1.06–1.20) in smoking, 0.73 (0.67–0.80) in alcohol, or 0.82 (0.75–0.90) in coffee. For the subgroup analyses, the adjusted RR of GSD was 1.10 (1.07–1.14) in former-smoking and 1.16 (1.12–1.20) in current-smoking. For the different types of alcohol, the adjusted RR of GSD was 0.70 (0.62–0.78) for beer, 0.72 (0.66–0.77) for wine, and 0.78 (0.70–0.87) for liquor, respectively.

Mendelian randomization analyses

As shown in **Figure 2** and **Supplementary Figure 11**, our MR found that genetically predicted current smoking and PAU both were associated with an increased risk of GSD, while genetically predicted tea consumption was associated with a decreased risk of GSD. However, there was no genetic association between smoking cessation, common alcohol use, coffee consumption, and the risk of GSD.

The causal association between cigarette smoking and incident gallstone disease

In the IVW method, using lifetime-smoking associated 120 independent SNPs as IVs, we found that it had a causal effect on diagnosed cholelithiasis (OR: 1.008, 95% CI: 1.003–1.013, $P = 0.001$) and patients underwent cholecystectomy [1.015 (1.008–1.023), $P = 6.9 \times 10^{-5}$], but not for self-reported gallstones [1.005 (1.001–1.009), $P = 0.024$] when

3 http://www.mrbase.org/

TABLE 1 Addictive behaviors risks for gallstone disease included in meta-analysis.

	No. of studies	Sample size	I^2 (%)	Pooled RR (95% *CI*)	$P_{between}$
Cigarette smoking	**17**	**4,584,729**	**96.98**	**1.17 (1.06–1.29)**	
Female	8	4,377,535	98.65	1.16 (0.99–1.35)	0.95
Male	4	94,985	0.00	1.15 (1.11–1.20)	
America	8	3,136,107	97.82	1.18 (1.01–1.37)	0.82
Europe	7	1,412,481	71.91	1.14 (1.06–1.22)	
Asia	2	36,141	0.00	1.19 (1.03–1.38)	
With cholecystectomy	3	2,858,469	99.52	1.28 (0.87–1.88)	0.55
Without cholecystectomy	2	102,284	0.00	1.13 (1.02–1.25)	
Smoking status	9	1,753,267			0.02
Former smoker			0.00	1.11 (1.09–1.14)	
Current smoker			46.68	1.18 (1.13–1.24)	
Smoking pack-years	2	152,240			0.01
≤ 20			0.00	1.15 (1.08–1.23)	
> 20			0.00	1.31 (1.21–1.40)	
Alcohol intake	**17**	**1,819,052**	**97.94**	**0.84 (0.78–0.91)**	
Female	8	1,566,845	94.29	0.85 (0.79–0.91)	0.38
Male	8	142,364	82.55	0.89 (0.82–0.97)	
America	7	391,307	69.50	0.85 (0.81–0.89)	0.21
Europe	8	1,391,604	99.22	0.79 (0.68–0.91)	
Asia	2	36,141	24.35	0.97 (0.81–1.17)	
With cholecystectomy	1	139,272	0.00	0.87 (0.84–0.89)	0.88
Without cholecystectomy	2	92,880	95.62	0.84 (0.58–1.22)	
Type of drinks	3	106,342			0.60
Beer			20.42	0.84 (0.73–0.96)	
Wine			0.00	0.87 (0.81–0.93)	
Liquor			0.00	0.82 (0.75–0.90)	
Frequency of intake (days/week)	2	104,380			0.02
1–2			0.00	0.94 (0.88–1.02)	
3–4			0.00	0.85 (0.77–0.95)	
5–7			62.49	0.77 (0.66–0.88)	
Grams intake per day	2	104,380			0.00
< 15			0.00	0.91 (0.85–0.96)	
≥ 15			1.47	0.71 (0.64–0.78)	
Coffee Consumption	**7**	**333,773**	**86.88**	**0.86 (0.79–0.93)**	
Female	4	127,384	78.11	0.87 (0.79–0.96)	0.98
Male	4	99,452	77.25	0.87 (0.74–1.02)	
America	2	126,906	73.51	0.83 (0.68–1.00)	0.80
Europe	5	206,867	85.43	0.85 (0.75–0.96)	
Type of Coffee	2	126,906			0.00
Caffeinated coffeeCaffeinated coffee intake			29.61	0.84 (0.82–0.87)	
Decaffeinated coffee intake			0.00	1.00 (0.96–1.05)	
Cups of Coffee per day	3	231,399			0.17
≤ 1			0.00	0.92 (0.87–0.97)	
2–3			65.03	0.81 (0.77–0.86)	
3–6			0.00	0.83 (0.71–0.97)	
≥ 6			0.00	0.77 (0.61–0.97)	
Tea consumption	**2**	**126,906**	**3.30**	**1.08 (1.04–1.12)**	
Female	1	80,898	0.00	1.07 (1.03–1.12)	0.71
Male	1	46,008	52.90	1.10 (0.98–1.23)	
America	2	126,906	0.00	1.07 (1.04–1.11)	—
Cups of tea per day	2	126,906			0.41
≤ 1			0.00	1.08 (1.03–1.13)	
2–3			44.76	1.05 (0.97–1.12)	
≥ 4			0.00	1.15 (1.02–1.29)	

The bold values here are meant to indicate the overall estimate of total studies.

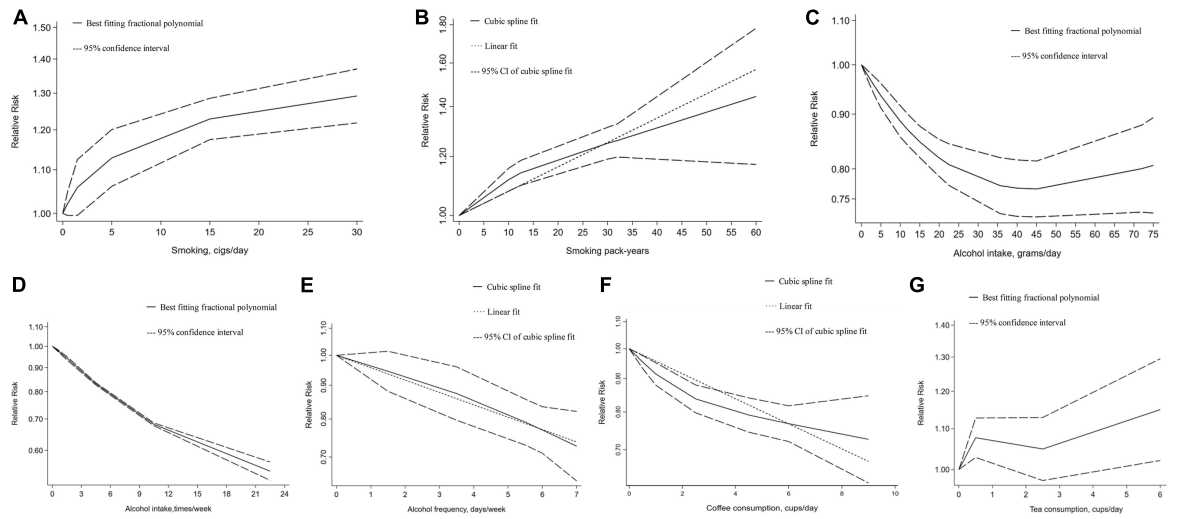

FIGURE 1
Dose–response relationship between addictive substance intake and the gallstone risk. **(A)** Smoking cigarettes per day, **(B)** smoking pack-years, **(C)** alcohol intake grams per day, **(D)** alcohol intake times per week, **(E)** alcohol drinking days per week, **(F)** coffee consumption cups per day, **(G)** tea consumption cups per day. Two-term best fitting fractional polynomial regression model indicated that a potential non-linear model fitting the observed outcomes is identified. A single cubic spline curve is fitted to the data and the goodness of non-linear fit is calculated. Since the non-linear fit was not significant and it was similar to the linear fit model, so linear regression is used.

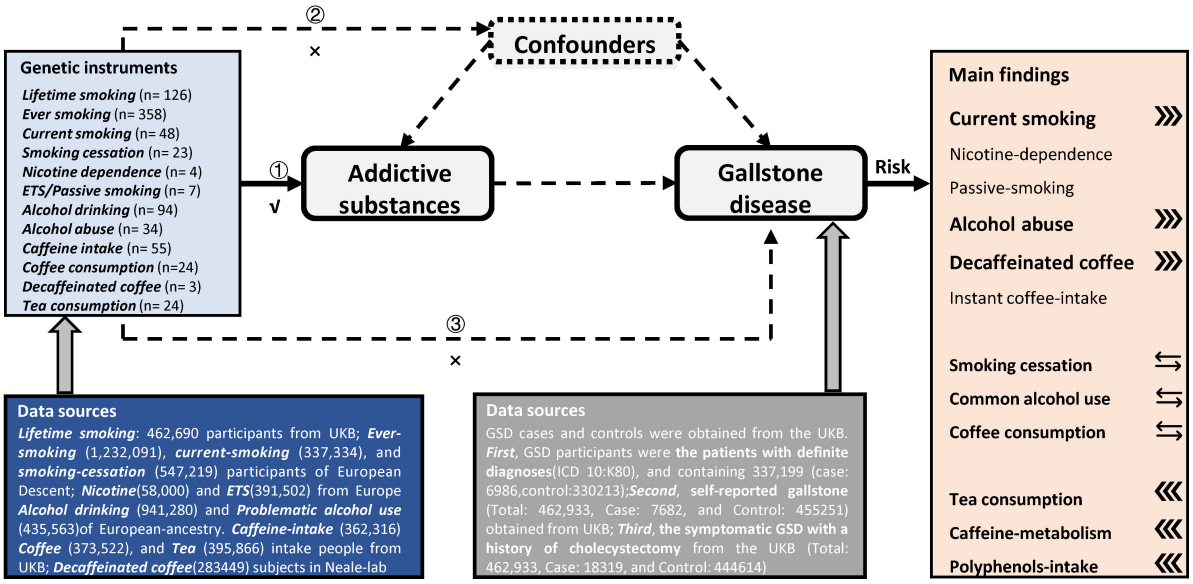

FIGURE 2
Overview of the design and main findings in this Mendelian-randomization study. Assumption 1 indicates that the genetic instruments are significantly genome-wide associated with these addictive substances of interest. Assumption 2 indicates that our genetic instruments should not be associated with confounders. Assumption 3 indicates that genetic instrument affect these outcomes only *via* the exposures.

compared with $P_{adjusted} < 0.013$. The estimates remained directional and consistent observed in MR-Egger regression, despite the causalities were not significant. Then, by using the intercept of MR-Egger, we observed no evidence of horizontal pleiotropy ($P_{pleiotropy}$ for the diagnosed cholelithiasis, self-reported gallstones, and cholecystectomy: 0.399, 0.658, and 0.693), and the weighted median and maximum likelihood methods yielded similar results, which illustrated the high stability of this causality.

This positive association of all outcomes was further confirmed by smoking initiation associated SNPs (N_{IV} = 345, 338, and 342). As for ever-smoking, it was associated with

an increased risk of diagnosed cholelithiasis [1.006 (1.003–1.008), $P = 2.2 \times 10^{-6}$], self-reported gallstones [1.003 (1.001–1.005), $P = 0.001$], and cholecystectomy [1.009 (1.005–1.012), $P = 4.1 \times 10^{-8}$] in IVW. There was no detected horizontal pleiotropy by using MR-Egger (all $P_{\text{pleiotropy}} \geq 0.05$), and similar effects were observed using the weighted median or maximum likelihood method. For the subgroup analyses, current-smoking also significantly increased the risk of diagnosed cholelithiasis [1.007 (1.002–1.011), $P = 0.004$], whereas it disappeared in smoking-cessation [1.000 (0.994–1.007), $P = 0.905$]. This study, for the first time, provided an explanation of the pathogeny of GSD with addictive behavior, the results of our MR showed that nicotine dependence was a major risk factor for GSD [1.012 (1.002–1.022), $P = 0.017$], and ETS was also causally associated with the risk of cholecystectomy [1.043 (1.002–1.086), $P = 0.038$] (see online **Supplementary Table 6**).

A potential relationship of the risk of gallstones with alcohol drinking

Regarding common alcohol use ($N_{\text{IV}} = 88$, 84, and 89), no causal association of GSD risk was found, and this result might be due to the presence of heterogeneity (all $P_Q \leq 0.001$). In this MR, we further found that PAU was potentially associated with the risk of diagnosed cholelithiasis ($N_{\text{IV}} = 28$, 1.014 [1.001–1.026], $P = 0.029$) and self-reported gallstones ($N_{\text{IV}} = 28$, 1.012 [1.001–1.023], $P = 0.028$), but not cholecystectomy [$N_{IV} = 29$, 1.019 (0.996–1.042), $P = 0.104$] in IVW. Moreover, the effects attenuated slightly in MR-Egger regression with the intercept (0.613, 0.204, and 0.170) confirming that pleiotropy was not detected in the three outcomes. Similarly, the positive estimate was identified for the risk of GSD with PAU in the maximum likelihood method.

A highly debated association between coffee consumption and the gallstone risk

No significant causal association of GSD risk with coffee consumption was found in this IVW. Even though removing an SNP (rs2472297), the null effect on GSD with consuming coffee was not altered [0.996 (0.987–1.005) for cholelithiasis, 0.999 (0.989–1.008) for gallstones, and 0.998 (0.980–1.017) for cholecystectomy]. While genetically predicted caffeine-intake significantly decreased the GSD risk in the weighted median [0.994 (0.989–0.999), $P = 0.012$] for self-reported gallstones; [0.991 (0.984–0.998), $P = 0.016$] for cholecystectomy. Although all of the $P_Q \leq 0.001$ and heterogeneity existed, no horizontal pleiotropy was detected by MR-Egger with the intercept of 0.136, 0.164, and 0.113 in our outcomes. Furthermore, we analyzed the associations between the metabolism of caffeine and the incidence of GSD (**Supplementary Table 7**). There was a negative causal association between habitual caffeine-intake [0.993 (0.988–0.997), $P = 0.003$], caffeine-metabolism [0.997 (0.995–0.999), $P = 0.013$], and the GSD risk; whereas decaffeinated coffee [1.127 (1.043–1.217), $P = 0.002$] or instant coffee [1.074 (1.016–1.135), $P = 0.012$] was related with the GSD risk increased.

The directly protective effect on the risk of gallstone disease with tea consumption

Consuming tea (an additional source of caffeine mainly in black tea) was significantly negatively associated with GSD risk in IVW (**Table 2**). For diagnosed cholelithiasis [$N_{IV} = 19$, 0.990 (0.982–0.997), $P = 0.008$], self-reported gallstones [$N_{IV} = 18$, 0.993 (0.987–0.999), $P = 0.014$] and cholecystectomy [$N_{IV} = 18$, 0.983 (0.974–0.992), $P = 0.000$]. Although an association was not significant, the estimate remained directional and consistent as observed in MR-Egger; and similar estimates were also obtained in the weighted median and maximum likelihood methods. For other sensitivity analyses, no heterogeneity existed with the funnel plot presented symmetrically in **Supplementary Figure 12**.

Discussion

As summarized in this study, the meta-analysis based on a longitudinal study indicates that addictive behaviors are significantly associated with the incidence of GSD. Compared with never smokers, current smokers have a positive dose-dependent response to GSD risk, and the evidence is further verified by the MR analysis. There are negative dose–response relationships between common alcohol use, coffee intake, and the GSD risk. However, the results of MR do not confirm the causal relationship between them. The novel finding of this study is that alcohol abuse may be causally associated with an increment in GSD risk, whereas tea consumption has a protective effect on the GSD risk in Europe.

Smoking has been shown to alter lipid metabolism, and the abnormal synthesis of bile may cause cholesterol supersaturation for the formation of gallstones (33). Consistent with a previous dose-response meta-analysis (8), the risk of GSD is found to be increased by smoking (cigarettes per day) with a non-linear relationship in our study. Moreover, a finding suggests that it is a linear dose-response association between pack years of smoking and risk of GSD and is further subjected to causality. It is also notable that the causal association of GSD is not significant in smoking cessation.

To date, evidence linking alcohol drinking with GSD is controversial (9). In this meta-analysis, we verify a negatively non-linear dose–response association between drinking alcohol grams per day and GSD risk, consistent with Cha et al. (34), but the strict study design and dose definition are used in our study. We conclude that the association of GSD risk reduction appears to reach the limit when the dose is higher than 45 g/day, and this finding (J-shaped) is similar to that of Figueiredo et al. (5), while the appropriate dose of alcohol-intake protects against GSD awaits future study.

TABLE 2 Mendelian randomization estimate of a causal association between addictive behaviors and the risk of gallstones.

Outcome disease	Exposure phenotype	Number of IVs	Inverse variance weighted	MR egger	Weighted median	Maximum likelihood	Horizontal pleiotropy	Q_pval
			OR (95% CI), *P*-value	OR (95% CI), *P*-value	OR (95% CI), *P*-value	OR (95% CI), *P*-value		
Diagnoses ICD10 K80: Cholelithiasis (Neale Lab)	Lifetime smoking [ac]	120	1.008 (1.003–1.013), 0.001	1.000 (0.982–1.020), 0.965	1.008 (1.001–1.015), 0.030	1.008 (1.004–1.013), 0.000	0.399	0.244
	Ever smoking [acd]	345	1.006 (1.003–1.008), 2.2E–06	1.000 (0.991–1.010), 0.954	1.004 (1.001–1.008), 0.007	1.006 (1.003–1.008), 4.2E–07	0.263	0.025
	Current smoking [ac]	47	1.007 (1.002–1.011), 0.004	1.004 (0.996–1.011), 0.353	1.006 (0.999–1.012), 0.096	1.007 (1.002–1.011), 0.004	0.382	0.719
	Smoking cessation	21	1.000 (0.994–1.007), 0.905	0.993 (0.977–1.010), 0.441	0.998 (0.991–1.005), 0.598	1.000 (0.995–1.005), 0.877	0.375	0.025
	Common alcohol use	88	0.997 (0.989–1.006), 0.531	0.992 (0.976–1.008), 0.344	0.999 (0.987–1.011), 0.885	0.997 (0.991–1.004), 0.420	0.466	0.000
	Problematic alcohol use [bc]	28	1.014 (1.001–1.026), 0.029	1.002 (0.959–1.048), 0.918	1.009 (0.998–1.020), 0.115	1.014 (1.007–1.022), 0.000	0.613	5.0E–07
	Caffeine intake	42	0.998 (0.992–1.004), 0.530	0.992 (0.983–1.002), 0.119	0.995 (0.989–1.002), 0.154	0.998 (0.994–1.002), 0.369	0.136	0.000
	Coffee consumption	18	0.996 (0.987–1.005), 0.418	0.989 (0.971–1.006), 0.228	0.993 (0.982–1.004), 0.199	0.996 (0.989–1.003), 0.272	0.344	0.017
	Tea consumption[ac]	19	0.990 (0.982–0.997), 0.008	0.986 (0.969–1.002), 0.113	0.992 (0.982–1.003), 0.159	0.990 (0.982–0.997), 0.009	0.601	0.733
Self–reported: Gallstones (MRC-IEU)	Lifetime smoking [bc]	117	1.005 (1.001–1.009), 0.024	1.009 (0.991–1.027), 0.327	1.004 (0.998–1.009), 0.175	1.005 (1.001–1.009), 0.007	0.658	0.002
	Ever smoking [ac]	338	1.003 (1.001–1.005), 0.001	0.998 (0.991–1.006), 0.675	1.003 (1.001–1.006), 0.017	1.003 (1.002–1.005), 0.000	0.218	0.023
	Current smoking	46	1.001 (0.997–1.005), 0.554	0.999 (0.992–1.007), 0.820	1.000 (0.995–1.006), 0.973	1.001 (0.998–1.005), 0.473	0.508	0.022
	Smoking cessation	20	1.002 (0.997–1.006), 0.477	0.990 (0.979–1.001), 0.101	1.002 (0.996–1.007), 0.597	1.002 (0.998–1.006), 0.417	0.044	0.157
	Common alcohol use	84	0.997 (0.990–1.005), 0.463	1.013 (0.992–1.035), 0.236	1.004 (0.995–1.014), 0.357	0.997 (0.992–1.003), 0.363	0.130	0.001
	Problematic alcohol use [bc]	28	1.012 (1.001–1.023), 0.028	0.988 (0.951–1.026), 0.533	1.003 (0.994–1.012), 0.537	1.012 (1.007–1.018), 2.4E–05	0.204	0.000
	Caffeine intake [d]	41	1.000 (0.994–1.005), 0.899	0.994 (0.985–1.004), 0.237	0.994 (0.989–0.999), 0.012	1.000 (0.996–1.003), 0.822	0.164	0.000
	Coffee consumption	17	0.999 (0.989–1.008), 0.809	0.993 (0.974–1.012), 0.477	0.994 (0.986–1.002), 0.131	0.999 (0.993–1.004), 0.665	0.490	1.3E–05
	Tea consumption [b]	18	0.993 (0.987–0.999), 0.014	0.989 (0.976–1.002), 0.124	0.991 (0.982–0.999), 0.024	0.993 (0.987–0.999), 0.015	0.558	0.614
Operation code: Cholecystectomy (UKBiobank)	Lifetime smoking [acd]	120	1.015 (1.008–1.023), 6.9E–05	1.021 (0.992–1.051), 0.165	1.014 (1.006–1.023), 0.001	1.016 (1.010–1.021), 3.6E–08	0.693	1.4E-08
	Ever smoking [acd]	342	1.009 (1.005–1.012), 4.1E-08	1.012 (0.999–1.025), 0.081	1.009 (1.005–1.013), 3.9E-06	1.009 (1.006–1.011), 5.1E-11	0.628	4.8E-08
	Current smoking [c]	48	1.007 (1.000–1.014), 0.068	0.998 (0.986–1.010), 0.749	1.004 (0.996–1.012), 0.282	1.007 (1.002–1.012), 0.011	0.098	0.000
	Smoking cessation	23	1.004 (0.998–1.010), 0.221	0.991 (0.975–1.006), 0.240	1.003 (0.995–1.011), 0.463	1.004 (0.998–1.010), 0.172	0.077	0.187
	Common alcohol use	89	0.994 (0.979–1.009), 0.410	0.991 (0.963–1.019), 0.535	1.001 (0.986–1.016), 0.943	0.993 (0.986–1.001), 0.088	0.832	1.0E–36
	Problematic alcohol use [c]	29	1.019 (0.996–1.042), 0.104	0.980 (0.925–1.039), 0.506	1.007 (0.993–1.020), 0.331	1.020 (1.011–1.029), 5.2E–06	0.170	0.000
	Caffeine intake	41	0.999 (0.988–1.010), 0.859	0.987 (0.970–1.005), 0.172	0.991 (0.984–0.998), 0.016	0.999 (0.994–1.004), 0.692	0.113	0.000
	Coffee consumption	17	0.998 (0.980–1.017), 0.854	0.979 (0.945–1.015), 0.269	0.988 (0.977–0.999), 0.027	0.998 (0.990–1.006), 0.664	0.240	2.6E–11
	Tea consumption [acd]	18	0.983 (0.974–0.992), 0.000	0.977 (0.956–0.999), 0.054	0.979 (0.967–0.991), 0.000	0.983 (0.974–0.992), 0.000	0.559	0.300

Significant associations reported are adjusted *p*-values at a Bonferroni corrected and *p*-values directly, [a]IVW method: $P_{adjusted} < 0.013$, [b]IVW method: $P_{val} < 0.050$, [c]Maximum Likelihood: $P_{adjusted} < 0.013$, [d]Weighted Median: $P_{adjusted} < 0.013$, If significant heterogeneity existed $P_Q < 0.05$, a random-effects model (IVW) was selected.

