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Nutrition and sustainable development goal 3: good health and wellbeing

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Nutrition and sustainable development goal 3: good health and wellbeing

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Table of contents

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Editorial: Nutrition and sustainable development goal 3: good health and wellbeing

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Editorial on the Research Topic
Nutrition and sustainable development goal 3: good health and wellbeing

Nutrition is recognized to play a pivotal role in human life and its existence. Investing in nutrition yields significant returns for individuals—enhancing school performance and workforce productivity—and societies—enhancing national economic growth. This creates a ripple effect that extends beyond individual health. Nutrition is deeply interconnected with at least 12 of 17 Sustainable Development Goals (SDGs) and is crucial for achieving these goals.

In this Research Topic on Nutrition and Sustainable Development Goal 3: Good Health and Wellbeing, we provide recent evidence on the role of nutrition in promoting good health and wellbeing, as well as the future of food. SDG 3 addresses a wide range of disease conditions across all age groups, encompassing maternal and child health, non-communicable diseases, communicable diseases such as AIDS, tuberculosis, malaria, neglected tropical diseases, hepatitis, water-borne diseases, and substance use disorders. The Global Burden of Diseases study in 2017 attributed 11 million (95% uncertainty interval [UI] 10–12) deaths and 255 million (234–274) DALYs to dietary risk factors (1).

With the economic, demographic, and epidemiological changes in both low- and middle-income countries, there has been a drastic shift in dietary consumption and energy expenditure, commonly referred to as the “nutrition transition” (2). This transition has contributed to the burden of malnutrition, characterized by overnutrition, undernutrition, and micronutrient deficiencies often called “hidden high hunger.” Sub-optimal intake of healthy food is one of the major drivers of non-communicable diseases (NCDs), such as diabetes, obesity, and other metabolic disorders, as well as cardiovascular diseases. Undernutrition, mostly due to poverty, hunger, and nutrition illiteracy, results in stunted growth, poor maternal and child health outcomes, and greater susceptibility to communicable diseases. Globally, malnutrition remains a persistent challenge. Several regions across the world are witnessing the paradoxical coexistence of undernutrition and overnutrition. Kumma et al. in a study involving 2,483 Ethiopian participants aged 25–64 years, reported changing dietary patterns within the Ethiopian population, with the co-existence of Western, traditional, and healthy dietary patterns. The study highlighted the association between these changing dietary patterns and one or more cardiovascular risk factors, with Western diet consumers being at a higher risk compared to traditional diet consumers. The authors emphasized the need to promote healthy and traditional dietary patterns, along with physical activity, to address the rising incidence of cardiovascular diseases in Ethiopia. Similarly, Chen et al. identified distinct dietary patterns, namely vegetable-rich, animal-food, and prudent dietary patterns, using data from the Nutrition and Health in Southwest China (NHSC) 2013–2018 survey. The prudent dietary pattern, characterized by ethnic foods, whole grain products, fruits, eggs, and dairy and wheat products, was associated with lower systolic and diastolic blood pressure among the Southwest Chinese population. Adeba et al. in their study in Western Ethiopia, reported high prevalence of unhealthy dietary practices (73.3%), which were found to be associated with low income, being unmarried, daily meal frequency, and poor knowledge about a healthy diet.

Sustainable Development Goals envisage holistic development and well-being from the perspective of a life-course approach. Good Nutrition lays the foundation of health, that begins at the preconception stage to proper growth and development in childhood and adolescence, and good health and wellbeing in the adulthood. Adequate nutrition during the first 1,000 days of life (3) is critical for physical growth and cognitive development. Childhood undernutrition causes nearly 45% of deaths among children under the age of 5 (3) (WHO) and results in long-term irreversible effects, including impaired physical growth, recurrent infections, and cognitive underdevelopment (4). In a randomized intervention study by Gsoellpointner et al. in Vienna, nutrient supplementation through the early introduction of solid foods improved zinc, calcium, and phosphorus intake in very low birth weight (VLBW) infants during the first year of life. The authors further recommended prolonged iron and vitamin D supplementation for least 12 months to meet the recommended levels. Larson et al., in a meta-analysis of trials on egg consumption and growth in children, found significant improvements in the height and weight of children who consumed eggs compared to the control group, suggesting that eggs are an affordable nutritional option in low- and middle-income countries. Beyond dietary supplementation, significant attention must be given to addressing the social determinants of health to combat malnutrition. A study by Sanin et al. explored the factors influencing childhood undernutrition in vulnerable regions of Bangladesh using data from the Bangladesh Demographic and Health Survey (2007–2018). A decadal reduction in stunted growth and underweight among children under five was observed. The study found that urban residence, the child's age and gender , morbidity, maternal body mass index, maternal and paternal education, decision-making ability, use of contraceptives, the occurrence of domestic violence, antenatal care, mode of delivery, birth interval, and geographic region were all associated with childhood malnutrition.

Adolescence is a transitional phase of human life, marking the shift from childhood to adulthood, and is characterized by physical, mental, and psychosocial changes. Survivors of childhood malnutrition often experience chronic energy deficiency (CED) and more than one type of malnutrition. Yulia et al. in a study conducted in Indonesia, found that half of the adolescents in urban (54%) and rural (61.7%) areas were at risk for CED and consumed inadequate macronutrients. The double burden of malnutrition (DBM), characterized by both overnutrition and undernutrition, is predominant in low- and middle-income countries due to the complex interaction of poor nutrition, biological factors, and environmental and social influences across the life course. A school-based study by Getacher et al. among individuals aged 10–19 years in Ethiopia found a DBM prevalence of 21.5% (14.8% thinness and 6.7% overweight/obesity), which was associated with age, gender, type of school, dietary diversity, meal frequency, home gardening practice, illness history, and knowledge of nutrition. Multi-sectorial interventions addressing the two contradictory nutrition paradigms are crucial for alleviating the growing burden of malnutrition. Healthy eating among adolescents can be promoted by creating supportive environments for healthy eating and physical activity in educational settings, starting from the early years. Preschool and school educators need to be trained with the necessary skills and expertise to support the holistic development of children, including fostering healthy behaviors related to diet and physical activity. Educational institutions are platforms for molding healthy future generations. In a study by Lafave et al., the CHEERS eHealth program improved nutrition and physical activity practices within early childhood education and care (ECEC) centers. They found that educators' personal nutrition-related knowledge, attitude, and behaviors were positively associated with their self-assessments of the nutrition environment and practices in ECEC centers. The CHEERS survey's Food Served subscale further showed a positive correlation with the objective measures of the EPAO-Foods Provided and Nutrition Policy subdomains.

Maintaining an optimal body mass index is critical at all stages of life. Globally, 49% of adults are overweight or obese. Several studies continue to explore the diet-related factors that contribute to obesity, as well as effective nutritional therapies to prevent it. Cattaneo et al., in an RCT, observed that a 4-week restricted Mediterranean diet was effective in improving anthropometric and blood parameters among participants with severe obesity. The authors recommend targeting taste as a new approach to prevent the risk of therapeutic failure. Another randomized controlled trial by Hooshiar et al., among women with obesity and overweight in Iran, showed that the alternate day modified fasting (ADMF) diet was effective in weight reduction [−5.23 (1.73) vs. −3.15 (0.88); $p < 0.001$] and body mass index [−2.05 (0.66) vs. −1.17 (0.34); $p < 0.001$] compared to the daily calorie restriction (CR) diet. There were significant improvements in sleep and daytime dysfunction. Future studies need to explore the long-term effects of the ADMF diet on overweight/obesity. Conversely, low BMI was found to be inversely related to mortality (5). A study by Ishikawa et al., found improvements in the physical and mental health scores of health-related quality of life within 12 weeks of medium-chain triglycerides (MCT) supplementation and moderate-intensity walking exercise among sedentary older adults aged 60–74 with low BMI values (<24 kg/m^2).

Nutritional epidemiological studies have established irrefutable evidence on the relationship between diet and chronic diseases, and several observational studies, trials, and meta-analyses have examined the complex relationship between diet and diseases. A prospective cohort study by Kityo et al. in Korea among 13,568 adults included in the Health Examinees-Gem (HEXA-G) reported high intake of processed red meat to be a risk factor for all-cause mortality [men: hazard ratio (HR) 1.21, 95% CI 1.07–1.37; women:

HR 1.32, 95% CI 1.12–1.56]. An increased risk of all-cause mortality (HR 1.21, 95% CI 1.05–1.39) and cancer mortality (HR 1.24, 95% CI 1.03–1.50) was observed in women with high intake of organ meat. Moderate intake of pork belly was associated with a reduced risk of all-cause mortality in men (HR 0.76, 95% CI 0.62–0.93) and women (HR 0.83, 95% 0.69–0.98), but high intake was associated with an increased risk of CVD mortality in women (HR 1.84, 95% CI 1.20–2.82). Xing et al., in a mendelian randomization analysis on a European cohort, showed genetically determined tea intake to have a causal impact on total body bone mineral density (TB-BMD), with an odds ratio (OR) of 1.204 (95% CI: 1.062–1.366, $p = 0.004$), especially in the age group of 45–60 years (OR = 1.360, 95% CI: 1.088–1.700, $p = 0.007$). Tea consumption was found to increase bone density and reduce the risk of osteoporosis in the age group of 45–60 years within the European population. The findings of a large-scale prospective cohort study in the US by Qi et al., involving 101,190 participants with a median follow-up of 12.2 years, suggested that dietary glycaemic index was associated with a higher risk for renal cancer (HR Q3 vs. Q1: 1.38; 95% CI: 1.09–1.74). Further studies are recommended in other populations to establish dietary GI as a modifiable risk factor for renal cancer prevention. Torabynasab et al. in a systematic review and meta-analysis of cross-sectional studies, found a protective effect of dietary caffeine against the development of depression, with no evidence linking tea consumption. The authors recommended further longitudinal studies to establish the causal relationship between coffee, tea, and caffeine and the risk of depression. Al-Maweri et al., in an updated meta-analysis, found a significant association between low serum levels of vitamin D and the risk of recurrent aphthous stomatitis (mean difference = – 8.73, 95% CI: – 12.02 to – 5.44) and recommended screening and supplementation of Vitamin D for the prevention and treatment of aphthous stomatitis. Wang X. et al. observed a relationship between vitamin K and metabolic dysfunction-associated fatty liver disease (MAFLD) among individuals from the United States using the National Health and Nutrition Examination Survey 2017–2018. The -MAFLD population had lower vitamin K intake than the non-MAFLD population, suggesting a protective effect. Further prospective studies or intervention trials are warranted to establish the causal relationship.

Recent advances in molecular epidemiology have paved the way for a greater understanding of the molecular determinants of nutritional imbalances and disorders. The Oxidative Balance Score (OBS), which assesses the impact of diet and lifestyle on oxidative stress, has been linked to lower risks of metabolic syndrome. Liu and Chen found the OBS to be inversely related to the risk of NAFLD among 6,341 adult participants using the US National Health and Nutrition Examination Survey 1999–2018. On a similar note, Park et al. found an inverse relationship between the OBS and metabolic syndrome among 2,735 adults over 19 years and 5,807 adults aged 40–69 years using data from the Korean National Health and Nutritional Examination Survey (KNHANES) and the Korean Genome and Epidemiology Study (KoGES), respectively. These findings suggest the benefits of a healthy lifestyle for the prevention of metabolic syndrome. Wang P. et al. observed a 24% decrease in the risk of non-cancer mortality (aHR = 0.76, 0.60–0.92) among 5,009 cancer patients who had higher dietary total antioxidant capacity (DAC) but no significant effect on all-cause or cancer mortality. Higher dinner DAC, rather than breakfast or lunch DAC, was associated with a 21% lower risk of all-cause mortality (aHR = 0.79, 95% CI: 0.65–0.98) and a 28% lower risk of non-cancer mortality (aHR = 0.72, 95% CI: 0.57–0.90). The authors emphasized the importance of advocating for DAC consumption at dinner to reduce mortality risk in cancer survivors.

Nutraceuticals—nutrient-based supplements and plant-derived products—are gaining recognition for their health benefits. Derbo et al. in a community-based study in Ethiopia, found that 49.6% of participants consumed *Moringa stenopetala* leaves (locally known for reducing the risk of malnutrition, low birth weight, and anemia) during pregnancy. Factors associated with the consumption included younger age (below 24 years), rural residence, antenatal care attendance, a history of contraceptive use, and having good knowledge about the importance of *Moringa stenopetala*. Studies have shown that omega-3 fatty acids (Gui et al.) improve nutritional status and reduce chronic inflammation in cancer patients, while flaxseed supplementation (Musazadeh et al.) had no significant effect on sex hormone levels. Li et al., in a meta-analysis of RCTs, found that oral intake of fruits or fruit extracts led to significant improvements in skin hydration and a decrease in transepidermal water loss (TEWL). Wang N. et al. in animal studies, reported that consuming fermented wax gourd, a traditional food of Eastern China, enhanced the presence of beneficial probiotics and reduced pathogenic *Helicobacter sp*. in the mouse gut.

Synbiotics, which combine probiotics and prebiotics, are another promising area of research. However, a study by Talebi et al. among women with polycystic ovary syndrome (PCOS) yielded mixed results, highlighting the need for more rigorous trials.

Nutritional literacy is recognized to have a positive influence on behavior change and the adoption of healthy eating practices. However, many populations, especially those living with HIV or in low-income regions, still face significant barriers to proper nutrition. Gemede et al. observed that poor nutritional knowledge and practices among HIV-positive adults in Ethiopia were 74.9 and 69.1%, respectively. Nutritional knowledge was associated with factors such as educational level, monthly income, occupation, and marital status. A study by Zhang et al. showed that knowledge, attitude, and practices regarding oil and salt intake were relatively poor, with factors such as region, ethnicity, urban and rural residence, education, taste preference, and the prevalence of chronic diseases influencing the oil- and salt-related KAP scores. Yang et al., in a study among elderly populations in rural China, found that higher dietary indices were associated with better quality of life scores, highlighting the important role of a healthy diet in improving overall wellbeing.

The COVID-19 pandemic has had both short- and long-term effects on health, particularly affecting eating habits and lifestyle behaviors. Rafraf et al., in their study, found that university students experienced weight gain, reduced physical activity, and worsened sleep quality during the pandemic. In Indonesia (Fatmah), food insecurity worsened, affecting many families' ability to access nutritious foods. These findings highlight the need to address nutritional needs during and after a pandemic.

At the population level, nutrition policies play a crucial role in promoting health. Kirk et al. examined the impact of the Affordable Care Act (ACA) on nutrient consumption in the United States, using data from the National Health and Nutrition Examination Survey (NHANES). The study found that the intake of micronutrients from nutrient-dense foods, such as fruits and vegetables, did not change significantly after the ACA was implemented. However, there was an increase in the use of nutritional supplements after the ACA ($p = 0.05$), particularly for magnesium (OR = 1.02), potassium (OR = 0.76), vitamin D (both D2 and D3, OR = 1.34), vitamin K (OR = 1.15), and zinc (OR = 0.83). This trend was observed in both the general population and specific subgroups, including cancer survivors and Medicaid recipients. Given the connection between increased supplement use and expanded insurance coverage, the authors call for further research to better understand how broader access to nutritional supplements might influence the intake of both micronutrients and macronutrients, helping to meet daily recommended nutritional requirements.

Achieving SDG 3 requires a comprehensive approach to nutrition that extends beyond health to include sustainable food systems and equitable food distribution. Advances in food production and technology are essential for improving food quality and ensuring access to healthy food. Unsustainable agricultural practices, food waste, and inequitable food distribution exacerbate environmental degradation and hinder equitable access to nutritious foods. To address this disparity, it is critical to focus on a new model of sustainable food production to improve food systems. Wang and Zhang in a review, shed light on the "big food view" to enhance food production, improve quality and diversity through scientific and technological innovation, and enable access to healthy food for better living.

The path to achieving SDG 3 is closely linked to the global commitment to improving nutrition. Without addressing the root causes of malnutrition and fostering sustainable food systems, the goal of ensuring good health and wellbeing for all will remain unmet. As we progress toward 2030, nutrition must not be merely an agenda item but the core of efforts to achieve health equity and environmental sustainability. A well-nourished population is the foundation of a prosperous and thriving world.

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EM: Writing – original draft, Writing – review & editing. AC: Writing – review & editing. EI: Writing – review & editing. MA: Writing – review & editing.

Conflict of interest

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Common factors influencing childhood undernutrition and their comparison between Sylhet, the most vulnerable region, and other parts of Bangladesh: Evidence from BDHS 2007–18 rounds

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Introduction: Undernourishment is disproportionately spread within Bangladesh, making some regions like Sylhet more vulnerable than the rest of the country. We aimed to assess the trend of diverse associated factors related to childhood stunting, wasting, and being underweight. Furthermore, we have compared the estimated factors between Sylhet, the most vulnerable region, and other parts of Bangladesh.

Methods: We performed a secondary data analysis where data were derived from the nationally representative cross-sectional surveys: Bangladesh demographic and health survey (BDHS) 2007, 2011, 2014, and 2017–18 rounds. The outcome variables were childhood undernutrition, including stunting, wasting, and being underweight. Descriptive statistics such as mean, standard deviation, frequency, and proportion were used to summarize the data. All variables were summarized by BDHS survey time points. We used multiple logistic regression models to measure the associated factors with childhood stunting, wasting, and being underweight.

Results: The percentage of children under the age of 5 years who were stunted declined from 40% in 2007 to 31% in 2018. Similar trends are observed in the decrease in the percentage of underweight children, dropping from 39% in 2007 to 22% in 2018. Wasting dropped to 8% in 2018 after years of critically high levels (17%). According to the results of the regression analyses, urban residence, child's age and gender, morbidity, maternal BMI, maternal and paternal education, decision-making ability, use of contraceptives, the occurrence of domestic violence, antenatal care, c-section, and birth interval, as well as geographic region, were all linked to childhood malnutrition.

Conclusion: The Sylhet division falls short in several critical associated indicators, including parental education, maternal BMI, obtaining at least four

ANC, women empowerment, and usage of contraceptives. Policymakers must concentrate on region-specific planning and proper intervention to achieve a more uniform improvement across the country.

KEYWORDS

undernutrition, stunting, wasting, underweight, Sylhet region, Bangladesh, trend

Background

Undernutrition includes wasting (low weight-for-height), stunting (low height-for-age), and underweight (low weight-for-age). Low weight-for-height is known as wasting, indicating acute and severe weight loss. Low height-for-age or stunting is a consequence of chronic undernutrition. Underweight or low-weight-for-age children are a combination of the above two outcomes and can be stunted, wasted, or both (1, 2). Undernourished children face greater vulnerability to disease and subsequent death as nearly 45% of deaths among children under 5 years of age could be directly related to this (3). A significant portion of these children come from low- and middle-income countries. However, the prevalence of undernourished children greatly varies within the same country. This is well evident that undernutrition is an adverse outcome modulated by multidimensional components (4). These encompass direct factors such as poor dietary habits and illness (5); lack of food security and inadequate water, sanitation and hygiene commodities (6); insufficient access to health services (7) and overarching social factors like poverty with economic and demographic disadvantages (8). All these elements vary between and within countries, which requires context-specific research to develop interventions supported by evidence (9).

Bangladesh has achieved impressive social and economic development growth over the past three decades. Yet, a hefty proportion of its population (43%) lives under the poverty line of 1.25 dollars per day (10). Such a vast population unable to afford nutritious food or access improved healthcare facilities, makes the goal of reducing the country's prevalence of undernourished children extremely challenging. Furthermore, undernourishment is disproportionately spread within the country, making some regions even more vulnerable than others (11, 12). Although the poverty rate is lower in the eastern part of Bangladesh, surprisingly, the undernutrition rate is much more prevalent in this region compared to the north-western part (12, 13). The Sylhet region is considered ecologically susceptible due to being a remote area, wetland ecosystems, and social dogmatism (7, 13). Such unique regional factors may create non-income obstacles, hindering the nutritional status of children (12). However, the change in these factors in Bangladesh over time has yet to be explored. Through our analysis, we aimed to assess the trend of childhood stunting, wasting, and underweight and explore the factors influencing these metrics. The second objective was to compare the estimated factors between Sylhet, the most vulnerable region, and other regions of Bangladesh.

Materials and methods

Data sources

For this paper, we used secondary data derived from the nationally representative cross-sectional surveys of the BDHS 2007 (14), 2011 (15), 2014 (16), and 2018 (17), undertaken by the authority of the Ministry of Health and Family Welfare's National Institute for Population Research and Training (NIPORT). The BDHS follows a similar study design that has been described in the published reports. Briefly, the BDHS sample was stratified and selected in two stages. Administratively, Bangladesh was divided into several divisions, and each division was further stratified into urban and rural areas. The whole list of enumeration areas (EAs) spanning the entire nation, created by the Bangladesh Bureau of Statistics for the People's Republic of Bangladesh population census, served as the sample frame for the BDHS.

To draw the sample, required EAs as a cluster were selected with a probability proportional to the EA in the first stage. Household listing was done based on the inclusion and exclusion criteria and prepared the sampling frame. In the second stage of selection, a fixed number of required households per cluster were selected using a systematic sampling procedure from the newly created sampling frame. We used Children's Record (KR) data for this analysis. Data for a total of 12,860 youngest children were used from four consecutive BDHS (2007–2011); of those 4,926 were from 2007, 7,325 samples from 2011, 6,855 samples from 2014, and the rest of the 7,562 were extracted from the 2017-18 BDHS.

Variable under study

The outcome variables of this paper focus on childhood undernutrition including stunting, wasting, and being underweight. These variables were derived from child's age and sex-specific composite indicators such as length/height-for-age z score (LAZ/HAZ), weight-for-length/height z score (WLZ/WHZ) and weight-for-age z score where the z score was defined as "(observe anthropometry value – average value of reference population)/standard deviation of reference population." Children were defined as stunted if LAZ/HAZ < -2, wasted if WLZ/WHZ < -2 and underweight if WAZ < -2 [2]. In the database, cases were treated as missing if LAZ/HAZ > 6 or LAZ/HAZ < -6, WLZ/WHZ > 5 or WLZ/WHZ < -5, and WAZ > 5 or WAZ < -6.

Based on the literature review as well as the bi-variate relationship with childhood nutritional status, several independent variables were selected such as geographical area, place of residence, wealth index, improved toilet, source of drinking water, religion, maternal BMI < 18.5, maternal education, empowerment, attitude toward domestic violence, receiving at least four ANC from a medically trained provider, delivery type, use of a contraceptive method, paternal education, birth interval, current age – respondent, partners age, child's age in months, child's sex, and having fever in last 2 weeks. The mother's empowerment was defined as the ability to make decisions about her own health care, major household purchases, and visits to family or relatives.

Statistical analyses

We performed analyses using Stata version 13.0 (StataCorp, College Station, TX, USA). Firstly, to visualize the outcome indicators, statistical plot like bar diagram was used. Several descriptive statistics such as mean, standard deviation, frequency, and proportion were used to summarize the data. All variables were summarized by BDHS survey time points. Due to binary outcomes, simple logistic regression was used to assess the bi-variate association between outcome variables and all independent variables. We used multiple logistic regression models to assess the associated factors with childhood stunting, wasting, and being underweight. The independent variables were included in the model based on the literature review as well as the bi-variate association. "svyset" option was used to allow for adjustments for the cluster sampling design, weights and the calculation of standard errors. Again, logistic regression was used to assess the status of wealth index, birth interval, cesarean delivery, ANC visit, attitude toward domestic violence, contraceptive methods, empowerment, paternal and maternal education, maternal underweight, and child's morbidity in the Barisal, Chittagong, Dhaka, Khulna, and Rajshahi regions compared with the Sylhet region. We selected the Sylhet region as the reference as several health indicators are performing poorly here. The odds ratios with 95% CIs were calculated as inferential statistics and $P < 0.05$ were considered as a significance level.

Results

Over 60% of the data was collected from rural areas, and the rest of the data was collected from urban areas over time. The background characteristics of the surveyed children from 0 to 59 months old in 2007, 2011, 2014, and 2018 BDHS are shown in **Table 1**. Our findings show the nutritional status of children has improved steadily over the past decade (**Figure 1**). The percentage of children under age of 5 years who were stunted declined from 40% in 2007 to 31% in 2018. The decline in the percentage of children who are underweight followed a similar pattern, falling from 39% in 2007 to 22% in 2018. After years at critically high levels (17%), wasting decreased to 8% in 2018.

Table 2 presents the factors associated with childhood stunting, wasting and being underweight. Childhood stunting was associated with urban residence [aOR: 1.12 (95% CI: 1.00, 1.25); *p*-value = 0.047], having a fever in the last 2 weeks [aOR: 1.17 (95% CI: 1.08, 1.26); *p*-value < 0.001], maternal BMI < 18.5 [aOR: 1.32 (95% CI: 1.22, 1.44); *p*-value < 0.001], maternal education below secondary [aOR: 1.16 (95% CI: 1.05, 1.27); *p*-value = 0.002], paternal education below secondary [aOR: 1.34 (95% CI: 1.23, 1.46); *p*-value < 0.001], not having decision-making power [aOR: 1.11 (95% CI: 1.02, 1.22); *p*-value = 0.022], using contraceptive [aOR: 1.21 (95% CI: 1.12, 1.31); *p*-value < 0.001], the occurrence of domestic violence [aOR: 1.09 (95% CI: 1.01, 1.18); *p*-value = 0.028], not receiving at least four ANC [aOR: 1.13 (95% CI: 1.02, 1.26); *p*-value = 0.017], non-cesarean delivery [aOR: 1.31 (95% CI: 1.18, 1.47); *p*-value < 0.001], and birth interval < 24 months [aOR: 1.22 (95% CI: 1.03, 1.44); *p*-value = 0.018].

Childhood wasting was associated with the male sex [aOR: 1.16 (95% CI: 1.05, 1.29); *p*-value = 0.005], having a fever in the last 2 weeks [aOR: 1.30 (95% CI: 1.17, 1.45); *p*-value < 0.001], maternal BMI < 18.5 [aOR: 1.59 (95% CI: 1.42, 1.77); *p*-value < 0.001], and maternal education below secondary [aOR: 1.30 (95% CI: 1.16, 1.45); *p*-value < 0.001].