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Concerning the types of alcohol, an RR of GSD is 0.82, 0.84, and 0.87 (liquor, beer, and wine) as the alcohol concentration decreased. One possible explanation is that alcohol may reduce cholesterol levels, improve HDL-C levels, and promote the secretion of bile acid, which in turn may inhibit gallstone formation (35). Meanwhile, we also propose two possible explanations for a non-causality found between common alcohol use and GSD risk in this MR. One possibility is that there may be a mediation effect for liver cirrhosis in the relationship. Some studies indicate that alcohol drinking increases the risk of liver cirrhosis, which has a close correlation with incident GSD (36, 37). Second, it may have a potential non-linear relationship between them that moderate drinking decreases the risk of GSD, whereas problematic drinking increases the GSD risk.

Interestingly, high coffee consumption was associated with a decrement in GSD risk. An MR also suggested a causal relationship between them in the Danish cohort (38). But, our results of MRs do not support such putative causality from a larger sample size in UKB, which agrees with a finding reported by Yuan et al. (13). In addition to population differences, one intriguing possibility is that self-reported coffee consumption includes decaffeinated coffee, coffee beverages, and others, which may weaken the effect of caffeine (39). Furthermore, this study provides the first report, to our knowledge, of a negative correlation of the GSD risk with drinking tea in Europe.

Tea consumption as another addictive behavior with caffeine intake (including black and green tea) has been associated with a GSD risk decreased in both genders within the population of Asia, and caffeine can stimulate cholecystokinin secretion and release bile acids into the intestine (40, 41). Our MR verified this causal association in a European population, and tea polyphenol (mainly found in green tea) was also found to be causally associated with GSD risk, despite only one instrument being used. Certainly, more studies need to be done in the future. Current knowledge shows that polyphenols may accelerate bowel movements, and promote lipolysis and absorption, which in turn decreases morbidity in GSD (42).

Here, some plausible mechanisms are explored for the causal associations between addictive substance use and GSD risk. For the association between active smoking or ETS and the risk of GSD, the nicotine-dependence may be a key factor in this relationship. Of note, electronic cigarette has not been reported in GSD-related research. In addition, caffeine and tea polyphenols are the most commonly consumed psychostimulants, and they both causally decrease the risk of GSD in our MR. However, coffee consumption (including decaffeinated coffee or other beverage) is not associated with GSD, which may weaken the effect of caffeine.

There is high heterogeneity in our meta-analysis, and existing research regarding this topic is relatively fewer, which may have yielded publication bias. For example, the positive association between tea consumption and GSD in the American population might be due to the smaller number of studies included, which is an important limitation of our study. The second limitation is vertical pleiotropy, which could be shown to mediate the effect within a relationship between exposure and outcome. Another limitation is that we could not explore a non-linear relationship using this MR approach. As for the heterogeneity in a different population, our IVs were all identified in GWAS of a European-origin sample, although these instruments can only explain the percent of 0.24–1.72 (smoking cessation, caffeine-intake, etc.) in total estimated heritability, which limits the generalizability of our finding to diverse populations.

Conclusion

In conclusion, tobacco smoking, PAU, and decaffeinated coffee directly confer high risks of GSD; nonetheless, habitual caffeine intake and tea consumption may have a protective role against GSD due to an effect of caffeine metabolism or polyphenol intake. Accordingly, we infer that changing addictive behavior may be necessary for reducing the risk of GSD.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Author contributions

BZ managed the project and study design. XW and YB read and abstracted the studies included in the meta-analysis. XJ and YB analyzed the data in the Mendelian randomization study. MZ and YB prepared the tables and figures. DG, MT, and YB did the statistical analyses. YB drafted the manuscript with HC, XS, YW, XW, and XJ. All authors reviewed and approved the article.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.940689/full#supplementary-material

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Association between dietary inflammation and erectile dysfunction among US adults: A cross-sectional analysis of the National Health and Nutrition Examination Survey 2001–2004

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Background: Although chronic low-grade inflammation has been linked to the development of erectile dysfunction (ED), the association between pro-inflammatory diets and ED is unclear. The dietary inflammation index (DII) is a novel method to quantify the inflammatory potential of a diet.

Objective: Our objective was to investigate the association between the DII and ED among US males.

Design: This cross-sectional study included 3,693 males 20–85 year of age from the National Health and Nutrition Examination Survey (NHANES) 2001–2004. Multivariable-adjusted logistic regression models were used to assess the association between the DII and ED. All analyses accounted for the complex sampling design.

Results: The mean ± SE of the DII was 0.8 ± 0.1 and 0.4 ± 0.1 among participants with and without ED, respectively. After adjusting for age, race/ethnicity, education, smoking status, physical activity, drinking status, hypertension, diabetes, cardiovascular disease, hypercholesterolemia, BMI, and eGFR, the DII score was associated with ED (odds ratio 1.12; 95% CI: 1.04–1.19). Moreover, this association was also stable in our subgroup analysis or sensitivity analyses.

Conclusion: Dietary inflammatory potential, as estimated by the DII score, is positively associated with ED among US males.

KEYWORDS

erectile dysfunction, inflammation, dietary score, dietary recall, NHANES, cross-sectional study

Introduction

Erectile dysfunction (ED) is one of the most common types of sexual dysfunction in men (1, 2) and is predicted to affect approximately 322 million men worldwide by 2025 (3). ED has led to a poorer quality of life and reduction of economic productivity in males, as well as a substantial financial burden on society (4, 5). Some ED risk factors have been identified, including aging, history of diabetes, cardiovascular disease, chronic kidney disease, and smoking (6, 7). However, those risk factors, in most cases, are limited or unmodifiable. Thus, the identification of modifiable risk factors for ED is important.

Diet is a potential source of chronic low-grade inflammation which is related to the pathogenesis of ED (8). Previously, some dietary patterns, especially anti-inflammatory diet, have been linked to ED (9, 10). For example, Mediterranean diet is a kind of anti-inflammatory diet, and recently a long-term randomized clinical trial by Maiorino et al. has noticed a protective effect of Mediterranean diet on erectile function, as well as a reduction of C-reactive protein levels in males with newly diagnosed type 2 diabetes (T2DM) (11). It can be inferred that dietary inflammatory potential is related to the development of ED. However, the impact of the dietary inflammatory potential on ED is still unclear. The DII is a novel method re-devised by Shivappa et al. to quantify the potential inflammatory levels of our daily diet (12). Since its development, DII has been validated against many inflammatory biomarkers in different ethnics and was widely used in a large number of studies (13–19). To our knowledge, the association between the level of dietary inflammation potential and ED has not been reported before. To best understand the influence of dietary inflammation on ED and provide clues for its prevention, this cross-sectional study explored the association between DII and ED among US adults, using data from the National Health and Nutrition Examination Survey (NHANES).

Materials and methods

Data sources

The NHANES is a nationally representative survey with a stratified, multistage probability cluster sampling design in the USA. It is administered by the National Center for Health Statistics (NCHS) and can be used to assess the health or nutritional status of the non-institutionalized US population (20). Data for erectile function is available in the NHANES 2001–2004 and all data were collected following standardized protocols from the NCHS. To provide reliable estimates, we utilized the 4-year data for analyses. The study was approved by the NCHS Research Ethics Review Board and written consents were obtained from the participants before their participating. We followed the Strengthening the Reporting of Observational Studies in Epidemiology – Nutritional Epidemiology (STROBE-nut) guidelines in reporting.

Study population

Of 4116 males (≥20 years) with available information for self-reported ED in the NHANES 2001–2004, 125 were excluded due to having or having received surgery/radiation/medicine treatment for prostate cancer. Eighteen men were further excluded for taking phosphodiesterase type 5 (PDE5) inhibitors or Yohimbine (21), and 83 participants were excluded due to incompleteness of data needed to calculate the dietary inflammatory index. According to a previous study, a daily calorie intake below 800 kcal or above 5,000 kcal is thought to be implausible (22). Thus, 199 participants were excluded for this reason. The flow chart for subject selection is presented in **Figure 1**.

Definition of erectile dysfunction and dietary inflammation index

In the NHANES, erection function was assessed by a question: "How would you describe your ability to get and keep an erection adequate for satisfactory intercourse?" The following response options were provided: "always or almost always able," "usually able," "sometimes able," or "never able." This single question has been validated in a sub-sample from the Massachusetts Male Aging Study (MMAS) and was considered as a practical tool for assessing ED (23). In the present study, ED was defined as a dichotomous variable where men who responded "sometimes able" or "never able" to maintain an erection were considered as with ED and those who responded "always or almost always able" or "usually able" were considered as without ED (24, 25).

Based on the previous study, the DII score was calculated based on 27 food parameters extracted from the NHANES 2001–2004 by a 24-h dietary recall interview (12, 26). Then, the DII score was calculated by the following steps. First, the *z*-score for each of the food parameters for each individual was calculated based on the world average and standard deviation. Second, to control the effect of "skewing," each *z*-score was converted to a centered percentile value. Third, the food parameter-specific DII score was calculated as the centered percentile value times its respective standardized overall inflammatory effect score (12). Finally, the parameter-specific DII scores were summed to get the final index for each individual. A more positive DII value indicates a more pro-inflammatory diet, and a more negative DII value indicates a more anti-inflammatory diet. **Supplementary Table 1** lists the respective world average, SD, and standardized overall inflammatory effect score of the 27 food parameters used in the present study.

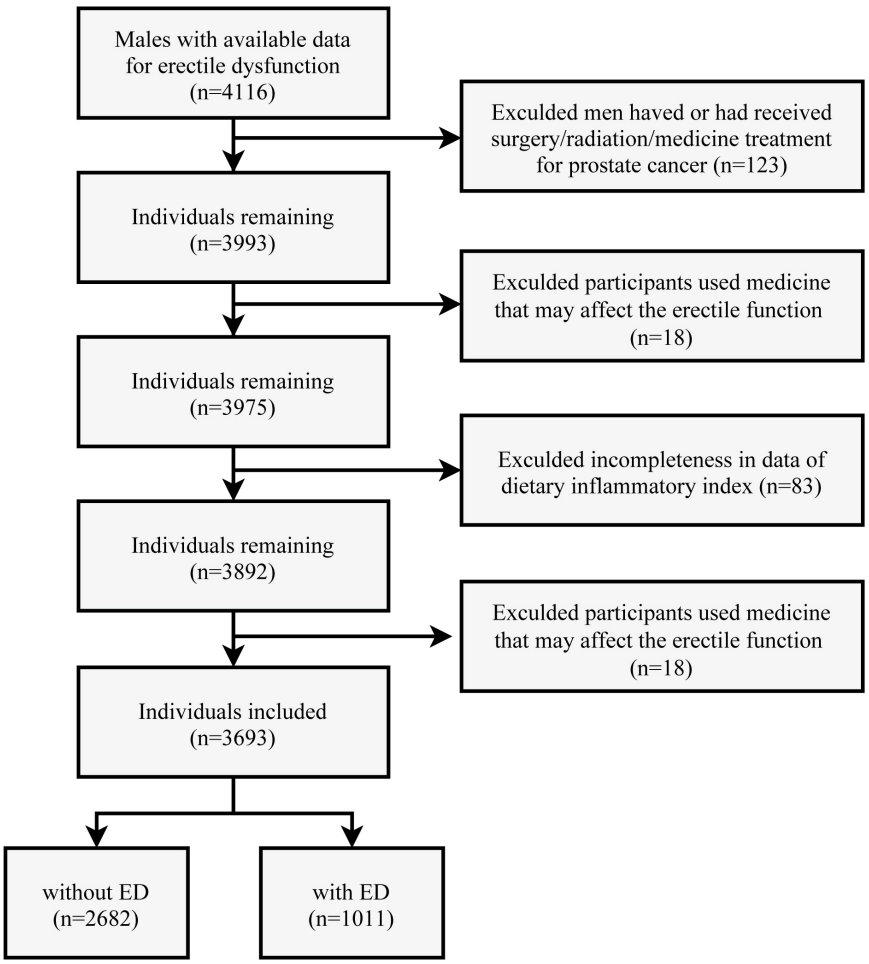

FIGURE 1
Flow diagram of the screening and enrollment of study participants.

Covariates

Potential confounders in the present study included age (27), race/ethnicity (non-Hispanic white, Mexican-American, non-Hispanic black, and others) (28), education (high school or less, some college, and college graduate or higher) (29), body mass index (BMI) (30), diabetes (31), hypertension (32), cardiovascular disease, hypercholesterolemia (33), estimated glomerular filtration rate (eGFR) (34, 35), smoking status (36), alcohol drinking status (37), and physical activity level (38). Diabetes was defined as any participant with self-reported diabetes or who had a fasting plasma glucose level of 126 mg/dl or greater or a glycated hemoglobin level of 6.5% or greater. Hypertension was defined as taking anti-hypertensive agents, systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg. Cardiovascular disease was defined as self-reported history of one of the following conditions: coronary heart disease, myocardial infarction, congestive heart failure, and stroke. Hypercholesterolemia was defined as being told to take cholesterol-lowering medications or total cholesterol ≥240 mg/dl. The eGFR was calculated based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. In the NHANES, self-reported smoking status can be assessed by the following two survey question: "Have you smoked at least 100 cigarettes in your entire life?" and "Do you now smoke cigarettes every day, some days or not at all?" According to the National Health Interview Survey, smoking status can be categorized into three groups: never smoker (smoked less than 100 cigarettes in the lifetime, or has never smoked), ex-smoker (smoked at least 100 cigarettes in the lifetime and responded that now do not smoke), current smoker (smoked at least 100 cigarettes in the lifetime and responded that now smoke cigarettes every day or some days) (39). Serum cotinine is a biomarker of current smoking. Considering that there is no minimum duration of smoking cessation, we took serum cotinine into consideration to reduce the misclassification of current smoker into ex-smoker. Specifically, self-reported never smokers and ex-smokers who having a

serum cotinine level above 10 ng/ml were corrected to be current smokers (40). Alcohol drinking status was determined by self-reporting. Those who drank at least 12 standard drinks in any one year were defined as "alcohol drinking," otherwise they were designated as "without alcohol drinking." Physical activity was divided into three groups based on self-reported leisure-time physical activity: inactive, moderate, and vigorous.

Statistical analysis

The sampling weights were applied in our analyses following the NHANES analytic guidelines (41). A 4-year sampling weight was calculated using the formula: Dietary day one 4-Year sample weight = 1/2 × Dietary day one 2-Year sample weight (WTDRD1). Weighted means/proportions and standard errors (SEs) were used to describe the characteristics of the participants. Continuous data were compared using the survey *t*-test, and categorical data were compared by the survey (Rao–Scott) χ^2 test. Since the missing data was small (missing rate ranged from 0 to 3.9%) for any variable, no imputation method was used in the present study. Odds ratio (OR) and 95% confidence interval (CI) were calculated to show the association between DII and ED by using logistic regression models. Four models were conducted using the logistic regression analyses and generalized variance-inflation factors (GVIF) $\geq$3 indicated the presence of multicollinearity in the analysis. Model 1 was the crude model with no covariate adjusted. Model 2 was adjusted for age, race/ethnicity. Model 3 was the main model. If a covariate changed the estimates of DII and ED by more than 10% when entered into the crude model or eliminated from the complete model, it was included as a potential confounder in model 3. Therefore, model 3 was adjusted for age, race/ethnicity, education levels, smoking status, physical activity levels and hypertension. A fully adjusted model

TABLE 1 Weighted distributions of characteristics of participants.[a]

	All participants		No ED		ED		*P*-value
	No.[b]	Mean ± SE	No.[b]	Mean ± SE	No.[b]	Mean ± SE	
AGE, year	3,693	44.8 ± 0.4	2,682	41.2 ± 0.3	1,011	61.4 ± 0.5	<0.001
BMI, kg/m^2	3,607	28.1 ± 0.1	2,646	27.9 ± 0.2	961	28.9 ± 0.3	<0.001
DII	3,693	0.4 ± 0.1	2,682	0.4 ± 0.1	1,011	0.8 ± 0.1	<0.001
eGFR, [ml/(min × 1.73m^2)]	3,550	93.2 ± 0.6	2,578	96.3 ± 0.6	972	79.8 ± 1.0	<0.001
Race/ethnicity, %							0.09
Mexican-American	774	7.7 ± 1.2	560	8.1 ± 1.2	214	6.1 ± 1.5	
Non-Hispanic black	681	9.9 ± 1.1	529	10.2 ± 1.2	152	8.4 ± 1.3	
Non-Hispanic white	1,994	74.6 ± 2.0	1,406	73.7 ± 2.0	588	78.4 ± 2.6	
Others	244	7.8 ± 1.1	187	7.9 ± 1.1	57	7.1 ± 1.5	
Education, %							<0.001
High school or less	1,023	16.4 ± 0.8	612	13.5 ± 0.8	411	29.1 ± 2.4	
Some college	915	25.9 ± 1.1	709	26.5 ± 1.3	206	23.4 ± 1.7	
College graduate or higher	1,753	57.7 ± 1.4	1,359	59.9 ± 1.4	394	47.5 ± 2.1	
Diabetes, %	513	10.2 ± 0.6	216	6.3 ± 0.6	297	27.6 ± 1.6	<0.001
Hypertension, %	1,247	30.8 ± 1.3	664	24.7 ± 1.3	583	57.5 ± 1.6	<0.001
Cardiovascular disease, %	489	9.7 ± 0.8	178	5.4 ± 0.7	311	29.0 ± 2.1	<0.001
Hypercholesterolemia, %	1,023	28.5 ± 1.1	646	25.3 ± 1.2	377	42.5 ± 2.5	<0.001
Alcohol drinker, %	3,068	82.6 ± 2.1	2,244	83.1 ± 2.3	824	80.4 ± 2.2	0.21
Smoker, %							<0.001
Never smoker	1,363	38.7 ± 1.8	1,087	41.5 ± 2.0	276	26.5 ± 2.0	
Ex-smoker	1,046	25.2 ± 1.1	594	21.4 ± 1.2	452	42.2 ± 2.1	
Current smoker	1,281	36.2 ± 1.8	1,000	37.1 ± 1.9	281	31.3 ± 2.9	
Physical activity, %							<0.001
Inactive	1,433	31.3 ± 1.2	915	28.1 ± 1.3	518	44.5 ± 2.1	
Moderate	1,004	28.8 ± 0.9	675	26.9 ± 1.1	329	37.1 ± 1.8	
Vigorous	1,256	39.9 ± 1.4	1,092	44.9 ± 1.6	164	17.8 ± 2.2	

BMI, body mass index; DII, dietary inflammation index; eGFR, estimated glomerular filtration rate; ED, erectile dysfunction.
[a]Data are presented as weighted percentages ± SE for categorical variables and weighted means ± SE for continuous variables.
[b]Unweighted numbers.

was done for model 4, which was adjusted for covariates in model 3 and drinking status, diabetes, cardiovascular disease, hypercholesterolemia, BMI, and eGFR. To further explore the potential associations, the DII score was also classified by tertiles for multivariable logistic regression analyses, and tests for trend were conducted by entering the median value of each DII tertiles as a continuous variable in the multivariable logistic regression models. Stratified and interaction analyses were performed according to age groups, race and ethnicity, hypertension, diabetes, and cardiovascular disease. Finally, two sensitivity analyses were additionally performed to assess the robustness of our findings. In the first sensitivity analysis, we excluded participants taking medicines that potentially affect erectile function, including antidepressants (42), antipsychotics (43), antihyperglycemic agents (9, 44), sex hormones and corticosteroids (45). In the second sensitivity analysis, a stricter criterion of ED was used. Only those who responded "never able to get and keep an erection adequate for satisfactory intercourse" were considered as having ED. All statistical analyses were performed with the statistical software R (The R Foundation)[1]. A *P*-value <0.05 (two-sided) was considered to indicate statistical significance. A *post hoc* power analysis was performed, which demonstrated that the power for the primary outcomes was sufficient (power >0.90).