Childhood underweight was associated with having fever in the last 2 weeks [aOR: 1.38 (95% CI: 1.28, 1.48); *p*-value < 0.001], maternal BMI < 18.5 [aOR: 1.79 (95% CI: 1.64, 1.94); *p*-value < 0.001], maternal education below secondary [aOR: 1.29 (95% CI: 1.18, 1.41); *p*-value < 0.001], paternal education below secondary [aOR: 1.22 (95% CI: 1.09, 1.35); *p*-value < 0.001], using contraceptive [aOR: 1.14 (95% CI: 1.04, 1.24); *p*-value = 0.005], not receiving at least four ANC [aOR: 1.22 (95% CI: 1.09, 1.37); *p*-value < 0.001], non-cesarean delivery [aOR: 1.22 (95% CI: 1.08, 1.39); *p*-value = 0.002], and birth interval < 24 months [aOR: 1.26 (95% CI: 1.05, 1.51); *p*-value = 0.012]. Geographical region and child's age were associated with both stunting and underweight. On the other hand, low socio-economic status had high prevalence of childhood stunting and being underweight.

TABLE 1 General characteristics of households by survey time points.

Indicators, *n* (%)	2007	2011	2014	2018
Geographical area				
Barisal	658 (13.4)	856 (11.7)	814 (11.9)	790 (10.4)
Chittagong	980 (19.9)	1393 (19)	1284 (18.7)	1224 (16.2)
Dhaka[1]	1051 (21.3)	1229 (16.8)	1222 (17.8)	2022 (26.7)
Khulna	623 (12.6)	877 (12)	778 (11.3)	828 (10.9)
Rajshahi[2]	829 (16.8)	1911 (26.1)	1732 (25.3)	1698 (22.5)
Sylhet	785 (15.9)	1059 (14.5)	1025 (15)	1000 (13.2)
Place of residence				
Urban	1748 (35.5)	2328 (31.8)	2215 (32.3)	2701 (35.7)
Rural	3178 (64.5)	4997 (68.2)	4640 (67.7)	4861 (64.3)
Wealth index				
Poorest	937 (19)	1526 (20.8)	1435 (20.9)	1610 (21.3)
Poorer	988 (20.1)	1400 (19.1)	1295 (18.9)	1467 (19.4)
Middle	910 (18.5)	1408 (19.2)	1332 (19.4)	1369 (18.1)
Richer	943 (19.1)	1473 (20.1)	1412 (20.6)	1534 (20.3)
Richest	1148 (23.3)	1518 (20.7)	1381 (20.1)	1582 (20.9)
Improved toilet (yes)	1906 (40.3)	3506 (49.5)	4286 (63.9)	4186 (58.7)
Source of drinking water (improved)	4280 (87.2)	6456 (88.1)	6078 (88.9)	6486 (86.4)
Religion				
Islam	4473 (90.8)	6600 (90.1)	6279 (91.6)	6892 (91.1)
Hinduism	419 (8.5)	699 (9.5)	523 (7.6)	624 (8.3)
Buddhism	17 (0.3)	14 (0.2)	39 (0.6)	32 (0.4)
Christianity	12 (0.2)	12 (0.2)	13 (0.2)	14 (0.2)
Other	4 (0.1)	0 (0)	1 (0)	0 (0)
Maternal BMI < 18.5	1514 (31.1)	1906 (26.6)	1513 (22.2)	1070 (14.4)
Maternal education				
No education	1268 (25.7)	1332 (18.2)	1040 (15.2)	531 (7)
Primary	1507 (30.6)	2193 (29.9)	1871 (27.3)	2132 (28.2)
Secondary	1742 (35.4)	3174 (43.3)	3189 (46.5)	3598 (47.6)
Higher	406 (8.2)	626 (8.5)	755 (11)	1301 (17.2)
Woman’s own health care	2703 (54.9)	4464 (62)	4183 (61.9)	5542 (74.7)
Making major household purchases	2557 (51.9)	4066 (56.5)	3786 (56.1)	5133 (69.2)
Visits to her family or relatives	2672 (54.2)	4286 (59.6)	3914 (58)	5327 (71.8)
None of the three decisions	1410 (28.6)	1794 (24.9)	1708 (25.3)	1024 (13.8)
Less occurrence domestic violence	3329 (67.6)	4941 (67.5)	4876 (71.1)	6184 (81.8)
At least 4 ANC from medically trained provider	997 (20.2)	1621 (22.1)	1182 (17.2)	2246 (29.7)
Delivery type				
Cesarean section	478 (9.7)	1168 (16)	1088 (24.2)	1671 (33.4)
Non-cesarean	4446 (90.3)	6146 (84)	3405 (75.8)	3336 (66.6)
Use contraceptive method	2853 (57.9)	4882 (66.6)	4702 (68.6)	5172 (68.4)

(Continued)

TABLE 1 (Continued)

Indicators, *n* (%)	2007	2011	2014	2018
Paternal education				
No education	1585 (32.2)	1959 (26.7)	1692 (24.7)	1096 (14.8)
Primary	1380 (28)	2126 (29)	2044 (29.8)	2469 (33.3)
Secondary	1319 (26.8)	2194 (30)	2098 (30.6)	2413 (32.5)
Higher	636 (12.9)	1040 (14.2)	1019 (14.9)	1422 (19.2)
Birth interval				
No previous birth	1543 (31.4)	2476 (33.9)	2575 (37.6)	2758 (36.6)
<24 months	478(9.7)	552 (7.6)	462 (6.8)	509 (6.8)
≥24 months	2895 (58.9)	4276 (58.5)	3803 (55.6)	4272 (56.7)
Current age – respondent[†]	25.9 ± 6.34	25.7 ± 6.06	25.6 ± 5.96	25.9 ± 5.8
Partners age[†]	35.2 ± 8.68	34.6 ± 8.88	34 ± 7.88	33.8 ± 7.26
Child's age in months[†]	26.5 ± 16.44	27.7 ± 17.21	27.5 ± 16.72	27.3 ± 17.21
Child's sex				
Male	2515 (51.1)	3790 (51.7)	3576 (52.2)	3973 (52.5)
Female	2411 (48.9)	3535 (48.3)	3279 (47.8)	3589 (47.5)
Child had fever in last 2 weeks	1847 (38.8)	2743 (38.6)	2518 (37.8)	2519 (34.1)

[1]Mymensingh was merged with Dhaka, [2]Rangpur was merged with Rajshahi, and [†]mean ± SD.

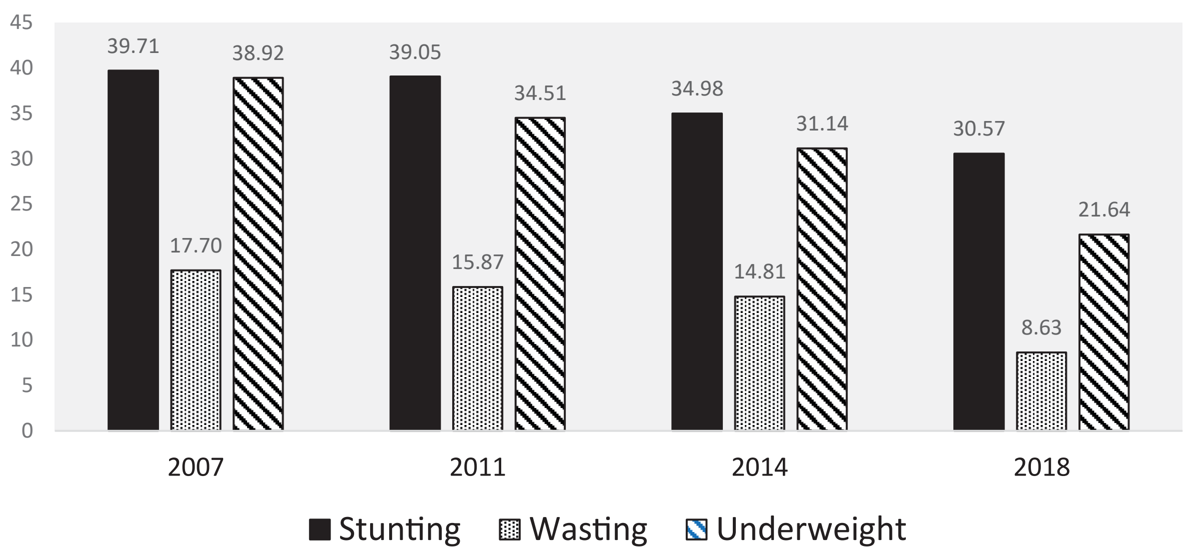

FIGURE 1
Proportion of childhood stunting, wasting, and underweight over the period.

We have compared the common predictors of undernutrition in Table 3 across the geographical regions. We found that status of cesarean delivery, receiving at least 4 ANC from a medically trained provider, use of contraceptive methods, empowerment, paternal education, maternal education, maternal BMI > 18.5, and not reporting fever in the last 2 weeks were better in those regions compared with the Sylhet region.

Discussion

With remarkable accomplishments in several health indicators, Bangladesh is yet to achieve the goal of reducing undernutrition among children under the age of 5 years, particularly in the Sylhet region. Through this analysis, we aimed to explore the factors influencing childhood undernutrition and compare the factors

TABLE 2 Factors associated with childhood stunting, wasting and underweight.

	Stunting		Wasting		Underweight	
	aOR (95 CI)	*P*-value	aOR (95 CI)	*P*-value	aOR (95 CI)	*P*-value
Geographical region						
Sylhet	Reference		Reference		Reference	
Barisal	0.85 (0.73, 0.98)	0.025	1.00 (0.81, 1.23)	0.981	0.86 (0.73, 1.02)	0.080
Chittagong	0.89 (0.78, 1.02)	0.090	1.11 (0.93, 1.31)	0.249	0.96 (0.84, 1.10)	0.568
Dhaka	0.84 (0.74, 0.96)	0.013	0.95 (0.79, 1.14)	0.569	0.80 (0.69, 0.93)	0.004
Khulna	0.68 (0.59, 0.78)	0.000	1.02 (0.85, 1.22)	0.824	0.72 (0.61, 0.84)	0.000
Rajshahi	0.72 (0.63, 0.82)	0.000	0.99 (0.83, 1.18)	0.907	0.82 (0.70, 0.95)	0.007
Place of residence						
Rural	Reference		Reference		Reference	
Urban	1.12 (1.00, 1.25)	0.047	0.99 (0.87, 1.11)	0.814	1.02 (0.92, 1.13)	0.676
Child's sex						
Female	Reference		Reference		Reference	
Male	1.07 (0.99, 1.15)	0.097	1.16 (1.05, 1.29)	0.005	0.96 (0.89, 1.04)	0.310
Child's age in months	1.02 (1.02, 1.03)	0.000	1.00 (1.00, 1.00)	0.514	1.02 (1.02, 1.03)	0.000
Having fever						
No	Reference		Reference		Reference	
Yes	1.17 (1.08, 1.26)	0.000	1.30 (1.17, 1.45)	0.000	1.38 (1.28, 1.48)	0.000
Maternal BMI < 18.5						
BMI ≥ 18.5	Reference		Reference		Reference	
BMI < 18.5	1.32 (1.22, 1.44)	0.000	1.59 (1.42, 1.77)	0.000	1.79 (1.64, 1.94)	0.000
Maternal education						
At least secondary	Reference		Reference		Reference	
Below secondary	1.16 (1.05, 1.27)	0.002	1.30 (1.16, 1.45)	0.000	1.29 (1.18, 1.41)	0.000
Religion						
Muslim	Reference		Reference		Reference	
Others	0.92 (0.80, 1.05)	0.206	0.95 (0.82, 1.11)	0.539	1.03 (0.89, 1.18)	0.717
Paternal education						
At least secondary	Reference		Reference		Reference	
Below secondary	1.34 (1.23, 1.46)	0.000	0.94 (0.84, 1.07)	0.355	1.22 (1.09, 1.35)	0.000
Decision making power						
At least one						
None of three*	1.11 (1.02, 1.22)	0.022	0.96 (0.85, 1.08)	0.473	1.05 (0.96, 1.15)	0.310
Contraceptive						
No	Reference		Reference		Reference	
Yes	1.21 (1.12, 1.31)	0.000	0.95 (0.86, 1.05)	0.292	1.14 (1.04, 1.24)	0.005
Domestic violence						
No	Reference		Reference		Reference	
Yes	1.09 (1.01, 1.18)	0.028	1.02 (0.92, 1.13)	0.759	1.01 (0.93, 1.09)	0.841
At least 4 ANC from medically trained provider						
Yes	Reference		Reference		Reference	
No	1.13 (1.02, 1.26)	0.017	1.10 (0.97, 1.26)	0.149	1.22 (1.09, 1.37)	0.000

(Continued)

TABLE 2 (Continued)

	Stunting		Wasting		Underweight	
	aOR (95 CI)	*P*-value	aOR (95 CI)	*P*-value	aOR (95 CI)	*P*-value
Mode of delivery						
Cesarean	Reference		Reference		Reference	
Non-cesarean	1.31 (1.18, 1.47)	0.000	1.08 (0.91, 1.28)	0.391	1.22 (1.08, 1.39)	0.002
Birth interval						
No previous birth	Reference		Reference		Reference	
<24 months	1.22 (1.03, 1.44)	0.018	1.02 (0.85, 1.23)	0.801	1.26 (1.05, 1.51)	0.012
≥24 months	0.95 (0.88, 1.03)	0.238	0.98 (0.88, 1.10)	0.773	0.97 (0.88, 1.07)	0.555
Wealth index						
Richest	Reference		Reference		Reference	
Poorest	2.01 (1.79, 2.45)	0.000	1.19 (0.97, 1.46)	0.088	2.00 (1.69, 2.37)	0.000
Poorer	1.85 (1.60, 2.14)	0.000	1.05 (0.87, 1.27)	0.631	1.64 (1.38, 1.96)	0.000
Middle	1.61 (1.40, 1.85)	0.000	1.03 (0.86, 1.24)	0.720	1.43 (1.21, 1.68)	0.000
Richer	1.40 (1.22, 1.60)	0.000	1.07 (0.89, 1.28)	0.457	1.25 (1.08, 1.44)	0.003
Round						
2007	Reference		Reference		Reference	
2011	0.94 (0.85, 1.05)	0.287	0.92 (0.81, 1.04)	0.197	0.82 (0.74, 0.90)	0.000
2014	0.89 (0.79, 1.01)	0.075	0.95 (0.82, 1.10)	0.460	0.85 (0.75, 0.97)	0.013
2018	0.95 (0.84, 1.08)	0.441	0.50 (0.42, 0.59)	0.000	0.56 (0.49, 0.63)	0.000

*Woman's own health care, making major household purchases, and visits to her family or relatives. Adjusted odds ratios (aOR) were estimated using multiple logistic regression analysis. The outcome variables were stunting, wasting, underweight, and independent variables were the indicators given in the first column.

TABLE 3 Comparison of the predictors of malnutrition among the geographical regions.

Indicators	†Adjusted OR (95% CI) for geographic areas compared to Sylhet				
	Barisal	Chittagong	Dhaka	Khulna	Rajshahi
Birth interval[1]					
<24 months	0.37 (0.29, 0.46)*	0.52 (0.42, 0.63)*	0.40 (0.33, 0.48)*	0.24 (0.19, 0.30)*	0.31 (0.25, 0.37)*
≥24 months	0.72 (0.63, 0.83)*	0.83 (0.73, 0.95)*	0.73 (0.64, 0.83)*	0.62 (0.54, 0.70)*	0.72 (0.63, 0.82)*
Cesarean delivery	1.08 (0.85, 1.37)	1.19 (0.95, 1.47)	1.84 (1.51, 2.25)*	2.47 (2.03, 3.02)*	1.43 (1.18, 1.74)*
At least 4 ANC from medically trained provider	1.17 (0.95, 1.45)	1.16 (0.92, 1.47)	1.30 (1.06, 1.61)*	1.88 (1.53, 2.30)*	1.50 (1.23, 1.84)*
Less occurrence of domestic violence	0.89 (0.74, 1.07)	1.09 (0.93, 1.28)	1.36 (1.16, 1.59)*	1.08 (0.90, 1.30)	0.98 (0.84, 1.15)
Use contraceptive method	2.20 (1.88, 2.58)*	1.25 (1.08, 1.45)*	1.96 (1.68, 2.28)*	2.80 (2.40, 3.26)*	2.96 (2.56, 3.42)*
None of the three decisions[2]	1.45 (1.24, 1.70)*	1.42 (1.24, 1.63)*	1.82 (1.58, 2.11)*	1.73 (1.48, 2.03)*	1.92 (1.68, 2.21)*
Paternal education	1.88 (1.57, 2.25)*	2.10 (1.76, 2.51)*	1.62 (1.37, 1.92)*	2.30 (1.94, 2.71)*	1.58 (1.35, 1.85)*
Maternal education	2.02 (1.63, 2.51)*	2.47 (2.00, 3.06)*	1.62 (1.33, 1.98)*	3.29 (2.72, 3.99)*	2.13 (1.77, 2.57)*
Maternal BMI ≥ 18.5	1.37 (1.19, 1.59)*	1.83 (1.59, 2.12)*	1.50 (1.30, 1.73)*	1.74 (1.50, 2.02)*	1.41 (1.23, 1.61)*
Did not have fever in last 2 weeks	1.01 (0.90, 1.15)	1.08 (0.96, 1.21)	1.28 (1.14, 1.44)*	1.38 (1.21, 1.58)*	1.14 (1.01, 1.27)*

[1]Base outcome was *No previous birth* in the multinomial logistic regression, [2]Woman's own health care, making major household purchases, visits to her family or relatives, and *p-value < 0.05. †Logistic regression was used to estimate the odds ratio comparing the Sylhet region with other regions where outcome variables were the indicators given in the first column after adjusting the place of residence, region, and survey time.

between Sylhet, the most vulnerable region, and other regions of Bangladesh.

We found that overall, child reporting fever in recent days, maternal BMI, type of birth, mother's education, father's education, household wealth, and geographical region were the common significant associated factors for childhood undernutrition. Having a fever in the 2 weeks leading up to the survey appears to be a significant determinant in childhood undernutrition. This has been established in previous studies in resource-poor settings (18, 19). Infections cause decreased food intake, nutritional losses due to poor digestion, and metabolic disturbances, all leading to undernutrition (19).

Our results show that children of women with no or only primary education are more likely to be affected by any kind of undernutrition. However, we also found a trend in the reduction of low educational qualifications among the surveyed mothers with increased percentages of mothers having secondary or higher secondary education over the decades. Other Bangladeshi studies have reported comparable results, showing that children of mothers with less educational qualification were significantly at greater risk of being undernourished (20, 21). Education enables a mother to receive and process information more effectively (22), empowering a mother to make informed decisions regarding health and nutrition. Also, it might increase their utilization of child health services (23).

Household wealth (the two measures of SES) appeared to be a strong predictor of child malnutrition outcomes. In Bangladesh and other underdeveloped nations, socio-economic indicators are protective factors for child health (12). Children residing in higher-income households are more likely to belong to comparatively food-secure families, have parents with relatively higher education and live in a better area with better access to health facilities (11, 24, 25). All these factors in combination possibly modulate the risk of undernutrition among the children residing in higher wealth index households.

One of our key objectives was to explore if there is any geographical variation in the established common associated factors of undernutrition. We found that the Sylhet division lags in several key risk indicators, namely parental education, maternal BMI, receiving at least four ANC from the medically trained provider, women empowerment, and use of contraceptive methods compared to other regions. UN reports (26), as well as several local studies (11, 13, 27–30), have confirmed the poor performance of the Sylhet division despite having the lowest poverty rate in the country. This has been well documented that along with the highest rates of chronic childhood undernutrition that is stunting, Sylhet also has the lowest female literacy rates, the worst school attendance rates for adolescent girls, the highest gender inequality scores, the worst performance against women's empowerment indicators, and overall the lowest proportion of empowered women in the nation (31), concurring with our findings. Such a result is very startling as Sylhet is considered a rich region as a vast number of its population lives abroad and sends remittances. However, this is a unique regional characteristic that potentially poses a barrier in several distinctive ways. Geographically, over a fourth of all arable land in Sylhet Division remains uncultivated, and only a single crop is produced on half of the remaining land, making the greater Sylhet region less productive. Furthermore, Haor (wetland) and tea estates are two significantly diverse geographical locations in the Sylhet region where a large portion of the marginalized population resides. It has been reported that about 54% of farmers in greater Sylhet are either marginal landholders or not at all. Affluent non-residents own a major portion of cultivable lands, keeping Sylhet's farming potential vastly underutilized (32, 33). Related to this, there is a notable temptation among the locals to migrate to a foreign country for a better livelihood. Hence, many poor families lack the willingness to send their children to schools, increasing the high incidence of child labor, paid or unpaid, until they grab any opportunity to go abroad (34, 35).

Limitations

Our analysis incorporates limitations, similar to other cross-sectional surveys. Due to the study design and cross-sectional data collection in the primary phase, our findings do not allow us to conclude any causal association between the factors and child undernutrition outcomes. Furthermore, the primary data did not include important indicators like child birthweight. We were unable to account for other regional factors such as community-level poverty, physical and financial barriers to health facilities, that may have influenced the associations.

Conclusion

We conclude that several common indicators play a critical role in regulating different aspects of the nutritional status of the under-five children in Bangladesh. Furthermore, some of the indicators are showing a trend of improvement, but significant regional variation still exists. To achieve a more homogenous improvement across the country, the policymakers must focus on region-specific planning and appropriate intervention.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://dhsprogram.com/Data/.

Ethics statement

The studies involving human participants were reviewed and approved by the Bangladesh Demographic and Health Survey received ethical approval from the Inner City Fund (ICF) Macro Institutional Review Board, Maryland, USA and the National Research Ethics Committee of Bangladesh Medical Research Council (BMRC), Dhaka, Bangladesh. Each participant gave informed consent before participating in the survey. The de-identified data for this study were obtained from the DHS online. Institutional ethical approval was not necessary as the study was conducted on anonymous public-use data, which had no identifiable information on the survey respondents. The patients/participants provided their written informed consent to participate in this study.

Author contributions

KS, MH, and MK: conceptualization. KS and MH: data curation. MH: formal analysis. KS and MK: software and writing – original draft. KS, MK, MH, RR, and TA: writing – review and editing. 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.

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Association between dietary caffeine, coffee, and tea consumption and depressive symptoms in adults: A systematic review and dose-response meta-analysis of observational studies

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Background: Recent studies have reported an association between dietary caffeine intake (coffee and tea) and the presence of depressive symptoms. However, the findings are not conclusive.

Purpose: This study aimed to examine the correlation between the consumption of dietary caffeine (coffee and tea) and the presence of depressive symptoms in adults.

Methods: PubMed and Scopus databases were searched until December 2021. Two investigators analyzed data from identified studies and rated the quality of the evidence using the GRADE approach. Using the random-effects models, we estimated the relative risks (RRs) and 95% confidence intervals (CIs). We also modeled the dose-response associations through a one-stage, weighted mixed-effects meta-analysis.

Results: A total of 29 eligible studies included a total of 422,586 participants. On comparing the highest with the lowest category in cohort studies, we identified an inverse association between the intake of coffee and depressive symptoms (RR: 0.89, 95%CI: 0.82–0.95; $I^2 = 63.7\%$, GRADE = low). There was a 4% reduction in the risk of depression associated with an increase in coffee intake of 240 ml/day (RR: 0.96, 95%CI: 0.95, 0.98; $I^2 = 22.7\%$). By comparing the highest category with the lowest category in cohort studies, we discovered that caffeine intake was inversely associated with depressive symptoms (RR: 0.86, 95%CI: 0.79, 0.93; $I^2 = 0.0\%$, GRADE = moderate). Based on our data analysis, no correlation exists between tea consumption and depressive symptoms.

Conclusion: According to our findings, coffee and dietary caffeine may have a protective effect against the development of depression. However, no evidence suggesting a link between tea consumption and reduced depressive symptoms has been found. Therefore, further longitudinal studies are needed to substantiate the causal relationship between coffee, tea, and caffeine and the risk of depression.

KEYWORDS
caffeine, coffee, tea, depressive symptoms risk, observational studies, dose response meta-analysis

1. Introduction

As a leading cause of disability, depression has received considerable attention as a common mental disorder. The prevalence of depression has progressively increased in recent years, making it a public health concern throughout the world. According to a study by Our Word in Data, approximately 3.4% (with a margin of error of 2–6%) of the global population suffers from depression (1).

There are some strong associations between lifestyle factors and depression. Several studies suggest that an unhealthy lifestyle, including poor dietary patterns, alcohol consumption, and a sedentary lifestyle, substantially leads to the risk of depression (2–7). Other studies also demonstrated that obesity and depression have a bidirectional relationship, suggesting that depression can contribute to the development of obesity and that obesity can exacerbate the symptoms of depression (8). It was also indicated that inflammation and oxidation have a fundamental role in depression pathophysiology (9), and subsequently, attention was paid to anti-inflammatory compounds and antioxidant-rich foods as depression relievers. Studies conducted to examine the relationship between anti-inflammatory compounds and antioxidant-rich foods supported the mentioned hypothesis by exploring the effect of Mediterranean or plant-based dietary patterns on the risk of developing depression, as it resulted in a significant reduction (10–12). It is worth considering that a recent systematic review had also revealed evidence in support of the aforementioned studies showing that depression is negatively correlated with high adherence to diet recommendations, including avoiding processed foods, eating an anti-inflammatory diet, consuming magnesium and folic acid various fatty acids, and consuming fish (13).

Coffee and tea are among the most popular beverages in the world, and they are common sources of caffeine (14). It is suggested that caffeine is related to depression as it modulates dopaminergic transmission and facilitates serotonin release (15, 16). In addition to caffeine, there are some anti-inflammatory and antioxidant compounds in coffee, such as chlorogenic acid and catechins, which demonstrate potential beneficial effects against depression. In addition to caffeine, tea contains neuroprotective compounds such as L-theanine and polyphenols (17–19). A recent study also illustrated that during the COVID-19 pandemic, tea consumption increased (70 vs. 30%), effectively improving mood, focus, and performance and, as a result, relieving stress; this improvement is believed to be due to the presence of the compounds theanine and caffeine in tea (20).

Therefore, we performed a systematic review of observational studies to examine the correlation and the dose-response relationship between depression risk and the intake of coffee, tea, and caffeine.

2. Methods

2.1. Protocol and registration

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-2020) guideline when we submitted this meta-analysis (21), and the protocol of the systematic review was registered at PROSPERO (registration number: CRD42022298824).

2.2. Literature search and selection

This investigation was carried out in accordance with the PRISMA reporting guidelines for systematic reviews and meta-analyses. The databases of PubMed and Scopus were systematically searched for relevant studies published in the English language up to December 2021. All the terms relevant to coffee, tea, caffeine, depression, and study design were applied to detect potentially eligible studies (Supplementary Table 1). We also checked the reference lists of the included studies and manually reviewed other studies to ensure that we did not miss any relevant studies.

2.3. Eligibility criteria

The inclusion and exclusion criteria were defined by adhering to the PICOS (population, intervention/exposure, comparator, outcome, and study design) framework. The criteria for inclusion were as follows: (1) cross-sectional, case-control, or prospectively designed studies conducted in the general population aged 18 years or older; (2) evaluating the association between coffee, tea, or caffeine intake as exposure and depression as the outcome; (3) assessing and reporting hazard ratios (HRs) or odds ratios (ORs) and the corresponding 95% confidence interval (CI) for $\geq$2 quantitative categories of dietary coffee, tea, and caffeine intake. For dose-response meta-analyses, it was also necessary to consider the number of cases and non-cases in each category of dietary exposures and ranges of intake. Studies reporting continuous estimation from the associations were also eligible. Review studies, interventional studies, and studies focusing on children, adolescents (under 18 years of age), and pregnant and lactating women were excluded.

2.4. Data extraction

Two independent investigators (KT and HSH) extracted the following data from the identified studies: the first author's last name, year of publication, country, and region where the study was conducted, the percentage of women who participated in the study, the sample size (total number of participants and number of cases), the study design, follow-up years, the age range of the study population at baseline, coffee, tea, and caffeine intake categories, the ORs with their 95% CI for each category of coffee, tea, and caffeine intake, exposure and outcome assessment tools, and covariates adjusted for in the multivariable analysis. We extracted the ORs that reflect the greatest degree of adjustment for potential confounders.

2.5. Quality assessment

Following the Robins-I tool framework, we evaluated the quality of the included (22). All studies were evaluated for confounding, participant selection, exposure assessment, misclassification during follow up, missing data, measurement of the outcome, and selective reporting of the results. The mentioned domains were classified as having a low, moderate, or serious risk of bias due to their characteristics. Supplementary Table 2 provides information about the domains of the Robins-I tool and how the judgment was made for each domain.

2.6. Statistical analysis

In the present meta-analysis, a random-effects model was performed to estimate the RR and its 95% CI as the effect size (23). For studies reporting the effect size as the odds ratio or hazard ratio, we considered them to be equal to HR (24). In the first step, a meta-analysis was conducted to compare the risk ratios of caffeine, coffee, and tea consumption in primary studies based on high vs. low intake categories. Whenever there are sex-specific effect sizes, we used a fixed-effect model to combine the estimates and then applied the combined effect size to the analysis. Cochran's Q (25) and I^2 statistics (26) were used to test for heterogeneity.

To identify potential sources of heterogeneity, subgroup analyses were conducted based on geography, follow-up duration, number of participants, and adjustments for main confounders such as energy intake, body mass index (BMI), smoking, drinking alcohol, and exercising. With at least ten primary studies available, we used Egger's test to determine the presence of publication bias (27) in addition to visually inspecting funnel plots (25). Additionally, we conducted a sensitivity analysis to assess the impact of individual studies on the overall estimate by excluding them one by one.

Based on the method presented by Greenland and colleagues, we also conducted a random-effect dose-response meta-analysis for studies whose data were sufficient for dose-response meta-analyses of depression for a specific amount of increase in coffee, caffeine, and tea consumption (28, 29). It was required to calculate the median point in each category, as well as the distribution of cases and person-years based on dietary caffeine, coffee, and tea. Our final approach was to perform a one-stage linear mixed-effects meta-analysis to clarify dose-response relationships (30). Based on Harrell's recommended percentiles of 10, 50, and 90%, we used restricted cubic splines with three knots to model the exposures (31). We combined the study-specific estimates by using a one-stage linear mixed effects meta-analysis to take into account correlations within each category of relative risks (30). As a result of this method, the study-specific slope lines were estimated in a single step and combined to obtain an overall average slope (28, 32). Due to the limited number of studies ($n \leq 2$) included in the analysis, we used the best-fitting second-order fractional polynomial to model curvilinear associations (30). Statistical analyses were conducted using STATA version 16.0. A *P*-value of less than 0.05 was considered significant.

2.7. Grading the evidence

The quality of the evidence was assessed by two independent authors (HSH and KT) using the GRADE approach (33). This tool classifies evidence as strong, moderate, low, or very low quality. The evidence used in this study was classified as low quality, as it was based on observational studies such as prospective cohort studies and cross-sectional studies. Depending on pre-specified criteria, they can be downgraded or upgraded. Evidence that has been downgraded includes limitations of the study, inconsistency, indirectness, imprecision, and publication bias, whereas evidence that has been upgraded includes a significant degree of association, a dose-response gradient, and attenuation caused by plausible confounding. Disagreements were resolved with a consensus.

3. Results

A total of 1,348 studies were identified at baseline from databases (PubMed 295 and Scopus 1,053), and five articles were found through manual search. We removed 228 duplicate studies. Of the 1,125 remaining studies, 1,063 were excluded on the basis of title and abstract screening. Sixty-two articles were screened by reading their full text. Of those, 33 were excluded as 11 were review articles and 22 articles reported irrelevant outcomes. Ultimately, our systematic review ultimately included 29 studies that met the inclusion criteria established for this study. The process through which studies were selected and identified is summarized in Supplementary Figure 1.

3.1. Study characteristics

The study included a total of 422,586 participants, ranging in age from 18 to 97 years. Eligible studies were published between 2005 and 2021. Table 1 shows detailed information extracted from articles. Among them, five studies considered coffee consumption as the main variable, seven studies considered tea (39, 41, 42, 44, 49, 58, 61) or coffee (40, 45, 51, 53, 62) as the main variable, seven considered caffeine (35, 38, 54, 55, 63), and 10 studies considered coffee, tea, or caffeine altogether (34, 36, 37, 43, 46, 48–50, 52, 56, 57). In addition, twenty studies had a cross-sectional design (34–36, 38, 42, 43, 45, 48–52, 54–57, 61–64), seven studies were prospective cohorts (37, 39, 40, 44, 46, 53, 58), and two studies evaluated both cross-sectional and prospective data (44, 47). Depression was identified by validated depression questionnaires such as the Beck Depression Inventory (BDI) (34), the Geriatric Depression Scale (GDS) (36, 41, 50), the Center for Epidemiological Studies Depression Scale (CES-D) (39, 42–44, 56, 61, 64), the Patient Health Questionnaire (PHQ-9) (49, 51, 54, 55), the Hospital Anxiety and Depression Scale (HADS) (38, 62), the Depression Anxiety Stress Scale (DASS-21) (48), and the Minimum Data Set-based Depression Rating Scale (MDS-DRS) (63). In 21 studies, depression was assessed using standardized scales, while in other studies, varied methods were used, such as physician diagnosis (40, 46, 53), self-report of depression (40, 45, 46, 57), the International Classification of Diseases (ICD) criteria (37), the Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria (35), the MINI (Mini-International Neuropsychiatry Interview) (47), and antidepressant use (40, 53). Coffee, tea, and caffeine intake were assessed with validated questionnaires such as the Food Frequency Questionnaire (FFQ) (40, 45, 46, 53, 57, 62), the 4-day food record (37), 24-hr recall (54, 55, 64), the Brief Dietary History Questionnaire (BDHQ) (43, 56), an interview or by asking participants (34–36, 38, 39, 42, 44, 47–52, 61, 63), and observation as well (58). Studies have been carried out in Finland (34, 37), Virginia (35), Japan (43, 56), the United Kingdom (38, 57), China (39, 42, 49, 50, 61), the United States (40, 46, 54, 55, 64), Japan (36, 57, 62), Taiwan (44), Korea, France (47), Tripoli (48), Atlantic Canada (51), Korea (45, 52), Navara (53), the Netherlands (57, 63), Germany (57), Spain, Singapore (41, 58), and Palestine (60). By gender, three studies included only women (39, 40, 56), and one study included only men (37), while 25 other

TABLE 1 Characteristics of included studies.

References	Country	Female, %	Age range (year)	Design	Sample size	Cases	Exposure	Effect size (95%CI)	Comparison	Exposure Assessment	Exposure (range of intake)	Outcome Assessment	Follow-up (year)	Adjustments
Hintikka et al. (34)	Finland	55.7	25–64	Cross-sectional	2,011	210	Coffee Tea	Tea: 0.47 (0.27–0.83) Coffee: 0.90 (0.54–1. 50)	Daily (≥5 cups/day) vs. Not daily	Questionnaire	0–≥5 cups/day	21-item BDI	1998–2005	Age, sex, current daily smoking, alcohol consumption patterns, marital status, employment status, length of basic education, having vocational training, economic hardship and poor subjective health, frequency of eating lake fish, sea fish, fresh vegetables, boiled vegetables, and fruits and use of multivitamin pills and fish oil capsules
Kendler et al. (35)	Virginia	51.8	37.9	Cross-sectional	3706	NA	Caffeine	1. 79 (1. 47–2.17)	At least several days per week vs. ≥625 mg of caffeine per day	Interview	0–>650 mg/day	DSM-III-R criteria	1995–1997	Age, gender
Niu et al. (36)	Japan	57.3 607 women	>70	Cross-sectional	1,058	361	Coffee Green tea Black or oolong tea:	Coffee: 0.82 (0.53– 1.27 Green tea: 0.56 (0.39– 0.81) Black or oolong tea: <1 cup/d: 0.82 (0.56– 1.20) ≥1 cups/d: 0.71 (0.49, 1.02)	NA	Questionnaire	Coffee: Almost never–≥1 cups/d Tea: ≤1–≥4 cups/d	30-item GDS	2002–2009	Age, sex, BMI, hypertension, diabetes, history of cardiovascular diseases, cancer, or arthritis, high C-reactive protein, smoking and drinking habits, physical activity, cognitive status, impaired instrumental activities of daily living, body pain, education, living alone, marital status, serum albumin concentration, total energy intake, intakes per 2,000 kcal of energy intake as protein and folate, tea consumption (for coffee analysis), coffee consumption (for tea analysis), perceived social support, visiting friends
Ruusunen et al. (37)	Finland	0	42–60	Prospective cohort	2,232	49	Coffee Tea Caffeine	Coffee 0.25(0.07-0.91) Tea 1.40 (0/78- 2.51) Caffein 0.85 (0.34–2.15)	Coffee: >813 ml/day vs. never Caffeine: > 781 mg/d	4d record	Coffee: none->813 ml/d Tea: yes/no Caffeine: >425 mg/d-781 mg/d	Diagnosed by a physician by ICD criteria ICD-9	17/5 years	Age, examination years, socio-economic status, smoking, alcohol consumption, maximal oxygen uptake, BMI, and daily intakes of folate and PUFA

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TABLE 1 (Continued)

References	Country	Female, %	Age range (year)	Design	Sample size	Cases	Exposure	Effect size (95%CI)	Comparison	Exposure Assessment	Exposure (range of intake)	Outcome Assessment	Follow-up (year)	Adjustments
Smith (38)	United Kingdom	57	49.6	Cross-sectional	3,223	NA	Caffeine	0.12 (0.1- 0.2)	>250 mg/day	Questionnaire	<140–>260 Mg/d	HADS	NA	NA
Chen et al. (39)	China	100	53.7	Prospective cohort	1,399	363	Tea	0.39 (0.19 to 0.84)	>100 g dried tea leaves/ mo. vs. never	Interview	0 to >100 g dried tea leaves	20-item CES-D	2002–2006	Age at diagnosis, education, income, marital status, exercise, comorbidity, menopausal symptoms, relapse/metastasis, radiotherapy, and quality of life (SF-36 mental health index scale score)
Lucas et al. (40)	USA	100	63	Prospective cohort	50,739	2,067	Coffee	0.82 (0.68–0.98)	≥4 cups/day vs. ≤1 cups/week	FFQ	0–≥4 cups/day	defined as self-reported physician-diagnosed depression and antidepressant use	1996–2006	Age, interval, total energy intake, menopausal hormones use, smoking, BMI, physical activities, marital status, social or community group involvement, self-reported history of diagnosis of diabetes, cancer, myocardial infarction or angina, high blood pressure, MHI score, a minimum latency of exposure of 8 years
Feng et al. (41)	Singapore	NA	55–93	Prospective cohort	1,615	73	Tea	0.30 (0.11–0.85)	≥6 cups/day vs. never	Interview	0–≥6 cups/ day	GDS-15	2005–2007	Age, education, housing type, marital status, physical exercise, social and productive activities summed score, MMSE total score, GDS total score
Feng et al. (42)	China	59.3	68.6	Cross-sectional	1,368	285	Tea	0.58 (0.42–0.80)	No or irregular consumption per month–daily consumption	Asking participants	No or irregular consumption per month–daily consumption	15-item CES-D	June 2010 to July 2011	Age, education, housing type, marital status, physical exercise, social and productive activities summed score, MMSE total score, GDS total score
Pham et al. (43)	Japan	40% 218 women	20–68	Cross-sectional	537	157	Coffee Green tea Caffeine	Coffee: 0.61(0.38-0.98) Green tea: 0.54 (0.29- 1) Caffeine: 0.57 (0.30–1.05)	Coffee: ≥2 cups/day vs. <1 cup/day Caffeine: ≤100 mg/d–>291 Green tea: ≥4 cups/day vs. ≤1 cup/day	BDHQ	Coffee: <1–≥2 cups/day Caffeine: ≤100 mg/day- >291 mg/day Green tea: ≤1–≥4 cups/ day	20-item CES-D	2009–2013	Age, sex, workplace, cancer, CVD, diabetes or chronic hepatitis, marital & living status, overtime work, BMI, job position, smoking, physical activity, alcohol drinking, n-3 PUFA · red meat · vegetable · fruit · coffee · green tea consumption, serum C-reactive protein concentration, serum folate concentration

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TABLE 1 (Continued)

References	Country	Female, %	Age range (year)	Design	Sample size	Cases	Exposure	Effect size (95%CI)	Comparison	Exposure Assessment	Exposure (range of intake)	Outcome Assessment	Follow-up (year)	Adjustments
Tsai et al. (44)	Taiwan	46.8	≥53-year-old	Prospective cohort Cross-sectional	Longitudinal: 2,145 Cross sectional: 4,122	Longitudinal: 31/8%= 682 Cross sectional: 36.8%= 1,516	Tea	Longitudinal: 0.83 (0.65–1.08) Cross-sectional: 0.63 (0.50–0.79)	≥3 times/ week vs. ≤2 times/week	Interview	≤2 to ≥3 times/week	10-item CES-D	1999–2007	Age, sex, level of education, psychological stress, diabetes, heart disease, IADL status, family support, audio acuity
Park and Moon (45)	Korea	59.6% 6,069 Women	20–97	Cross-sectional	10,177	425	Coffee	0.58 (0.44–0.76)	≥3 cups/day vs.≤ 0.14 cups/day	FFQ	0.14–3 cups/d	Self-reported depression	2010–2011	Diseases and stroke, perceived stress level, coffee · green tea · soft drink · vegetable · fruit · blue-backed fish · bean · red meat consumption
Omagari et al. (36)	Japan	13.3	41–82	Cross-sectional	89	15	Coffee	0.082 (0.009–0.711)	Coffee: 0–2 vs. ≥3 cups/d	FFQ	0–≥3 cups/day	HADS	April to September 2013	Sex, lipids, and n-6 PUFAs, the lipid and carbohydrate energy ratios
Guo et al. (46)	USA	51.5	50–71	Prospective cohort (nested case-control)	252,612	11,311	Coffee Tea	Tea: M: 1.21 (0.95–1.53) F:1.01 (0.92–1.32) Coffee M:0.90 (0.80–1.01) F:0.93 (0.84–1.04)	None vs. ≥4 cups per day	FFQ	0–≥4 Cups/day	self-reported physician-diagnosed depression	1995–2006	Age, sex, race, education, marital status, smoking, alcoholic beverage intake, physical activity, BMI, energy intake
Ritchie et al. (47)	France	Cross-sectional: 61.3 longitudinal: 56.3	≥65	Cross-sectional longitudinal	Cross-sectional: 8,125 longitudinal: 5,785	Cross-sectional: 1,973 longitudinal: 1,076	Caffeine	Cross-sectional: M: 0.94 (0.76- 1.18) F:0.92 (0.80- 1.06) Longitudinal: M: 0.85 (0.66- 1.08) F: 0.86 (0.74- 1.01)	No comparison	Interview	≥3 Cups/day (≥3 units of caffeine, each unit = 100 mg 1 cup of coffee = 100 mg 1 cup of tea = 50 mg)	MINI	NA	Age and center, education, cardiovascular pathologies, hypertension, BMI, HDL cholesterol, triglycerides, mobility, baseline depressive symptoms
Taher et al. (48)	Tripoli	68.5	38.7 ± 8.5	Cross-sectional	200	89	Tea or coffee	2.48 (1.36–4.54)	Yes/no	Questionnaire	Yes/no	DASS-21	July to October 2014	NA

(Continued)

TABLE 1 (Continued)

References	Country	Female, %	Age range (year)	Design	Sample size	Cases	Exposure	Effect size (95%CI)	Comparison	Exposure Assessment	Exposure (range of intake)	Outcome Assessment	Follow-up (year)	Adjustments
Li et al. (49)	China	51.8	70.7	Cross-sectional	9,371	979	Tea	Green tea: 0.97 (0.80- 1.18) Black tea: 0.39 (0.23- 0.66)	None vs. ≥3 cups/day	interview based on a self-designed questionnaire	0–≥3 cups/day	PHQ-9	NA	Age and gender, race, education level, marital status, living status, income, vegetable intake, fruits intake, red meat intake, fish intake, eggs intake, smoking, alcohol drinking, physical activity, hypertension, diabetes, coronary heart disease, Activities of Daily Living Scale scores and Mini-Mental State Examination scores.
Chanda et al. (50)	China	69.7	60–93	Cross-sectional	614	NA	Tea Coffee	Tea: 0.82 (0.71–0.95) Coffee: 0.86(0.71–1.04)	Drinking coffee or tea for Less or more than 15 years	Interviewer-administered questionnaire	Drinking coffee or tea for Less or more than 15 years	GDS-15	2011–2015	NA
Yu et al. (51)	Atlantic Canada	68.9	35–69	Cross-sectional	18,838	3,217	Coffee	Male: 1.11(0.85–1.45) Female: 1.38(1.15–1.64)	Never vs. ≥ 4 cups/day	Questionnaire	0–4 cups of coffee	PHQ-9	2009–2013	Age, ethnicity, education, province of residence, smoking status, alcohol drinking, self-reported cardiovascular disease and diabetes, healthy eating index (in tertiles), total physical activity (in MET-min/week tertiles), and BMI
Kim et al. (52)	Korea	59.7	≥19	Cross-sectional	9,576	1,443	Green tea Coffee Caffeine	Green tea: 0.79 (0.63–0.99) Coffee: 0.68 (0.55–0.85) Caffeine: 0.76(0.62–0.92)	Green tea never vs. ≥3 Cups/Week Coffee: never vs. ≥2 cups/day Caffeine: ≤22 mg/day vs. >122.9 mg/day	FFQ	Green tea: 0–≥3 Cups/Week Coffee: 0–≥2 cups/day Caffeine: ≤22–>122.9 mg/day	Assessed by some questions	NA	Adjusted for age and sex, BMI, income level, education level, alcohol intake, smoking status, physical activity, intake of energy, vegetable, fruit, red meat, fish, and green tea (or coffee)
Navarro et al. (53)	Navara	60	36.4 years	Cohort study	14,413	199	Coffee	0.37 (0.15–0.95)	<1 vs. ≥4 cups/day	FFQ	<1–≥4 cups/day	two criteria simultaneously: (a) validated physician-diagnosed depression together with (b) new onset of habitual antidepressant use	10 years	Adjusted for sex, alcohol intake (linear and quadratic term), years of university education, marital status, smoking, body mass index, total energy intake, adherence to the Mediterranean diet, between-meal snacking and following special diets, leisure-time physical activity (METS-h/week), hours of TV watching, hypertension

(Continued)

TABLE 1 (Continued)

References	Country	Female, %	Age range (year)	Design	Sample size	Cases	Exposure	Effect size (95%CI)	Comparison	Exposure Assessment	Exposure (range of intake)	Outcome Assessment	Follow-up (year)	Adjustments
														at baseline, baseline high blood cholesterol, self-perception of competitiveness, anxiety, and psychological dependence, and use of anxiolytics, and stratified for age (decades) and recruitment period
Pogoda et al. (54)	USA	50	47.3	Cross-sectional	1,342	132	Caffeine	1.40 (0.63- 3.11)	First vs. forth quartile	24- h recall	First-forth quartile	PHQ-9	2009–2010	Adjusted for gender, race/ethnicity, smoking status, and use of antidepressants.
Iranpour et al. (55)	USA	52.8	Aged ≥18	Cross-sectional	4,737	305	Caffeine	0.23 (0.06–0.8)	First vs. forth quartile	Dietary recall	First-forth quartile	PHQ-9	2005–2006	Age, sex, family PIR, education, marital status, disease history, sleep disorders, thyroid problems, physical activity, social support, smoking, total energy, cholesterol, retinol, vitamin A, beta-carotene, beta-cryptoxanthin, vitamin B1, iron, and phosphorus levels
Kimura et al. (56)	Japan	100	65–94	A multi-center cross-sectional study	1,992	NA	Coffee Green tea Caffeine	Coffee: 0.64 (0.46–0.88) Caffeine: 0.75 (0.55–1.02) Green tea: 0.85 (0.62–1.17)	Coffee: 0–3 vs. 107–619 g/1,000 kcal Green tea: 0–99 vs. 320–788 g/1,000 kcal Caffeine: 0–119.2 vs. 234.9–758 mg/1,000 kcal	BDHQ	Coffee: 0–619 g/1,000 kcal green tea: 0–788 g/1,000 kcal) caffeine: 0–758 mg/1,000 kcal	CES-D	2011–2012	Adjusted for age, residential block, living status(alone or not alone), current smoking (yes or no), alcohol drinking (yes or no), marital status(married or nit married), physical activity level (total metabolic equivalents-hour/day: METs), size of residential area (city with a population ≥1 million, a city with a population, BMI and education(junior high school, high school, junior college, and university and higher), EPA+DHA intake (mg/1000 kcal), folate intake(mcg/1000 kcal) dietary supplement (yes/no)
Ángeles Pérez-Ara et al. (57)	Netherlands, United Kingdom, Germany, and Spain	75.3	18–75	Cross-sectional	941	312	Coffee Tea	Coffee: 1.00 (0.60–1.65) Tea: 1.10 (0.63–1.92)	Coffee: <1 cup/d vs. > 3 cups/d Tea: <1 cup/d vs. > 3 cups/d	FFQ	<1 cup/d–>3 cups/d	30-item self-administered questionnaire	September 2015 and October 2016.	Adjusted for the site, age, gender, marital status, level of education, BMI, MooDFOOD diet score, smoking, alcohol use, physical activity, high blood pressure, diabetes, and stomach or intestinal ulcer

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TABLE 1 (Continued)

References	Country	Female, %	Age range (year)	Design	Sample size	Cases	Exposure	Effect size (95%CI)	Comparison	Exposure Assessment	Exposure (range of intake)	Outcome Assessment	Follow-up (year)	Adjustments
Ng et al. (58)	Singapore	NA	Mean age 67 years	Prospective cohort study	3,177	57	Tea	0.34 (0.13- 0.90)	None or <1 cup/d vs. ≥3 cups/d	Reported habitual intake of common tea types using indigenous references	None–≥3 cups/d	GDS-15	Four years	Age, sex, ethnicity, education, housing type, single/divorced/widowed, living alone, physical and social activity, smoking, alcohol, number of comorbidities, MMSE, and baseline GDS level
Kromhout et al. (59)	Netherlands	59	82 years +9	Cross-sectional	206	145	Caffeine	0.6 (0.2–2.1)	High vs. low	Cups of coffee, tea, and cola consumed were observed and recorded six times a day.	Low-normal-high	MDS-DRS	NA	Age, gender, and stage of cognitive decline together with any of the following variables that were significantly related to the specific outcome (the use of psychotropic medication, marital status, Barthel Index total score, the presence of pain, cohort, and kidney function)
Safarini et al. (60)	Palestine	61.2	NA	Cross-sectional	1,051	598	Caffeine	Coffee: 0.573 (0.261–1.255) Tea: 0.567 (0.270–1.189)	NA	Questionnaire	NA	BDI-II	October 2020 and January 2021	Study year, gender, and academic field
Yao et al. (61)	China	54.2%	83.7	Cross-sectional	13,115	NA	Green tea	0.85 (0.76–0.95)	Never or <1 cup/month vs. ≥1 Cup/daily	Self-reported	Never or <1 cup/month <1 cup/day but ≥1 Cup/month ≥1 Cup/daily	CES-D-10	NA	The demographic factors included age and sex. Socioeconomic conditions included education, socioeconomic status, rural residence, and geographical regions. Family/social support included marital status and living arrangements. Health behaviors included social and leisure activity index, smoking, alcohol drinking, BMI (as a proxy for unhealthy behaviors), and regular dietary (vegetable/fruit/fish/nut) intake. Health status were measured by self-rated health, of 13 cognitive impairment, medical illness, comorbidity, and disability in activities of daily living (ADL)

BMI, Body Mass Index; BDI, Beck Depression Inventory; GDS, Geriatric Depression Scale; CES-D, Center for Epidemiological Studies Depression Scale; PHQ-9, Patient Health Questionnaire; HADS, Hospital Anxiety and Depression scale; DASS, Depression Anxiety Stress Scale; MDS-DRS, Minimum Data Set-based Depression Rating Scale; MINI, Mini International Neuropsychiatric Interview; ICD, International Classification of Diseases; DSM, Diagnostic and Statistical Manual of Mental Disorders; FFQ, Food Frequency Questionnaire; BDHQ, Brief Dietary History Questionnaire.