Results

Table 1 shows the weighted characteristics stratified by ED status. There were 3,693 males included in our analyses and 1,011 of them had ED. The weighted number of all participants is 3,809,255,599, and the weighted prevalence of ED is 33.7%. For all participants, the weighted mean age was 44.8 years old (SE = 0.4), and most of them were non-Hispanic whites (74.6%, SE = 2.0). The DII scores ranged from −5.15 (most anti-inflammatory) to +4.93 (most pro-inflammatory), and the mean of DII score was higher in participants with vs. without ED (0.8 vs. 0.4, $P < 0.001$). Participants with ED were more likely to be older and have a higher BMI, lower educational level, lower eGFR, lower physical activity level, and diabetes, hypertension, cardiovascular disease, and hypercholesterolemia.

Table 2 summarizes results from sample-weighted logistic regression analyses. The association between DII and ED was stable in different adjusted models. In the crude model (model 1), the odds ratio of DII on ED was 1.13 (95% CI, 1.08–1.19). Males in the highest DII tertiles vs. those the lowest DII tertiles were at a higher risk of ED [OR 1.64 (95% CI, 1.30–2.08)]. In the main model (model 3) adjusted for age, race and ethnicity, education levels, hypertension, smoking status, and physical activity levels, the odds ratio was 1.11 (95% CI, 1.05–1.18). The odds ratios were 1.19 (95% CI, 0.85–1.66) and 1.47 (95% CI, 1.12–1.94) for DII tertiles 2 and 3, respectively (*p* for trend = 0.01). Furthermore, this association was stable in the fully adjusted model and the trend was robust.

TABLE 2 Association between DII and ED.

	Model 1[a]	Model 2[b]	Model 3[c]	Model 4[d]
DII (continuous)	1.13 (1.08, 1.19)	1.12 (1.05, 1.18)	1.11 (1.05, 1.18)	1.12 (1.04, 1.19)
DII categories				
Tertile 1	Ref.	Ref.	Ref.	Ref.
Tertile 2	1.34 (1.00, 1.78)	1.22 (0.88, 1.69)	1.19 (0.85, 1.66)	1.16 (0.80, 1.67)
Tertile 3	1.64 (1.30, 2.08)	1.48 (1.13, 1.93)	1.47 (1.12, 1.94)	1.51 (1.09, 2.10)
P for trend	<0.001	0.01	0.01	0.02

DII, dietary inflammation index; ED, erectile dysfunction.
[a]Model 1: crude model.
[b]Model 2: adjusted for age, race and ethnicity, and education.
[c]Model 3 (the principal model): model 2 + physical activity, smoking status, and hypertension.
[d]Model 4 (the fully adjusted model): model 3 + drinking status, diabetes, cardiovascular disease, hypercholesterolemia, BMI, and eGFR.

Figure 2 shows the results of subgroup analysis. The DII score was associated ED among those aged 20 to 40 years (OR, 1.33; 95% CI, 1.05–1.68), and those with (OR, 1.10; 95% CI, 1.00–1.21) and without hypertension (OR, 1.13; 95% CI, 1.01–1.26), without diabetes (OR, 1.13; 95% CI, 1.04–1.22). No significant interaction was detected in the interaction analysis. Results of sensitivity analyses are presented in Table 3. After excluding participants taking medicines that potentially affect erectile function, the odds ratio was 1.11 (95% CI, 1.03–1.20) after adjusting for age, race and ethnicity, education levels, hypertension, smoking status, and physical activity levels. After re-defining ED to self-reported "never able" to maintain an erection, the odds ratio was 1.16 (95% CI, 1.06–1.27) in the adjusted model.

Discussion

This nationally representative study found robust association between DII and ED in US adult males. In the present study, after adjusting for the baseline imbalance, participants in the highest DII tertiles still had an approximately 1.5-time higher odds of having ED compared with those without ED. Besides, this relationship remained stable in the full-adjusted model that additionally adjusted for a large set of covariates. Notably, the slight variations of the odds ratio between the full-adjusted model and model 2 may indicate that some risk factors of ED (like diabetes, cardiovascular disease, and hypercholesterolemia) may not play a key role here. However, it should be interpreted cautiously, due to the potential residual confounding. Interestingly, in our subgroup analyses, males who are younger and without diabetes seemed to have a higher risk of ED, although no significant interaction was

1 http://www.R-project.org

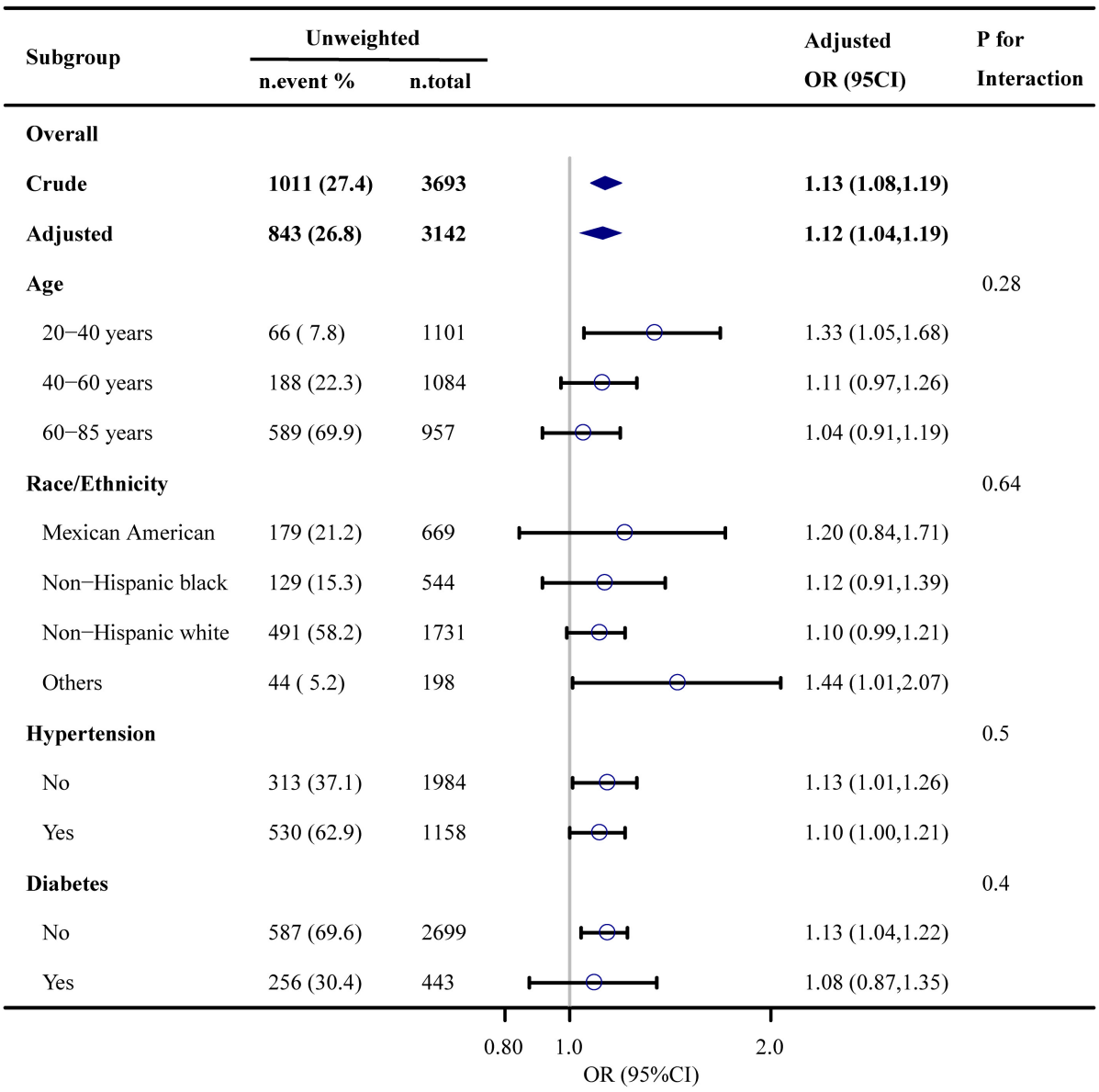

FIGURE 2
Association between dietary inflammation index and erectile dysfunction. Each stratification was adjusted for age, race and ethnicity, educational level, physical activity, smoking status, drinking status, BMI, hypertension, diabetes, cardiovascular disease, hypercholesterolemia, and eGFR, except the stratification factor itself.

detected. Considering the cross-sectional nature, this may be explained by the reverse causation. ED is a common situation in the elderly persons who are more prone to chronic diseases, like diabetes. The higher risk of developing many chronic diseases may encourage the old to choose a healthier dietary pattern which may content more anti-inflammatory component. In our sensitivity analysis that ruled out the impact of some medication on ED, the odds ratio remained. In the other sensitivity analysis that redefined ED by using a stricter criterion, we noticed a higher odds ratio. This could be explained by a reduction of the misclassification of cases into non-cases which can bias the odds ratio to null. To conclude, the present study provides evidence of a robust association between DII and ED.

Most existing studies focus on the protective effect of specific anti-inflammatory nutrients or diet patterns on ED. As listed in **Supplementary Table 1**, both caffeine and flavonoids are anti-inflammatory food parameters. A study by Lopez et al. noticed a protective effect of caffeine against the risk of developing ED (22), and Cassidy et al. reported a protective effect of flavonoids against the risk of developing ED (46). Current evidence also suggested an anti-inflammatory effect of vitamin D (47, 48), and Farag et al. found a positive association between vitamin

TABLE 3 Sensitivity analyses.

	DII	T1	T2	T3	*P* for trend
Sensitivity analysis 1[a]					
Crude model	1.14 (1.08, 1.21)	Ref.	1.33 (0.98, 1.81)	1.70 (1.30, 2.24)	<0.001
Adjusted model[b]	1.11 (1.03, 1.20)	Ref.	1.31 (0.88, 1.94)	1.53 (1.08, 2.17)	0.02
Sensitivity analysis 2[c]					
Crude model	1.18 (1.10, 1.25)	Ref.	1.86 (1.21, 2.86)	2.25 (1.67, 3.05)	<0.001
Adjusted model[b]	1.16 (1.06, 1.27)	Ref.	2.01 (1.24, 3.24)	2.12 (1.41, 3.18)	0.002

[a]Sensitivity analysis excluding participants taking medication that potentially affect erectile function.
[b]Adjusted for age, race and ethnicity, education, physical activity, smoking status, and hypertension.
[c]Sensitivity analysis that re-defines ED to self-reported "never able" to maintain an erection.

D deficiency and ED (21). These studies support our findings very well. For pro-inflammatory dietary components, it is hard to judge its impact on ED since only limited relevant studies. Saturated fatty acids (SFA) and fat are widely considered as pro-inflammatory dietary components. Medeiros Júnior et al. has found that a high-SFA diet could led to an increase in collagen fibers and decline in corpus cavernosum cell proliferation in rat penile tissue (49). Nguyen et al. also found that a high-fat diet in combination with marijuana can lead to an accelerated corporal fibrosis in mouse (50). However, those are all indirect evidence and further studies are needed in this field. Both Mediterranean diet and plant-based diet are previously found to be inversely associated with DII score and they are also suggested to play a role in maintaining erectile health (51, 52). In 2010, Giugliano et al. found that a greater adherence to Mediterranean diet was associated with a lower risk of ED in Italian male that with T2DM (53). To further assess the effect of Mediterranean diet on ED, they conducted a long-term dietary trial in participants with newly diagnosed T2DM (11). It shows that over the entire follow-up, men in the Mediterranean diet group had a better ED as well as a lower C-reactive protein levels compared with those in the low-fat group. However, since those studies were conducted in participants with T2DM, application of the results to the general population should be carefully considered. Recently, a cross-sectional study by Carto et al. has reported that a healthful plant-based diet was negatively associated with ED among the US population (54). A cohort study by Yang et al. also found that healthy plant-based diet indices was inversely associated with incident ED and unhealthy plant-based diet indices was positively associated with incident ED among US elderly males (55). A possible explanation for those studies could be both of those two diet patterns share some similar anti-inflammatory food groups, which may have protective effect on ED. Results of our study complement the previous research and may provide valuable information for understanding the pathology of diet on ED.

Although previous studies have noticed an elevation of inflammatory biomarkers in both animal models and humans with ED (56, 57), the exact mechanism is still unclear. A possible explanation is that the pro-inflammatory diet mediated ED by increasing the vascular endothelial injury. As is known, endothelial nitric oxide is a molecule that regulates vascular tone and can protect endothelial cells from oxidative damage. Previous studies showed that inflammatory biomarkers, such as TNF-α, can inhibit endothelial nitric-oxide synthase (eNOS) gene expression in endothelial cells (58–60), leading to vascular endothelial injury and a higher risk of ED. In addition, a pro-inflammatory diet can contribute to the pathogenesis of diabetes and cardiovascular disease. Both are potential risk factors of ED.

The present study has serval strengths. First, it used large high-quality data from the NHANES and considered many potential covariates, which strengthens the reliability of the results. Furthermore, since all analyses were accounted for the NHANES complex sampling design, these findings are generalizable to general US males. Finally, to our knowledge, this is the first study to explore the association between DII and ED. It provides data for future studies. However, some limitations should be mentioned in this study. First, due to the cross-sectional nature, causal inference about the association between DII and ED could not be established. Thus, further well-designed cohort studies are needed for future studies. Second, although the single question to access ED was validated in the previous study, there could still be a recall bias. Thus, we also conducted a sensitivity analysis that re-defined ED by using a stringent criterion to further verify the reliability of our results. Third, although we adjusted for potential confounders as far as possible, there could be some residual or unmeasured confounders, such as glucocorticoid use (45). Thus, we conducted a sensitivity analysis that excluded participants taking medicines that potentially affect erectile function. Finally, the DII was calculated based on a 24-h dietary recall interview in the present study. Although a prospective investigation found that DII is relatively constant during several years of observation in females (61), it is not necessarily generalizable to males. Nevertheless, since the present study is the first one to investigate the DII–ED relationship, it still provides preliminary evidence in this direction.

Conclusion

In summary, this cross-sectional analysis suggests that dietary inflammatory potential, as estimated by the DII score, is positively associated with ED in non-institutionalized US males. Since a pro-inflammatory diet may be a modifiable risk factor of ED, we expect more studies on this field.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: CDC National Center for Health Statistics NHANES database: https://wwwn.cdc.gov/nchs/nhanes/Default.aspx.

Author contributions

ZR contributed to study planning, data analyses, and drafting of the manuscript. XX, HY, RL, WJ, and TL contributed to study planning and manuscript development. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.930272/full#supplementary-material

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Memory function performance in individuals classified as overweight, obese, and normal weight

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Evidence accumulated to date about the relationship between cognitive impairments and adults who are overweight and obese suggests that excess weight has a great impact on memory function. Nevertheless, most of the literature has focused only on studying the influences on working memory and episodic memory. This study aimed to examine the potential associations of clinical and anthropometric measures [body mass index (BMI), WHR, body fat, visceral fat, muscle mass, and hypertension] with six memory domains, including contextual memory, short-term visual memory, short-term memory, non-verbal memory, short-term phonological memory, and working memory, in a sample of 124 individuals classified as overweight (n = 33), obese (n = 53), and normal weight (n = 38). The results obtained showed that, after controlling for employment situations, people classified as obese had poorer short-term phonological memory and working memory than those with normal weights. Bivariate correlations showed that measures of weight, BMI, waist–hip ratio index, body fat, and visceral fat were inversely associated with memory function. However, muscle mass was not a significant predictor of memory function. Higher systolic blood pressure was also associated with worse memory function. The study provides evidence of the importance of adiposity in health and memory function.

KEYWORDS

overweight, obesity, normal weight, memory function, adiposity

Introduction

According to the World Health Organization (WHO) (1), overweight and obesity are conditions defined as an excess or abnormal accumulation of fat that can be harmful to health. Being overweight or obese depends on body mass index (BMI) classification, where a score of over 25 is considered overweight, and that over 30 is obese. The

prevalence of overweight and obesity has tripled in the last three decades, turning into an important world health problem. In 2016, more than 1.900 billion adults were overweight and more than 650 million were obese (2). According to the European Health Interview Survey, the prevalence of overweight and obesity in Europe has increased dramatically over the past decades in many regions (3). In Spain, the proportions of men and women who were overweight were 44.9 and 30.6%, respectively, while the prevalence of obesity was 16.5% for males and 15.5% for females (4).

Epidemiological studies have shown a clear association between overweight and obesity and the occurrence of chronic diseases, such as diabetes mellitus (5), musculoskeletal disorders (6), high blood pressure, cardiovascular diseases (7), and cancer (8). Furthermore, it has been found that eating habits characterized by the intake of fat and refined foods not only have a negative impact on physical health but also contribute to greater cognitive decline and the appearance of neurodegenerative pathologies, including cognitive impairment and dementia (9–11).

Recent studies have shown a bidirectional relationship between obesity and cognitive function. Detrimental associations between anthropometric measures of obesity (e.g., BMI or waist circumference) and some cognitive domains were reported (12). Nevertheless, alterations in neuropsychological processes, such as poor performance in attention, memory, or executive functions, can also have an impact on behaviors prone in individuals with obesity (e.g., appetite dysregulation, decision-making, poor dietary choices, and a tendency toward uncontrolled eating) (13). The literature shows that memory is essential for food-related decision-making and has a great impact on appetite control and weight gain (13), especially working memory (14, 15) and episodic memory (16). This bidirectional relationship between obesity and cognitive function shows two pathways. The present study is focused on the first, which is the association between anthropometric measures of obesity and neurocognitive performance. Recent researchers have reported that cerebral inflammation produced by the accumulation of adipose tissue, activation of the immune system, and gray matter atrophy could be some of the mechanisms involved in this relationship (17–19).

In connection with these findings, executive functions and memory are two domains that are affected by a high level of BMI (15). The executive functions (EFs) constitute a set of cognitive capacities necessary to control and self-regulate an individual's behavior (20). The EFs include cognitive processes, which are cognitive flexibility, monitoring, inhibition, planning, working memory, and processing speed (21). This set of cognitive abilities allows us to organize, integrate and manipulate information acquired, giving us the ability to make decisions, create, anticipate, and plan future goals (22). The literature has demonstrated that EFs can be affected by the accumulation of adipose tissue in different organs, tissues, and systems of the body (23). A previous meta-analysis of 72 studies demonstrated that higher BMI contributed to the appearance of deficits in the cognitive abilities of inhibition, decision-making, working memory, planning, and cognitive flexibility (23).

Furthermore, recent research on brain dysfunction in individuals who are overweight or obese reported that excess weight had a greater effect on memory function (13), which is a cognitive process through which information is encoded, stored, and retrieved (24). According to the literature, there exist several types of memory based on classification criteria. Regarding recall time, immediate memory, working memory, short-term memory, and long-term memory can be distinguished. However, referring to the voluntariness of memory, implicit memory, unconscious process memory, explicit memory, episodic, and semantic voluntary memory can be differentiated (25). The evidence indicates that each type of memory and each process that we use has neural activations associated with different brain areas. The medial temporal cortex, the hippocampus, the prefrontal cortex, and the cerebellum are some of the zones involved in memory function (26). For example, a study about neural activity in low- and high-BMI participants reported that individuals with higher BMI had poorer activity in memory structures than those with normal weights (27). A study by Prickett et al. (28) also found that obesity was predictive of poorer performances in verbal memory and working memory. Other neuropsychological studies have demonstrated lower performances in visual memory, prospective memory, and verbal memory (29). Nevertheless, to date, the majority of studies have focused on the study of episodic and working memory, neglecting other essential memory types for correct day-to-day performance.

Since the great influence of BMI on memory has been demonstrated, it is important to study how the effect of excess fat affects memory subcomponents. For this reason, to expand the spectrum of memory functions, the present study aims to analyze the potential associations between clinical and anthropometric measures and memory subcomponents (contextual memory, short-term visual memory, short-term memory, non-verbal memory, and short-term phonological memory) in individuals classified as overweight, obese, and normal weight. We hypothesize that higher clinical and anthropometric measures are associated with poorer memory subcomponents in individuals classified as obese and overweight compared to adults with normal weight.

Materials and methods

Participants and procedure

The sample included a total of 124 male and female Spanish participants between the ages of 22 and 63 (M = 46.02 years;

SD = 9.31). The sample size was estimated with a power calculation using G*Power3 (30). Calculations of 80% power with an alpha of 0.05 suggested that 35 participants per group were needed to detect an effect with a medium effect size of 0.25. Participants were recruited by advertisements on the website of the Tech4Diet project: 4D modeling and visualization of the human body. The inclusion criteria were: (i) a BMI greater than 24.9 kg/m^2 [overweight (25 $\geq$ BMI $<$ 30) and obese (BMI $\geq$ 30)] or (ii) a BMI smaller than 24.9 (normal weight (18.5 $\leq$ BMI $<$ 25), according to the BMI classification of the WHO; (iii) the ability to read and write fluently; and (iv) Spanish as a mother tongue. The exclusion criteria were (i) currently being or having been in dietetic–nutritional treatment supervised by a nutritionist in the last year; (ii) the presence of an endocrine–metabolic disorder (including thyroid, pituitary gland, and adrenal gland problems and metabolic syndrome); (iii) the presence of a previous history of neurological disease (e.g., stroke or Parkinson's disease) or a history of head trauma; (iv) the presence of a history of severe psychopathology according to the diagnostic criteria of the DSM-IV-TR; and (v) currently receiving psychiatric treatment. Initial participants were recruited from September to November 2020. Normal-weight participants were recruited in June 2021. Of the 126 initial volunteers, two were excluded from the study for having histories of endocrine–metabolic disorder. The final sample included 124 male and female participants classified as overweight, obese, or normal weight. All the measurements were conducted on one testing day. Additionally, all the participants completed a neuropsychological battery of executive function tests. Data were collected at ALINUA, a nutrition and food cabinet endorsed as a health center dependent on the Faculty of Health Sciences of the University of Alicante. The duration of neuropsychology sessions lasted approximately 40 min.