10.3389/fnut.2023.1051444

TABLE 2 ROBINS-I judgment for each domain and overall.

Study	Bias due to confounding	Bias due to the selection of participants	Bias due to exposure assessment	Bias due to misclassification during follow-up	Bias due to missing data	Bias due to measurement of the outcome	Bias due to selective reporting of the results	Overall judgment
Hintikka et al. (34)	Serious	Low	Serious	Moderate	Low	Moderate	Low	Serious
Kendler et al. (35)	Serious	Low	Serious	Moderate	Low	Moderate	Low	Serious
Niu et al. (36)	Moderate	Low	Serious	Moderate	Low	Moderate	Low	Serious
Ruusunen et al. (37)	Serious	Low	Moderate	Moderate	Low	Moderate	Low	Serious
Smith (38)	Serious	Low	Serious	Moderate	Low	Moderate	Low	Serious
Chen et al. (39)	Serious	Low	Serious	Moderate	Low	Moderate	Low	Serious
Lucas et al. (40)	Serious	Moderate	Moderate	Low	Moderate	Serious	Low	Serious
Feng et al. (41)	Serious	Low	Serious	Moderate	No information	Moderate	Low	Serious
Feng et al. (42)	Serious	Low	Serious	Moderate	Low	Moderate	Low	Serious
Pham et al. (43)	Serious	Moderate	Moderate	Moderate	Low	Moderate	Low	Serious
Tsai et al. (44)	Serious	Low	Serious	Moderate	Low	Moderate	Low	Serious
Park and Moon (45)	Serious	Low	Moderate	Moderate	Low	Moderate	Low	Serious
Omagari et al. (36)	Serious	Moderate	Moderate	Moderate	Low	Moderate	Low	Serious
Guo et al. (46)	Moderate	Low	Moderate	Moderate	Moderate	Moderate	Low	Moderate
Ritchie et al. (47)	Serious	Low	Serious	Low	Moderate	Moderate	Low	Serious
Taher et al. (48)	Serious	Moderate	Serious	Moderate	Low	Moderate	Low	Serious
Li et al. (49)	Serious	Low	Serious	Moderate	Low	Moderate	Low	Serious
Chanda et al. (50)	Serious	Low	Serious	Moderate	Low	Moderate	Low	Serious
Yu et al. (51)	Serious	Low	Serious	Moderate	No information	Moderate	Low	Serious
Kim et al. (52)	Moderate	Low	Moderate	Moderate	Moderate	Serious	Low	Serious
Navarro et al. (53)	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Low	Moderate
Pogoda et al. (54)	Serious	Low	Serious	Moderate	Low	Moderate	Low	Serious
Iranpour et al. (55)	Serious	Low	Serious	Moderate	Low	Moderate	Low	Serious
Kimura et al. (56)	Serious	Moderate	Moderate	Moderate	Low	Moderate	Low	Serious
Ángeles Pérez-Ara et al. (57)	Serious	Moderate	Moderate	Moderate	Low	Moderate	Low	Serious
Ng et al. (58)	Serious	Low	Serious	Low	Moderate	Moderate	Low	Serious
Kromhout et al. (59)	Serious	Serious	Moderate	Moderate	Low	Moderate	Low	Serious
Safarini et al. (60)	Serious	Serious	Serious	Moderate	Low	Moderate	Low	Serious
Yao et al. (61)	Serious	Low	Serious	Moderate	Low	Moderate	Low	Serious

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studies included both genders (34–36, 38, 41–46, 48–52, 54, 55, 57, 58, 61–64).

Due to confounding, four studies were rated to have a moderate risk of bias (36, 46, 52, 53), while the others were rated to have a serious risk of bias. Regarding the exposure assessment, 11 studies were classified as moderate risk (37, 43, 45, 46, 52, 53, 56, 57, 59, 62, 65), and others presented a serious risk of bias. For the selection of participants, seven studies were rated as having a moderate risk (43, 48, 53, 56, 57, 62, 65), two studies were rated as having a serious risk (59, 60), and others were rated as having a low risk of bias. Considering misclassifications during follow-up, almost all studies were classified as having a moderate risk of bias, while three studies were at low risk (47, 58, 65). Due to missing data, a moderate risk of bias was assessed in six studies (46, 47, 52, 53, 58, 65) and a low risk in the remaining studies. Two studies had serious outcome measurement bias (52, 65), while other studies were at moderate risk. All 29 studies were judged to be at low risk of bias due to selective reporting of results, and, ultimately, the overall judgment of two studies showed a moderate risk of bias (46, 53), while 27 were at serious risk (Table 2).

3.2. Association of coffee consumption with depression

Four cohort studies with a total of 319,996 participants and 13,583 cases reported information about coffee consumption and depressive symptoms (37, 46, 53, 65). Comparing the highest with the lowest category, there was an inverse association between intake of coffee and depressive symptoms (RR:0.89, 95% CI: 0.82–0.95; $I^2 = 63.7\%$, $P_{heterogeneity} = 0.04$; Figure 1). Four studies were eligible for the linear dose-response analysis (37, 46, 53, 65). An increase in coffee intake of 240 ml per day was associated with a 4% lower risk of developing depression (RR: 0.96, 95% CI: 0.95, 0.98; $I^2 = 22.7\%$, $P_{dose-response} < 0.001$) (Figure 2). We did not find a nonlinear association between coffee intake and depression risk ($P_{non-linearity} = 0.89$) (Figure 2). Our subgroup analyses suggested that there were potential sources of heterogeneity based on geographical region, follow-up duration, and the number of participants (Supplementary Table 3). Based on a visual inspection of the funnel plot, we found some asymmetry (Supplementary Figure 2). Egger's regression test indicated possible publication bias ($P = 0.001$). Findings from other sensitivity analyses revealed that excluding any single study from the analysis did not appreciably alter the pooled effect sizes.

Ten cross-sectional studies with a total of 45,883 participants reported information about coffee consumption and depressive symptoms (34, 36, 43, 45, 50, 51, 57, 62, 66, 67). Comparing

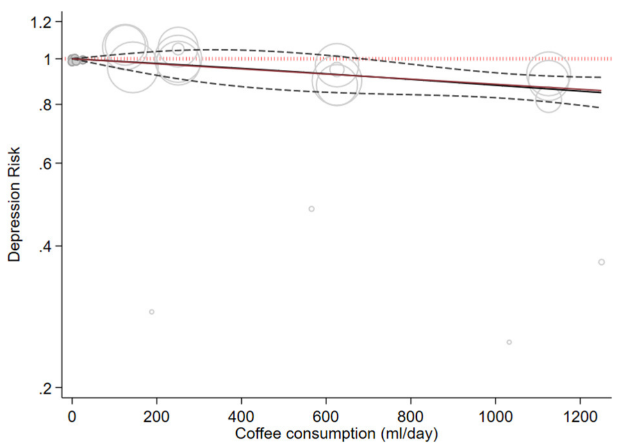

FIGURE 2
Dose-response association between coffee intake and depressive symptoms. The solid line represents a non-linear dose response, and the dotted lines represent a 95% confidence interval. Circles represent hazard ratio point estimates for coffee intake categories from each study with circle size proportional to the inverse of standard error.

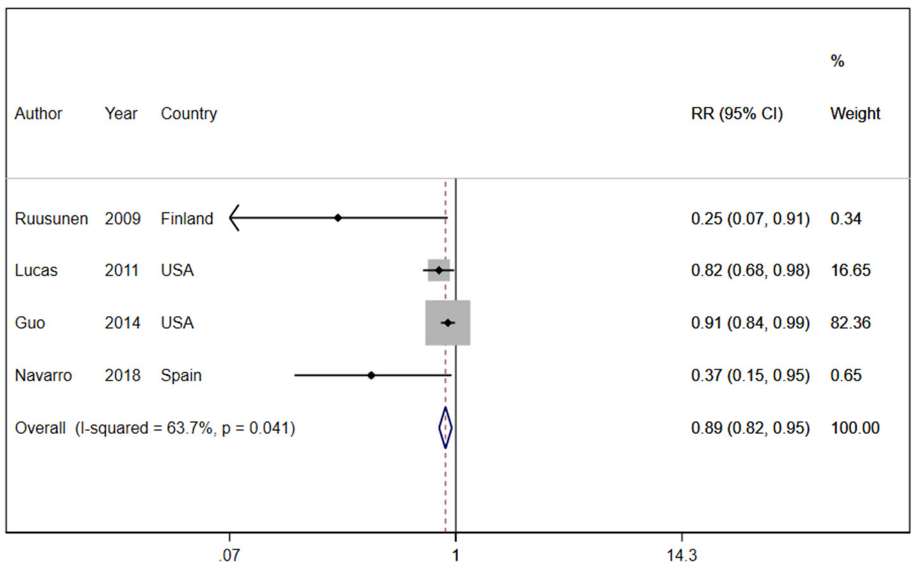

FIGURE 1
Relative risk of depressive symptoms for the highest compared with the lowest category of coffee intake. RR, relative risk.

the highest with the lowest category, coffee intake had an inverse association with depressive symptoms (RR: 0.78, 95% CI: 0.62, 0.98; I^2 = 81.3%, $P_{heterogeneity}$ = 0.001; Supplementary Figure 3). Our subgroup analyses showed heterogeneity based on geographical region, smoking status, physical activity, energy intake, alcohol consumption, and BMI (Supplementary Table 4).

3.3. Association of tea consumption with depression

Six cohort studies with a total of 263,180 participants and 12,471 cases reported information about tea consumption and depressive symptoms (37, 39, 41, 44, 46, 58). On comparing the highest category with the lowest category in random effects analysis, there was no significant inverse association between tea consumption and depressive symptoms (RR: 0.74, 95%CI: 0.51, 1.08; I^2 = 77.6%, $P_{heterogeneity}$ = 0.001; Figure 3). In the linear dose-response analysis of tea consumption and depression risk, based on four cohort studies, an increase in tea intake of 240 ml/day was not associated with the risk of depression (RR: 0.99, 95%CI: 0.97, 1.01; I^2 = 58%). We did not find a dose-response association between tea consumption and the risk of depression ($P_{dose-response}$ = 0.405) (Figure 4). There was no non-linear association between tea intake and the risk of depression ($P_{non-linearity}$ = 0.89) (Figure 4). Our subgroup analyses showed heterogeneity based on the BMI, alcohol consumption, geographical region, follow-up duration, number of participants, and smoking status. The subgroup analysis indicated a significant inverse association in studies conducted in Asia (RR: 0.73, 95%CI: 0.58, 0.92; I^2 = 67%, n = 3) as well as a significant inverse association in studies that did not adjust for alcohol intake (RR: 0.77, 95%CI: 0.60, 0.98; I^2 = 71%, n = 2), BMI (RR: 0.73, 95%CI: 0.58, 0.92; I^2 = 67%, n = 3), or smoking (RR: 0.77, 95%CI: 0.60, 0.98; I^2 = 71%, n = 2) (Supplementary Table 5). Based on the visual inspection of the funnel plot, we found asymmetry (Supplementary Figure 4); however, when we did Egger's regression tests, no significant publication bias was observed (P = 0.07). Upon excluding the study by Ruusunen et al. (37) from the analysis, there was a significant negative association between tea consumption and depression (RR: 0.64, 95% CI: 0.41–0.99).

Twelve cross-sectional studies with a total of 47,882 participants reported information about tea consumption and depressive symptoms (34, 36, 42–44, 49, 50, 52, 56–58, 61). On comparing

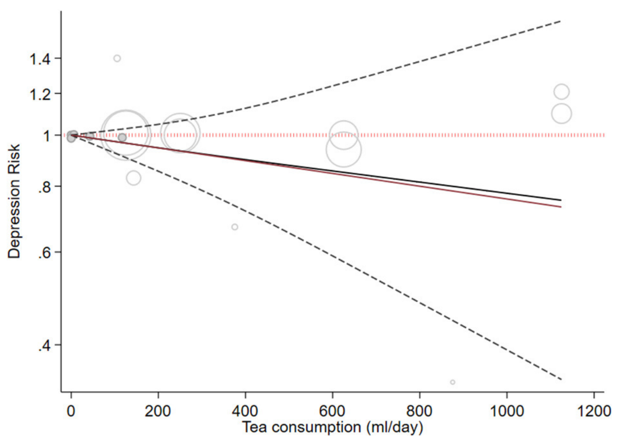

FIGURE 4
Dose-response association between tea intake and depressive symptoms. The solid line represents a non-linear dose response, and the dotted lines represent a 95% confidence interval. Circles represent hazard ratio point estimates for coffee intake categories from each study with circle size proportional to the inverse of standard error.

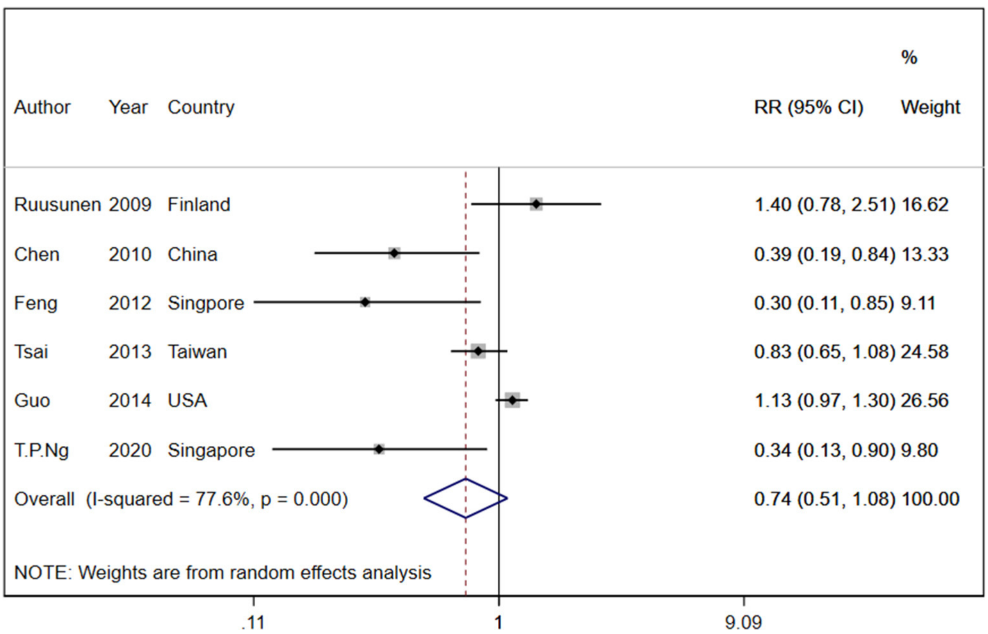

FIGURE 3
Relative risk of depressive symptoms for the highest compared with the lowest category of tea intake. RR, relative risk.

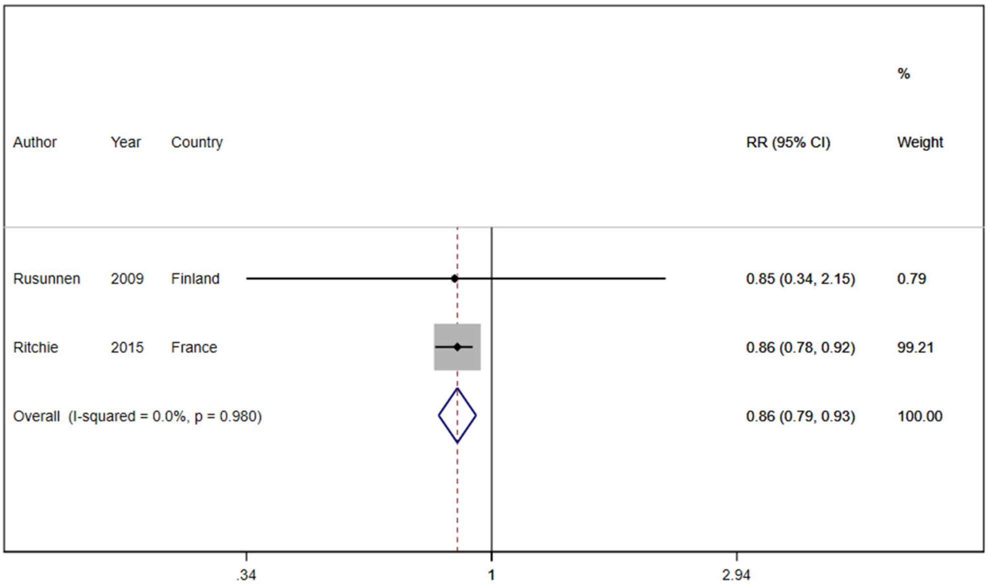

FIGURE 5
Relative risk of depressive symptoms for the highest compared with the lowest category of caffeine intake. RR, relative risk.

the highest category with the lowest category, total tea, green tea, and black tea consumption had an inverse association with depressive symptoms (RR:0.73, 95%CI: 0.65, 0.81; $I^2 = 61.0\%$, $P_{heterogeneity} = 0.025$, RR:0.84, 95%CI: 0.78, 0.90; $I^2 = 45.3\%$, $P_{heterogeneity} = 0.103$, RR:0.58, 95%CI: 0.43, 0.79; $I^2 = 70.1\%$, $P_{heterogeneity} = 0.067$; respectively Supplementary Figure 5). Our subgroup analyses showed heterogeneity based on the number of participants (Supplementary Table 6).

3.4. Association of caffeine consumption with depression

Two cohort studies with a total of 8,017 participants and 1,125 cases reported information about caffeine intake and depressive symptoms (37, 47). On comparing the highest category with the lowest category, caffeine intake was inversely associated with depressive symptoms (RR: 0.86, 95%CI: 0.79, 0.93; $I^2 = 0.0\%$, $P_{heterogeneity} = 0.980$; Figure 5). One cohort study was eligible for dose-response analysis (37). An increase in caffeine intake of 200 mg/day was not associated with the risk of depression (RR: 1.05, 95%CI: 0.80, 1.36; $I^2 = 0\%$). A non-linear dose-response meta-analysis performed on one cohort study indicated a non-statistically significant increasing association with intake of 200–600 mg of caffeine ($P_{dose-response} = 0.163$ Figure 6) and an inverse association with higher intake ($P_{dose-response} = 0.18$, Figure 6); however, there was a nonlinear association ($P_{non-linearity} = 0.01$ Figure 6).

Ten cross-sectional studies with a total of 34,495 participants reported information about caffeine intake and depressive symptoms (35, 38, 43, 47, 52, 54–56, 59). Comparing the highest with the lowest category, caffeine intake did not have an inverse association with depressive symptoms (RR: 0.64, 95% CI: 0.40, 1.01; $I^2 = 95.3\%$, $P_{heterogeneity} < 0.001$; Supplementary Figure 6). Based on a visual inspection of the funnel plot, we found some asymmetry (Supplementary Figure 7); however, when we did Egger's regression tests, no significant publication bias was observed ($P = 0.34$). The overall effect size depended on the studies of Kendler et al. (35) and Pogoda et al. (54). By excluding these studies, a significant negative association was found between caffeine and depression risk (RR: 0.55, 95% CI: 0.34–0.88, RR: 0.58, 95%CI: 0.36–0.95, respectively).

3.5. Grading the evidence

We applied the GRADE tool to make a judgment on the quality of the evidence. The quality of evidence was rated low for coffee intake due to devaluations for risk of bias and inconsistency, very low for tea intake due to downgrades for risk of bias, inconsistency, and imprecision, and moderate for dietary caffeine intake associated with downgrades for risk of bias (Supplementary Table 7).

4. Discussion

Our systematic review and dose-response meta-analysis of observational studies revealed that a higher intake of caffeine, coffee, and tea was inversely associated with the risk of depressive symptoms. Higher coffee consumption was associated with an 11 and 22% lower risk of depressive symptoms in the cohort and cross-sectional studies, respectively, and there was also a significant linear dose-response relationship. When we compared higher tea consumption to lower consumption in cohort studies, we found that the risk of depressive symptoms was reduced by 26%, and this inverse association was replicated in cross-sectional studies; however, we did not find a dose-response association. An analysis of cohort and cross-sectional study results investigating the association between caffeine intake and the risk of depressive symptoms found that a higher caffeine intake was associated with a 14 and 13% lower risk of depressive symptoms, respectively.

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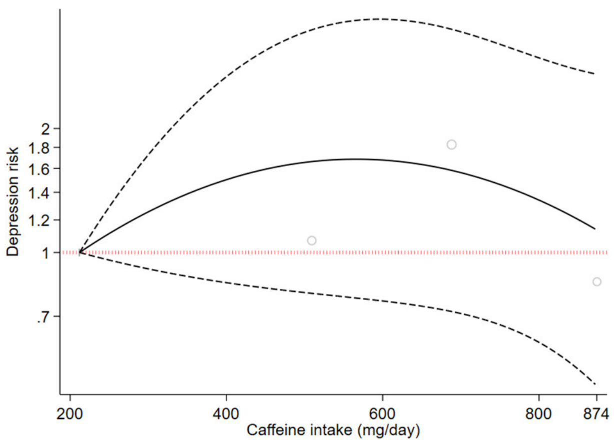

FIGURE 6
Dose-response association between caffeine intake and depressive symptoms. The solid line represents a non-linear dose response, and the dotted lines represent a 95% confidence interval. Circles represent hazard ratio point estimates for coffee intake categories from each study with circle size proportional to the inverse of standard error.

These results provide supportive evidence consistent with the findings of previous meta-analyses of observational studies examining the association between coffee, tea consumption, and depression. Grosso et al. suggested that coffee and tea consumption act as protective factors for depression. It was noted that the caffeine content may account for some of the beneficial effects, but this does not fully explain the established relationship (68). Kang et al. also showed quantitative evidence for the inverse association between high coffee and tea consumption and the risk of developing depression but concluded that it is difficult to determine the causality of this association because of the observational design of the studies and insufficient prospective studies for this topic (69). Regarding the association between coffee and caffeine consumption and depression, Wang et al. conducted a meta-analysis of observational studies and also identified a strong inverse association (70). Dong et al. also performed a meta-analysis of observational studies focusing on the relationship between tea consumption and depression, which led to the identification of tea as a potential preventive factor against depression (71). A recent study by Fisicaro et al. (72) found that higher mocha coffee consumption was associated with a higher level of psycho-cognitive functioning in elderly non-smokers in Italy with chronic subcortical ischaemic vascular disease (SIVD) and cognitive profile of mild vascular cognitive impairment (mVCI). This finding is consistent with previous findings and further supports the fact that coffee has positive effects on the cognitive process as well (72).

Moreover, the results of some studies are inconsistent with the results of the present meta-analysis. In the study conducted by Kendler et al., it was shown that caffeine consumption by people who are highly vulnerable to caffeine dependence may make them more prone to chronic symptoms of anxiety and depression (35). A meta-analysis by Pogoda et al. did not report caffeine to be protective against depression (54). Jee et al. (73) showed that the beneficial and/or harmful effects of caffeine on several neurological and psychiatric disorders may differ depending on gender. Women may be more likely to experience a reduction in stroke risk with caffeine consumption. However, caffeine consumption may also increase the risk of sleep disorders for both sexes equally. A greater reduction in dementia risk was also observed in women than in men, according to a review. However, caffeine had a greater protective effect against Parkinson's disease in men than in women (73). A cohort study on 3,323 students aged 11 to 17 years found that the impact of caffeine on anxiety varied between sexes, with no significant effects observed in girls, while in boys, caffeine consumption was associated with an increase in anxiety levels (73, 74). Different kinds of disease, different disease types, and different dosages of caffeine or different kinds of tea or coffee may be the reasons for these contradictions. Therefore, further research is needed to expand the existing knowledge in this field.

The mechanisms underlying the inverse association between the consumption of caffeine (coffee and tea) and the risk of developing depression are not yet fully determined; however, some possible biological explanations have been suggested. The first favorable effect can be mediated by caffeine. Caffeine stimulates the central nervous system since it is a non-specific adenosine A1/A2A receptor antagonist, generating psychostimulant effects through modulating dopaminergic transmission by increasing calcium signaling (15, 75, 76). Caffeine metabolites have an effect on adenosine receptors in the brain, which helps to alleviate depression (76). The second important mechanism that had been suggested to play a protective role against depression is related to a complex mixture of chemicals with anti-inflammatory activities. It is common knowledge that the pathophysiology of depression is correlated with low-grade inflammation and oxidative stress dysregulation (9, 77–79). Specific phenolic compounds in coffee, called chlorogenic acid and caffeic acid, play an anti-inflammatory and antioxidant role, leading to the reduction of oxidative stress (Supplementary Figure 8) (80–82).

Tea shares similar effects as it is the main source of polyphenols, particularly catechins. Epigallocatechin gallate (EGCG) is known to be the most potent tea component, counteracting depression through powerful antioxidant activity (17). Another antidepressant measure taken by EGCG is monoamine oxidase (MAO) inhibition, leading to a higher dopamine and serotonin concentration in the brain (83, 84). In addition to the properties mentioned, some studies demonstrated a probable antidepressant effect of EGCG *via* an increase in brain-derived neurotrophic factor, neuronal survival, and plasticity (83). The benefits of tea are not only related to its caffeine and polyphenol content. Theanine, the main amino acid found in the tea has beneficial effects against depression by increasing serotonin and dopamine in the brain, which are considered to be two key neurotransmitters in the etiology of depression (Supplementary Figure 8) (18, 19).

The strengths of our study deserve consideration. First, we applied the best-fitting second-order fractional polynomial to model curvilinear associations when restricted cubic splines could not be calculated because of the limited number of studies ($n \leq 2$) included in the analyses. Second, we also considered all studies carried out from 2015 to 2021, including 12 cross-sectional studies and two prospective cohort studies in our analysis, which were not considered in previous systematic reviews and meta-analyses. Finally, the GRADE system was applied to rate the overall quality of the evidence.

Our study also has some potential limitations. The current study is based on observational studies with potentially unknown confounders that affect estimates of effects, but most studies were adjusted for potential confounders such as age, gender, health status, dietary intake, smoking status, physical activity, and socioeconomic conditions. Nonetheless, unmeasured confounders might have biased evaluations and results, such as family or social support. Due to the cross-sectional design, we cannot ensure whether caffeine, coffee, and tea consumption have reduced the risk of developing depression or whether individuals with depression symptoms consume less than non-depressed individuals. Various methods have been used to measure caffeine, tea, and coffee consumption (e.g., grams, cups, and times). Regarding depression assessment, a variety of instruments were used, from specific questionnaires to patient reports.

5. Conclusion

In summary, our results suggest that consuming coffee, tea, and dietary caffeine may have protective effects against depression and may lower the risk of developing depression. However, it is crucial to conduct well-designed prospective studies using harmonized tools in the future for both exposure and outcome assessments.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The datasets generated or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request. Requests to access these datasets should be directed to SJ, jazayeri.sh@iums.ac.ir.

Ethics statement

This research was conducted in accordance with the Research Ethics Committees of the Iran University of Medical Sciences (Ethics No. IR.IUMS.REC.1401.814).

Author contributions

KT and HS designed the project, performed the literature search, and wrote the first draft of the manuscript. HS analyzed the data and interpreted the results. KT, NP, and SJ revised the subsequent drafts for important intellectual content. SJ was the guarantor. All authors 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

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Total and cause-specific mortality associated with meat intake in a large cohort study in Korea

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Background: Asia has experienced a large increase in meat intake in the past decade, yet the health impact of meat intake is not well studied.

Objective: We examined the association of meat intake with all-cause, cancer and cardiovascular disease (CVD) mortality in an Asian country.

Methods: Participants were 113,568 adults with dietary data at recruitment (2004–2013) of the Health Examinees-Gem (HEXA-G) study, a prospective cohort study conducted in 8 regions of Korea. Participants were followed until 31 December 2020. Total, red, white, and organ meat intake were computed based on a 106-item questionnaire. Multivariable Cox proportional hazard models were implemented using the lowest quintile of meat intake as the reference category.

Findings: For 1,205,236 person-years, 3,454 deaths were recorded. High intake of processed red meat was positively associated with all-cause mortality [men: hazard ratio (HR) 1.21, 95% confidence interval (95% CI) 1.07–1.37; women: HR 1.32, 95% CI 1.12–1.56]. Increased risk of all-cause mortality (HR 1.21, 95% CI 1.05–1.39) and cancer mortality (HR 1.24, 95% CI 1.03–1.50) was observed in women with high intake of organ meat. Moderate intake of pork belly was associated with reduced risk of all-cause mortality in men (HR 0.76, 95% CI 0.62–0.93) and women (HR 0.83, 95% 0.69–0.98) but high intake was associated with increased risk of CVD mortality in women (HR 1.84, 95% CI 1.20–2.82). Low beef intake decreased the risk of CVD mortality in men (HR 0.58, 95% CI 0.40–0.84), but roasted pork increased cancer mortality in women (HR 1.26, 95% CI 1.05–1.52).

Conclusion: There was increased risk of all-cause mortality associated with intake of processed red meat in men and women, increased risk of all-cause and cancer mortality with intake of organ meat in women, and increased risk of cancer mortality with intake of roasted pork intake in women. High intake of pork belly increased the risk of CVD mortality in women, but moderate intake was inversely associated with mortality from all-causes in both men and women.

KEYWORDS

meat intake, processed red meat, all-cause mortality, cancer mortality, CVD mortality, cohort study, cox model

1. Introduction

Estimates from the 2022 Global Nutrition Report indicated that close to two-thirds of avoidable deaths were attributed to sub-optimal dietary composition, including 9% attributed to high intake of meat, and 8% attributed to processed meat (1). The Asian region has experienced the third largest increase in red and processed red meat intake in the past decade (1), suggesting that this region is experiencing a nutritional transition toward a

Westernized dietary pattern. Nonetheless, various aspects of the traditional dietary pattern that emphasize high intake of plant foods and moderate intake of fat have been retained in Asian countries like Korea (2, 3). Accordingly, there is need to understand the health impacts of meat intake in Asian countries considering their unique dietary culture. Cohort studies and meta-analyses have reported a positive association of red and processed meat intake with all-cause and cause-specific mortality mainly in Europe and North America (4–12).

However, pooled data from Asian countries indicated an inverse association between red meat intake and mortality (13). Conversely a recent cohort study from Japan reported an increased risk of total and CVD mortality with heavy intake of total and red meat, a low risk of stroke mortality with high intake of total meat and a reduced risk of all-cause and cancer mortality in men with moderate intake of processed meat (14). Different results between Asian and Western countries have been explained by low intake of meat in Asian vs. Western countries (11, 13, 14). However, red and processed meat intake in an Adventist population with low intake of meat, increased the risk of mortality (4). Previous studies in Asian populations neither differentiated processed and unprocessed red meat (13), nor accounted for methods of preparation which are also different from those in the West (15–17). On the other hand, the association between white meat consumption and mortality is inconclusive with some studies reporting inverse associations (7, 8, 10, 13, 14) and others reporting null associations (9, 11, 12).

The Health Examinees (HEXA) cohort is a large prospective study of over 170,000 adults aged above 40 years who were recruited from 38 regions of Korea between 2004 and 2013. Using this data set, we comprehensively examined meat intake by type, degree of processing, and method of preparation in relation to death from all-causes and specific causes.

2. Materials and methods

2.1. The Health Examinees-Gem Study

We conducted an observational analysis of the HEXA cohort, a prospective population-based sub-cohort within the Korean Genome and Epidemiology study (KoGEs) that was established to investigate the etiological factors of complex diseases (18). The HEXA recruited participants between 2004 and 2013 at 38 health examination centers and training hospitals located in the eight regions of Korea. The study details have been published elsewhere (19). Ethical approval was obtained from the Ethics Committee of the Korean Health and Genomic Study of the Korean National Institute of Health and the Institutional Review Boards of all participating hospitals (IRB no. E-1503-103- 657). All participants provided written informed consent before their participation.

2.2. Measures

2.2.1. Assessment of dietary intake

Dietary intake was assessed once at baseline using a 106-food item semiquantitative food frequency questionnaire (SQFFQ) that had been tested for reproducibility and validity using 12-day dietary records obtained from 124 participants (18). Food consumption frequencies were classified into nine levels (from "never" to "three times or more a day"), and portion sizes were classified into three levels (one-half, one, and one and a half servings). Energy and macronutrient content of each item was estimated using a food composition table developed by the Korean Rural Development Administration (RDA) (19). Meat items on the SQFFQ were pork (roasted and braised), and pork belly; edible viscera/organ meat; processed meat; beef (steak/roasted), beef soup with bones, and beef soup with vegetables; dog meat; and chicken (fried/stew). For mixed dishes which contained meat, we extracted meat weight by applying weights using the food recipe information. The applied weights represent the % weight contributed by a meat item to the mixed dish. Red meat included beef, pork, and dog meat while white meat included chicken.

2.2.2. Baseline covariates

Educational level, and household income were assessed in addition to demographic characteristics such as age and sex. Other covariates included lifestyle factors such as: smoking, where current smokers were defined as participants who had smoked more than four hundred cigarettes during their lifetime and were still smoking (20); drinking-categorized into current alcohol drinkers, past drinkers and never drinkers. Current alcohol drinkers were those who reported that they had ever drunk alcohol and were still drinking at the time of the interview. Regular physical exercise was assessed by asking participants to report (1) whether they engage in regular physical exercise that causes body sweating; (2) the number of times they engage in these exercises in a week (1–2 times/week to everyday); and (3) the duration of the exercise. Regular exercise was defined as engaging in activities that caused body sweating for at least five times a week lasting at least 30 min per session.

Weight and height were objectively measured at baseline by trained medical staff. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). BMI was categorized into four classes based on the WHO classification of BMI for Asian adults: <18.5, 18.5–22.9, 23–24.9, 25.0–29.9, and ≥ 30.0 kg/m^2 (21). The information about diseases and use of medication was reported by participants through a standardized questionnaire that was administered by trained staff, and was used to define prevalent cancer, cardiovascular, cerebral vascular, respiratory and gastrointestinal diseases. Chronic kidney disease was diagnosed using estimated glomerular filtration rate (eGFR < 60 $mL/min/1.73\ m^2$) that was estimated using the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) (22). Diabetes was defined as fasting blood glucose $\geq$126 mg/dL or drug treatment for elevated fasting blood glucose, hypertension was defined as systolic blood pressure $\geq$130 mmHg or diastolic blood pressure $\geq$85 mmHg or drug treatment for elevated blood pressure. Abdominal obesity was defined as waist circumference $\geq$90 cm for men and $\geq$80 cm for women (23). The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria was used to define metabolic syndrome (24). For each chronic disease, participants were assigned a score of 1 (one) in the presence and a score of zero (0) in the absence of each disease. These scores were summed across all the diseases to create a disease score.

The disease score was then classified as: zero (no disease) and ≥ 1 (having atleast a disease).

2.2.3. Ascertainment of mortality

The date and causes of death from 2004 to 31 December 2020, were ascertained through linkage to the death certificate data base of the Korean National Statistical Office. The deaths of participants on Medicaid were ascertained through linkage to the National Health Insurance Service. Participants' unique identifiers were used to add mortality data from Statistics Korea. For cause-specific mortality, the 10th revision of the International Classification of Disease (ICD-10) codes was used. ICD-10 code C00-C97 and I00-I99 were used to classify cancer and CVD-specific deaths, respectively.

2.2.4. Statistical analysis

Participants were divided into sex-specific quintiles of total meat intake and intake of meat subtypes. The main outcome variable was death from any cause, cancer or CVD. Missing data were replaced by the mode for categorical variables and by the median for BMI. The distribution of participant characteristics according to quintiles of total meat intake was described using percentages for categorical variables or least square means for continuous variables and was stratified by sex.

Follow-up time was calculated for each participant starting from the date of recruitment until the date of death. Participants who did not experience the event were censored on 31st December 2020. We examined the association of meat intake with all-cause, cancer and CVD mortality using multivariable cox proportional hazard models. Adjusted hazard ratios (HRs) and 95% confidence intervals for meat intake, using quartile one (Q1) as the reference category, were computed and adjusted for age (continuous), demographic factors (marital status, educational level, job, and monthly household income); lifestyle factors (total energy intake, smoking, drinking, and regular physical exercise); and health-related factors (BMI and history of chronic disease). The proportional hazards assumption was assessed by including multiplicative terms between variables and follow-up time and was not violated ($p > 0.05$ for all categories).

We stratified the analyses by sex since meat intake is higher in men than women. In addition, we conducted sensitivity analysis (1) by excluding participants who died within 1 year of follow-up, to avoid latent period bias and reverse causation; (2) adjusting

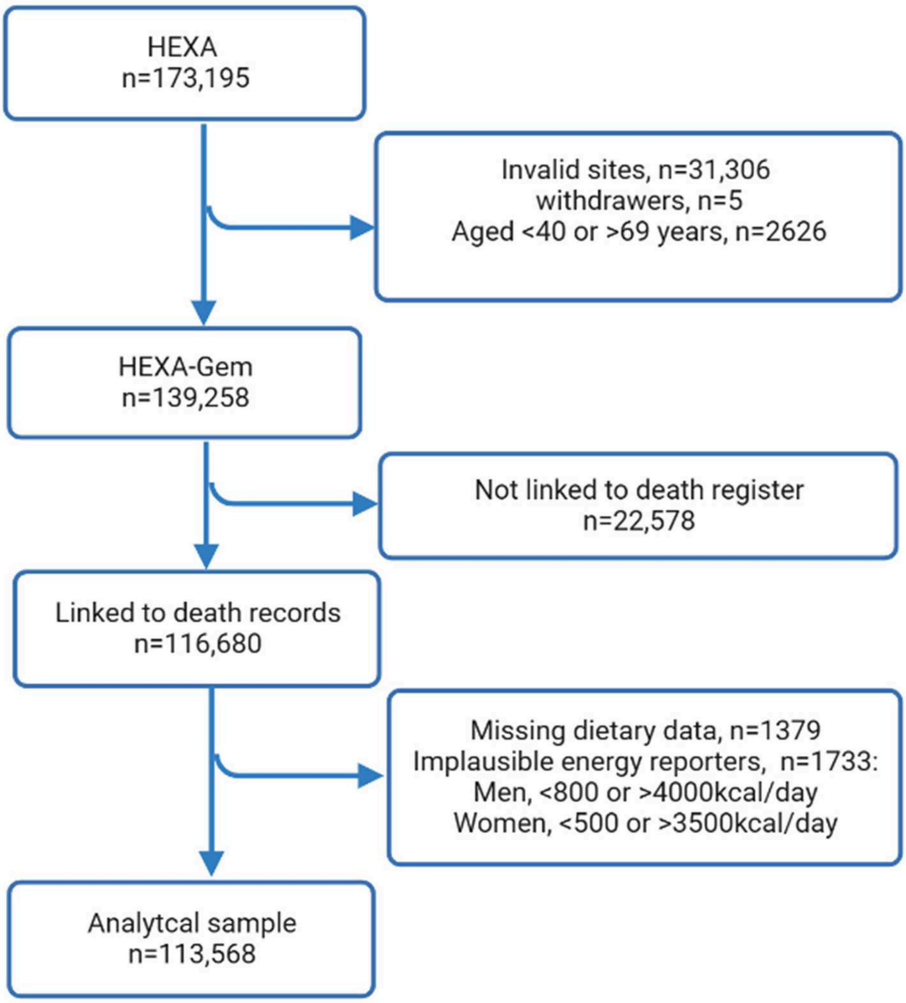

FIGURE 1
Selection of study participants.

for alcohol intake in grams/day (continuous) to test residual confounding from alcohol intake; and (3) adjusting for selected dietary variables that are associated with meat intake (vegetables, fish, seafood, and legumes).

All data were analyzed using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA), and P<0.05 was used to define statistical significance.

3. Results

The HEXA-Gem (HEXA-G) ($n = 141{,}968$) sample was derived from the original HEXA study ($n = 173{,}195$) by excluding: (1) sites that only participated in the pilot study from 2004 to 2006; (2) sites that did not meet the HEXA standards for biospecimen quality control; (3) sites that participated in the study for < 2 years (25) ($n = 31{,}306$), those who withdrew from the study ($n = 5$), and those younger than 40 or older than 69 years ($n = 2{,}626$). A total of 22,578 participants did not consent to record linkage and were excluded from the analysis. Furthermore, 3,112 participants were excluded due to missing data on dietary intake ($n = 1{,}379$) and implausible energy reporting ($n = 1{,}733$) leaving 113,568 participants for primary analysis (Figure 1).

The mean age at baseline was 53.5 years (SE 8.2), 38,847 (34%) were men and 74,721 (66%) were women. For 1,205,236 person-years [median follow-up of 10.6 (9.5–11.9) years], 3,454, 1,720, and 539 total, cancer and CVD deaths, respectively, were recorded. The highest consumers of total meat were more likely to be younger, highly educated, and had high income. In addition, individuals in

TABLE 1 General characteristics of participants according to quintiles of total meat intake in the HEXA-G study.

	Quintiles of total meat intake (g/day)									
	Men					Women				
	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
n	7,756	7,783	7,769	7,770	7,769	14,945	14,943	14,942	14,940	14,951
Age, years, mean ± SEM	56.8 ± 0.1	54.6 ± 0.1	53.2 ± 0.1	52.3 ± 0.1	51.7 ± 0.1	55.4 ± 0.1	53.4 ± 0.1	52 ± 0.1	51 ± 0.1	50.3 ± 0.1
Education, ≥college	35.4	40.3	43.9	45.4	44.2	16.3	20.0	23.5	27.0	27.8
Income, ≥3,000 USD	37.6	44.5	49.5	51.0	51.3	29.2	36.6	42.0	46.3	46.2
Married	94.1	94.3	95.0	94.0	94.3	82.3	86.4	88.0	89.1	90.5
Seoul resident	32.8	37.9	39.5	38.8	36.3	30.2	33.7	35.7	37.3	34.5
Current smoker	44.0	42.6	41.4	40.1	37.7	1.2	1.1	1.2	1.3	1.4
Past smoker	24.4	28.8	31.7	33.8	37.8	1.9	1.9	2.4	2.3	2.9
Current drinker	60.1	70.7	76.4	77.0	79.6	19.6	27.6	32.3	35.8	38.6
Past drinker	10.9	7.8	6.0	5.4	5.4	2.2	1.8	1.7	1.8	1.8
Regular exercise	39.4	36.0	36.0	34.4	33.5	38.1	37.1	36.1	35.2	33.1
BMI, ≥25.0 kg/m^2	36.7	37.8	39.4	41.3	43.9	30.1	28.5	27.6	27.1	26.9
History of disease										
Cancer	3.6	2.4	1.9	1.7	1.6	5.6	4.4	3.2	3.0	2.7
Hypertension	54.5	53.2	51.8	52	50.9	44.5	38.9	36.1	33.3	32.2
Diabetes	12.9	11.7	10.5	9.6	10.7	7.9	5.8	5.4	4.7	4.6
Metabolic syndrome	20.4	19.8	20.0	20.2	20.5	23.6	19.5	17.9	16.0	15.6
CKD	7.5	6.5	5.6	5.6	5.3	7.5	6.5	5.6	5.6	5.3
Heart disease	1.5	0.9	0.7	0.8	0.6	0.8	0.4	0.3	0.3	0.2
Disease score, 1	6.8	5.0	4.1	3.8	3.0	6.8	5.0	4.1	3.8	3.0
Total deaths, *n*	566	424	372	396	308	395	278	264	228	223
Cancer deaths, *n*	251	199	176	191	135	198	152	151	137	130
CVD deaths, *n*	86	63	65	69	50	62	40	36	35	33
Energy intake, Kcal/d, mean	1569.8	1682.1	1801.6	1941.8	2214.9	1423.5	1,528	1634.5	1766.8	2044.3
Total meat, g/d, median	12.7	28.2	44.3	68.7	123.1	6.3	17.5	29.8	48.8	94.3
Menopausal women						77.3	68.0	60.6	55.4	51.5
Hormonal therapy user						4.5	4.2	3.9	4.0	3.7
Oral contraceptive user						82.0	82.5	83.5	84	84.6

Values are % unless otherwise indicated. CKD, chronic kidney disease.

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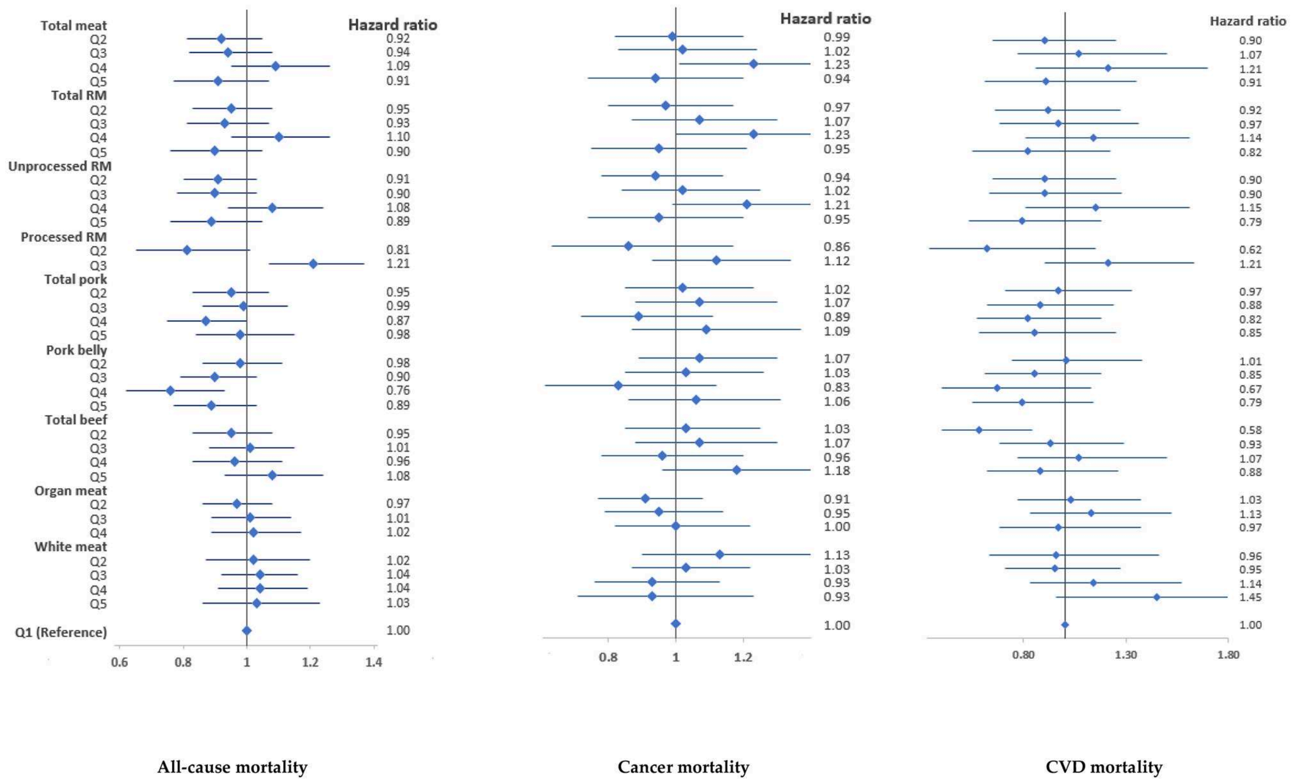

FIGURE 2
Associations of meat intake with all-cause and cause-specific mortality in the HEXA-G study in men. Points are hazard ratios, and lines represent 95% confidence interval. Models were adjusted for baseline age, marital status, education, income, job, smoking, drinking, regular physical exercise, total energy intake, total meat intake (for specific meat types), history of chronic diseases, and body mass index. RM, Red meat. 95% confidence intervals, sample sizes and person years are shown in Supplementary Table 3.

the highest quartiles of total meat intake were current drinkers, less likely to engage in regular exercise and had a higher BMI in men and low BMI in women (Table 1).

Total energy intake was high in the highest quintile of meat intake. The proportion of individuals with chronic diseases, menopausal women, and users of postmenopausal hormonal therapy was low among highest consumers of total meat (Table 1). In addition, vegetable, fish, and sea food intake were high among highest consumers of total meat (Supplementary Table 1). The distribution of meat intake by sex are displayed in Supplementary Table 2. The median intake of total meat was 44.3 g/day in men and 29.7 g/day in women. Red meat contributed over 90% to total meat intake, and 98% of red meat intake was unprocessed in both genders.

Total meat, total red meat or white meat intake was not associated with all-cause mortality in both men and women (Figures 2, 3). However, high intake of processed red meat was positively associated with all-cause mortality in men [hazard ratio (HR) 1.21, 95% confidence interval (95% CI) 1.07–1.37] and women (HR 1.32, 95% CI 1.12–1.56). Moderate intake of pork belly was inversely associated with all-cause mortality (men, HR 0.76, 95% CI 0.62–0.93; women, HR 0.82, 95% CI 0.69–0.89) (Figures 2, 3), but high intake was positively associated with CVD mortality in women (HR 1.84, 95% CI 1.20–2.82) (Figure 3). There was a low risk of CVD mortality in men with low beef intake (HR 0.58, 95% CI 0.40–0.84). However, women with high intake of organ meat/meat viscera had a high risk of all-cause (HR 1.21, 95% CI 1.05–1.39) and cancer mortality (HR 1.24, 95% CI) 1.03–1.50 (Figure 3). These associations persisted in sensitivity analyses (Supplementary Tables 7, 8).

When the analyses were extended to meat intake according to preparation method, a high risk of cancer mortality was observed in women with the highest intake of roasted pork (HR 1.26) (Table 2).

4. Discussion

Although the associations of meat intake with mortality, particularly red meat and processed meat are well recognized in Western populations where meat intake is a dominant part of the diet, the relationship between meat intake and mortality in Asian populations that have low absolute meat intake and diverse meat preparation methods is not well characterized. In this large cohort from an Asian population, the novel aspect is the evaluation of meat intake by type, and preparation method in relation to total and cause-specific mortality.

High intake of processed red meat was positively associated with all-cause mortality in men and women, and high intake of organ meat was positively associated with all cause and cancer

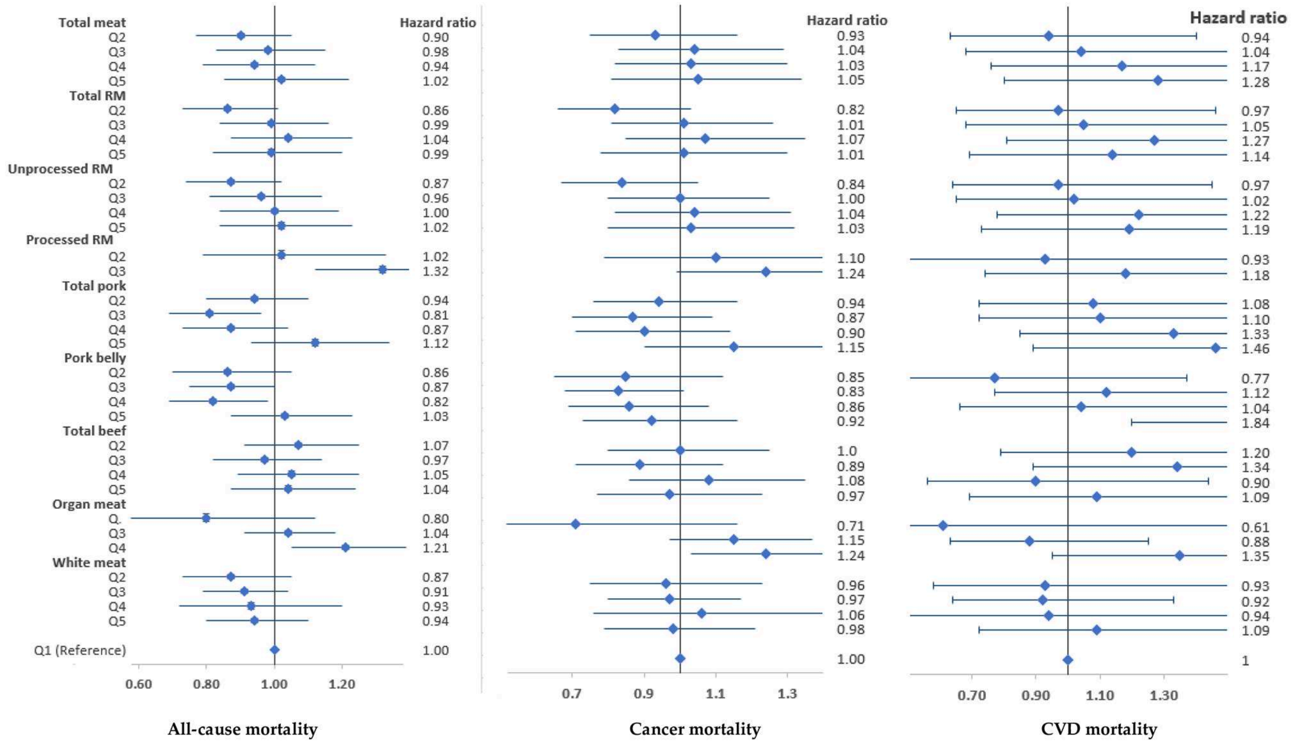

FIGURE 3
Associations of meat intake with all-cause and cause-specific mortality in the HEXA-G study in women. Points are hazard ratios, and lines represent 95% confidence interval. Models were adjusted for baseline age, marital status, education, income, job, smoking, drinking, regular physical exercise, total energy intake, total meat intake (for specific meat types), history of chronic diseases, body mass index, and menopausal status. RM, Red meat. 95% confidence intervals, sample sizes and person-years are shown in Supplementary Table 4.

specific mortality in women. However, moderate intake of pork belly was inversely associated with all-cause mortality, but high intake was positively associated with a high risk of CVD mortality in men. Moreover, low beef intake was inversely associated with CVD mortality in men. Considering different meat dishes, roasted pork ribs and pork belly were associated with an increased risk of cancer mortality in women. No evidence of association was reported with white meat.