Ethical considerations

The study was approved by the Ethical Committee of the University of Alicante, as well as by the Ethics Committee of the Instituto de Investigación Sanitaria y Biomédica de Alicante [ISABIAL (Health and Biomedical Research Institute of Alicante)] (CEIm: 180380). The study is also part of two ongoing research projects funded by the Ministry of Science and Innovation: "*4D modeling and visualization of the human body for the improvement of adherence to dietary-nutritional treatment of obesity through low-cost technologies*" (TIN2017-89069-R) and "*Predictive models of the morphological evolution of the human body to improve adherence*" (PID2020-119144RB-100). After participants were informed about the voluntary nature of their participation and the fact that they could withdraw from the study whenever they wanted and without consequences, informed consent was obtained from all the subjects involved.

Measures

Anthropometrics, body composition, and clinical parameters

A TANITA MC-780MA P digital weight scale (TANITA Corporation, Arlington Heights, IL, USA) and a 213 SECA portable stadiometer (SECA, Hamburg, Germany) were used to carry out the weight (0.1 kg precision), body fat (0.1 kg precision), visceral fat (cm), muscle mass (0.1 kg precision), and height (0.1 cm precision) measurements. Body mass index (BMI) was calculated as weight/height squared (kg/m^2). According to the WHO classification, we established the cut-off point for overweight as 24.99 kg/m^2, while obesity was defined as a BMI over 30 kg/m^2 and normal weight as 18.5–24.99 kg/m^2.

The waist and hip circumferences were measured using a flexible measuring tape (measurement precision, 0.1 cm). To ensure accurate results, all the measurements were performed twice, and the waist–hip ratio (WHR) value was calculated as the ratio of the waist to hip circumference.

Blood pressure (systolic and diastolic) was measured using an M7 Intelli IT blood pressure monitor (OMROM, M7, Corp., Kyoto, Japan). Capillary cholesterol (mg/dl), glucose (mg/dl), and triglyceride (mg/dl) concentrations were also examined with an Accutrend®Plus instrument using two drops of blood (15–40 μl) collected from different fingers with a lancing device (Accuchek® Softclix® Pro, Roche Diagnostics GmbH, Mannheim, Germany).

Memory (cognitive function)

Memory was examined using the CogniFit General Cognitive Assessment (CAB). It is a computer-assessed neuropsychological test battery commonly used in protocols of cognitive skills research. The CogniFit neuropsychological battery has been widely used for clinical and research purposes (31) since the tests that it offers have been validated against various standard neuropsychological tests (32). Furthermore, scientific studies using CogniFit activities in healthy children, adults, and older people with the aim of improving cognitive function are numerous and have high methodological quality, giving CogniFit the highest level of empirical evidence (31, 33, 34). CogniFit scores range from 0 to 800 points, where high scores refer to increased cognitive performance. For scores between 0 and 200 (red), cognitive abilities are considered cognitive weaknesses. Patients with scores of 200–400 (yellow) are considered patients with cognitive abilities within what is expected for people of their age and gender, but they are still improvable. Higher scores in the range of 400–600 (green) mean that cognitive abilities with these scores are in good

condition. Cognitive abilities that show scores above 600 (green) are considered strengths or cognitive skills since they are in better condition than those of other people of the same sex and age. Scores on the six cognitive abilities[1] are assigned using weights previously derived from factor analyses performed on normative data and are standardized into *Z*-scores. In the present study, we used the memory measures of the General Cognitive Assessment (CAB). Specifically, we examined the following: contextual memory (ability to memorize and discriminate the real source of a specific memory), short-term visual memory (ability to temporarily retain a small amount of visual information), short-term memory (ability to retain a small amount of information to be used in a short period of time), non-verbal memory (ability to store and retrieve non-verbal information by nature), short-term phonological memory (ability to remember phonological information for a short period of time) and working memory (ability to temporarily store and handle information in order to perform complex cognitive tasks). CogniFit offers a wide battery of exercises designed not only to evaluate cognitive function but also to rehabilitate problems in memory or other cognitive functions with practice and cognitive training.

The following are the names and descriptions of the tasks in the cognitive training program (CAB):

1. ***Numbers:*** A series of numbers is displayed, from 2 to 10 digits. The task consists of memorizing them to exactly reproduce them later. *Working memory, short-term memory, and short-term phonological memory.*
2. ***Three figures:*** Three figures are shown for a short period of time. Subsequently, four possible trios of figures are shown. The task is to select the one that corresponds exactly to the first sequence shown. *Working memory and non-verbal memory.*
3. ***Illuminated circles:*** Circles light up in a specific order. The task is to exactly memorize the order and execute it when it is the individual's turn. *Short-term memory, working memory, non-verbal memory, and short-term visual memory.*
4. ***Objects seen or heard:*** This task requires sound. Objects are presented one after another. If it is the first time that an object appears as an image or its name is heard, the patient must press "not presented". If the object was last presented as an image, the patient presses "presented as an image", and if it was last heard, the patient presses "presented orally". *Contextual memory, working memory, and non-verbal memory.*
5. ***Images and words:*** This task requires sound. For a short period of time, some objects appear one after another. Then some words appear, either written or heard. The task consists of determining whether they were previously displayed by pressing the appropriate button (presented or not presented). At the end of each level, the exercise asks the patient to estimate how many questions were answered correctly. *Contextual memory and non-verbal memory.*

1 http://www.cognifit.com (accessed on 30 December 2021).

Data analysis

First, participants were classified into different groups considering their BMI. This classification was conducted following the BMI classification of the WHO. After group classification, differences in the prevalence of participants in each group depending on sociodemographic data (sex, marital status, educational level, and employment situation) were analyzed employing Chi-squared statistics, as well as ANOVA for age. Anthropometric, body composition, and clinical parameter comparisons between subjects classified as overweight, obese, and normal weight were evaluated using ANCOVAs and controlling for the effects of the employment situation. Furthermore, to identify the possible differences between groups in the different types of memory (contextual memory, short-term visual memory, short-term memory, non-verbal memory, short-term phonological memory, and working memory), ANCOVAs were conducted controlling for the effects of employment situation separately for each type of memory. Bonferroni correction was used in *post hoc* comparisons. A value of $p < 0.05$ was considered significant in all cases. Partial eta square was used as the effect size measure. Multiple clinical, anthropometric, and memory features were assessed, and Pearson's correlation was used to explore possible correlations among them. All the statistical analyses were performed using SPSS, Version 24.0 (Armonk, NY, USA). The descriptive values were expressed as the mean and standard deviation (*M* and SD, respectively).

Results

Frequency and percentage of sociodemographic variables

The sociodemographic data are presented in Table 1. There were no significant differences between groups in sex, age, marital status, and educational level. The sample differed in terms of employment situation ($p < 0.05$), as shown in Table 1. Although small, the differences between groups in employment situation ($p = 0.04$) could be explained by the "unemployed" individuals, as in the obesity group there were 10, while in the others

TABLE 1 Frequency and percentage of sociodemographic characteristics for participants in each group.

		Obese (n = 53)	Overweight (n = 33)	Normal weight (n = 38)	p
Sex	Male	19 (39.6%)	12 (25.0%)	17 (35.4%)	0.65
	Female	34 (44.7%)	21 (27.6%)	21 (27.6%)	
Age		48.43 (8.37)	45.82 (9.52)	42.84 (9.63)	0.45
Marital status	Single	6 (30.0%)	6 (30.0%)	8 (40.0%)	0.75
	Married	41 (46.1%)	23 (25.8%)	25 (28.1%)	
	Divorced	6 (40.0%)	4 (26.7%)	5 (33.3%)	
	Other	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Educational level	Non-studies/primary studies	6 (66.7%)	1 (11.1%)	2 (22.2%)	0.09
	Secondary studies	24 (54.5%)	9 (20.5%)	11 (25.0%)	
	University studies	23 (32.4%)	23 (32.4%)	25 (35.2%)	
Employment situation	Full-time/part-time job	43 (38.4%)	33 (29.5%)	36 (32.1%)	0.04*
	Unemployed	10 (83.3%)	0 (0.0%)	2 (16.7%)	
	Other	0 (0.0%)	0 (0.0%)	0 (0.0%)	

*$p < 0.05$.

there were 0 and 2, showing a small difference between these groups.

Bivariate correlations

Bivariate correlations among weight, height, BMI, WHR, body fat, visceral fat, muscle mass, systolic blood pressure, diastolic blood pressure, glucose, cholesterol, triglycerides, contextual memory, short-term visual memory, short-term memory, non-verbal memory, short-term phonological memory, and working memory are presented in Table 2. Significant correlations were found between weight and short-term memory (p = 0.03), non-verbal memory (p = 0.04), short-term phonological memory (p = 0.01), and working memory (p = 0.01). Height was associated with contextual memory ($p < 0.01$). In the case of BMI, significant correlations were found in short-term visual memory (p = 0.01), short-term memory ($p < 0.01$), non-verbal memory ($p < 0.01$), short-term phonological memory ($p < 0.01$) and working memory ($p < 0.01$). In WHR, correlations were associated with two memory types: short-term memory (p = 0.04) and working memory (p = 0.03). Body fat was found to have significant correlations with short-term memory ($p < 0.01$), non-verbal memory (p = 0.03), short-term phonological memory ($p < 0.01$), and working memory ($p < 0.01$). Visceral fat had significant correlations with short-term memory (p = 0.03), short-term phonological memory ($p < 0.01$), and working memory ($p < 0.01$). Finally, systolic blood pressure was associated with short-term visual memory (p = 0.03), short-term memory (p = 0.03), non-verbal memory (p = 0.01), and working memory (p = 0.01). The bivariate correlations were small or moderate, ranging from.18 to.34.

Differences in anthropometrics, body composition, and clinical parameters between individuals classified as overweight, obese and normal weight

Table 3 presents differences in anthropometrics, body composition, and clinical parameters among the participants. The obese group had higher weight [BMI: F (2) = 116.17; $p < 0.01$; η^2 = 0.65], higher WHR [BMI: F (2) = 23.97; $p < 0.01$; η^2 = 0.28], higher body fat [BMI: F (2) = 67.05; $p < 0.01$; η^2 = 0.52], higher visceral fat [BMI: F (2) = 60.44; $p < 0.01$; η^2 = 0.50], higher muscle mass (kg) [BMI: F (2) = 6.09; $p < 0.01$; η^2 = 0.09], higher systolic blood pressure [BMI: F (2) = 21.24; $p < 0.01$; η^2 = 0.26], and diastolic blood pressure [BMI: F (2) = 31.35; $p < 0.01$; η^2 = 0.34] than those classified as overweight and normal weight. No significant intergroup differences were found in height or the clinical parameters of glucose, cholesterol, and triglycerides. The means and standard deviations for each group, as well as *post-hoc* analyses, are presented in Table 3.

Differences in memory between individuals classified as overweight, obese, and normal weight

Differences in the types of memory among participants classified as overweight, obese, and normal weight are presented in Table 4. There were no significant intergroup differences in contextual memory, short-term visual memory, short-term memory, and non-verbal memory. However, individuals with normal weights demonstrated better short-term phonological memory [BMI: F (3) = 6.00; $p < 0.01$; η^2 = 0.09]

TABLE 2 Bivariate correlations between clinical and anthropometric measures and memory.

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Weight (Kg)	1	0.411**	0.892**	0.586**	0.554**	0.770**	0.507**	0.504**	0.517**	0.064	0.012	0.134	0.012	−0.129	−0.186*	−0.185*	−0.217*	−0.231**
		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.479	0.891	0.137	0.893	0.154	0.039	0.040	0.015	0.010
2. Height (m)		1	−0.025	0.269**	−0.293**	0.181*	0.588**	0.222*	0.047	−0.048	−0.014	0.012	0.252**	0.103	0.134	0.112	0.136	0.150
			0.785	0.003	0.001	0.044	0.000	0.013	0.603	0.599	0.874	0.892	0.005	0.255	0.137	0.215	0.133	0.097
3. BMI			1	0.497**	0.751**	0.742**	0.272**	0.453**	0.545**	0.104	0.042	0.156	−0.129	−0.213*	−0.294**	−0.277**	−0.323**	−0.347**
				0.000	0.000	0.000	0.002	0.000	0.000	0.249	0.645	0.083	0.153	0.017	0.001	0.002	0.000	0.000
4. WHR				1	0.186*	0.626**	0.470**	0.545**	0.370**	0.163	−0.085	0.139	0.029	−0.152	−0.180*	−0.174	−0.139	−0.195*
					0.038	0.000	0.000	0.000	0.000	0.070	0.351	0.123	0.746	0.092	0.046	0.054	0.125	0.030
5. Body fat					1	0.494**	−0.203*	0.171	0.379**	0.060	0.095	0.097	−0.088	−0.148	−0.237**	−0.192*	−0.300**	−0.234**
						0.000	0.024	0.057	0.000	0.508	0.296	0.285	0.331	0.100	0.008	0.033	0.001	0.009
6. Visceral fat						1	0.296**	0.451**	0.438**	0.179*	0.059	0.129	−0.065	−0.110	−0.186*	−0.145	−0.242**	−0.292**
							0.001	0.000	0.000	0.047	0.517	0.152	0.475	0.225	0.039	0.108	0.007	0.001
7. Muscle mass (Kg)							1	0.384**	0.252**	0.067	−0.055	0.044	0.138	−0.010	−0.012	−0.002	−0.032	−0.044
								0.000	0.005	0.459	0.545	0.624	0.126	0.916	0.894	0.984	0.728	0.628
8. Systolic blood pressure								1	0.756**	0.258**	0.012	0.074	−0.042	−0.191*	−0.195*	−0.222*	−0.146	−0.229*
									0.000	0.004	0.892	0.416	0.645	0.034	0.030	0.013	0.106	0.010
9. Diastolic blood pressure									1	0.139	−0.019	0.101	0.111	−0.118	−0.151	−0.14	−0.157	−0.119
										0.122	0.837	0.264	0.219	0.192	0.095	0.121	0.081	0.188
10. Glucose										1	0.178*	0.264**	−0.122	−0.12	−0.143	−0.088	−0.101	−0.15
											0.048	0.003	0.178	0.185	0.112	0.330	0.265	0.095
11. Cholesterol											1	0.062	−0.032	0.120	0.107	0.168	0.027	0.024
												0.492	0.723	0.184	0.238	0.062	0.767	0.791
12. Triglycerides												1	0.006	0.035	0.057	0.06	0.057	0.016
													0.950	0.702	0.533	0.508	0.533	0.862
13. Contextual memory													1	0.389**	0.382**	0.556**	0.234**	0.797**
														0.000	0.000	0.000	0.009	0.000
14. Short-term visual memory														1	0.889**	0.904**	0.352**	0.569**
															0.000	0.000	0.000	0.000
15. Short-term memory															1	0.841**	0.719**	0.686**
																0.000	0.000	0.000
16. Non-verbal memory																1	0.390**	0.720**
																	0.000	0.000
17. Short-term phonological memory																	1	0.586**
																		0.000
18. Working memory																		1

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

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TABLE 3 Means and standard deviations in clinical and anthropometric measures.

Variables	Obese (n = 53)	Overweight (n = 33)	Normal weight (n = 38)	*Post-hoc*	p	η^2
Clinical measures	**M (SD)**	**M (SD)**	**M (SD)**			
Weight (kg)	99.28 (14.98)	76.94 (10.00)	62.74 (8.97)	OB > OV, N OB, OV > N OB > N	<0.01***	0.65
Height (m)	166.47 (8.82)	166.06 (9.46)	166.32 (7.83)	OB > N, OV N > OV	0.51	0.01
WHR[1]	0.91 (0.10)	0.87 (0.08)	0.79 (0.08)	OB > OV, N OB, OV > N OB > N	<0.01***	0.28
Body fat	38.21 (6.30)	30.99 (6.62)	21.12 (7.18)	OB > OV, N OB, OV > N OB > N	<0.01***	0.52
Visceral fat	13.63 (4.99)	8.94 (4.10)	4.32 (2.49)	OB > OV, N OB, OV > N OB > N	<0.01***	0.50
Muscle mass (Kg)	55.06 (9.34)	51.30 (8.96)	49.77 (10.03)	OB > OV, N OB, OV > N OB > N	<0.01**	0.09
Systolic blood pressure	131.09 (13.81)	126.97 (16.30)	111.68 (15.48)	OB > OV, N OB, OV > N OB > N	<0.01***	0.26
Diastolic blood pressure	86.60 (8.45)	82.15 (9.99)	71.94 (8.21)	OB > OV, N OB, OV > N OB > N	<0.01***	0.34
Glucose	90.91 (14.60)	82.79 (20.86)	79.08 (21.08)	OB > OV, N OB, OV > N OB > N	0.69	0.00
Cholesterol	201.58 (31.57)	207.61 (38.79)	198.74 (43.06)	OV > OB, N OV > N	0.61	0.00
Triglycerides	262.28 (153.63)	235.00 (105.49)	228.84 (188.95)	OB > OV, N OB, OV > N OB > N	0.58	0.00

[1]WHR, waist–hip ratio. **$p < 0.01$; ***$p < 0.001$.

and working memory [BMI: F (3) = 3.37; p = 0.02; η^2 = 0.05] than those classified as obese and overweight. The means and standard deviations of each group for memory, as well as *post-hoc* analyses, are presented in Table 4.

Discussion

The present study aimed to identify the influence of clinical and anthropometric measures in six memory domains. In particular, we examined neuropsychological performances in contextual memory, short-term visual memory, short-term memory, non-verbal memory, short-term phonological memory, and working memory in adults classified as obese, overweight, and normal weight. Participants classified as overweight and obese performed with similar levels of cognitive functions in every type of memory domain. Participants with normal weight showed similar memory function results, with the exception of short-term phonological memory and working memory, for which those presenting obesity obtained worse scores.

Short-term phonological memory, which is one of the registers of sensorial memory, is defined as the ability to remember phonological information that we receive from an environment for a short period of time (35). It is registered in the primary auditory cortex, which is located in the temporal lobe; it is involved in auditory and language processing, and it is also responsible for memory functions and the management of emotions (35). The short-term phonological memory store extends to several brain areas, most of them located in the prefrontal cortex (PFC), since this is where executive control takes place and attentional control is monitored (36). Our results suggest that a higher BMI was related to lower memory performance in individuals who are obese. In addition, working

TABLE 4 Means and standard deviations in memory subcomponents for each group.

Variables	Obese (n = 53)	Overweight (n = 33)	Normal weight (n = 38)	*Post-hoc*	p	η^2
Memory	**M (SD)**	**M (SD)**	**M (SD)**			
Contextual memory	463.51 (189.38)	534.15 (164.43)	450.34 (154.96)	OV > OB, N OB > N	0.13	0.03
Short-term visual memory	394.87 (243.50)	448.88 (221.17)	442.16 (224.09)	OV > N, OB N > OB	0.48	0.01
Short-term memory	399.04 (223.51)	460.64 (177.59)	493.05 (189.80)	N > OV, OB N, OV > OB N > OB	0.08	0.04
Non-verbal memory	374.17 (180.93)	416.64 (162.08)	438.66 (168.67)	N > OV, OB N, OV > OB N > OB	0.26	0.02
Short-term phonological memory*	363.70 (181.82)	415.55 (160.62)	487.61 (157.81)	N > OV, OB N, OV > OB N > OB	<0.01**	0.09
Working memory*	360.66 (161.25)	434.55 (127.84)	444.13 (132.43)	N > OV, OB N, OV > OB N > OB	0.02*	0.05

M, mean; SD, standard deviation; OV, overweight; OB, obesity; *N*, normal weight. *$p < 0.05$; **$p < 0.01$.

memory, which is defined as the ability to temporarily store and handle the information to perform complex cognitive tasks, was found to be affected in those with higher BMIs. Studies with functional magnetic resonance imaging have shown how the dorsolateral prefrontal cortex plays an essential role in working memory. This area acts as a mediator between information from posterior sensory areas and the limbic system, thus integrating and providing feedback between sensory and emotional information with the purpose of organizing behavior to achieve a specific goal (37). The association between obesity and lower memory performance found in our study is in line with previous studies that have suggested that working memory is frequently affected by higher BMI (15, 23, 27). Some mechanisms may explain these results. In particular, recent research reported that the accumulation of adipose tissue as a result of being overweight and obesity produced chronic inflammation in organisms that were able to disrupt the structure of essential organs, such as the brain, producing a significant impact on cognitive functions (10). An increased BMI was associated with gray matter atrophy in the temporal, frontal, and occipital cortices, as well as the thalamus and midbrain (38). In particular, previous studies involving patients with dementia and laboratory studies in rodents have related obesity to structural and metabolic changes in the hippocampus (39–41), which is directly involved in memory processes. Furthermore, structural modifications in the prefrontal cortex caused by the activation of the immune system due to inflammatory processes were associated with working memory impairments (42). A recent study about eating behavior reported that lower working memory was associated with a loss of control in eating behavior and the choice of highly calorie-dense foods, particularly with higher snack food and fat intakes (43). Thus, a deficit in working memory may lead to impulsive, excessive, and less flexible eating behavior (44). However, there is limited literature about the implications of short-term phonological memory and BMI. A recent meta-analysis by Cheke et al. (27) found that the volume of the temporal lobe in patients with adiposity was lower due to cerebral atrophy (45, 46). Due to short-term phonological memory being located in the primary auditory cortex, a lower temporal lobe volume may lead individuals who are obese and overweight to worse short-term phonological memory performance.