Total meat was not associated with all-cause mortality in both men and women in line with pooled data (13) and previous meta-analyses in Asian populations (7, 11). Thus, it has been suggested that meat types should be treated separately when analyzing their health effects (7, 11). Total red meat intake was inversely associated with all-cause mortality using pooled data from Asian studies (13) but was not associated with mortality in our analysis. In the former, the study did not distinguish processed from unprocessed red meat, and failed to account for differences in preparation methods. In previous studies, the low intake of red meat and processed meat in Asia has been suggested as one of the possible explanations for null or inverse associations between meat intake and health outcomes (11, 26, 27), with some authors arguing that the consumption of processed and red meat in Korea is not a cause for health concerns. Yet, even in the Adventist community with low intake of meat, the intake of red and processed meat was associated with all-cause and CVD mortality (4). In the current study using data from the Korean population with low meat intake, processed red meat was positively associated with all-cause mortality suggesting that processed meat may have detrimental health effects even at lower intake amounts. The positive association of processed meat with all-cause and CVD mortality is widely reported in Western populations (4, 6, 10–12, 27, 28). Processed, but not unprocessed red meat intake was associated with total and CVD mortality using data from 21 countries (9), and with all-cause mortality in two meta-analyses (27, 28).

Iron mutagens generated by high-temperature cooking (29, 30), N-nitroso compounds formed in processed meat and endogenously from heme iron (31, 32) are some of the mechanisms that may explain the detrimental health impacts of processed red meat. In animal studies, metabolism by intestinal microbiota of dietary L-carnitine-a trimethylamine abundant in red meat, also produces Trimethylamine Oxide (TMAO) and accelerates atherosclerosis (33). Processed red meats are also high in food additives especially nitrite/nitrates. Nitrates/nitrites in processed meat mediated up to 72% of the association between processed meat intake and mortality (10).

Moderate intake of pork belly was inversely associated with all-cause mortality, and low intake of beef appeared to be protective against CVD mortality in men. These associations could be attributed to beef preparation methods in the Korean population. Meat is consumed roasted or in soup or stews, preferably with

TABLE 2 Meat intake, all-cause mortality and cause-specific mortality according to meat preparation methods in the HEXA-G study in men.

	All-cause mortality			Cancer mortality		CVD mortality	
Men	Person-years	Deaths	HR (95% CI)	Deaths	HR (95% CI)	Deaths	HR (95% CI)
Roasted pork	89,519	362	0.95 (0.82–1.09)	163	0.92 (0.75–1.13)	57	0.81 (0.57–1.14)
Braised pork	100,294	416	1.06 (0.94–1.19)	197	1.12 (0.94–1.33)	69	1.05 (0.78–1.42)
Beef steak	106,160	448	0.97 (0.86–1.10)	221	1.06 (0.89–1.27)	66	0.83 (0.60–1.13)
Beef soup	126,615	483	1.00 (0.89–1.13)	225	0.93 (0.78–1.11)	85	1.01 (0.76–1.36)
Beef in vegetable soup	85,649	404	1.01 (0.88–1.15)	136	0.99 (0.81–1.20)	69	1.12 (0.80–1.57)
Fried chicken	131,345	505	1.02 (0.90–1.17)	163	0.96 (0.79–1.17)	94	1.25 (0.91–1.72)
Women							
Roasted pork	240,774	362	1.10 (0.95–1.26)	222	1.26 (1.05–1.52)	53	1.16 (0.81–1.67)
Braised pork	327,391	191	1.05 (0.93–1.19)	284	1.08 (0.92–1.26)	66	1.01 (0.74–1.38)
Beef steak	190,597	283	0.98 (0.84–1.14)	168	0.99 (0.81–1.20)	39	1.12 (0.75–1.67)
Beef soup	340,717	207	1.04 (0.93–1.17)	328	1.06 (0.91–1.23)	79	0.99 (0.74–1.33)
Beef in vegetable soup	137,751	227	1.02 (0.86–1.20)	207	1.06 (0.85–1.32)	29	0.96 (0.61–1.50)
Fried chicken	209,790	280	0.93 (0.79–1.09)	178	0.96 (0.77–1.20)	41	1.07 (0.70–1.62)

HR, (hazard ratios) are for highest vs. lowest intake of meat, CVD, cardiovascular disease. Models were adjusted for baseline age, marital status, education, income, job, smoking, drinking, regular physical exercise, total energy intake, total meat intake, history of chronic diseases, body mass index, and menopausal status in women. HR, hazard ratio. 95% confidence intervals, sample sizes and person-years are shown in Supplementary Tables 5, 6.

soybean paste. Beef is popularly prepared as "Bulgogi"- grilled beef flavored with garlic, onions, soy sauce, and sesame oil; and pork is prepared by steaming, stewing, boiling, or smoking (16). In Korea, pork is the most consumed red meat and there is a unique preference for pork belly ("*Sam-gyeop-sal*") among Korean consumers (34). In Western countries, pork belly is primarily cured and processed as bacon, but consumers in South Korea favor grilled or roasted bellies rather than cured or processed bacon (16). Thus, pork belly consumed in South Korea is lower in saturated fat than that consumed in Western countries due to different preparation methods (34). It should also be noted that consumption of pork belly and beef are common at social gatherings among the middle- and high-income class in the Korean society. Thus, the consumption of these meats could reflect high socio-economic status and high social capital in the Korean population. Our results suggest that moderate intake of unprocessed pork belly and beef may offer protective benefits against premature mortality.

When we considered meat preparation methods, the intake of roasted pork increased the risk of cancer mortality in women. Iron mutagens generated by high-temperature cooking are possible explanations of these associations (29, 30). In addition, direct frying or grilling of meat generates mutagenic Heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) (30) which have been linked to the development of several cancers (35–40).

Results from a recent meta-analysis reported a 6% reduction in all-cause mortality with high intake of white meat and a null association with CVD-mortality in Asian populations (7). Furthermore, a recent study from Japan reported a reduced risk of cancer mortality with increased intake of chicken in men (14). In the NIH-AARP Diet and Health Study, white meat intake was inversely associated with all-cause and cause-specific mortality (10). Poultry meat contains more unsaturated fat, and has a lower content of saturated fatty acids, heme iron, glycotoxins and sodium, which may be involved in oxidative stress, and atherosclerosis (10, 31, 32, 41). Unlike red meat, white meat does not form N-nitroso compounds (42, 43), and it has been suggested that this could possibly explain the inverse association between white meat intake and mortality risk. Our finding that white meat intake is not associated with mortality does not agree with pooled data from Asian studies that showed that chicken intake was inversely associated with reduced risk of all-cause mortality in men (13), but agrees with several studies that reported a null association between white meat intake and all-cause or CVD mortality (6, 9, 11, 12).

Several limitations should be considered while interpreting these findings. The observational study design precludes confirmation of causal relationships. The inclusion of old adults limits generalizability of our findings to young individuals. The possibility of residual confounding cannot be ruled out even though we adjusted for multiple confounders. We relied on a single dietary assessment at recruitment, and changes in meat intake over time were not evaluated. Nevertheless, we conducted sensitivity analyses by excluding participants with shorter follow-up durations. Dietary data were self-reported, which could have introduced measurement error and biased our results toward the null.

The main strengths of our study include the use of a large sample size from a population-based survey which increases generalizability to the Korean population, the comprehensive evaluation of meat intake by type, cooking and degree of processing in a less-studied population with low meat intake, the prospective study design, adjustment for potential confounding variables, use of a validated SQFFQ, and conducting several sensitivity analyses.

5. Conclusion

This study highlighted the unique features of meat consumption patterns in the Korean population, and that the type of red meat, and the preparation methods should be considered in future studies and in designing public health guidelines pertaining to meat intake in this population. The results suggested that processed red meat increased mortality risk from all-causes in men and women, and high intake of organ meat is positively associated with all-cause and cancer specific mortality in women. However, moderate intake of pork belly was inversely associated with all-cause mortality, and low beef intake was inversely associated with CVD mortality in men. Considering different meat dishes, roasted pork ribs and pork belly were associated with increased risk of cancer in women. No evidence of association was reported with white meat.

Data availability statement

The datasets presented in this article are not readily available because the dataset used for the analysis in this study is maintained and managed by the Division of Population Health Research at the National Institute of Health, Korea Centers for Disease Control and Prevention. The Health Examinees Study dataset has been merged with the cancer registry data provided by National Cancer Center of Korea in a collaborative agreement. It contains some personal data that may potentially be sensitive to the patients, even though researchers are provided with an anonymized dataset that excludes resident registration numbers. Other researchers may request access to the data by contacting the following individuals at the Division of Population Health Research, National Institute of Health, Korea Centers for Disease Control and Prevention: Requests to access the datasets should be directed to Dr. Kyoungho Lee (khlee3789@korea.kr).

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Korean Health and Genomic Study of the Korean National Institute of Health and the Institutional Review Boards of all participating hospitals (IRB no. E-1503-103- 657). The patients/participants provided their written informed consent to participate in this study.

Author contributions

AK, S-AL, and DK designed the research. AK conducted the research, analyzed the data, and wrote the paper. S-AL and DK had primary responsibility for final content. All authors 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.

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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.2023.1138102/full#supplementary-material

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Association between the prudent dietary pattern and blood pressure in Chinese adults is partially mediated by body composition

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High blood pressure or hypertension is one of the major risks of cardiovascular disease, which is the leading cause of death in China. This study aimed to assess the relationship between dietary patterns and blood pressure among Chinese adults. Using factor analysis of 66-item food frequency questionnaire to identify dietary patterns. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured according to standardized guidelines. Multivariate linear regressions were performed in 6849 Chinese adults (46.5% female) aged 21–70 years considering sociodemographic characteristics, lifestyle behaviors, and anthropometry data. The vegetable-rich pattern, animal-food pattern, and prudent dietary pattern were identified. After adjustment for potential confounders including age, gender, alcohol consumption, smoking status, energy intake, and physical activity, only prudent dietary pattern was negatively related to SBP (β =−2.30, p for trend =0.0003) and DBP (β =−1.44, p for trend =0.0006). Body mass index, waist circumstance and body fat percentage explained, respectively, 42.5%/47.8, 14.8%/17.6 and 26.0%/29.1% of the association between prudent pattern and SBP/DBP in mediation analysis. There were no association were observed between other dietary patterns and blood pressure. In conclusion, Prudent dietary pattern was associated with lower SBP and DBP among Southwest Chinese and this association was partially explained by body composition.

KEYWORDS

blood pressure, dietary pattern, factor analysis, body mass index, mediation effect

Introduction

Hypertension is the primary risk factor for cardiovascular disease (CVD) (1), which is the leading cause of death in China (2). Recently, the prevalence of hypertension in China increased from 24.9% in 2004 to 38.1% in 2018 (3), and the prevalence was as high as 44.7% among adults aged 35–75 from 2014–2017 (4). In addition, the awareness, treatment, and control rates of hypertension are at a relatively low level (3, 4), increasing the burden of CVD morbidity and mortality. Thus, an improved understanding of hypertension prevention and control from the perspective of modifiable risk factors is of significant public health interest (5). Accumulating studies have suggested that diet is an established risk factor influencing blood pressure (6, 7).

Besides these associations of individual nutrients or foods and food groups with blood pressure (8–11), clinical trials found that total dietary patterns, which describes the combinations of foods and nutrients consumed in totality by individuals, were better on lowing blood pressure (12). Recent observation studies also found that dietary patterns were associated with the prevalence of hypertension (13–18), for instance, traditional or western dietary pattern was associated with high blood pressure. However, these previous studies among Chinese adults have been based on earlier data (from 2002 to 2013) (16–18) or have limited study sample (ranging from 2,518 to 3,591) (16, 17), discouraging the optimal practical value for such evidence. An updated investigation on dietary pattern and blood pressure based on a larger sample of Chinese adults is urgently needed.

Notably, body composition, indicated body mass index (BMI), waist circumstance (WC), and percentage of body fat (BF%) could be attributed to dietary patterns and simultaneously has been indicated to be positively associated with blood pressure (19–21). In that way, body composition might confound or mediate the impact of dietary pattens on hypertension. For example, Shi et al. found the association between dietary pattern and hypertension was attenuated after the addition of BMI to the model (22). Livingstone et al. reported the relevance of dietary pattern with hypertension was stronger in individuals with overweight/obese (23). A recent study considered the effect of the Dietary Approaches to Stop Hypertension (DASH) diet for hypertension may be fully influenced by BMI (24). These studies suggest the relationship between diet pattern, body composition, and hypertension is complex and further research is needed. Over the past decades, China has been under a drastic transition in the diet, leading to unfavorable changes in dietary pattern characterized with increasing consumption of animal-source food, oils, and sugar-sweetened beverages (25). Given the emerging epidemic of hypertension as well as obesity, it is valuable to consider the potential effect of body composition in the dietary pattern-blood pressure association.

Therefore, using the cross-sectional data 2013 to 2018 from a population-based cohort among Southwest Chinese adults, we aimed to determine the association between dietary patterns derived by factor analysis and blood pressure and investigate the possible mediation effect of body composition.

Methods

Study sample

We obtained data from the Nutrition and Health in Southwest China (NHSC) 2013–2018 baseline survey to investigate the role of diet, lifestyle, genetic background, and their interactions on non-communicable diseases among Chinese adults. Details on study protocol have been described previously (26). In brief, 54 study sites (23 communities and 31 villages) from Sichuan, Guizhou, Yunnan provinces were included until 2018. At each site, we used a 2-stage (household person) sampling. Individuals who accepted the invitation to participate were invited to the study center for interviews. All assessments of participants in the NHSC Study have included questionnaires, anthropometric measurements, medical examinations, biochemical measurements, and face-to-face interviews by trained investigators about nutrition-related behaviors, lifestyles, and social status. To facilitate follow-up, participants who volunteered to participate, and signed an informed consent form were included in the final study. The study was approved by the Research Ethics Committee of Sichuan University. All participants had signed informed written consent.

This cross-sectional study used the data of 7,858 adults. Among them, adults with incomplete information regarding blood pressure data or other confounding variables ($n = 497$), with implausible energy intake (i.e., >4,200 or < 800 kcal/d for men and > 3,500 or < 500 kcal/d for women) (27) ($n = 125$), and with diagnosed cardiovascular diseases or hypertension at baseline ($n = 387$) were excluded. Finally, 6,849 participants (3,664 men and 3,185 women) aged 21–70 y were included in the present analysis (Figure 1).

Dietary intake data

Usual dietary intake information during the previous year was obtained from 66-item food frequency questionnaire (FFQ), this FFQ was developed based on the validated questionnaire (28, 29) that was used in the 2002 China National Nutrition and Health Survey, and be additionally modified added a few individual food groups, e.g., coffee (30) and tea (31), as their suspected biological effects; split some food groups into more clearly precise categories, e.g., wheat flour products were split into noodles, steamed rolls, dumplings and steamed stuffed bun. Participants were asked to recall the frequency of the consumption of each food item using daily, weekly, monthly, annually, or never and the estimated portion size, using local weight in China, i.e., 1 liang = 50 g or natural units, e.g., bowls. To enhance the accuracy of the estimated serving sizes, standard tableware including bowls, plates, and glasses were provided. Total energy intake and nutrients intake were assessed based on the China Food Composition Table (32).

Dietary pattern derivation

The food items from FFQ were categorized into main food groups based on similar sources, nutrient profiles (32), or hypothesized biological effects. As a result, 24 food groups were entered into the analysis in absolute weights (Table 1). Factor analysis was conducted in the SAS software (version 9.4, SAS Institute Inc., Cary, NC, United States.) with the PROC FACTOR procedure to identify dietary patterns. Kaiser-Meyer-Olkin test was 0.67 and the Bartlett's test of sphericity reaching statistical significance ($p < 0.0001$) indicated suitability of food intake data for factor analysis. The factors were orthogonally rotated to achieve a simpler structure with greater interpretability. Three factors (dietary patterns) to be retained were determined based on eigenvalues >1, the inspection of scree plot, and the interpretability of factors. Factor loadings reflect the correlations of each food group with the corresponding dietary pattern. Food groups with factor loadings ≥0.30 or ≤ −0.30 were considered as the most important contributors to each factor (33), and thus descriptive of that dietary pattern. Furthermore, the factor score for each pattern was constructed by summing standardized intakes of each food group weighted by their factor loadings, so that higher factor scores indicated better adherence to dietary patterns.

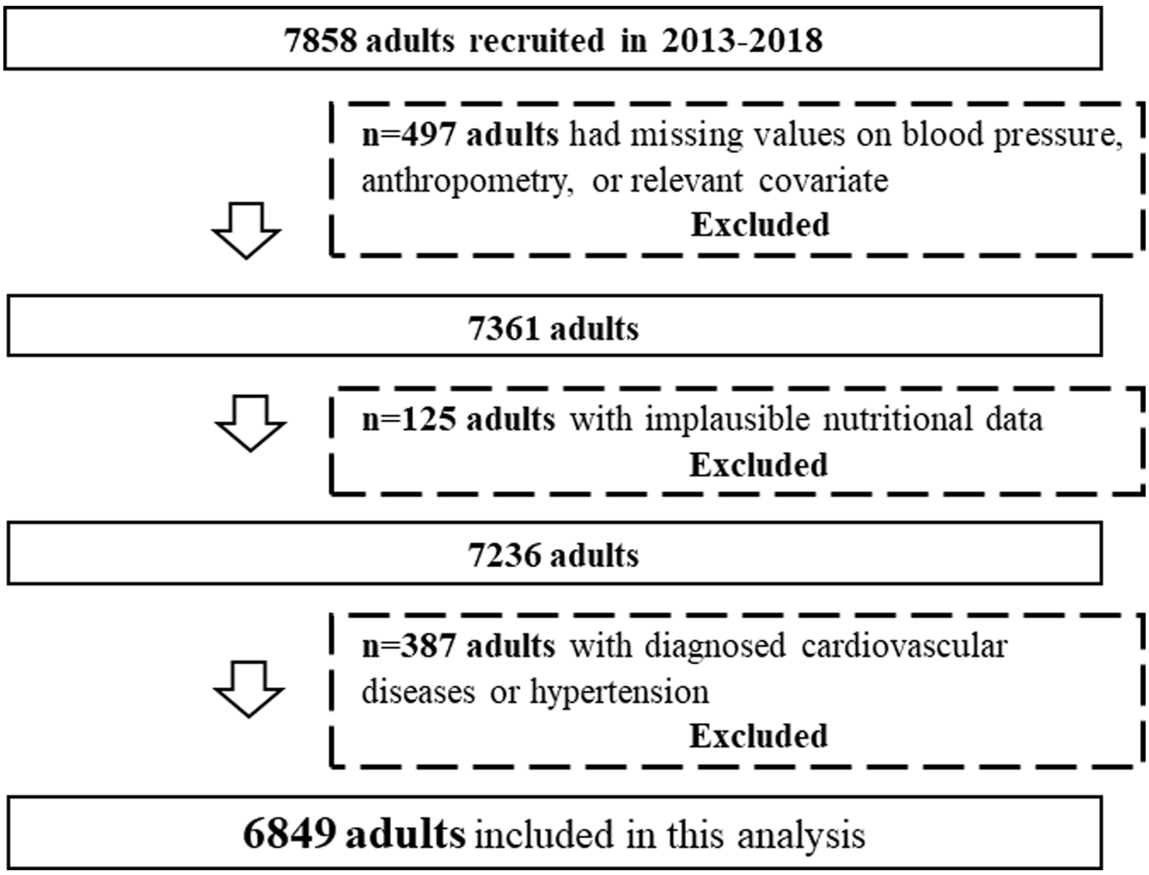

FIGURE 1
Flowchart for the study sample.

Blood pressure measurement

Participants' systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice by trained nurses with a standard mercury sphygmomanometer in their right arm, after a rest for about 5–10 min in the sitting position. SBP and DBP were recorded for each participant. If the difference in SBP and/or DBP between the first and second measurement was larger than 5 mmHg, the third measurement was taken. The average SBP and DBP were calculated from the nearest two values.

Covariates

Detailed information on participants' socio-demographic characteristics such as sex, age (years), and lifestyle behaviors including smoking status (current smokers, former smokers who quit smoking or non-current smokers who never smoked), alcohol consumption (yes: at least once in the past year; or no) was collected from a questionnaire-based interview. The physical activity was estimated in metabolic equivalents-hours per week (MET-hours/week) from moderate-to-vigorous physical activity (MVPA) (34).

Anthropometric measurements were performed by trained investigators according to standard procedures, with the subjects dressed lightly and barefoot. Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, using an Ultrasonic instrument (Weight and Height Instrument DHM-30; Dingheng Ltd., Zhengzhou Province, China). WC was measured at a point midway between the lowest rib margin and the iliac crest in a horizontal plane using non-elastic tape to the nearest 0.1 cm. All anthropometric measurements were performed twice for each participant. BMI was calculated as weight in kilograms divided by height in meters squared of the individual. %BF was calculated from BMI and WC using the equations from Liu, X. et al. (35) Body composition data were used as continuous variable in this analysis.

Statistical analysis

All statistical analyses were conducted using SAS procedures (version 9.4, SAS Institute Inc., Cary, NC, United States.) and R version 4.1.1. Results were considered statistically significant when a two-sided *value of p* <0.05.

Dietary pattern scores were grouped into tertiles (T1, T2, and T3) to obtain three categories indicating a low, moderate, and high adherence to a dietary pattern. Socio-demographic characteristics were described across tertiles in each dietary pattern. Difference in continuous variables was estimated using the generalized linear model, and categorical variables were compared by the chi-square test. To further investigate the nutrient composition of each dietary

TABLE 1 Food or food groups used in the dietary pattern analysis.

Food or food groups	Food items
Rice and its products	Rice, brown rice, black rice, sticky rice, rice noodles
Noodles	Noodles, pasta
Wheat and its products	steamed bun, steamed stuffed bun
Whole grains products	Graham Bread, multigrain biscuits
Ethnic foods	Tangyuan, spring rolls, mooncake, mung bean cake
Corn	Corn
Tubers	Potato, cassava, taro, yam
Sweet potato	Sweet potato
Legumes and its products	Dried legumes, tofu, soya-bean milk, dried bean curd, mung bean, red bean
Dark Vegetables	Leafy and flowering vegetable, aquatic vegetable
Light-colored vegetables	Root vegetable, leguminous vegetable and sprout, cucurbitaceous and solanaceous vegetable
Fungi and algae	Mushroom, agaric, tremella, laver, sea-tangle
Fruits	Kernel fruit, drupe fruit, berry, orange fruit, tropic fruit, melons
Nuts	Walnut, melon seeds, cashew, hazelnut, almond, pistachio
Meat and its products	Pork, beef, mutton, rabbit meat, processed pork, sausage
Poultry and its products	Chicken, duck, goose, turkey, pigeon
Animal organ	Animal heart, animal kidney, animal liver, animal lung
Fish and shellfish	Fish, shrimp, crab, shellfish
Eggs	Chicken eggs, duck eggs, goose eggs, partridge eggs
Preserved eggs	Salt eggs, year eggs
Pickled vegetables	Fermented soybean curd, salted vegetables, Chinese sauerkraut
Dairy products	Milk, dried milk, yoghurt, cheese
Tea and coffee	Green tea, black tea, oolong, coffee
Beverages	Carbonated drink, fruit juice, Yakult

pattern, linear correlations were performed with confounders in regards to the association between nutrients intakes and dietary patterns including age, gender, and energy intake.

We used multivariate linear regression models (PROC GLM procedure in SAS software) to explore the relationship of dietary pattern scores (continuous variable) with SBP and DBP. Three models were used in our study: model 1 was adjusted for age and gender, Model 2 was further adjusted for smoking status, alcohol consumption, energy intake and physical activity, and models 3–5 was additionally adjusted for body composition (In order, BMI, WC, BF%) to determine if the effects were independent of body size.

Furthermore, we created linear models to measure the association between dietary pattern scores (exposure), blood pressure (outcome), and body composition (mediator). Mediation analysis was performed only if the mediator variable was significantly associated with both the exposure and outcome. Mediation by body composition was estimated using the mediation R package (36) to calculate the average direct effect (ADE), the average causal mediation effect (ACME), the total effect (TE), and the proportion of mediated effect (PME). This effect is estimated by conducting 10,000 simulations using a quasi-Bayesian Monte Carlo method based on normal approximation (37).