The results of the present study suggest that weight, BMI, WHR index, body fat, and visceral fat were inversely associated with memory function. However, muscle mass was not a significant predictor of memory function. Evidence from independent studies showed a negative association between anthropometric measures (weight, BMI, and WHR) and cognitive performance (47). Recent cross-sectional evaluations have also demonstrated how increased WHR and visceral adiposity are associated with reduced cognitive scores (48–51). Studies in obese rodents and individuals have found considerable evidence for reduced memory performance (52, 53). In longitudinal studies of patients classified as normal weight, overweight, and obese, higher levels of BMI have been related to hippocampal atrophy and cortical thinning (54, 55). For example, Debette et al. (56) found that WHR was associated with changes in the total brain volume. Evidence also suggests that higher levels of body fat might produce adverse effects on health, including cognitive and neuroanatomical changes. A study by Nyberg et al. (53) with

a sample of 581 healthy individuals found that higher body fat was negatively related to subcortical and hippocampal volume and memory. Furthermore, visceral fat is considered an important risk factor in the development of resistance to insulin and is present in various stages of obesity-induced hippocampal dysfunction, which is a brain area involved in memory processes (57). Research has demonstrated that memory processes are of critical importance, as they have a great impact on appetite regulation and weight gain (43). Memories of specific recent eating episodes play an important role in directing food choices and influencing when and how much a person eats. Interrupting memory processes may lead to overconsumption and obesity since it is the remembered experience rather than the actual experience that is more strongly associated with future choices (44). In the present study, WHR and visceral fat were as strong predictors as body fat for memory function. These results might indicate that visceral fat and WHR, apart from increasing the adipose tissue surrounding the intra-abdominal organs (58), might also have a significant impact on organs, such as the brain. There is consistent evidence that suggests body fat plays a more direct role in the brain, rather than visceral fat and WHR (15, 59). This suggests that it is important to study the adiposity continuum and to use complementary measures rather than only BMI. Interestingly, higher systolic blood pressure in participants was negatively associated with worse memory function. These results might indicate that hypertension, which has been associated with an increased risk of cognitive decline (60, 61), may play a direct role in memory. This phenomenon is thought to occur because hypertension disrupts the structure and function of cerebral blood vessels and leads to ischemic damage to white matter regions, which are critical for cognitive function (60). Evidence from previous studies found an association between hypertension and memory deficit (62).

There are several limitations in the current study that suggest areas for future research. First, the study was cross-sectional, precluding the establishment of causal inferences. Second, researchers must be careful when generalizing and interpreting the findings, as we used a small sample size from a single city in Spain, and the effect sizes were small. Third, the participants in our study were voluntarily recruited from the community; therefore, these individuals might be more highly motivated to lose weight and less resistant to change than the general community. Fourth, CogniFit evaluates cognitive domains that may not fit different memory classifications, neglecting memory subtypes that may also be relevant. The evaluation and analysis of memory functions entail great complexity since the domains of memory, while distinct, still share similar functional and structural pathways in the brain, and it is unclear why or how some domains are expected to differ while others are not. Further investigations are needed to clarify these questions, and it would be helpful to use additional neuropsychological tests to measure other interesting types of memory function. As suggested in previous studies, there is a bidirectional relationship between obesity and cognitive function since impaired cognition can hinder eating self-regulation and obesity can generate changes at the neurological level (14). Memory is a domain that can be affected by higher BMI, but it is also possible that deficits in memory may lead individuals to worse dietary choices. While looking for evidence, it was difficult to assess the relevance of previous neuroscientific findings to understanding short-term phonological memory function in obesity. Therefore, further investigations are needed to understand the neural mechanisms underlying short-term phonological memory deficits in obese individuals. Regarding the relationship between anthropometric measures (weight, BMI, body fat, WHR, and visceral fat) and memory, the literature shows diverse results, so deeper investigations are required. Furthermore, recent studies demonstrated that hypertension produced an increased risk of cognitive decline (61). However, there is still a lack of consistent findings on the impact of hypertension on memory function, so future studies should test this hypothesis. Finally, as far as we know, this is one of the first studies conducted in Spain assessing different types of memory function between individuals classified as overweight, obese, and normal weight. Despite these limitations, this study provides evidence of the importance of adiposity in health and memory function since the findings serve to strengthen this association, as well as claim and propose the importance of cognitive functions in clinical nutritional processes. The presence of a cognitive stimulation protocol that has the objective of preventing dysfunction in premature cases and recovering performance in more advanced cases is considered to be essential. For this reason, it would also be interesting to analyze whether, through an anti-inflammatory nutritional dietary protocol (e.g., the Mediterranean diet) (63), cognitive dysfunctions caused by obesity-induced inflammation can be reversed.

Conclusion

The results provided evidence of the influence of anthropometric measures on memory function in individuals classified as obese, overweight, and normal weight. In particular, our findings suggested the importance of examining the independent roles of body fat, visceral fat, and WHR in the memory function of participants considered overweight and obese, since it has been demonstrated that the accumulation of fat in different regions of the body might suggest different memory impairments.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

This study was approved by the Ethical Committee of the University of Alicante, and also by the Ethics Committee of the Instituto de Investigación Sanitaria y Biomédica de Alicante [ISABIAL (Health and Biomedical Research Institute of Alicante)] (CEIm: 180380). The patients/participants provided their written informed consent to participate in this study.

Author contributions

MB: conceptualization, formal analysis, data curation, and writing—original draft preparation. MB, MS-S, AZ-M, and MT: methodology and writing—review and editing. MB, MT, MS-S, AZ-M, and JH-S: investigation. MB and MT: resources. JH-S: supervision. MS-S and AZ-M: funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Dietary diversity is associated with nutrient adequacy, blood biomarkers and anthropometric status among preschool children in poor ethnic minority area of Northwest China

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Introduction: This study aimed to evaluate the status of dietary diversity, nutrient adequacy, blood biomarkers of nutrients, and anthropometric status, as well as to determine the predictors of dietary diversity score (DDS) and mean adequacy ratio (MAR) among preschool children in poor ethnic minority areas of northwest China.

Methods: A total of 578 healthy preschool children aged 3–6 from 17 kindergartens were selected to assess dietary intake, anthropometric status (height-for-age z-score (HAZ), weight-for-age z-score (WAZ), weight-for-height z-score (WHZ), and blood biomarkers. DDS and nutrient adequacy ratio (NAR) were adopted to assess dietary diversity and micronutrient adequacy, respectively.

Results: The mean DDS (ranging from 1 to 9) was relatively low (4.67 ± 1.56). Most participants consumed starchy staples, but few participants consumed organ meat. DDS was associated with serum potassium, serum iron, WAZ, HAZ, all NARs, and MAR (all $p < 0.05$). Living in urban areas (β = 0.158), higher household wealth (β = 0.116), and more caregivers' nutritional knowledge (β = 0.022) were positively associated with DDS (all $p < 0.01$), while living in urban areas (β = 0.031), higher education of caregivers (β = 0.0.027), and higher DDS (β = 0.049) were positively associated with MAR (all $p < 0.01$).

Discussion: In conclusion, dietary diversity was associated with nutrient adequacy and other health outcomes. Nutritional education and poverty alleviation are integral to improving the nutritional status of preschool children.

KEYWORDS

preschool children, dietary diversity, nutrient adequacy, blood biomarkers, anthropometric status

Introduction

Dietary diversity score (DDS) is an indicator to evaluate whether the respondents have a diverse diet, which is defined as the number of different food groups consumed during a reference period (1). It is a widely used tool to assess dietary quality rapidly, especially nutrition and food security, because investigators can collect enough information to calculate it with only a brief questionnaire (2). Inadequate dietary diversity is a global problem, and is a prevalent concern among low-income individuals and families (3). In addition, the lack of dietary diversity is one of the leading causes of malnutrition in preschool children (4).

Preschool children are in a critical period of growth, cognitive, social, and psychological development creating a high demand for various nutrients in their life (5, 6). However, malnutrition and dietary imbalance in the preschool period will not only cause severe growth problems but also adversely affect their health in adulthood, such as intellectual disability and increased risk of chronic diseases (7, 8). Chinese children's nutritional status has greatly improved in the past two decades. But like many developing countries, China is also facing dual challenges of overnutrition and undernutrition (9, 10). According to the China Health and Nutrition Survey 2010–2013, the prevalence of stunting was 8.1% nationwide and 11.3% in rural areas for children aged 0–5 years (11). Preschool children's dietary pattern in some poor ethnic minority areas of northwest China differs from that in other areas, such as Linxia County and Gansu Province. The nutritional status of preschool children was worse than the national level in this area (12). For a long time, the government has issued many policies and made many positive attempts to improve children's dietary intake. However, currently there is no corresponding nutrition improvement plan specifically for preschool children aged 3–6 years (13, 14). Additionally, China's inherent urban-rural gap of economy and education, which makes families in rural low-income areas and ethnic minority areas cannot obtain enough diversified food, has led to the nutritional problems of preschool children (15, 16).

Many studies have shown that dietary diversity has beneficial effects on nutritional status at different ages, especially in children (3, 17–19). For instance, dietary diversity has been proven to be related to micronutrient adequacy in multiple age groups in both developing and developed countries (1, 2, 20–23). Some other studies have shown significant correlations between dietary diversity and anthropometric status [e.g., height-for-age z-score (HAZ) and weight-for-age z-score (WAZ)] or blood biomarkers (e.g., Plasma vitamin B12 and folate) in children (20, 24). In northwest China, it is difficult to carry out nutritional surveys among preschool children for many reasons, such as the underdevelopment of the economy and the lack of scientific researchers in the region. Additionally, the caregivers of preschool children are more traditional and unwilling to cooperate with completing the survey (for example, they believe that blood collection will hurt their children), which can also present difficulties in the research process. Therefore, as an intuitive and food-based indicator, maybe it is a good way to evaluate preschool children's nutritional status. However, research on dietary diversity among preschool children in China has primarily been conducted in the eastern and central regions (19, 25), and few studies have been conducted in the northwest region, where preschool children historically have a poorer nutritional status. Additionally, DDS is influenced by various sociodemographic factors and the correlation between DDS and nutrient adequacy in poor ethnic minority areas of northwest China has not been verified yet; thus, confirming this association could be helpful to simplify the dietary surveys from complicated 24-h dietary recalls to only grasp the food groups they consumed (26). Therefore, this study aimed to (1) evaluate the status of dietary diversity, nutrient adequacy, blood biomarkers of nutrients, and anthropometric status among preschool children in poor ethnic minority areas of northwest China, (2) examine the association between dietary diversity and nutrient adequacy or other health outcomes; and (3) determine the predictors of dietary diversity and nutrient adequacy, especially local-specific predictors. It could provide some theoretical support for nutrition intervention, nutritional education, and poverty alleviation as integral aspects to improve the nutritional status of preschool children.

Materials and methods

Study design and study population

We used baseline data from the GSPNIP study, a preschool nutrition improvement pilot program launched by the Gansu Provincial government and the World Food Program in November 2020 in Linxia County, Gansu Province in northwest China, which aimed to improve the nutritional status of preschool children by supplying breakfast to them. Linxia County is a region with the most underdeveloped economy and complex ethnic minority situation in China. The majority of ethnic minorities were Hui and Dongxiang and observe Islam as their primary religion. The enrollment number of preschool children in the kindergartens was 758. Preschool children aged 3–6 and their caregivers agreed to participate in this study and were included in this cross-sectional study. However, the following participants were excluded from the cohort study: (1) No history of acute or chronic illness (cancer, kidney disease, liver disease, etc.). (2) No gastrointestinal symptoms (constipation, diarrhea, etc.) and fever symptoms in the past three months. (3) No infectious diseases (AIDS, hepatitis B, syphilis, etc.). (4) No history of intake of antibiotics, antiviral, antifungal, or analgesic drugs in the past three months. Caregivers of preschool children were invited to the classroom for a questionnaire (mainly includes sociodemographic information and 24-h dietary recall) while the child(ren) had an anthropometric measurement and blood collection in another classroom. To ensure that the investigators can understand the dialects of the participants, the government and kindergarten staff assisted in the translation and explanation of the responses for the investigators. Since the government and kindergartens introduced our study to the caregivers before the research began, almost all of the caregivers were willing to take their children to participate in this study. In this way, we could easily obtain the written informed consent from children's legal guardians. The study was approved by the Medical Ethics Committee of School of Public Health, Lanzhou University, China (No. GW-20200910-1) and registered on the WHO International Clinical Trials Registry Platform (protocol code ChiCTR2200056916).

We used cluster sampling to select a representative sample of kindergartens from a sample pool of 150 kindergartens. A power analysis was conducted to ensure optimum power was achieved, resulting in a minimum sample size of 17. A total of 608 healthy preschool children aged 3–6 years from 17 kindergartens were randomly selected. All questionnaires were responded to with a response rate of 100%. Children who were ill (diseases mentioned in the exclusion criteria, $n = 5$) and had too many missing measurements (sociodemographic data had more than 3 missing blanks or dietary/anthropometric/blood biomarkers data was incomplete, $n = 25$) were excluded from the study, leading to the final sample size of 578 for analysis in this study with a valid questionnaire rate of 95.07%.

Sociodemographic data

Sociodemographic data were collected by interviewing preschoolers' caregivers with questionnaires. This questionnaire was adapted from the previous questionnaire of our group (3). The Cronbach test and the Kaiser-Meyer-Olkin (KMO) test were used for reliability and validity (Cronbach's $\alpha = 0.735$ and KMO = 0.802), respectively. Study variables included preschoolers' sex, ethnicity, family's current residence, education of caregivers, left-behind children, an only child, premature baby, feeding method, poor households, whether caregivers were engaged in farming, caregivers' nutritional knowledge, and picky eating behavior. Left-behind children were defined as at least one parent who had gone out to work for more than 3 months, a common phenomenon in poor areas of China (27). Whether they were poor households was determined after government investigations, which avoided the bias caused by people in the region who generally like to conceal their income. Feeding methods included breastfeeding, artificial feeding, and mixed feeding, while caregivers who answered 9 questions correctly (60%) and above were considered to pass the nutritional knowledge test. Picky eating behavior was evaluated by the caregivers themselves.

Dietary data

Throughout the study, we collected dietary intakes using face-to-face 24-h dietary recall. Participants were asked to report all foods and beverages they had consumed during the preceding 24 h. Considering many children had meals in kindergarten, we used two questionnaires to collect dietary information. One questionnaire was completed with the help of kindergartens chefs to collect information related to children's food consumption at schools. The other questionnaire was conducted by investigators interviewing the caregivers of preschool children and aimed to collect information on what the children ate away from kindergarten. Trained investigators from Lanzhou University were responsible for collecting information on the recipes, types, and brands of all reported food items. During the interview, standard serving bowls, plates, and glasses were used to help respondents estimate the portion sizes of foods and beverages as accurately as possible. More than 50 food models for foods commonly consumed by local people were provided to help clarify the dietary intakes. Additionally, a food photo book containing photos of 135 common food and beverage items, marked with their name and its weight, was used to improve dietary recall. Nutrient analysis software (Nutrition Calculator version 2.8.0.8, Beijing, China), based on the continuously updated in-house nutrient database (China food composition), was used to calculate daily nutrient intakes and food weights from 24 h recall. Currently, the database contains information on energy and 36 nutrients for 2,876 entries.

Values of energy intake and nutrient intake were used in the analysis.

Measurement of dietary diversity and nutrient adequacy

Dietary diversity score was used to evaluate children's dietary diversity based on the consumption of nine food groups [starchy staples (comprised of cereals and white roots and tubers); dark green leafy vegetables; other vitamin A-rich fruits and vegetables (comprised of vitamin A-rich vegetables and tubers and vitamin A rich fruit); other fruits and vegetables; organ meat; meat and fish; eggs; legumes, nuts, and seeds; milk and milk products] in the 24-h dietary recall, which was according to the guidelines of the Food and Agriculture Organization of the United Nations (28). Each food group consumed was scored 1 point and DDS was the sum of the scores (ranging from 1 to 9). The scoring did not include other food groups such as beverages, sugars, and preserves. In this study, DDS < 5 was defined as low DDS, and DDS ≥ 5 was defined as high DDS (20).

Nutrient adequacy ratios for the intake of energy, calcium, potassium, sodium, magnesium, iron, zinc, phosphorus, selenium, vitamin A, vitamin B_1, vitamin B_2, vitamin C, vitamin D, vitamin E, and niacin were calculated by dividing the daily intake of the nutrient by the recommended daily intake (EAR) for that nutrient according to the Chinese Dietary Reference Intakes (DRIs) (29). Because adequate intake of one nutrient cannot compensate for the lack of other nutrients, the maximum value of NAR was set to 1. MAR was used to assess the average condition of children's nutrient adequacy and was calculated by dividing the sum of all micronutrient NARs by the quantities of nutrients evaluated.

Anthropometric status and blood biomarkers

Height was measured to the nearest 0.1 cm using a height and sitting height measuring instrument (model SZ-200, Suheng, Shanghai, China). In contrast, weight was measured to the nearest 0.1 kg using a digital scale (model HD382, TANITA, Tokyo, Japan). WHO Anthro and WHO AnthroPlus, which the WHO officially recommended, were used to calculate the anthropometric status [HAZ, WAZ, weight-for-height z-score (WHZ)] of preschool children under 5 years old and over 5 years old, respectively. Based on this, the prevalence of stunting, wasting, and being underweight were calculated to understand the undernutrition status of preschool children (30).

To measure blood routine, 200 μl of the child's peripheral blood sample was collected into EDTA vacutainers by automated hematology analyzer (model XS-500i, SYSMEX, Tokyo, Japan) and 500μL of the child's peripheral blood sample was collected into lithium heparin evacuated blood collection tube to measure blood elements by atomic absorption spectrometer (model BH7100S, Bohui, Beijing, China). Hemoglobin (HGB), calcium, potassium, sodium, magnesium, iron, and zinc were measured in this study.

The following cutoffs were applied to derive binary outcomes:

- Stunting, wasting, and underweight: HAZ < -2, WHZ < -2, and WAZ < -2, respectively.
- Anemia: HGB <118 g/L (children younger than 5 years old) and HGB < 123 g/L (children aged 5 years and older) based on the standards of Gansu Provincial Maternity and Child-care Hospital (altitude-adjusted).
- Blood trace elements deficiency: Low serum calcium (<1.5 μmol/L), low serum potassium (<30 μmol/L), low serum sodium (<64 μmol/L), low serum magnesium (<1.12 μmol/L), low serum iron (<7.5 μmol/L), low serum zinc (<55.9 μmol/L) based on the standards of Gansu Provincial Maternity and Child-care Hospital.

Statistical analysis

The database was established with EpiData version 3.1 (EpiData Association, Odense, Denmark). Descriptive statistics were taken to count the basic information of the participants. The Shapiro–Wilk test was conducted to verify the normality of the distribution of data. For normally distributed data, they were presented as mean and standard deviation (SD); For non-normally distributed data, they were presented as medians (25th and 75th percentile). To analyze the difference in DDS among different sociodemographic factors, Student's *t*-test and one-way ANOVA were used for two-group comparisons and multiple comparisons, respectively. Differences in other variables between the high DDS and low DDS groups were tested using Student's t-test for normally distributed continuous variables and Mann–Whitney U-test for not normally distributed continuous variables, respectively. Furthermore, to measure the correlation between DDS and other variables, Spearman's correlation coefficients were used. Finally, Poisson regression was used to determine the predictors of DDS since it was the count data; and multiple linear regression was used to determine the predictors of MAR since it was the continuous data. Statistical significance was determined at $p < 0.05$ (two-tailed tests). All analyses were conducted with IBM SPSS (predictive analytics software and solutions) version 22.0 (International Business Machines Corporation, New York, State of New York, USA).

TABLE 1 The dietary diversity score (DDS) and participants' basic characteristics (n = 578).

Basic characteristics	*N* (%)	DDS (Mean ± SD)	*p*
Total	578 (100)	4.67 ± 1.56	
Gender			
Male	321 (55.54)	4.63 ± 1.55	0.422
Female	257 (44.46)	4.73 ± 1.58	
Ethnicity			
Han	307 (53.11)	4.68 ± 1.39	0.857
Non-Han	271 (46.89)	4.66 ± 1.74	
Family's current residence			
Rural	317 (54.84)	4.31 ± 1.49	<0.001***
Urban	261 (45.16)	5.11 ± 1.54	
Education of caregivers			
Primary school and below	360 (62.28)	4.39 ± 1.56[a]	<0.001***
Junior high school	150 (25.95)	5.12 ± 1.43[b]	
High school	45 (7.79)	4.98 ± 1.55[b]	
Bachelor degree and above	23 (3.98)	5.52 ± 1.56[b]	
Left behind children			
Yes	359 (62.11)	4.47 ± 1.54	<0.001***
No	219 (37.89)	5.00 ± 1.55	
Only child			
Yes	119 (20.59)	4.61 ± 1.45	0.575
No	459 (79.41)	4.69 ± 1.59	
Premature baby			
Yes	26 (4.50)	5.27 ± 1.37	0.032*
No	552 (95.50)	4.64 ± 1.57	
Feeding methods			
Breast milk	248 (42.91)	4.83 ± 1.58	0.109
Artificial feeding	153 (26.47)	4.54 ± 1.57	
Mixed feeding	177 (30.62)	4.56 ± 1.53	
Poor households			
Yes	199 (34.43)	4.18 ± 1.48	<0.001***
No	379 (65.57)	4.93 ± 1.55	
Engaged in farming			
Yes	380 (65.74)	4.45 ± 1.52	<0.001***
No	198 (34.26)	5.12 ± 1.57	
Caregivers' nutritional knowledge			
Pass	60 (10.38)	5.23 ± 1.65	0.003**
Failed	518 (89.62)	4.61 ± 1.54	
Picky eating behavior			
Yes	325(56.23)	4.67 ± 1.46	0.988
No	253(43.77)	4.67 ± 1.69	
Stunting			
Yes	39(6.75)	4.23 ± 1.56	0.067
No	539(93.25)	4.71 ± 1.56	
Wasting			
Yes	24(4.15)	4.46 ± 1.32	0.492
No	554(95.85)	4.68 ± 1.57	
Underweight			
Yes	40(6.92)	4.25 ± 1.41	0.076
No	538(93.08)	4.70 ± 1.57	
Anemia			
Yes	33(5.71)	4.00 ± 1.67	0.011*
No	545(94.29)	4.71 ± 1.5s	
Low serum zinc			
Yes	73(12.63)	4.79 ± 1.51	0.478
No	505(87.37)	4.66 ± 1.57	
Low serum calcium			
Yes	129(22.32)	4.72 ± 1.67	0.693
No	449(77.68)	4.66 ± 1.53	
Low serum iron			
Yes	90(15.57)	4.20 ± 1.62	0.002**
No	488(84.43)	4.76 ± 1.54	

DDS, dietary diversity score; SD, standard deviation. [a,b]Different superscript letters suggested a significant difference between the groups. p value was calculated using Student's t-test or one-way ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Results

Dietary diversity score based on basic characteristics

A total of 578 children (about 46.89% are ethnic minorities) aged 3–6 years old were included for analysis with a response rate of 100% and a valid questionnaire rate of 95.07% and the mean DDS was 4.67 ± 1.56. In this study, 62.28% of the caregivers had education at primary school or below, and 89.62% of them did not pass the nutritional knowledge test, while nearly two-thirds of the children were left-behind children and 79.41% of them were not an only child. Preschool children who lived in rural areas, whose parents had lower education, who were left-behind children, whose households were poor, whose caregivers' nutritional knowledge was lacking, who were anemic, and who had low serum iron had significantly lower DDS ($p < 0.05$). However, children who had ever been a premature baby had a higher DDS (Table 1).