Results

The three main dietary patterns identified by factor analysis and the factor loadings for each dietary pattern are presented in Table 2. Factor 1 was characterized by vegetables, tubers, nuts, corn, legumes and its products, and was named "the vegetable-rich pattern." The "animal-food pattern" (factor 2) was loaded heavily for poultry and its products, meat and its products, animal organ, beverages, fish and shellfish, wheat and its products and rice and its products. Factor 3 (the "prudent dietary pattern") was marked by high intakes of fruits, whole grains products, dairy products, eggs, wheat and its products, and ethnic foods, with inverse loading for rice and its products. These three dietary patterns explained 24.6% of the total variation in dietary intake (11.1%, 6.9%, and 6.6% for factor 1, factor 2, and factor 3, respectively).

General characteristics of study participants according to tertiles of each dietary pattern are presented in Table 3. The mean age of the study population was 45.0 ± 13.7 years. 46.5% of the study participants were female, with mean values of SBP and DBP being 123.7 ± 16.5 and 78.1 ± 10.6 mmHg, respectively. The vegetable-rich pattern was positively associated with age, SBP, and DBP. Participants with a higher score for vegetable-rich pattern were more likely to drink alcohol compared with those with a lower score. There were no associations between vegetable-rich pattern and gender, BMI, WC, BF% or smoking status. Animal-food pattern score was inversely associated with age and BF%, and positively associated with BMI and WC. Participants with a higher score for the animal food pattern were more likely to smoke or drink alcohol than participants with a lower score. Prudent dietary pattern was inversely associated with BMI, WC, SBP, DBP, and a greater percentage of not current smokers. All three patterns were positively correlated with energy intake.

Nutrient intakes across tertile of each dietary pattern are shown in Table 4. A higher vegetable-rich pattern score was associated with a higher protein, potassium, calcium, fiber, and most vitamins, including Vitamin A, Vitamin B1, Vitamin B2, Vitamin B3, Vitamin C, and Vitamin E. A higher animal food pattern score was associated with a higher intake of protein and vitamin A. The carbohydrate, calcium, Vitamin B1, Vitamin C, Vitamin E, and Fiber intake were significantly lower as the animal food pattern scores increased. Prudent pattern score was positively correlated with carbohydrate, potassium, calcium, Vitamin B1, Vitamin B2, Vitamin C, Vitamin E, and fiber, and inversely correlated with fat, protein, and vitamin B3.

Table 5 presents the associations of tertiles of each dietary pattern with SBP and DBP. Multiple linear regression analysis showed that prudent dietary pattern was inversely related to SBP ($\beta = -1{,}73$, p for trend =0.004) and DBP ($\beta = -1.20$, p for trend =0.002) after

TABLE 2 Orthogonally rotated factor loadings for three dietary patterns derived from factor analysis[a].

Food or food groups	Factor 1	Factor 2	Factor 3
	Vegetable food	Animal food	Prudent
Rice and its products	0.25	**0.32**	**−0.39**
Noodles	0.20	0.06	−0.04
Wheats and its products	0.14	**0.34**	**0.38**
Whole grains products	0.04	0.08	**0.47**
Ethnic foods	0.03	0.04	**0.35**
Corn	**0.41**	−0.14	0.04
Tubers	**0.55**	0.06	0.10
Sweet potato	**0.35**	−0.20	−0.16
Legumes and its products	**0.41**	0.25	0.03
Dark Vegetables	**0.65**	0.11	−0.01
Light-colored vegetables	**0.54**	0.20	0.09
Fungi and algae	**0.37**	0.29	0.29
Fruits	0.16	−0.02	**0.48**
Nuts	**0.42**	−0.22	0.00
Meat and its products	0.18	**0.56**	−0.28
Poultry and its products	0.08	**0.67**	0.11
Animal organ	−0.04	**0.51**	−0.03
Fish and shellfish	0.15	**0.46**	0.07
Eggs	0.28	−0.04	**0.38**
Preserved eggs	0.20	0.09	0.14
Pickled vegetables	0.19	0.18	−0.28
Dairy products	0.01	−0.06	**0.46**
Tea and coffee	0.14	0.01	0.06
Beverages	−0.15	**0.50**	0.12
% Variance explained	11.1%	6.9%	6.6%

[a]Factor loadings ≥ 0.30 or ≤ −0.30 are bolded.

adjustment for age and gender (model 1). Further adjustment for smoke state, alcohol consumption, energy intake and physical activity (model 2) did not change these inverse associations (for SBP, β = −2.30, p for trend =0.0003; for DBP, β = −1.44, p for trend =0.0006). In models 3–5, the strength of relation between prudent dietary pattern score with SBP (β = −1.35, p for trend =0.03, model 3; β = −1.65, p for trend =0.007, model 4; β = −1.89, p for trend =0.02, model 5) and DBP (β = −0,79, p for trend =0.045, model3; β = −0.99, p for trend =0.01, model 4; β = −1.14, p for trend =0.004, model 5) was attenuated but remained significant with additional adjustment for body composition. While the vegetable-rich pattern and animal-food pattern were not associated with either SBP and DBP in all models (all $p > 0.05$).

Since the strength of the association between prudent dietary pattern scores with SBP and DBP was attenuated by additional adjustment for body composition., we conducted a mediation analysis between prudent dietary pattern, body composition., and blood pressure (Figure 2). The results of mediation analyses indicated that BMI WC and BF% partially mediated the association between prudent dietary pattern and blood pressure, respectively. 42.5%/47.8% of the association between prudent dietary pattern scores and SBP/DBP was mediated by BMI (Figure 2A). In Figure 2B, the WC contributed to 14.8%/17.6% of the total effect of prudent dietary pattern on SBP/DBP. The between prudent dietary pattern scores and SBP/DBP was explained 26.0%/29.1% by BF% (Figure 2C).

Discussion

In this sample of Chinese adults, we identified the prudent dietary pattern, vegetable-rich pattern, and animal-food pattern, among them, only the prudent pattern was found to be inversely associated with SBP and DBP. In addition, BMI, WC and BF% contributed to, respectively, 42.5%/47.8, 14.8%/17.6 and 26.0%/29.1% of these associations of prudent pattern with SBP/DBP.

Compared with studies focusing on single nutrients or foods, our findings about the negative association between the prudent pattern with blood pressure have a more direct public health implication. Each unit increase in prudent pattern scores was associated with a 2.30 mmHg lower SBP and 1.44 mmHg lower DBP after adjusted for age, gender, alcohol consumption, current smoker, energy intake and physical activity. Although this change is relatively small, it is significant on a population level, for example, a 5 mm Hg reduction in blood pressure would prevent around 200,000 deaths per year among individuals younger than 70 years of age (38) and associated with around 4% lower mortality from coronary heart disease (39). Our result was in line with the study conducted in Korean adults aged 20–64 years (40). Moreover, we tried to find some clues in the loading foods of prudent pattern, and fruits were independently associated with blood pressure (data not shown) in our sample. Additionally, high-correlated nutrients, calcium (41), potassium (42), and magnesium (43) in prudent pattern were found to play crucial roles in the prevention of hypertension. Therefore, the impact of prudent pattern on lowing DBP and SBP could be considered according to a synergistic effect of these foods and nutrients. The prudent dietary pattern characterized by higher intakes of fruits, whole grains and low intakes of red meat was similar to the DASH diet, which is a healthy eating pattern have demonstrated positive effects on blood pressure (44).

Body composition played a critical mediating role in the association between prudent dietary pattern and blood pressure. Interestingly, the mediating effect of BMI is the largest with approximately half of the total effect of prudent pattern on blood pressure (42.5% for SBP and 47.8% for DBP), which might be partly explained by the evidence that measures of general adiposity had been found to be more strongly related to blood pressure than measures of central adiposity in Chinese adults (19, 45). These findings showed an

TABLE 3 Participant characteristics according to categories of dietary patterns[a].

	All subjects	Vegetable-rich pattern			Animal-food pattern			Prudent dietary pattern		
		T1	T3	*p*	T1	T3	*p*	T1	T3	*p*
N	6,849	2,283	2,283		2,283	2,283		2,283	2,283	
Age (y)	45.0 ± 13.7	41.6 ± 13.7	47.5 ± 13.3	<0.0001	52.0 ± 13.7	38.5 ± 12.1	<0.0001	46.2 ± 11.0	44.9 ± 15.8	0.09
Gender (%)										
Female	46.5	55.5	44.7	0.4	74.2	21.1	<0.0001	42.0	51.5	0.1
Male	53.5	44.5	55.3		25.9	78.9		58.1	48.5	
BMI (kg/m^2)	24.0 ± 3.1	23.8 ± 3.2	24.2 ± 3.1	0.2	23.5 ± 3.0	24.3 ± 3.3	0.02	24.6 ± 3.1	23.1 ± 3.0	<0.0001
WC	85.6 ± 8.6	85.2 ± 8.9	85.8 ± 8.2	0.3	84.5 ± 8.3	86.5 ± 9.1	0.007	86.9 ± 8.2	83.6 ± 8.4	0.02
BF%	27.2 ± 6.6	26.7 ± 6.4	27.2 ± 6.7	0.8	30.3 ± 6.0	24.4 ± 5.7	<0.0001	27.3 ± 6.8	27.0 ± 6.4	0.1
Alcohol consumption (%)										
Yes	52.3	53.8	57.0	0.048	34.3	70.9	<0.0001	52.1	49.8	0.5
No	47.8	46.2	43.0		65.7	29.1		47.9	50.2	
Smoking status (%)										
Current smoker	23.8	25.4	21.9	0.7	8.9	38.4	<0.0001	32.2	15.6	0.0001
Non-current smoker	76.2	74.6	78.1		91.1	61.6		67.8	83.4	
Energy intake (kcal/d)	1376.0 ± 523.5	1100.3 ± 475.0	1698.8 ± 532.1	<0.0001	1154.7 ± 503.0	1687.8 ± 526.0	<0.0001	1,304 ± 489.3	1558.5 ± 586.2	<0.0001
Physical activity (MET-h/wk)	17.5 ± 9.8	14.7 ± 7.0	18.9 ± 8.4	0.08	18.9 ± 9.9	16.8 ± 7.7	<0.0001	16.8 ± 7.0	17.5 ± 9.6	<0.0001
SBP (mmHg)	123.7 ± 16.5	120.6 ± 15.6	126.0 + 16.8	0.0001	124.0 + 18.0	123.7 + 14.9	0.7	127.4 ± 17.7	120.9 ± 16.2	0.0007
DBP (mmHg)	78.1 ± 10.6	77.2 ± 10.1	79.0 ± 10.9	0.04	76.7 ± 10.2	79.2 ± 10.5	0.07	81.2 ± 10.3	76.2 ± 10.8	0.0006

[a]Data were presented as means ± SD or percent; T1, tertile 1, including individuals with lowest dietary pattern score; T3, tertile 3, including individuals with highest dietary pattern score; difference in proportions were measured by Chi-square analysis (categorical variables) or linear trends were measured by analysis of variance (continuous variables) across tertiles.
SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WC, waist circumstance; BF%, body fat percentage.

TABLE 4 Mean nutrient intakes for tertile of dietary pattern score[a].

	Vegetable-rich pattern			Animal-food pattern			Prudent dietary pattern		
	T1	T3	*p*	T1	T3	*p*	T1	T3	*p*
Carbohydrate (%E)	57.0 ± 1.2	56.2 ± 1.2	0.2	58.2 ± 1.2	54.2 ± 1.2	<0.0001	55.5 ± 1.1	56.8 ± 1.1	0.002
Fat (%E)	26.1 ± 1.1	25.5 ± 1.0	0.8	25.6 ± 1.0	26.7 ± 1.0	0.3	26.4 ± 0.8	26.3 ± 0.8	0.04
Protein (%E)	17.0 ± 0.2	18.3 ± 0.4	<0.0001	16.2 ± 0.4	19.2 ± 0.4	<0.0001	18.1 ± 0.4	17.0 ± 0.4	<0.0001
K (mg/1000 kcal)	1119.5 ± 38.8	1527.1 ± 38.9	<0.0001	1343.5 ± 43.8	1286.7 ± 45.2	0.2	1190.9 ± 39.1	1380.2 ± 39.5	<0.0001
Ca (mg/1000 kcal)	267.8 ± 15.7	314.6 ± 15.7	<0.0001	324.8 ± 15.7	259.6 ± 16.1	0.0002	223.2 ± 13.4	338.1 ± 13.5	<0.0001
Mg (mg/1000 kcal)	163.3 ± 4.9	216.7 ± 5.4	<0.0001	199.4 ± 5.0	177.5 ± 4.1	<0.0001	179.6 ± 5.4	190.5 ± 5.5	0.006
Vitamin A (mg RAE/1000 kcal)	313.3 ± 29.5	424.0 ± 29.6	<0.0001	344.5 ± 30.1	405.4 ± 30.9	<0.0001	339.6 ± 27.8	359.9 ± 28.0	0.2
Vitamin B_1 (mg/1000 kcal)	0.47 ± 0.01	0.55 ± 0.01	<0.0001	0.54 ± 0.01	0.47 ± 0.01	<0.0001	0.47 ± 0.01	0.53 ± 0.01	<0.0001
Vitamin B_2 (mg/1000 kcal)	0.62 ± 0.02	0.69 ± 0.02	<0.0001	0.67 ± 0.02	0.65 ± 0.02	0.07	0.58 ± 0.02	0.71 ± 0.02	<0.0001
Vitamin B_3 (mg/1000 kcal)	11.3 ± 0.3	12.3 ± 0.3	0.001	9.8 ± 0.3	13.8 ± 0.3	<0.0001	13.6 ± 0.3	10.1 ± 0.3	<0.0001
Vitamin C (mg/1000 kcal)	59.8 ± 5.9	103.0 ± 5.9	<0.0001	87.9 ± 6.3	74.2 ± 6.4	0.001	66.2 ± 5.6	92.6 ± 5.7	<0.0001
Vitamin E (mg/1000 kcal)	7.7 ± 0.5	9.8 ± 0.5	<0.0001	10.5 ± 0.5	7.2 ± 0.5	<0.0001	7.4 ± 0.4	9.8 ± 0.5	<0.0001
Fiber (g/1000 kcal)	7.4 ± 0.4	10.7 ± 0.4	<0.0001	9.9 ± 0.4	8.0 ± 0.4	<0.0001	7.6 ± 0.4	10.0 ± 0.4	<0.0001

[a]Values are least-squares means±SD for tertile 1 and tertile 3 of dietary pattern score, from models adjusting for age, gender, energy intake; *p*-values refer to general linear regression.
E, energy; K, potassium; Ca, calcium; Mg, magnesium.

TABLE 5 Association between dietary patterns and blood pressure[a].

	SBP		DBP	
	β (SE)	p for trend	β (SE)	p for trend
Vegetable-rich pattern				
Model 1[b]	1.06 (0.62)	0.09	0.38 (0.40)	0.3
Model 2[c]	1.12 (0.76)	0.1	0.56 (0.50)	0.3
Model 3[d]	0.98 (0.71)	0.2	0.42 (0.46)	0.4
Model 4[e]	1.29 (0.72)	0.07	0.63 (0.47)	0.2
Model 5[f]	1.30 (0.73)	0.08	0.64 (0.47)	0.2
Animal-food pattern				
Model 1[b]	0.78 (0.67)	0.2	0.50 (0.44)	0.3
Model 2[c]	0.92 (0.76)	0.2	0.73 (0.50)	0.1
Model 3[d]	0.04 (0.72)	0.9	0.12 (0.47)	0.8
Model 4[e]	−0.23 (0.73)	0.8	−0.06 (0.48)	0.9
Model 5[f]	−0.05 (0.74)	0.9	0.00 (0.48)	0.9
Prudent dietary pattern				
Model 1[b]	−1.73 (0.59)	**0.004**	−1.20 (0.39)	**0.002**
Model 2[c]	−2.30 (0.64)	**0.0003**	−1.44 (0.42)	**0.0006**
Model 3[d]	−1.35 (0.61)	**0.03**	−0.79 (0.39)	**0.045**
Model 4[e]	−1.65 (0.61)	**0.007**	−0.99 (0.39)	**0.01**
Model 5[f]	−1.89 (0.62)	**0.002**	−1.14 (0.40)	**0.004**

[a]Values are estimate (SE, standard error). Linear trends ($p_{for\ trend}$) were obtained with SBP, DBP as continuous variables. Significant data are in bold.
[b]Model 1 adjusted for age, gender.
[c]Model 2 adjusted for variables in model 1 and alcohol consumption, current smoker, energy intake, physical activity.
[d]Model 3 adjusted for variables in model 2 and BMI.
[e]Model 4 adjusted for variables in model 2 and WC.
[f]Model 5 adjusted for variables in model 2 and BF%.
SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WC, waist circumstance; BF%, body fat percentage.

independent effect of prudent pattern on reducing blood pressure, which could not be ignored in the first place, and provided evidence for diet recommendations to prevent elevated blood pressure. In addition, the disclosed mediator effect of body composition indicated that obesity status seemed to be important for the dietary recommendation to hypertension control and reinforced the need to maintain a healthy weight, WC, and body fat in public health practice. However, the underlying biologic mechanisms remains unknown, and further research are needed to address the prospective interplay among dietary patterns, body composition, and blood pressure in Chinese adults.

Although some studies have suggested that a vegan or vegetarian diet may be protective against obesity, type 2 diabetes, or CVD (46, 47), vegetable-rich pattern in our study did not significantly affect blood pressure. This discrepancy might lie in the characteristic of our sample. Participants in the highest tertile had an approximately 14% higher age compared to those in the lowest tertile, and most of the elderly tend to change their eating habits. Moreover, it cannot be denied that the efficacy of BP lowering using the vegetable-rich dietary modification was inconsistent recently (44). For animal food pattern, our finding is consistent with the results reported in the previous study (48) among Chinese population, but its positive association with blood pressure in subjects from other countries (49, 50), suggesting that the effect of the animal food pattern on blood pressure may be race specific. Further prospective research is needed to confirm the impact of these dietary patterns on blood pressure.

The strengths of our study included its representative study sample and detailed measurement of blood pressure, dietary and anthropometric measurements with the ability to adjust for several major potential confounders. Meanwhile, we considered the mediation effect of body composition to optimize public health practice. Nevertheless, our study had several limitations. Given the cross-sectional design of this study, we were unable to evaluate the causal relationship between dietary patterns and blood pressure. Future prospective cohort studies are warranted to verify our findings. In addition, there was a lack of information on foods (e.g., types of meats, beverages, cooking methods, and seasoning), which play an important role in the regulation of blood pressure (51–53), so it is difficult to characterize dietary patterns in more specific details. Furthermore, although we have adjusted for demographics and lifestyle factors, residual confounding unmeasured factors might be present.

In this study, prudent dietary pattern characterized by higher intake of fruits, whole grains products, dairy products, eggs, and wheat and its products, and lower intakes of rice and its products was associated with lower SBP and DBP among Chinese adults. This association was partially explained by body composition.

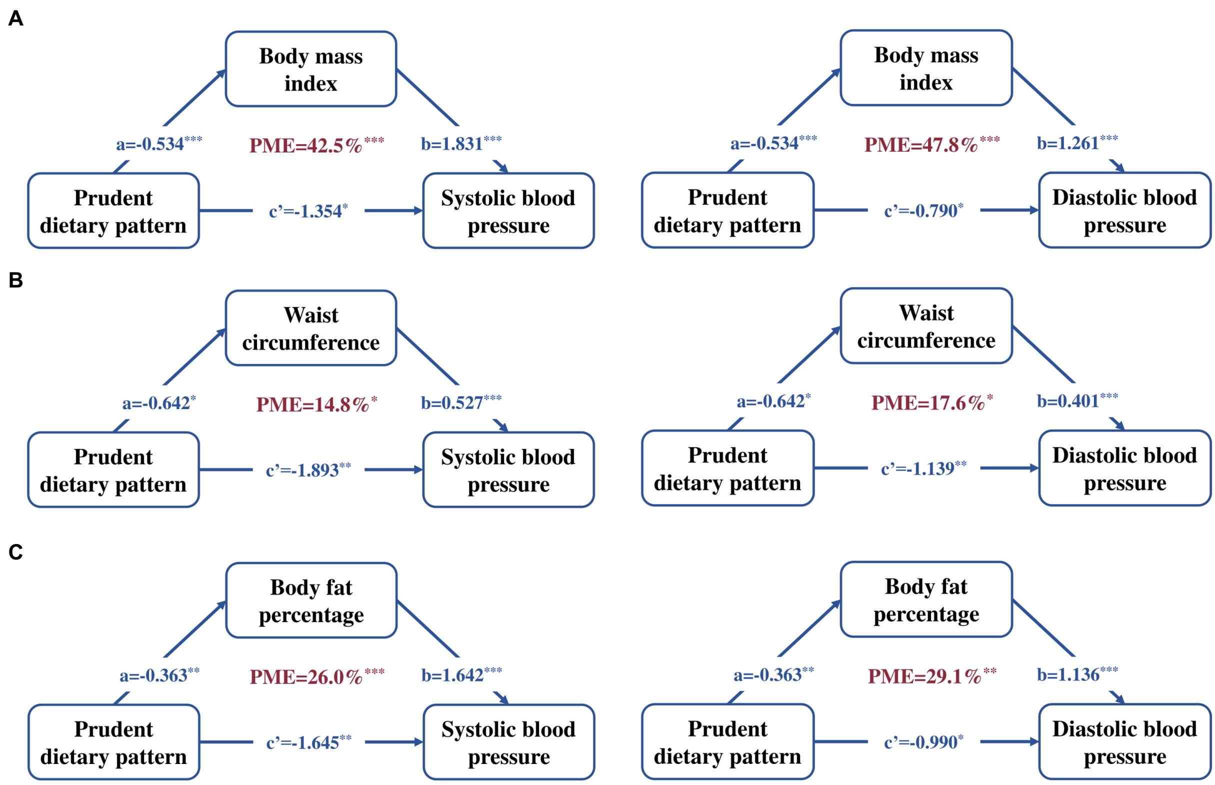

FIGURE 2
Mediation linkages among the prudent patterns scores (continuous exposure), blood pressure (continuous outcome) and body composition (continuous mediator). Adjusted for age, gender, alcohol consumption, current smoker, energy intake and physical activity. **(A)** BMI as the independent variable, **(B)** WC as the independent variable, and **(C)** BF% as the independent variable. Regression coefficients are presented, with the path a representing the effect of prudent patterns scores on body composition, path b representing the effect of body composition on blood pressure, path c' representing the direct effect of prudent patterns scores on blood pressure, The PME reflects the proportion of the total effect of the prudent patterns scores on the blood pressure that is explained by body composition. *p <0.05, **p <0.01, ***p <0.001. BMI, body mass index; DBP, diastolic blood pressure; PME, the proportion of mediated effect; SBP, systolic blood pressure; WC, waist circumstance; BF%, body fat percentage.

Data availability statement

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

Author contributions

GC conceived the project. MC and YX performed the analyses and wrote the manuscript. MC performed the initial data analyses. XW and SS coordinated the study centers. GC supervised the study. 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

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Dietary patterns and their association with cardiovascular risk factors in Ethiopia: A community-based cross-sectional study

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Purpose: To identify the dietary patterns and their association with cardiovascular risk factors among adult people in urban and rural areas of Wolaita, southern Ethiopia.

Methods: A total of 2,483 participants aged 25–64years were selected using a three-stage random sampling. Data for this study were collected using structured questionnaires, the previous 24-h dietary intake assessment, anthropometric, blood pressure, and biochemical measurements. We used factor analysis to identify dietary patterns. Factors associated with dietary patterns were analyzed using multiple linear regression models. The adjusted regression coefficients with their 95% CI were used to ascertain the association.

Result: We identified three major dietary patterns that explained 51% of the variance in food consumption. The *western dietary pattern* was characterized by the consumption of meat/organ meat, biscuits/sweets, chicken stew, pasta-macaroni recipes, butter, white wheat bread, egg recipe, and Ethiopian dish *shiro-wet*, and was positively associated with urban residence, obesity, hypertension, blood glucose, and total cholesterol levels. Adherence to the consumption of tubers, whole-grain maize products, coffee leaves-and-herbs beverage, legumes, and sweet potatoes featured the *traditional dietary pattern*. The *traditional dietary pattern* showed a positive relationship with rural residence, physical activity, and obesity, and it had a negative relationship with hypertension. The *healthy dietary pattern* was characterized by the intake of green leafy vegetables, green pepper, and whole-grain maize products, and negatively related to obesity, and hypertension, while positively related to urban residence.

Conclusion: The coexistence of *western, traditional, and healthy dietary patterns* in the present study indicates the transition to a new dietary pattern in the study area. All dietary patterns were associated with one or more cardiovascular risk factors, but the western dietary pattern was associated with most of these, while the traditional diet showed fewer such associations. Therefore, it might be useful to promote *healthy and traditional dietary patterns* along with physical activity. Interventions related to the current findings, if initiated early in life, may benefit the public in preventing cardiovascular risk factors such as obesity, hypertension, and type 2-diabetes.

KEYWORDS

dietary patterns, coexistence, cardiovascular disease risk factors, Wolaita, southern Ethiopia

Introduction

The global average for dietary quality is low with the Alternative Healthy Eating Index (AHEI) ranging from 0 to 100, where 100 represent the healthiest diet. Among children and adults in 2018, the mean global AHEI was 40.3. The diet quality has increased from 1990 to 2018 in most parts of the world, but not in Sub-Saharan Africa (SSA). This clearly indicates the need of more focus on dietary issues in countries in SSA (1). Diet is an important modifiable risk factor associated with non-communicable diseases (2). Individual nutrients and foods, however, cannot be considered in isolation due to the complex interactions among nutrients (3, 4). Dietary pattern is an essential factor for the health of individuals and populations, which is also a key factor in the pattern of energy and nutrient intake (5). Changes in dietary patterns are not limited to the satisfaction of basal physiologic needs, but also are affected by social and cultural factors, including eating behavior (6). The discrepancy in increased energy intake and reduced expenditure results in energy imbalance, and when this is coupled with the reduction in physical activity becomes the underlying cause of overweight and obesity (7).