Consumption of each food group

The most frequently consumed food groups were starchy staples (99.65%), followed by dark green leafy vegetables (75.78%), other fruits and vegetables (68.17%), meat and fish (54.50%), and eggs (54.33%). In comparison, the groups of other vitamin A-rich fruits and vegetables (44.98%); milk and milk products (39.10%); legumes, nuts, and seeds (30.97%) were

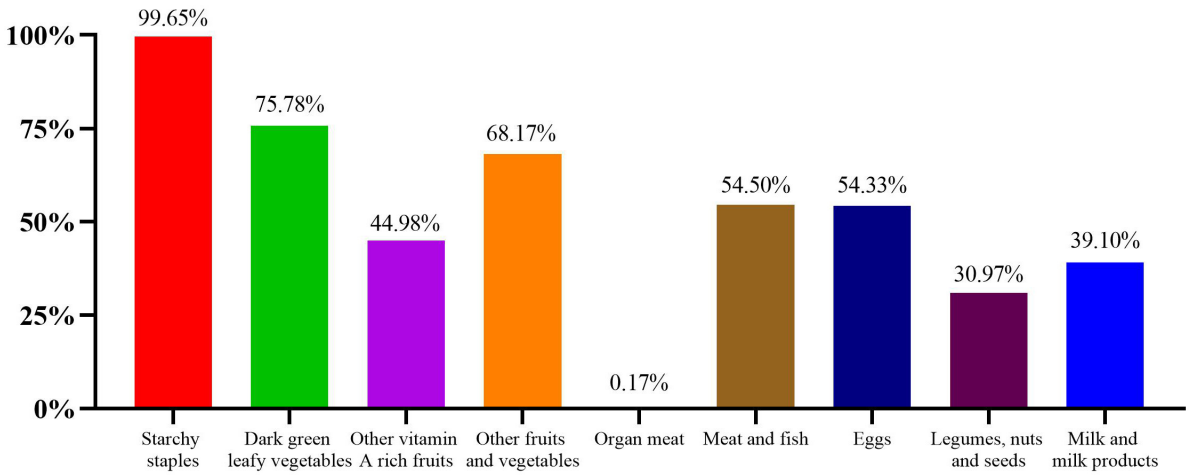

FIGURE 1
Percentage of participants consuming each food group.

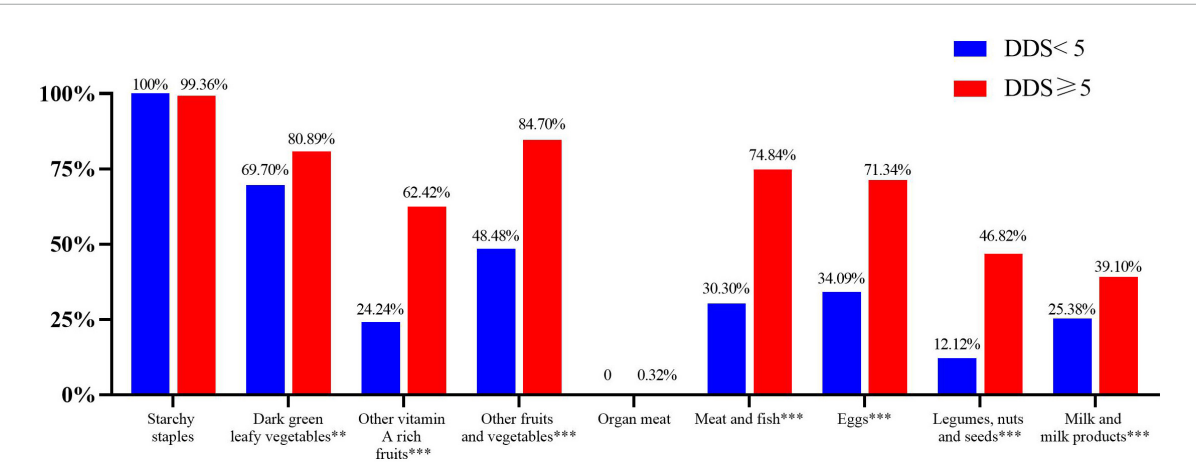

FIGURE 2
Percentage of participants consuming each food group in low and high DDS groups. $^{**}p < 0.01$, $^{***}p < 0.001$.

less likely to be consumed. The least consumed food group was organ meat, with only 0.17% of participants consumed (Figure 1).

With the exception of starchy staples and organ meat, the percentage of participants consuming each food group in the high DDS group was significantly higher than the low DDS groups ($p < 0.05$) (Figure 2).

Blood biomarkers, anthropometric measures, and nutrient adequacy ratios in low and high dietary diversity score groups

Blood biomarkers and anthropometric status in low and high DDS groups are shown in Table 2. Serum potassium, serum iron, WAZ, and HAZ were higher in high-DDS participants than those in low-DDS participants ($p < 0.05$). Spearman correlation coefficients for blood biomarkers and anthropometric status associated with DDS were determined for all participants. Serum potassium ($r = 0.201$, $p = 0.001$), serum iron ($r = 0.208$, $p = 0.001$), WAZ ($r = 0.098$, $p = 0.019$) and HAZ ($r = 0.097$, $p = 0.020$) were significantly positively correlated with DDS.

In the same way, of the 15 micronutrients NAR values assessed, all of them and MAR were higher in the high-DDS participants than in the low-DDS participants ($p < 0.01$). Moreover, energy and macronutrients NAR values were significantly higher in the high DDS group than those in the low DDS group ($p < 0.01$). In the correlation study, the NARs of all micronutrients and MAR were significantly positively correlated with DDS (r value range = 0.124–0.593, $p < 0.001$).

TABLE 2 Blood biomarkers and anthropometric status in low and high DDS groups.

	DDS < 5	DDS ≥ 5	p^a	r^b	p
HGB	141.00 (133.75, 147.00)	141.50 (136.00, 148.00)	0.097	0.076	0.067
Serum calcium	1.60 ± 0.14	1.59 ± 0.13	0.671	−0.008	0.845
Serum potassium	41.27 ± 3.07	42.08 ± 3.43	0.003**	0.201	< 0.001***
Serum sodium	75.55 ± 5.92	75.77 ± 5.42	0.628	0.042	0.314
Serum magnesium	1.48 ± 0.13	1.49 ± 0.14	0.533	0.074	0.077
Serum iron	8.09 ± 0.74	8.29 ± 0.70	< 0.001***	0.208	< 0.001***
Serum zinc	67.65 ± 10.66	68.96 ± 11.04	0.151	0.061	0.145
WHZ	−0.59 (−1.26, 0.08)	−0.48 (−1.11, 0.09)	0.257	0.044	0.295
WAZ	−0.74 ± 0.96	−0.55 ± 0.90	0.020*	0.098	0.019*
HAZ	−0.57 ± 1.02	−0.36 ± 1.05	0.015*	0.097	0.020*

Values are mean ± SD or medians (25th and 75th percentile). HGB, hemoglobin; WHZ, weight-for-height z-score; WAZ, weight-for-age z-score; HAZ, height-for-age z-score. [a]*p*-Values were calculated by Mann–Whitney U-test for non-normally distributed continuous variables and Student's t-test for normally distributed continuous variables. [b]Spearman's correlation coefficients (*r*) were calculated between participants' DDS and blood biomarkers or anthropometric status. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

TABLE 3 NARs and MAR in low and high DDS groups.

NARs	DDS < 5	DDS ≥ 5	p^a	r^b	p
Energy	0.56 ± 0.21	0.72 ± 0.21	< 0.001***	0.439	< 0.001***
Protein	0.74 ± 0.31	1.07 ± 0.36	< 0.001***	0.549	< 0.001***
Fat	0.41 (0.26, 0.60)	0.64 (0.50, 0.81)	< 0.001***	0.524	< 0.001***
Carbohydrate	0.67 ± 0.27	0.79 ± 0.27	< 0.001***	0.247	< 0.001***
Calcium	0.17 (0.11, 0.31)	0.32 (0.19, 0.54)	< 0.001***	0.511	< 0.001***
Potassium	0.54 ± 0.21	0.73 ± 0.21	< 0.001***	0.456	< 0.001***
Sodium	0.94 ± 0.20	0.98 ± 0.12	0.004**	0.124	0.003**
Magnesium	0.66 ± 0.22	0.82 ± 0.17	< 0.001***	0.429	< 0.001***
Iron	0.72 ± 0.23	0.88 ± 0.16	< 0.001***	0.421	< 0.001***
Zinc	0.57 ± 0.23	0.80 ± 0.20	< 0.001***	0.561	< 0.001***
Phosphorus	0.89 ± 0.19	0.93 ± 0.15	0.009**	0.157	< 0.001***
Selenium	0.71 ± 0.26	0.77 ± 0.25	0.005**	0.213	< 0.001***
Vitamin A	0.19 (0.05, 0.43)	0.55 (0.34, 0.88)	< 0.001***	0.587	< 0.001***
Vitamin B_1	0.43 (0.33, 0.59)	0.60 (0.45, 0.77)	< 0.001***	0.415	< 0.001***
Vitamin B_2	0.39 (0.23, 0.68)	0.74 (0.49, 1.00)	< 0.001***	0.550	< 0.001***
Vitamin C	0.29 (0.18, 0.46)	0.37 (0.23, 0.60)	< 0.001***	0.202	< 0.001***
Vitamin D	0.00 (0.00, 0.06)	0.10 (0.04, 0.16)	< 0.001***	0.494	< 0.001***
Vitamin E	0.78 ± 0.27	0.94 ± 0.14	< 0.001***	0.387	< 0.001***
Niacin	0.41 (0.28, 0.59)	0.65 (0.47, 0.82)	< 0.001***	0.428	< 0.001***
MAR	0.54 ± 0.14	0.69 ± 0.12	< 0.001***	0.593	< 0.001***

Values are mean ± SD or medians (25th and 75th percentile). NAR, nutrient adequacy ratio; MAR, mean adequacy ratio. [a]*p*-values were calculated by Mann–Whitney U-test for non-normally distributed continuous variables and Student's *t*-test for normally distributed continuous variables. [b]Spearman's correlation coefficients (*r*) were calculated between participants' DDS and NARs. **$p < 0.01$, ***$p < 0.001$.

Similar results also occurred in energy and macronutrients NAR values (Table 3). In this study, the relationship between DDS and the NARs of energy, selected minerals, and vitamins showed an increase in NARs for all these nutrients as DDS increased (Figures 3, 4). When DDS reached its maximum value of 9, NARs of iron, zinc, vitamin A and vitamin B2 reached 100%. However, NARs of the other nutrients did not meet 100% whatever the DDS was. In addition, the most common nutrients were calcium and vitamin D, which were only 40% at the highest.

Predictors of dietary diversity score and mean adequacy ratio

In the Poisson regression model (Table 4), living in urban areas [β = 0.158, 95%*CI*: (0.075, 0.241)], higher household wealth [β = 0.116, 95%*CI*: (0.030, 0.202)], and more caregivers' nutritional knowledge [β = 0.022, 95%*CI*: (0.006, 0.038)] were positively associated with DDS. Similarly, in the multivariable linear regression models (Table 5), being

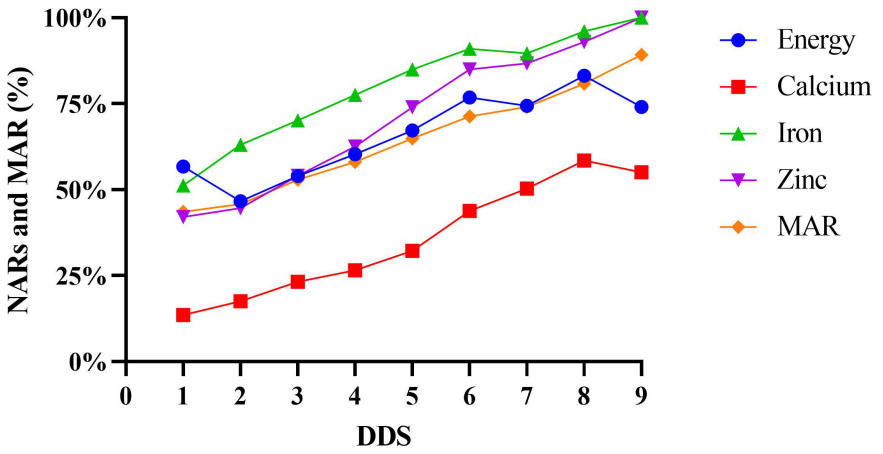

FIGURE 3
NARs and MAR at different levels of dietary diversity score.

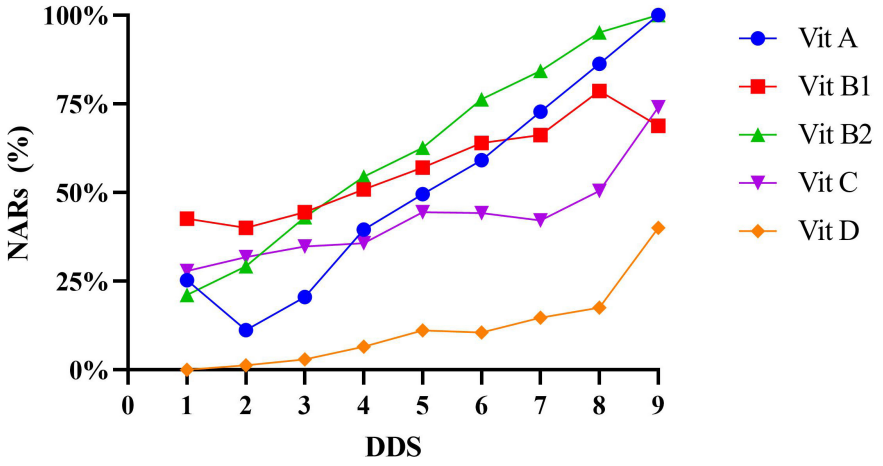

FIGURE 4
NARs of selected vitamins at different levels of dietary diversity score.

female [β = 0.025, 95%*CI*: (0.006, 0.043)], living in urban areas [β = 0.031, 95%*CI*: (0.010, 0.051)], higher education of caregivers [β = 0.027, 95%*CI*: (0.013, 0.040)] and higher DDS [β = 0.049, 95%*CI*: (0.042, 0.055)] were positively associated with MAR, while preschool children's age [β = −0.057, 95%*CI*: (−0.078, −0.035)] and being non-Han Chinese [β = 0.030, 95%*CI*: (−0.050, −0.010)] were negatively associated with MAR.

Discussion

This study assessed the status of anthropometric status, blood biomarkers of nutrients, nutrient adequacy, and dietary diversity, as well as their relationships and the possible influencing factors of DDS and NAR among preschool children in poor ethnic minority areas of northwest China. The present study demonstrated that dietary diversity was positively associated with all nutrients (both macro- and micro- nutrients) and several other health outcomes.

The results showed that the mean DDS of preschool children was 4.67 ± 1.56, which was lower than the DDS reported in other areas of Chinese studies [5.77 in Chen et al., 6.10 in Meng et al., 6.80 in Zhao et al., 7.4 in Jiang et al. (19, 25, 31, 32)]. Compared to other developing countries, their DDS was higher than the preschool children in South Africa [3.60 in Steyn et al. (33)] and Zambia [4.39 in Caswell et al. (2)] but lower than those in the Philippines [5.62 in Modjadji et al. (34)] and Sri Lanka [5.4 in Perkins et al. (24)]. DDS has been used for many years, but the scoring standard of DDS is still not uniform. Different food groupings, 1-day or 3-day dietary recall, and whether there was a threshold of food intake when calculating DDS all affected the

TABLE 4 Poisson regression model of predictors of DDS.

Variables	β	95%CI	*p*
Age	−0.066	(−0.155, 0.023)	0.148
Sex	0.022	(−.055, 0.098)	0.581
Female (vs. male)			
Ethnicity	0.058	(−0.023, 0.139)	0.160
Non-Han (vs. Han)			
Family's current residence	0.158	(0.075, 0.241)	< 0.001***
Urban (vs. rural)			
Education of caregivers[a]	−0.008	(−0.061, 0.045)	0.760
Left behind children	0.044	(−0.038, 0.126)	0.291
No (vs. yes)			
Only child	0.023	(−0.071, 0.117)	0.628
No (vs. yes)			
Premature baby	−0.088	(−0.251, 0.075)	0.292
No (vs. yes)			
Poor households	0.116	(0.030, 0.202)	0.008**
No (vs. yes)			
Engaged in farming	0.056	(−0.027, 0.139)	0.184
No (vs. yes)			
Caregivers' nutritional knowledge[b]	0.022	(0.006, 0.038)	0.006**

[a]Primary school and below, junior high school, high school, bachelor degree and above (education of caregivers) were assigned as 1, 2, 3, 4 respectively. [b]Caregivers' nutritional knowledge was analyzed using continuous variable nutritional knowledge score. $^{**}p < 0.01$, $^{***}p < 0.001$.

TABLE 5 Linear regression model of predictors of MAR.

Variables	β	95%CI	*p*
Age	−0.057	(−0.078, −0.035)	< 0.001***
Sex	0.025	(0.006, 0.043)	0.010*
Female (vs. male)			
Ethnicity	−0.030	(−0.050, −0.010)	0.003**
Non-Han (vs. Han)			
Family's current residence	0.031	(0.010, 0.051)	0.004**
Urban (vs. rural)			
Education of caregivers[a]	0.027	(0.013, 0.040)	< 0.001***
Left behind children	0.005	(−0.015, 0.025)	0.647
No (vs. yes)			
Only child	−0.020	(−0.043, 0.001)	0.056
No (vs. yes)			
Premature baby	−0.014	(−0.045, 0.017)	0.380
No (vs. yes)			
Poor households	0.012	(−0.008, 0.033)	0.245
No (vs. yes)			
Engaged in farming	0.006	(−0.013, 0.025)	0.555
No (vs. yes)			
Caregivers' nutritional knowledge[b]	0.002	(−0.002, 0.006)	0.247
DDS	0.049	(0.042, 0.055)	< 0.001***

[a]Primary school and below, junior high school, high school, bachelor degree and above (education of caregivers) were assigned as 1, 2, 3, 4, respectively. [b]Caregivers' nutritional knowledge was analyzed using continuous variable nutritional knowledge score. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

DDS value. Similarly, the prevalence of stunting, underweight, and wasting were 6.75, 6.92, and 4.15%, respectively, which were higher than in other studies conducted in Guangdong Province [1.31, 1.03, and 2.06% in Mu et al. (35)] or Shaanxi Province [1.24, 0.06, and 0.07% in Ding et al. (36)]. One possible explanation is that previous studies have concentrated on the central or eastern regions, where people have better access to higher socioeconomic status and nutritional concepts, which may contribute to the observed gap between this study and other studies. Additionally, the prevalence of low serum zinc, calcium, and iron were 12.63, 22.32, and 15.57%, respectively. Conversely, the prevalence of anemia was only 5.71%. The results of this study are generally similar to other studies, the iron deficiency rate is higher than that of Zhejiang Province (one of the most economically developed provinces in southeast China) but lower than that of Qinghai Province (in northwest China) (37, 38). Furthermore, the prevalence of anemia is quite low compared to other studies [19% in Wang et al., 11.19% in Zeng (39, 40)]. As of 2020, the "Nutrition Package" program of "1,000 days in early life" had been implemented in impoverished areas in China for many years. With the help of the "Nutrition Package," Chinese preschool children could supplement more micronutrients in their early life, as one of the reasons for the low prevalence of anemia in local children (13).

We also found that participants with key different characteristics had varying DDS. Specifically, participants who lived in rural areas had lower DDS than those living in urban areas, which was consistent with many studies (18, 19, 24, 31, 32). This is probably due to reduced access to diverse foods and can be more costly compared to individuals living in urban areas (18, 41). Studies also reported that participants with caregivers' that had a higher education level and family economic status in turn, had a higher DDS (18, 26, 42–44). Our present study also supports this finding. Left-behind children were a common phenomenon in impoverished areas in China, where at least one parent had gone out to work for more than 3 months. This may lead to children's lack of dietary diversity, as evidenced by the findings of this study and other similar studies (45). Additionally, we also found that caregivers with more nutritional knowledge could contribute to higher DDS of preschool children, as other studies pointed out (25). In our study, participants who had anemia and low serum iron had a lower DDS, which has not been investigated in many studies. However, contrary to our hypothesis, preschool children who were born prematurely had a higher DDS. One possible explanation is that caregivers of premature babies used to give them more care and better food due to the poor condition when they were born.

Many reasons have been proposed to explain why people consume certain food groups instead of others (46). Two important reasons may include costs and accessibility (25, 38,

46). In our study, most of the food preschool children ate was planted by their own families, such as potatoes, Chinese cabbage, spinach, and tomatoes. This is the reason why the consumption of starchy staples, dark green leafy vegetables, and other fruits and vegetables were high. In addition, animal foods can provide various nutrients such as iron, calcium, and vitamin D, which cannot be replaced by plant-based foods (47). However, animal product consumption in preschool children was lower than plant-based foods consumption, especially organ meat, which was minimally consumed by anyone. Ethnic differences could explain this result, as half of the participants were ethnic minorities and Muslims, which prevented them from consuming organ meats. Thus, these foods were rare in local markets (48). After grouping according to DDS, the proportion of participants consuming all food groups except starchy staples and organ meat in the high DDS group was higher than that in low DDS group, which was also similar to other studies (23, 49).

To our knowledge, the present study is the first to assess the relationship between dietary diversity and blood biomarkers or anthropometric status among preschool children in a poor ethnic minority area of China. DDS is positively associated with serum potassium, serum iron, WAZ, and HAZ. For blood biomarkers, Vyncke et al. found that Diet Quality Index (similar to DDS) was positively associated with 25-hydroxyvitamin D, holo-transcobalamin and n-3 fatty acid serum levels in European adolescents (50), while Ganpule-Rao et al. found that DDS was positively associated with vitamin B_{12}, folate, and Hb in Indian rural youth (20). For anthropometric status, only a positive association between DDS and HAZ in Sri Lankan young children was observed by Perkins et al. (24). Compared with dietary intake, blood biomarkers or anthropometric status were medium and long-term indicators that reflected the nutritional status of preschool children, so the link with DDS is not too strong. However, the results of this study suggested that DDS could also assess blood biomarkers or anthropometric status in preschool children to some extent.

Simultaneously, a positive correlation was also found between DDS and NARs in all macro- and micronutrients, which was in accordance with but much stronger than previous findings (1, 3, 19, 21, 49). Although there were very few participants with a DDS of 1 and 9 (only 4 and 1, respectively) and random errors would be introduced in studying the relationship between DDS and NARs and MAR, we can generally infer that even when the DDS was 9, part of NARs and MAR were still less than 100% (2, 18, 33, 51). However, many NARs (such as calcium and vitamin D) were quite low in both high DDS and low DDS groups, suggesting that preschool children were at risk of hidden hunger. In our study, both the number of consumers and the consumption of milk and dairy products, as well as meat and fish, were very limited, which was undoubtedly a key factor in the observed low NARs.

According to the Poisson regression model of predictors of DDS, living in urban areas, higher household wealth and more caregivers' nutritional knowledge were positive influential factors of DDS, which was consistent with many studies (25, 32). Similarly, living in urban areas, higher education of caregivers, and higher DDS were positively associated with MAR, illustrating the positive association between DDS and NAR. Thanks to the grassroots poverty alleviation work vigorously promoted by the Chinese government, we can expect that the nutritional status of preschool children will be improved accordingly. Also, in part with the help of the constantly increasing level of economic development and living quality of individuals in poor ethnic minority areas dietary intake will be improved in the near future. In addition, nutritional education for caregivers could also be given attention to improve the nutritional status of preschool children.

China is an expansive country with diverse lifestyles and nutrition intake. To the best of our knowledge, our study is the first survey focused on the association between dietary diversity and nutrient adequacy, blood biomarkers, anthropometric status as well as exploring the predictors of DDS and MAR in preschool children in poor ethnic minority area of northwest China. The findings could help evaluate the nutritional status of preschool children via an alternative approach that could simplify the workload of dietary surveys and provide preschool children with much more balanced diets, particularly making up for the lack of using only one type of indicator (e.g., blood trace element) in such poor areas. There were also several limitations to this study. First, the study design was a cross-sectional investigation and it could not infer a causal relationship. Second, it was conducted only in one county of one province, and the data might be not representative. Third, due to the local caregivers' traditional thinking and religious beliefs, only peripheral blood was collected instead of venous blood, which was a limitation that we could not analyze biomarkers such as plasma vitamins. Fourth, it is difficult to control some confounders such as biological variations.

Conclusion

In conclusion, this study indicated that the dietary diversity was relatively low with starchy staples being the most consumed food group and the intakes of many nutrients were inadequate. While the prevalence of stunting, wasting, and underweight was high among preschool children in poor ethnic minority area of Northwest China. Additionally, DDS was associated with serum potassium, serum iron, WAZ, HAZ, all NARs, and MAR. Efforts are warranted to increase the dietary diversity of preschool children in such areas to improve their nutritional status. Furthermore, living in urban areas, higher household wealth, not engaging in farming, and more caregivers' nutritional knowledge were positively associated with DDS while living in

urban areas, higher education of caregivers, and higher DDS were positively associated with MAR. Nutritional education for caregivers and policies such as poverty alleviation and nutrition improvement to promote the dietary diversity of preschool children are necessary.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of School of Public Health, Lanzhou University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

BH and YZ designed research. BH, ZW, ST, YC, YJ, QZ, XC, MS, and CZ conducted research. BH analyzed data and wrote the manuscript. BH and CK edited the manuscript. LW and YZ had primary responsibility for final content. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Association between daily eating frequency and mortality in people with diabetes: Findings from NHANES 1999–2014

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Background: Previous studies have shown that increasing the frequency of eating is beneficial in terms of cardiovascular metabolic risk factors; however, limited evidence is available for the association between daily eating frequency and mortality, especially in people with diabetes. Therefore, we aimed to explore the association between eating frequency and long-term mortality in populations with diabetes.

Methods: We selected 4,924 individuals suffering from diabetes (mean age: 57.77 years; 51.3% men) from the National Health and Nutrition Examination Survey (NHANES) between 1999 and 2014. Daily eating frequency was used as the exposure factor in this study. We extracted the mortality data from the National Death Index records and matched them with the population of NHANES. All participants were followed up from the date of getting enrolled in NHANES to 31 December 2015. Multivariate Cox proportional hazards regression, Kaplan–Meier survival curves, and restricted cubic spline were used to assess the associations between eating frequency and all-cause and cause-specific mortality among people with diabetes.

Results: During 34,950 person–years of follow-up, 1,121 deaths were documented, including 272 cardiovascular disease (CVD)-related deaths and 156 cancer-related deaths. After adjusting for confounding factors, the daily eating frequency was linearly inversely associated with all-cause and CVD-related mortality, and the HR (95% CIs) for per one-time increment of eating frequency was 0.88 (0.80–0.98) and 0.77 (0.63–0.93), respectively. Sensitivity analyses showed that the main results and statistical significance were still stable.

Conclusion: Higher eating frequency was independently related to lower all-cause and CVD-related mortality in people with diabetes, which can be used as a potential strategy for daily-diet management among populations suffering from diabetes.

KEYWORDS

diabetes, eating frequency, all-cause mortality, CVD-related mortality, National Health and Nutrition Examination Survey

1. Introduction

With the improvement of global economic conditions and the diversification of diet in recent years, more people are suffering from diabetes. The number of people with diabetes was close to 500 million in 2019. It is expected that the number will reach nearly 600 million by 2030, and by 2045, this number will increase by 51%, and also the number of diabetes-related deaths will reach millions each year, which will pose a huge burden on social, financial, and health systems around the world (1–3). Therefore, it is extremely necessary to identify the controllable factors of diabetes as early as possible to prevent the premature death of patients with diabetes.

As we all know, diet plays a very important role in the daily management of patients with diabetes, and the quality and quantity of each diet may lead to great fluctuations in blood glucose, which in turn leads to the progression of diabetes. However, because people with diabetes are more likely to feel more hungry than ordinary people with normal blood glucose, they eat more frequently than normal people to prevent hypoglycemia, while it is unknown whether the increase in eating frequency will benefit the prognosis of patients with diabetes. There was evidence that Chen et al. (4) conducted a long-term follow-up survey of 6,884 participants from the third National Health and Nutrition Examination Survey (NHANES 1988–1992) and found that after adjusting for confounding factors, participants who ate more frequently every day had a 32% lower risk of cardiovascular disease (CVD) death than those who ate less frequently, which was still consistent among female participants. In addition, another prospective cohort study involving 63,999 eligible participants showed that unrestrained eating, a proxy for diet frequency, timing, and caloric intake, was associated with an increased risk of all-cause and cancer-specific mortality, but a decreased risk of cardiovascular disease-specific mortality (5). Although Carew et al. (6) found that the frequency of eating was associated with numerous established risk and preventative factors for coronary artery disease (CAD) at baseline, there was no direct association with the risk of CAD hospitalization or mortality in the 13,328 adult cohorts in Canada. The evidence regarding the effect of eating frequency remains inconclusive. The reasons for this situation may be the different methods of eating frequency assessment and the heterogeneity of the study population, and they failed to perform a subgroup analysis among participants with diabetes, so the association between eating frequency and mortality in people with diabetes remains unknown.

In addition, because diabetics have a faster metabolism and are prone to hunger, they tend to eat more frequently than individuals without diabetes. As we all know, diabetes not only causes a great economic burden to the country and people but also greatly affects the total mortality rate. However, it is unknown whether the eating frequency of patients with diabetes is also related to the effect of diabetes on mortality, so, in this study, we aimed to use the eating frequency of individuals with diabetes as an exposure variable to evaluate its effect on mortality in a nationally representative sample of American adults from NHANES.

2. Materials and methods

2.1. Study population

For this analysis, we only included people with diabetes and collected the NHANES data set from 1999 to 2014. According to the American Diabetes Association criteria, NHANES defined diabetes through self-reported diagnosis, use of insulin or oral hypoglycemic medication, fasting glucose ≥7.0 mmol/L, or glycated hemoglobin A1c (HbA1c) ≥6.5% (7). We excluded participants with unreliable dietary recall status or unrealistic total daily calorie intake (<800 or >8,000 kcal for men, <600 or >6,000 kcal for women) (8), and we additionally excluded those who were self-reported as pregnant, having cancer at baseline, or were less than 18 years of age. We also excluded participants with ineligibility status for mortality. Finally, we had an analytic sample of 4,924 participants (**Supplementary Figure 1**). All participants provided written informed consent, and the study protocol was approved by the National Center for Health Statistics of the Center for Disease Control and Prevention Institutional Review Board and in line with the Declaration of Helsinki.

2.2. Covariates

Demographic characteristics, health behaviors, dietary habits, medical history, and clinical indicators were considered covariates. Education level was categorized as <9th grade, 9–11th grade, 12th grade, and >12th grade. The family income-to-poverty ratio was classified as 0–1.0, 1.0–3.0, or >3.0. Smoking status was classified as never smoker, former smoker, or current smoker. Alcohol users were defined as those who had at least 12 drinks in the last 12 months. Ideal physical activity was defined as ≥150 min of moderate-intensity activities per week, ≥75 min of vigorous-intensity activities per week, or an equivalent combination of both. activities per week, or an equivalent combination Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a sphygmomanometer after people had rested in a seated position for 5 min. Healthy eating index (HEI), total daily calorie intake, breakfast consumption, and the day of intake were obtained from the 24-h dietary recall interviews, and HEI scores were calculated by R language using the "hei" package. Medical history was self-reported using interviewer-administered questionnaires. Clinical indicators, including fasting glucose, insulin, HbA1c, triglyceride, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured in the NHANES laboratory. Insulin resistance (HOMA2-IR) was calculated with the HOMA calculator (University of Oxford, Oxford, UK) (9), and the estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD- EPI) study equation based on serum creatinine (10).

2.3. Determination of eating frequency

In this study, eating frequency was defined as the number of eating episodes per day, and an eating episode was defined as food or beverage items (>5 kcal) consumed within 15 min of one another

over the 24-h recall (11). The frequency of eating was calculated from the dietary data: Individual Foods files. Detailed information about the time and energy of each occasion reported by each participant is included in the Individual Foods files. From 1999 to 2002, only one dietary recall interview was conducted, but from 2003 to 2014, a second dietary recall interview was added approximately 3–10 days after the first recall. Dietary data from the first recall were included in the present analysis (12).

2.4. Ascertainment of mortality

We obtained mortality data for NHANES (1999–2014) from the public-use linked mortality files (LMFs), and the LMFs provide mortality follow-up data from the date of survey participation through 31 December 2015. Mortality outcomes of interest include all-cause, CVD-, and cancer-related mortality. Follow-up time for the present study was defined as the period between the first dietary interview date and the last known date about each participant, living or dead (13).

2.5. Statistical analysis

Dietary sampling weights were used in the statistical analysis, and new weights were created for the combined NHANES cycles, as recommended by the National Center for Health Statistics (NCHS). Total eating frequency was categorized as <3, =3, =4, and >4/day (14). Baseline characteristics were presented as mean ± SE or proportions. To calculate the differences between various groups, the weighted Chi-square test was used for categorical variables and the weighted linear regression model was used for continuous variables (15). To examine the associations of eating frequency levels with cardiometabolic biomarkers at baseline, the least squares mean and generalized linear models were used. Multivariate Cox proportional hazard models were constructed to obtain the hazard ratios (HRs) to evaluate the risk of all-cause, CVD-, and cancer-related mortality. In the multivariate models, we adjusted for age, gender, and race/ethnicity in model 1. In model 2, we further adjusted for BMI, education level, family income–poverty ratio, alcohol user, smoking status, ideal physical activity, healthy eating index (HEI) scores, daily calorie intake, breakfast skipping, and diet record days. In model 3, we further adjusted for duration of diabetes, diabetes medication use (16), self-reported hypertension, hypercholesterolemia, and CVDs, and self-reported hypertension and hypercholesterolemia medication use. In model 4, we further adjusted for HbA1c, HOMA2-IR, SBP, DBP, TC, triglyceride, HDL-C, LDL-C, and eGFR. Multiple imputations were used for missing covariates. The linear trend was tested by assigning a median value to each category as a continuous variable.

Restricted cubic spline regression with three knots (5th, 50th, and 75th) was used to check if there existed a non-linear relationship between the frequency of eating and mortality. Stratified analyses were also conducted by age (≤65 or >65 years), gender (male or female), race/ethnicity (White or non-White), alcohol use (Yes or No), ideal physical activity (Yes or No), smoking status (never, ever, current smoker), BMI (<30.0 or ≥30.0 kg/m^2), and diabetes duration (≤10 or >10 years). The *p*-values for the product terms between frequency of eating and stratification variables were used to estimate the significance of interactions. Sensitivity analyses were performed to test the robustness of our findings. First, to verify the stability of the results, we excluded participants with unrealistic total daily calorie intake (<500 or >3,500 kcal/day for women, and <800 or >4,200 kcal/day for men) based on an existing reference (17). Second, to reduce the potential reverse causation bias, we excluded those who died within the first 1 or 2 years of follow-up. All statistical analyses were performed in R software (4.1.0).

3. Results

3.1. Baseline characteristics by quartile of eating frequency

Among the 4,924 participants with diabetes (mean age: 57.77 years; 51.3% male), the daily eating frequency ranged from 1 to 8. The baseline characteristics of the different eating frequency subgroups are shown in **Table 1**. Compared with participants who ate less frequently (eating frequency <3 times), participants who ate more frequently (eating frequency > 4 times) were more non-Hispanic-White, more alcohol users, more ever smokers, more people with higher education levels and family income–poverty ratio, had more ideal physical activity, had higher daily calorie intake and healthy eating index (HEI) score, more people who ate breakfast and had diet record in weekdays, and had more hypercholesterolemia medication use (all $P < 0.05$). Importantly, participants with more eating frequency had lower all-cause and CVD-related mortality (all $P < 0.01$). **Table 2** shows the least squares means of cardiac metabolic risk factors grouped based on eating frequency. Participants who ate more frequently had lower levels of HOMA2-IR than those who ate less ($P = 0.014$), and there was no significant association between eating frequency and glucose, insulin, HbA1c, TC, HDL-C, LDL-C, triglycerides, and eGFR (all $P > 0.05$).

3.2. Association between eating frequency and mortality

During 34,950 person-years of follow-up, 1,121 deaths were documented, including 272 CVD-related deaths and 156 cancer-related deaths. **Table 3** shows the results of multivariate Cox proportional hazard regression analyses of the association between eating frequency and mortality. After adjusting for confounding factors including age, sex, race/ethnicity, BMI, education level, family income–poverty ratio, alcohol consumption, smoking status, ideal physical activity, healthy eating index (HEI) score, daily calorie intake, breakfast skipping, diet record days, duration of diabetes, diabetes medication use, self-reported hypertension, hypercholesterolemia, CVDs, self-reported hypertension, hypercholesterolemia medication use, HbA1c, HOMA2-IR, SBP, DBP, TC, triglyceride, HDL-C, LDL-C, and eGFR, regardless of whether the eating frequency was used as a continuous variable or a classified variable, it was significantly associated with all-cause and CVD-related mortality. When eating frequency was used as a continuous variable, the HR was evaluated per one time increment of eating frequency, and HRs (95% CIs) for all-cause and CVD-related mortality were, respectively, 0.88 (0.80–0.98) and 0.77 (0.63–0.93); when eating frequency was used as a classified variable, the HR was evaluated based on a subgroup (eating frequency <3) as a reference,

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TABLE 1 Baseline characteristics of participants with diabetes classified according to eating frequency in National Health and Nutrition Examination Survey (NHANES) 1999–2014.

	Total	Eating frequency < 3	Eating frequency = 3	Eating frequency = 4	Eating frequency > 4	*P*-value
Age (mean ± SE) (years)	57.77 ± 0.33	54.84 ± 1.01	57.78 ± 0.56	58.38 ± 0.41	57.09 ± 0.78	0.004
Gender						0.005
Male	2,526 (51.3)	174 (58.0)	736 (50.7)	1,129 (48.5)	487 (57.7)	
Female	2,398 (48.7)	126 (42.0)	717 (49.3)	1,199 (51.5)	356 (42.3)	
Race/Ethnicity						< 0.001
Mexican American	491 (10.0)	32 (10.8)	162 (11.1)	206 (8.8)	92 (10.9)	
Other Hispanic	316 (6.4)	20 (6.8)	102 (7.1)	157 (6.7)	36 (4.3)	
Non-Hispanic white	2,883 (58.6)	132 (44.2)	784 (54.0)	1,418 (60.9)	548 (65.0)	
Non-Hispanic black	839 (17.0)	97 (32.4)	309 (21.3)	350 (15.0)	83 (9.8)	
Other race	394 (8.0)	17 (5.8)	96 (6.6)	197 (8.5)	84 (9.9)	
BMI (mean ± SE) (kg/m^2)	33.02 ± 0.18	34.02 ± 0.55	33.08 ± 0.32	32.89 ± 0.24	32.92 ± 0.47	0.248
SBP (mean ± SE) (mmHg)	131.25 ± 0.48	133.37 ± 1.59	130.98 ± 0.72	130.91 ± 0.63	131.89 ± 1.50	0.407
DBP (mean ± SE) (mmHg)	69.91 ± 0.41	72.82 ± 0.96	70.03 ± 0.70	68.94 ± 0.52	71.35 ± 0.85	< 0.001
Alcohol user						0.030
Yes	3,245 (65.9)	192 (63.9)	933 (64.2)	1,513 (65.0)	607 (72.0)	
No	1,679 (34.1)	108 (36.1)	520 (35.8)	815 (35.0)	236 (28.0)	
Smoking status						0.032
Never smoker	2,411 (49.0)	133 (44.3)	690 (47.5)	1,207 (51.9)	380 (45.0)	
Ever smoker	1,678 (34.1)	94 (31.2)	516 (35.5)	773 (33.2)	295 (35.0)	
Current smoker	836 (17.0)	73 (24.5)	246 (16.9)	348 (14.9)	169 (20.0)	
Education levels						< 0.001
<9th grade	616 (12.5)	44 (14.6)	223 (15.3)	255 (11.0)	94 (11.1)	
9–11th grade	850 (17.3)	76 (25.4)	279 (19.2)	381 (16.4)	114 (13.5)	
12th grade	1,273 (25.9)	89 (29.8)	364 (25.1)	596 (25.6)	224 (26.5)	
>12th grade	2,185 (44.4)	91 (30.2)	587 (40.4)	1,095 (47.0)	412 (48.9)	
Family income-poverty ratio						< 0.001
≤1.0	961 (19.5)	101 (33.5)	352 (24.2)	370 (15.9)	138 (16.3)	
1.0–3.0	2,079 (42.2)	116 (38.7)	636 (43.8)	973 (41.8)	354 (41.9)	
>3.0	1,884 (38.3)	83 (27.8)	465 (32.0)	984 (42.3)	352 (41.8)	
Ideal physical activity						0.011
Yes	1,905 (38.7)	79 (26.3)	543 (37.4)	946 (40.7)	336 (39.9)	
No	3,019 (61.3)	221 (73.7)	910 (62.6)	1,381 (59.3)	507 (60.1)	
HEI score, (mean ± SE)	55.80 ± 0.31	50.47 ± 1.04	53.96 ± 0.47	57.39 ± 0.38	56.48 ± 0.73	< 0.001
Daily calorie intake (mean ± SE), calories	1,976.15 ± 17.93	1,557.59 ± 56.46	1,807.98 ± 30.57	2,015.04 ± 24.98	2,307.35 ± 47.95	< 0.001
Breakfast skipping						< 0.001
Yes	964 (19.6)	182 (60.7)	417 (28.7)	276 (11.9)	89 (10.5)	
No	3,960 (80.4)	118 (39.3)	1,036 (71.3)	2,052 (88.1)	755 (89.5)	
Diet record days						0.012
Weekdays	4,292 (87.2)	249 (82.9)	1,240 (85.4)	2,038 (87.5)	765 (90.8)	
Non-weekdays	632 (12.8)	51 (17.1)	213 (14.6)	290 (12.5)	78 (9.2)	
Duration of diabetes						0.815
≤3 years	1,568 (31.8)	102 (34.1)	458 (31.5)	732 (31.4)	277 (32.8)	
3–10 years	1,662 (33.7)	90 (29.9)	511 (35.2)	795 (34.1)	267 (31.6)	

(Continued)

TABLE 1 (Continued)

	Total	Eating frequency < 3	Eating frequency = 3	Eating frequency = 4	Eating frequency > 4	*P*-value
>10 years	1,694 (34.4)	108 (36.1)	484 (33.3)	802 (34.4)	300 (35.6)	
Hypertension						0.987
Yes	3,047 (61.9)	189 (62.9)	899 (61.9)	1,442 (62.0)	517 (61.3)	
No	1,877 (38.1)	111 (37.1)	554 (38.1)	886 (38.0)	326 (38.7)	
Hypercholesterolemia						0.118
Yes	2,772 (56.3)	152 (50.8)	800 (55.0)	1,308 (56.2)	512 (60.7)	
No	2,152 (43.7)	147 (49.2)	653 (45.0)	1,020 (43.8)	331 (39.3)	
CVD						0.884
Yes	1,171 (23.8)	69 (22.9)	358 (24.6)	553 (23.8)	192 (22.7)	
No	3,753 (76.2)	231 (77.1)	1,095 (75.4)	1,775 (76.2)	652 (77.3)	
Diabetes medication use						0.075
No insulin or pills	1,750 (35.5)	141 (47.2)	553 (38.1)	773 (33.2)	282 (33.5)	
Only diabetes pills	2,150 (43.7)	112 (37.3)	607 (41.8)	1,058 (45.4)	373 (44.3)	
Only insulin	549 (11.1)	22 (7.2)	167 (11.5)	268 (11.5)	92 (10.9)	
Pills and insulin	475 (9.6)	25 (8.3)	126 (8.6)	229 (9.8)	96 (11.4)	
Hypertension medication use						0.827
Yes	2,645 (53.7)	152 (50.6)	779 (53.6)	1,264 (54.3)	450 (53.4)	
No	2,279 (46.3)	148 (49.4)	674 (46.4)	1,064 (45.7)	393 (46.6)	
Hypercholesterolemia medication use						0.003
Yes	2,009 (40.8)	85 (28.4)	573 (39.4)	974 (41.9)	377 (44.7)	
No	2,915 (59.2)	215 (71.6)	880 (60.6)	1,354 (58.1)	467 (55.3)	
Outcomes						
All-cause mortality						< 0.001
Yes	988 (20.1)	70 (23.2)	341 (23.5)	459 (19.7)	118 (14.0)	
No	3,936 (79.9)	230 (76.8)	1,112 (76.5)	1,869 (80.3)	725 (86.0)	
CVD-related mortality						< 0.001
Yes	242 (4.9)	21 (7.0)	101 (7.0)	91 (3.9)	28 (3.3)	
No	4,682 (95.1)	279 (93.0)	1,352 (93.0)	2,236 (96.1)	815 (96.7)	
Cancer-related mortality						0.170
Yes	134 (2.7)	6 (2.1)	42 (2.9)	73 (3.1)	13 (1.6)	
No	4,790 (97.3)	293 (97.9)	1,411 (97.1)	2,255 (96.9)	830 (98.4)	

Data are numbers (percentages) unless otherwise indicated. All estimates accounted for complex survey designs.

and HRs (95% CIs) of subgroup (eating frequency > 4) for all-cause and CVD-related mortality were, respectively, 0.67 (0.45–1.01) and 0.53 (0.26–1.08); the P trend values were, respectively, 0.013 and 0.005. Additionally, as shown in **Figure 1**, the Kaplan–Meier survival curve stratified according to the eating frequency showed that the cumulative incidence of all-cause and CVD-related death decreased with the increase in eating frequency (log-rank test, $P < 0.05$). **Figure 2** shows the restricted cubic spline results of the association between eating frequency and mortality. We found that there was a linear association between eating frequency and the risk of all-cause and CVD-related mortality (all non-linear $P > 0.05$).

Sensitivity analyses showed that the main results and statistical significance were still consistent with **Table 3** where participants with unreliable dietary recall status or unrealistic total daily calorie intake (<500 or >3,500 kcal/day for women, and <800 or >4,200 kcal/day for men), or participants who died during the 1-year follow-up period were excluded (**Supplementary Tables 1, 2**). However, when we excluded individuals who died within 2 years of follow-up, the association of eating frequency with all-cause mortality and CVD-related mortality in participants with diabetes was weakened or even no longer significant (**Supplementary Table 3**).

3.3. Subgroup analyses

Supplementary Table 4 showed stratified analyses of the association between eating frequency and all-cause mortality among

TABLE 2 Least squares mean of cardiometabolic markers according to eating frequency among diabetes.

	Eating frequency < 3	Eating frequency = 3	Eating frequency = 4	Eating frequency > 4	*P* trend
Glucose (mmol/L)	9.43 ± 0.36	9.13 ± 0.22	9.01 ± 0.22	8.71 ± 0.27	0.101
Insulin (pmol/L)	162.89 ± 20.56	138.99 ± 8.52	144.24 ± 9.68	136.94 ± 26.95	0.686
HOMA2-IR	3.28 ± 0.36	2.91 ± 0.24	2.93 ± 0.17	2.37 ± 0.23	0.014
HbA1c (%)	7.38 ± 0.14	7.55 ± 0.08	7.50 ± 0.07	7.35 ± 0.09	0.125
Total cholesterol (mmol/L)	4.75 ± 0.13	4.80 ± 0.07	4.72 ± 0.06	4.71 ± 0.08	0.350
HDL-C (mmol/L)	1.29 ± 0.05	1.21 ± 0.02	1.18 ± 0.02	1.20 ± 0.03	0.149
LDL-C (mmol/L)	2.69 ± 0.12	2.71 ± 0.07	2.67 ± 0.07	2.72 ± 0.1	0.947
Triglyceride (mmol/L)	2.09 ± 0.19	2.32 ± 0.13	2.38 ± 0.11	2.51 ± 0.22	0.185
eGFR, ml/min 1.73m2	81.65 ± 1.75	82.24 ± 0.84	83.68 ± 0.82	83.52 ± 1.17	0.152

The least square (mean ± SE), and *P* trend were estimated with adjustment of age, sex, race/ethnicity, BMI, education level, family income-poverty ratio, alcohol user, smoking status, ideal physical activity, healthy eating index (HEI) score, daily calorie intake, breakfast skipping, diet record days, duration of diabetes, diabetes medication use, self-reported hypertension, hypercholesterolemia, and CVD, and self-reported hypertension, hypercholesterolemia medication use.

TABLE 3 HR (95% CIs) for all-cause and cause-specific mortality according to eating frequency among diabetes.

	Eating frequency (per 1 time increment)	Eating frequency < 3	Eating frequency = 3	Eating frequency = 4	Eating frequency > 4	*P* trend
All-cause mortality						
Model 1*	0.85 (0.77, 0.93)	1.00	0.81 (0.55, 1.20)	0.67 (0.47, 0.95)	0.60 (0.41, 0.89)	< 0.001
Model 2†	0.89 (0.80, 0.99)	1.00	0.94 (0.59, 1.49)	0.81 (0.51, 1.28)	0.71 (0.44, 1.15)	0.028
Model 3‡	0.89 (0.80, 0.99)	1.00	0.98 (0.62, 1.56)	0.84 (0.53, 1.34)	0.73 (0.45, 1.18)	0.032
Model 4§	0.88 (0.80, 0.98)	1.00	0.89 (0.61, 1.29)	0.77 (0.54, 1.11)	0.67 (0.45, 1.01)	0.013
CVD-related mortality						
Model 1*	0.72 (0.61, 0.86)	1.00	0.84 (0.45, 1.54)	0.46 (0.26, 0.83)	0.49 (0.26, 0.94)	< 0.001
Model 2†	0.80 (0.66, 0.96)	1.00	1.01 (0.53, 1.93)	0.61 (0.32, 1.16)	0.67 (0.34, 1.35)	0.015
Model 3‡	0.78 (0.64, 0.95)	1.00	1.02 (0.52, 1.98)	0.61 (0.31, 1.19)	0.62 (0.30, 1.29)	0.009
Model 4§	0.77 (0.63, 0.93)	1.00	0.89 (0.49, 1.60)	0.54 (0.30, 0.97)	0.53 (0.26, 1.08)	0.005
Cancer-related mortality						
Model 1*	0.92 (0.75, 1.13)	1.00	1.13 (0.46, 2.83)	1.21 (0.49, 3.01)	0.71 (0.27, 1.87)	0.416
Model 2†	0.90 (0.72, 1.13)	1.00	1.08 (0.42, 2.76)	1.13 (0.45, 2.89)	0.67 (0.24, 1.85)	0.360
Model 3‡	0.92 (0.74, 1.15)	1.00	1.24 (0.49, 3.11)	1.32 (0.52, 3.36)	0.77 (0.27, 2.16)	0.485
Model 4§	0.91 (0.73, 1.14)	1.00	1.17 (0.46, 2.98)	1.25 (0.48, 3.22)	0.73 (0.26, 2.05)	0.430

*Model 1: Adjusted for age, sex, and race/ethnicity; †Model 2: Further adjusted (from Model 1) for BMI, education level, family income-poverty ratio, alcohol user, smoking status, ideal physical activity, healthy eating index (HEI) score, daily calorie intake, breakfast skipping, and diet record days; ‡Model 3: Further adjusted (from Model 2) for duration of diabetes, diabetes medication use, self-reported hypertension, hypercholesterolemia, and CVD, and self-reported hypertension, hypercholesterolemia medication use; § Model 4: Further adjusted (from Model 3) for HbA1c, HOMA2_IR, systolic blood pressure, diastolic blood pressure, total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, and estimated glomerular filtration rate (eGFR).

participants with diabetes. The results showed that there was a negative association between eating frequency and all-cause mortality in the subgroups of age ≤65 years, male, non-White, alcohol drinking, having ideal physical activity, never smoking, BMI > 30kg/m^2, and diabetes duration of ≤10 years. Stratified analyses of the association between eating frequency and CVD-related mortality in participants with diabetes are shown in **Supplementary Table 5**, and we found that eating frequency was negatively associated with CVD-related mortality in participants with age >65 years, male, non-White, alcohol drinking, with and without ideal physical activity, never and current smoking, BMI ≤ 30kg/m^2, and BMI > 30kg/m^2. No significant interaction was found between eating frequency and these stratification variables for both all-cause mortality and CVD mortality.

4. Discussion

Our study filled the knowledge gap of the association between eating frequency and mortality in people with diabetes. In this study, we found eating frequency was linearly inversely associated with all-cause and CVD-related mortality in people with diabetes, and this association was stable in many subgroups. These findings added some potentially effective clues to the daily diet management of patients with diabetes.

As early as the 1960s, Fábry et al. (18) found that the increase in eating frequency could not only change the overall energy metabolism and increase the production of glycogen and fat in rats but also improve the overweight, blood lipid, and glucose tolerance of normal people. However, the eating frequency they defined only

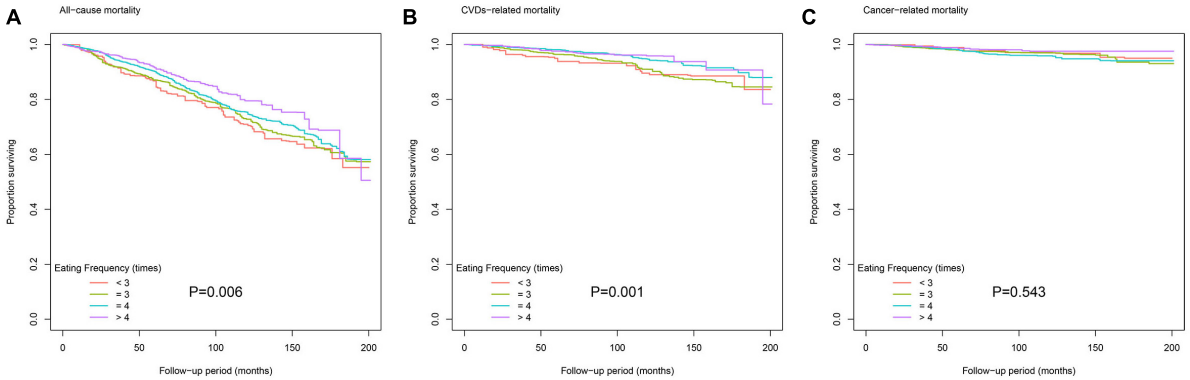

FIGURE 1
Kaplan–Meier survival curve for **(A)** all-cause mortality, **(B)** cardiovascular disease (CVD)-related mortality, and **(C)** cancer-related mortality according to eating frequency.

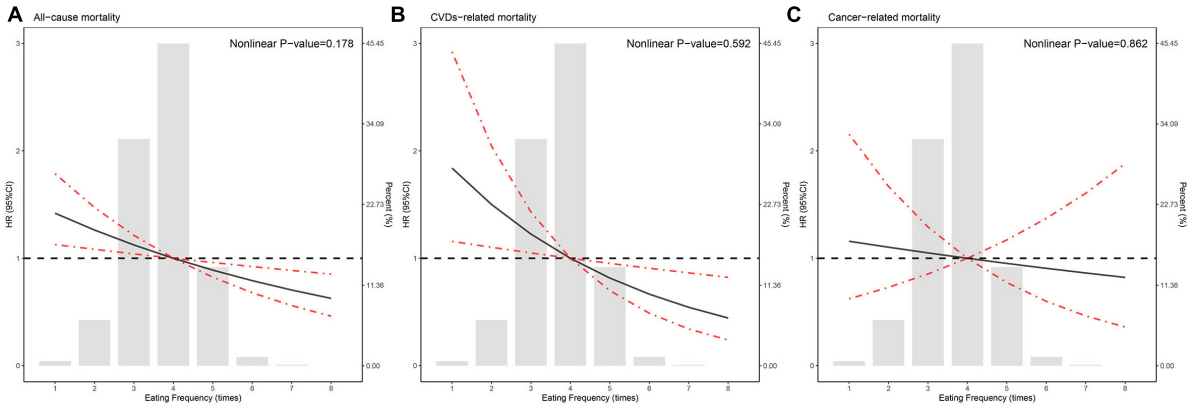

FIGURE 2
Hazard ratios for **(A)** all-cause mortality, **(B)** cardiovascular disease (CVD)-related mortality, and **(C)** cancer-related mortality according to eating frequency and the histogram of the probability distribution is presented in the background. Hazard ratios were calculated by Cox models after adjusting for age, sex, race/ethnicity, Body mass index (BMI), education level, family income-poverty ratio, alcohol consumption, smoking status, ideal physical activity, healthy eating index (HEI) score, daily calorie intake, breakfast skipping, diet record days, duration of diabetes, diabetes medication use, self-reported hypertension, hypercholesterolemia, and CVD, self-reported hypertension, hypercholesterolemia medication use, HbA1c, HOMA2_IR, systolic blood pressure, diastolic blood pressure, total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, and estimated glomerular filtration rate (eGFR).

came from the records of meals, not including the intake times of snacks and other non-meals. Subsequently, Stote et al. (19) showed in an 8-week randomized controlled trial that at the end of the experiment, when the total daily calorie intake of the two groups was the same, compared with the subjects who ate 1 meal/day, the SBP, DBP, TC, and LDL-C of the subjects who ate 3 meals/days were lower, while the urea nitrogen was higher, which indicated that the change of eating frequency could lead to the adaptive changes of some cardiovascular metabolic factors without affecting the daily energy needs of the body. In addition, several observational studies have proved that higher eating frequency can improve blood lipids, blood pressure, BMI, and waist circumference (14, 20–25). However, a recent review, after summarizing the results of nine previous clinical trials drew an opposite conclusion, that is, without changing the daily total calorie intake, increasing the frequency of eating might not help improve the traditional metabolic risk factors of CVDs or help in losing weight (26). Besides, another epidemiological study showed that the increase in eating frequency among children aged 9–10 was related to poor diet quality, bad behavior, and obesity (27). However, there was also no consistent conclusion in clinical diseases. For instance, there were pieces of evidence that higher eating frequency was associated with lower risks of metabolic syndrome and hypertension (28–30), whereas another study demonstrated that higher frequency of eating was related to the progression rate of blood pressure and new-onset hypertension among adults free from CVDs and diabetes (31). At present, the studies on the associations between eating frequency and dietary quality and cardiovascular metabolic markers are still widely concerned, while the report on the association between eating frequency and mortality is not only rare but also has no unified conclusion. For example, in a cohort study of 13,328 participants from Canada (2004–2013), Carew et al. (6) found that there was no direct association between eating frequency and the risk of CAD mortality. Nevertheless, another cohort survey involving 6,884 participants from NHANES (1988–1992) showed that more eating frequency was closely associated with lower CVD-related mortality in the fully adjusted model (4). Moreover, not only eating frequency is associated with mortality but a previous study has also shown that inappropriate eating environments may

also be associated with other diet-related health problems, such as increased intake of hyper-processed foods (32). Although our study found no differences in glucose, insulin, serum lipid, and renal function among different groups of eating frequency, we got another exciting result, that is, there was a significant negative association between eating frequency and insulin resistance among patients with diabetes.

Although this study has achieved the expected results, the mechanism was still unclear. After investigating the related literature, we found that there might be several mechanisms to mediate the association between eating frequency and mortality. For example, several similar studies have found that increasing the frequency of eating can improve the fat production and insulin resistance of mice and the cardiovascular metabolic risk factors of subjects (14, 18, 20, 21, 24, 33, 34), and the reduction of these risk factors can benefit the long-term prognosis. Additionally, Zhang et al. (35) found in a large cohort study that an uncontrolled diet is associated with an increase in all-cause mortality and cancer-related mortality (especially gastrointestinal cancer), but is associated with a decrease in CVD-related mortality, while insulin resistance is closely associated with mortality, which suggests that the insulin resistance pathway may be potentially important in the relationship between dietary behavior and major health outcomes (5). In addition, some studies have shown that the increase in the frequency of eating is related to the improvement in the quality of diet (22, 25, 36–38), and the daily diet quality is closely related to human health, which may indirectly improve the long-term death risk. Finally, metabolic syndrome, obesity, and hypertension are recognized independent risk factors of CVDs and deaths, while previous studies have shown that higher eating frequency is associated with a lower prevalence of metabolic syndrome, obesity, and hypertension (28, 29), which explained why increasing the frequency of eating can reduce all-cause and CVD-related mortality. However, most of the above possible mechanisms come from randomized controlled trials and epidemiological investigations, while related cell and animal tests are still rare, so more basic studies are needed to determine the potential mechanisms.

Despite the exciting findings of this study, there were still several limitations. For example, as an observational study, we were unable to determine the causal link between eating frequency and mortality. Additionally, there was currently no accepted standard for the definition of eating frequency, and we only referred to previous studies to define eating frequency, so the promotion of the results was limited to people with a similar eating frequency. Furthermore, in this study, due to the limited NHANES data, the assessment of eating frequency and other covariates is a one-off, and there are no repeated measurements, so we were unable to assess the impact of dynamic changes in eating frequency on mortality. In addition, although we took into account the nature of the day in which participants ate, such as weekdays or weekends, we did not assess the main places where eating frequency occurred, and eating frequency too much outside might increase mortality. Also, we failed to assess the effect of snack frequency on eating frequency in our analysis, which was also a restriction of our study. Moreover, we failed to explore the association between eating frequency and mortality in other populations, such as those without diabetes or those prone to hypoglycemia. Although according to the current data, we are temporarily unable to determine why dietary frequency can help diabetics prevent premature death, and the mechanism is unknown, our findings in this study also provided some reference and theoretical basis for the management of diabetes patients. As we all know, the management of patients with diabetes is complicated. The diet of patients with diabetes is not only related to the frequency of diet but also the quantity and type of food. When we analyze the impact of dietary factors on mortality in patients with diabetes, we should fully consider all dietary factors, including food type, composition, and frequency of eating, but our data do not have such detailed dietary data. Therefore, we can only analyze the relationship between eating frequency and mortality in patients with diabetes, which is one of the limitations of our study. Finally, we might not be able to control some non-man-made confounding factors, such as genetic susceptibility.

5. Conclusion

We found that higher eating frequency was related to lower all-cause and CVD-related mortality in people with diabetes, and the association between eating frequency and mortality was independent of overall diet quality, total calorie intake, and the timing of eating, which can be used as a potential strategy for the daily diet management among populations suffering from diabetes, as well as providing valuable insights for formulating preventive measures against premature death.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Ethics statement

The National Center for Health Statistics of the Center for Disease Control and Prevention Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JX and ZW conducted the analyses and wrote the first draft of the manuscript. XZ, JW, WF, and YH collected and assembled the data. NL and YL conceived the study design. All authors contributed to the interpretation of the results and the critical revision of the manuscript for important intellectual content and read and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.937771/full#supplementary-material

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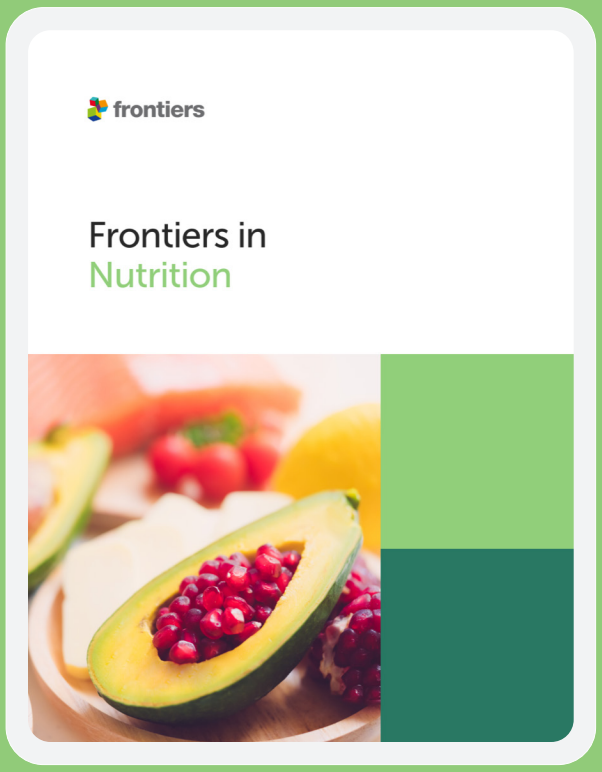

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