Dietary patterns that contain above the recommended quantity of energy-dense food items had an association with an increased burden of cardiovascular diseases (CVD) (2, 8). Globally, CVD remains the leading cause of mortality among middle-aged adults, and in high-income countries, it is the main cause of death next to cancer (8, 9). The dietary patterns in high-income countries are characterized by a high quantity of added sugars, fats, refined carbohydrates, and animal-source foods, which are termed a *western diet* (5, 8, 10).

Low- and middle-income countries (LMICs) are not immune to this problem. They are facing a double burden of diseases; and illnesses resulting from both under and over-nutrition (10). The incidence of non-communicable diseases such as cardiovascular diseases and type-2 diabetes is increasing due to changing lifestyles, urbanization, and increasing life expectancy (2, 11, 12). The dietary patterns in the LMICs, especially those with emerging economies are changing from the *traditional dietary pattern* with a high intake of fruits, vegetables, cereals, tubers, and legumes to *western diets* characterized by a high intake of energy-dense food items such as animal food products (13–15). The consumption of calories from meat, sugar, and vegetable oils increased significantly in developing countries between 1963 and 2003 (16).

Diets are determined by several factors, including individual and environmental factors. Urbanization independently or in combination with other factors is associated with changes in dietary patterns (17, 18). The urban food consumption pattern is generally more diversified; contains more animal products and sugar (7, 17, 18). A systematic review of data from forty countries in sub-Saharan Africa indicated variation in the dietary patterns between rural and urban areas (19). Factors including hypertension, blood cholesterol level, smoking, and physical activity were also related to dietary patterns (2, 5, 20, 21).

Ethiopia was frequently attacked by drought and famine during the past decades (22, 23). The total population living under the poverty line in 1994/95 was around 49.5%. However, after the application of various poverty reduction measures, the level of poverty in Ethiopia is decreasing (24). Succeeding economic growth in Ethiopia, the rate of urbanization is progressively increasing (24–27). Evidence indicates the coexistence of economic growth and urbanization results in lifestyle changes including changes in dietary patterns, which in turn may lead to increased obesity and nutrition-related NCDs diseases. This community-based study is the first of its kind in Ethiopia involving exploratory factor analysis to examine the dietary patterns among the adult population in the urban and rural areas of the study. Globally, the incidence of CVD is rising (2, 11, 12), and information on the association of cardiovascular risk factors with dietary patterns particularly in Ethiopia remains scant. Therefore, the current study aimed to assess the dietary patterns and study their association with cardiovascular risk factors among adult people in urban and rural areas of Wolaita, southern Ethiopia. This information might be useful for the promotion of healthy and traditional dietary patterns.

Materials and methods

Setting

The study was carried out in Wolaita, southern Ethiopia from May 2018 to February 2019. Wolaita has experienced rapid urbanization in the past 20 Years (28). We selected a town with the largest population size undergoing rapid urbanization, and a rural district with a relatively traditional lifestyle from Wolaita Zone.

Wolaita was one of the famine-affected and vulnerable areas in Ethiopia during 1983–1986 (22, 23). The livelihood of the urban population in Wolaita is based on employment, trade, or daily labor, while the livelihood of the rural population is based on crop production and animal husbandry (29). Access to food in rural areas depends on subsistence farming and is influenced by farm size, rainfall patterns, and crop production culture (29, 30).

Study design, participants, and sampling technique

We conducted a community-based cross-sectional study. Two thousand four hundred eighty-three people aged 25–64 years participated in the study, and all invited to the study participated, except 3 people who were not available during three visits. The residents of randomly selected households from urban and rural areas were considered the study subjects. We selected the study participants by employing a three-stage survey. First, the survey *kebeles* (villages) were chosen randomly from a series of all registered *kebeles* in both study sites. Eleven out of 54 urban and ten out of 52 rural *kebeles* were included in our study. Secondly, we used a random integer generator to randomly choose households in the chosen *kebeles* from a list given to us by the community health workers (31). The list also had the names of the people living in each household. Thirdly, the number of study participants was decided proportionately to the size of their *kebeles* and households, and the participants were finally chosen from the eligible household members using a lottery method.

Sample size

The sample size for this study was computed using Epi Info version 7 StatCalc software. This project is a part of a larger study, titled nutritional changes, and chronic diseases in Wolaita in southern Ethiopia (32, 33), and the number of participants was 2,486. We also considered assumptions from the study entitled prevalence of high blood pressure, hyperglycemia, dyslipidemia, metabolic syndrome and their determinants in Ethiopia: evidence from the national NCDs STEPS survey, 2015 to compute the sample size in one of the studies in our project (34). Accordingly, with a 14.9% prevalence of hypertension in rural, 19.7% prevalence of hypertension in urban, 95% confidence level, 80% power, one for the ratio of unexposed and exposed groups, and 10% non-response rate the total sample size became 2,233. In this study, since we aimed at assessing the dietary patterns, and their association with cardiovascular risk factors considering residence as the main exposure variable, we have computed posthoc power for the mean difference using OpenEpi version 3.03 software with 95% CI. Accordingly, the sample size to assess the dietary patterns (*western, traditional, and healthy*), and their association with cardiovascular risk factors taking residence as a primary exposure variable was adequate with the power of the study >90%.

Data collection procedure and techniques

A total of 2,483 adult household members randomly selected for the survey were interviewed by trained data collectors, using a structured questionnaire about the socio-demographics such as age, education, wealth, diet, and other lifestyle factors. Additionally, we measured the anthropometric, blood pressure, and biomedical parameters of the study participants. The data collection process was undertaken within the participants' homes, and people in the study obtained information about the data collection such as dates and overnight fasting from the supervisor and coordinator of the data collection before the data were collected. The blood samples were collected in the morning before eating breakfast.

The questionnaire was first designed in English and then translated into Amharic and Wolaitato. For validation, a re-translation was conducted by another expert. We provided training for the data collection team including nurses, laboratory technologists, field supervisor, coordinator, and data clerks for one week. The training consisted purpose of the survey, ethical conduct, and data collection techniques such as 24-h dietary recall assessment, interviewing skills, calibration of data collection instruments, and anthropometry. Following this, we conducted a pretest on 5% of our sample size among the population which was not selected for the survey. Subsequently, the inputs obtained from the pretest were incorporated into our data collection tool.

We adapted a quantitative 24-h dietary recall technique to serve as the data collection instrument for the previous day's 24-h dietary intake assessment (35). To measure the dietary intakes at a population level, we employed single-day dietary histories on different individuals, the study population was selected randomly and all the days of the week were represented in the sample, and this was in line with the recommendation given by the principles of nutritional assessment (36). Since the previous day's history is a recent memory, the interviewers asked the study participants to tell all the foods and beverages they consumed with their specific information such as preparation. We interviewed the participants by probing them recall all foods and beverages consumed during the previous day (from sunrise to sunrise) before the survey. The study participants were requested to provide specific information on foods and drinks including their product names and preparation techniques. We prepared a finite list of foods and beverages such as cereals, pulses, dairy products, vegetables, fruits, tubers, roots, meat and meat products, poultry, fish, egg, fats and oils, sugars, salt, coffee, and tea that helped to recall the previous day food and beverage consumption, and ticked off the mentioned items. In the end, the interview was finalized with the study participants confirming that all the foods and beverages they consumed during the previous day had been mentioned.

A participant's physical activity was assessed by asking about activities during work, for instance carrying or lifting heavy loads. In addition, they were asked about their travel to and from places; e.g. walking and bicycling, and they were also asked about sports, fitness, and recreational activities (e.g., running, football, swimming). The activities were categorized as time spent on moderate-intensity and vigorous-intensity activities. The metabolic equivalent (MET) was calculated as each activity had a predefined value. MET-minutes/week of the specific activity was the product of the number of days in a week used to accomplish a given activity, the average time spent in minutes in a day, and the corresponding MET value. The overall MET-minutes/week was computed by summing the MET-minutes/week value of each activity (37, 38).

We also asked four questions about smoking habits (do you currently smoke any tobacco products; do you currently smoke tobacco products daily; do you currently use smokeless tobacco; do you currently use smokeless tobacco products daily).

A person's BMI is computed by dividing weight in kg by height in m^2. We quantified weight to the closest 0.1 kg by employing a mobile digital weighing scale (Seca electronic scale). We used a movable stadiometer comprising a suitable triangular headboard to quantify height (Seca stadiometer). The participants' weight and height were measured while they stood straight, held their heads upright, and wore light clothing and shoes. During the height measurement, the external auditory of the ear and the bottom border of the eye were aligned in a single horizontal plane. In addition, the heels, shoulder blades, and buttocks touched the scale as the knees of the legs stayed together. Along with it, the arms were kept side by side. The participants' heights were finally measured to the nearest 0.1 cm.

We measured blood pressure using a digital sphygmomanometer (Riester richampion®N, Germany). The participant's blood pressure was taken three times following ten minutes of rest with the right upper arm positioned at the level of the heart. Systolic and diastolic blood pressures were determined by taking the average of the last two readings.

We took whole venous blood samples from each participant in the morning at their homes following an overnight fast. We collected blood samples in vacutainer tubes consisting of ethylenediaminetetraacetic acid, after cleaning the skin with a 70% alcohol swab. Then, the blood samples were kept in an icebox and transported to Wolaita Sodo University Hospital for analysis. Analysis of the blood samples was accomplished within twelve hours duration after acquisition. We used a BS-200 chemistry analyzer to investigate lipid profiles. Assessment of blood glucose (BG) was performed using a glucose meter (SensoCard®) at the site of blood sample collection.

Operational definitions and categories for the analyses

The dietary patterns in our study were named after the food items or groups with the highest loadings in factor analysis, and related literature (2, 15, 39, 40). The *Western dietary pattern* consisted of meat/organ meat, biscuits/sweets, chicken stew, pasta-macaroni recipes, butter, white wheat bread, egg recipe, and *shiro-wet* food items or groups. *Shiro-wet* is an Ethiopian traditional dish mainly prepared using a mixture of the following ingredients: chickpea flour, red pepper flour, tomatoes, onions, garlic, oil, and sometimes butter. A *traditional dietary pattern* was characterized by the consumption of tubers, whole-grain maize products, coffee leaves-and-herbs beverage, legumes, and sweet potatoes. The food items or groups categorized under the *traditional dietary pattern* are culturally popular in the rural part of the study areas. In this study, green leafy vegetables, green peppers, and whole-grain maize products made up the *healthy dietary pattern* and were found in the urban area along with the food items or groups found in the *western dietary pattern*. Hyperglycemia was defined as having a blood glucose level ≥ 7.0 mmol/l, and/or self-reported use of medication for diabetes (41). A participant with total cholesterol (TC) level ≥ 5.2 mmol/L was categorized as having raised TC (42). A blood triglyceride (TG) level ≥ 1.7 mmol/l was defined as an elevated TG level (42). Hypertension was characterized by having a systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, and/or using medication for lowering the blood pressure (43). A body mass index (BMI) of 30 kg/m^2 or greater indicates obesity (44). Having a level of physical activity <600 MET minutes per week was considered physically inactive (37, 38). Age was categorized into four using 10-year groups based on the WHO STEPS recommendation (37).

Assessment of dietary patterns

Generally, dietary patterns are identified by using foods or nutrients, or a combination of both, and foods or food groups are often used as nutrients are composite food scores (45). Since the aim of this study was the identification of dietary patterns and associated cardiovascular factors, we used the previous day's 24-h dietary intake to assess the dietary patterns. The dietary patterns were determined using factor analysis based on the intake of 24 food items or groups (45, 46). Some of the food items were categorized into groups depending on their similarities such as legumes, tubers, green leafy vegetables, and pasta and macaroni. Food items or groups with factor loadings ≥0.3 or ≤ −0.3 were considered as significantly contributing to the pattern. The number of factors that were retained in the analysis was determined based on the *eigenvalue* > 1.0, evaluation of the *scree plot*, and the plausibility of the factors. We used *orthogonal transformation* (*varimax rotation*) to identify uncorrelated factors and facilitate interpretability. Therefore, factor analysis and subsequent vari*max rotation* were used to determine the dietary patterns.

Positively loaded food items or groups contributed to a given dietary pattern, whereas negatively loaded foods have an opposite relation with a particular dietary pattern. A high factor score demonstrates a high intake of foods comprising a particular food pattern, whereas low scores demonstrate a low intake. Dietary patterns were named after the food items or groups with the highest loadings of those dietary patterns. Factor scores for each dietary pattern and participant were estimated by summing the consumption of each food item or group weighted by their factor loadings. Subsequently, the *tertiles* of the dietary patterns scores were generated by classifying the scores into three categories: first tertile (lowest), second tertile, and third tertile (highest) to show the frequencies in relation to other variables in the descriptive tables. The association of dietary patterns with CVD risk factors was analyzed using bivariate and multiple linear regressions, and cross-tabulation was used for descriptive analysis.

Data entry and analysis

Data entry was accomplished using Epi-Data version 3.1 and excel-template, and exported to the STATA 15 software for analysis. We have performed residence-specific principal component analysis to build the wealth index, using 40 variables for rural and 28 variables for urban areas. Detailed information on wealth index construction was reported in a previous publication from the project, and in this study, it was categorized as poor, medium, and rich (33).

The prevalence and frequencies of tertiles of dietary patterns were calculated. The outcome variables were dietary patterns that were identified using factor analysis. The covariates used in this analysis include residence, education, physical activity, obesity, hypertension, hyperglycemia, total cholesterol, and triglyceride levels. The associations between covariates and dietary patterns were assessed using bivariable and multivariable linear regression models building a separate model for each of the identified dietary patterns. The data analysis was started after declaring the data set as a three-stage cluster survey to account for the effect of clustering on the estimated standard errors. The assumption of normality of the continuous variables was checked objectively using *sktest* (skewness-kurtosis test) and subjectively using histograms. Based upon this the natural logarithmic transformation was made for the outcome variables to satisfy the assumption. The result demonstrated the normal distribution of the residuals. Variables with the *p* values <0.2 in the bivariable analysis were considered a candidate for multiple linear regression analysis. The adjusted regression coefficient with its 95% CI is presented, and the absence of 0 within the 95% CI declared the presence of association.

Ethical considerations

This study was approved by both the Institutional Review Board at Hawassa University in Ethiopia (IRB/005/10) and the Regional

Committee for Medical Research Ethics Northern Norway, REK North (2017/2248/REK nord). The study subjects provided written informed consent following the introduction of the purpose of the study. Individuals in the study remained anonymous, except those having hyperglycemia, hypertension, or other serious ailments that were referred to the closest health facility.

Results

Socio-demographic characteristics of the participants

A total of 2,483 adults participated in the study of 2,486 invited people. The number of male people involved in the study was 1,313 (52.9%). There was a relatively equal level of participation between urban (50.1%) and rural (49.9%) study areas. Of the total study participants 1,085 (43.7%), 674 (27.1%), 441 (17.8%), and 283 (11.1%) were aged between 25–34, 35–44, 45–54, and 55–64 years, respectively. Concerning the educational level of the study participants: 1410 (56.8%) had a primary level of education or below, 397 (16.0%) had high school, and 676 (27.2%) had college or education above this level. Calculating the wealth index analysis, we have found 784 (31.6%) poor participants, 793 (31.9%) medium level, and 906 (36.5%) rich.

Of the total study participants, 47.2% (1172) had a physical activity status of ≥600 MET minutes per week. One hundred nine (4.4%) of the overall study participants were obese. The prevalence of hypertension was 32.9% (818), while hyperglycemia was 4.4% (110). Hypercholesterolemia was detected in 5.7% (142), and hypertriglyceridemia in 15.8% (393) of the study participants. Eighteen (0.7%) people were reported to be daily smokers.

Dietary patterns

Three dietary patterns were distinguished, describing 51% of the total variance in food consumption using factor analysis. The dietary patterns with their rotated factor loadings are illustrated in Table 1. The first pattern comprised animal-source foods with added sugars and refined carbohydrates that resemble the *western type dietary pattern*. This pattern explained 21.5% of the total variance in food intake. Food items or groups with the highest factor loadings such as meat/organ meat, and biscuits/sweets positively contributed to the *western dietary pattern*, whereas whole grain maize products had a negative contribution. The second pattern demonstrated a high intake of plant-based food sources that matches a *traditional dietary pattern* explaining 18.1% of the total variance. This pattern consists of tubers having the highest positive factor loading. The third pattern was termed the *healthy dietary pattern* which explained 11.4% of the total variance and featured the consumption of green vegetables and whole-grain maize products (See Table 1).

TABLE 1 Dietary patterns found in factor analysis, with their rotated factor loadings in an Ethiopian population (*n*=2,483).

Food items/ groups	Dietary patterns			H^2
	Western	Traditional	Healthy	
Meat/ organ meat	0.8014	−0.0827	−0.1815	0.3179
Biscuits/ sweets	0.7479	0.0347	0.0086	0.4393
Chicken stew	0.7287	0.0084	0.0220	0.4684
Pasta/ macaroni recipe	0.6327	−0.2366	−0.1959	0.5054
Butter	0.6235	−0.4004	−0.3489	0.3292
White wheat bread	0.6106	−0.3523	−0.2858	0.4214
Egg/ egg recipe	0.5742	−0.0298	−0.0103	0.6693
Shiro-wet	0.4199	−0.6664	−0.0985	0.3699
Whole grain maize products	−0.4290	0.5735	0.3546	0.3613
Coffee beverage	0.1005	−0.1015	−0.5522	0.6747
Green leafy vegetables	−0.1956	−0.0890	0.7690	0.3625
Green pepper	−0.1115	0.0885	0.6705	0.5302
Tubers	−0.1021	0.7064	0.0648	0.4864
Legumes	−0.0290	0.5340	0.2611	0.6458
Sweet potato	−0.0084	0.5028	0.0635	0.7431
Teff injera	−0.0063	−0.7722	0.2832	0.3234
Coffee leaves-herb beverage	−0.0003	0.5509	0.1407	0.6767

H^2: Communality; Shiro-wet: Ethiopian dish mainly prepared using a mixture of the following ingredients: chickpea flour, red pepper flour, tomatoes, onions, garlic, oil, and sometimes butter.

Description of dietary patterns scores and CVD risk factors

Participants with the highest tertile of the *western and healthy dietary patterns* tended to reside in the urban study area, while those with the highest tertile of *traditional dietary pattern* resided in the rural area. We observed a higher level of physical activity among participants with the highest tertiles of the *traditional dietary pattern* and those who lived in rural areas. A higher occurrence of obesity was observed among participants with the upper tertile of the *western* and the second but not the third tertile of the *traditional dietary pattern*, while obesity was lower among participants with the upper tertile of both *traditional and healthy dietary patterns*. Hypertension increased among the adult people with the highest tertiles of the *western dietary pattern* and decreased among people with the highest tertiles of the *traditional and healthy dietary patterns*. Similarly, people in the highest tertiles of the *western dietary pattern* had an increased level of hyperglycemia and elevated total cholesterol levels. We found no difference in smoking rates across the increasing tertiles of dietary patterns (Table 2).

Cardiovascular factors and association with dietary patterns

The study participants having obesity were positively associated with western [β = 1.2; 95% CI: 0.9–1.5], and traditional [β = 1.1; 95% CI:

TABLE 2 Socio-demographic, behavioral, and biochemical characteristics across tertiles of the three dietary patterns scores identified among adults in Wolaita, southern Ethiopia.

Variables (*n*=2,483)	Dietary patterns								
	Western			Traditional			Healthy		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Age (year)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
25–34	365 (33.6)	404 (37.2)	316 (29.1)	372 (34.3)	321 (29.6)	392 (36.1)	310 (28.6)	360 (33.2)	415 (38.3)
35–44	239 (35.5)	219 (32.5)	216 (32.1)	184 (27.3)	202 (30.0)	288 (42.7)	229 (34.0)	240 (35.6)	205 (30.4)
45–54	125 (28.3)	118 (26.8)	198 (44.9)	133 (30.2)	150 (34.0)	158 (35.8)	175 (39.7)	141 (32.0)	125 (28.3)
55–64	95 (33.6)	82 (29.0)	106 (37.5)	76 (26.9)	103 (36.4)	104 (36.8)	112 (39.6)	85 (30.0)	86 (30.4)
Gender									
Female	366 (31.3)	386 (33.0)	418 (35.7)	390 (33.3)	358 (30.6)	422 (36.1)	364 (31.1)	403 (34.4)	403 (34.4)
Male	458 (34.9)	437 (33.3)	418 (31.8)	375 (28.6)	418 (31.8)	520 (39.6)	462 (35.2)	423 (32.2)	428 (32.6)
Gender									
Rural	560 (45.2)	393 (31.7)	287 (23.2)	26 (2.1)	312 (25.2)	902 (72.7)	445 (35.9)	462 (37.3)	333 (26.9)
Urban	264 (21.2)	430 (34.6)	549 (44.2)	739 (59.5)	464 (37.3)	40 (3.2)	381 (30.7)	364 (29.3)	498 (40.1)
Residence									
≤ Primary	545 (38.7)	461 (32.7)	404 (28.7)	225 (16.0)	414 (29.4)	771 (54.7)	479 (34.0)	478 (33.9)	453 (32.1)
High school	116 (29.2)	141 (35.5)	140 (35.3)	139 (35.0)	147 (37.0)	111 (28.0)	119 (30.0)	140 (35.3)	138 (34.8)
College+	163 (24.1)	221 (32.7)	292 (43.2)	401 (59.3)	215 (31.8)	60 (8.9)	228 (33.7)	208 (30.8)	240 (35.5)
Wealth index									
Poor	273 (34.8)	258 (32.9)	253 (32.3)	235 (30.0)	258 (32.9)	291 (37.1)	281 (35.8)	252 (32.1)	251 (32.0)
Medium	263 (33.2)	248 (31.3)	282 (35.6)	222 (28.0)	251 (31.7)	320 (40.4)	272 (34.3)	245 (30.9)	276 (34.8)
Rich	288 (31.8)	317 (35.0)	301 (33.2)	308 (34.0)	267 (29.5)	331 (36.5)	273 (30.1)	329 (36.3)	304 (33.6)
Physical activity									
No	436 (33.3)	393 (30.0)	482 (36.8)	573 (43.7)	476 (36.3)	262 (20.0)	490 (37.4)	385 (29.4)	436 (33.3)
Yes	388 (33.1)	430 (36.7)	354 (30.2)	192 (16.4)	300 (25.6)	680 (58.0)	336 (28.7)	441 (37.6)	395 (33.7)
Smoking									
No	817 (33.1)	818 (33.2)	830 (33.7)	764 (31.0)	767 (31.1)	934 (37.9)	820 (33.3)	818 (33.2)	827 (33.6)
Yes	7 (38.9)	5 (27.8)	6 (33.3)	1 (5.6)	9 (50.0)	8 (44.4)	6 (33.3)	8 (44.4)	4 (22.2)
Obesity									
No	824 (37.4)	816 (34.4)	734 (30.9)	737 (31.0)	720 (30.3)	917 (38.6)	778 (32.8)	772 (32.5)	824 (34.7)
Yes	0 (0)	7 (6.4)	102 (93.4)	28 (25.7)	56 (51.4)	25 (22.9)	48 (44.0)	54 (49.5)	7 (6.4)
Hypertension									
No	548 (32.9)	697 (41.9)	420 (25.2)	498 (29.9)	403 (24.2)	764 (45.9)	301 (18.1)	576 (34.6)	788 (47.3)
Yes	276 (33.7)	126 (15.4)	416 (50.9)	267 (32.6)	373 (45.6)	178 (21.8)	525 (64.2)	250 (30.6)	43 (5.3)
Hyperglycemia									
No	795 (33.5)	819 (34.5)	759 (32.0)	743 (31.3)	709 (29.9)	921 (38.8)	783 (33.0)	773 (32.6)	817 (34.4)
Yes	29 (26.4)	4 (3.6)	77 (70.0)	22 (20.0)	67 (60.9)	21 (19.1)	43 (39.10)	53 (48.2)	14 (12.7)
TC									
Low	794 (33.9)	794 (33.9)	753 (32.2)	723 (30.9)	700 (29.9)	918 (39.2)	748 (32.0)	783 (33.5)	810 (34.6)
High	30 (21.1)	29 (20.4)	83 (58.5)	42 (29.6)	76 (53.5)	24 (16.9)	78 (54.9)	43 (30.3)	21 (14.8)
TG									
Low	699 (33.4)	725 (35.0)	666 (31.9)	651 (31.2)	605 (29.0)	834 (39.9)	651 (31.2)	685 (32.8)	754 (36.1)
High	125 (31.8)	98 (24.9)	170 (43.3)	114 (29.0)	171 (43.5)	108 (27.5)	175 (44.5)	141 (35.9)	77 (19.6)

T1: Tertile 1; T2: Tertile 2; T3: Tertile 3.

0.5–1.6] dietary patterns; while they were inversely associated with the *healthy dietary pattern* [$\beta = -0.48$; 95% CI: −0.94, −0.02]. Similarly, participants who developed hypertension were positively associated with the *western dietary pattern* [$\beta = 1.0$; 95% CI: 0.7–1.3], as they were negatively associated with the *traditional* [$\beta = -1.2$; 95% CI: −1.4, −0.9], *and healthy* [$\beta = -0.7$; 95% CI: −0.9, −0.4] dietary patterns. Being a resident in the urban area was positively related to the *western dietary pattern* [$\beta = 0.6$; 95% CI: 0.3–0.8]. Urban residence was also positively associated with the *healthy dietary pattern* [$\beta = 0.8$; 95% CI: 0.5–1.0]. Meanwhile, urban residence had an inverse relationship with the *traditional dietary pattern* [$\beta = -0.9$; 95% CI: −1.6, −0.2]. We found a positive linear association between the *traditional dietary pattern* and physical activity [$\beta = 0.4$; 95% CI: 0.2–0.6]. Moreover, there was an increasing linear association between blood glucose levels and the *western dietary pattern* [$\beta = 0.15$; 95% CI: 0.11–0.18]. We also observed an increased *western dietary pattern* with the increasing total cholesterol level [$\beta = 0.15$; 95% CI: 0.07–0.23], after adjusting for the other factors in the model (See Table 3; Figures 1A–C).

As only 18 people were smoking, this variable was not included in the main analyses shown in Table 3. However, an analysis including this variable was performed, but this did not influence the results (data not shown).

Discussion

This community-based study is the first of its kind in Ethiopia involving exploratory factor analysis to examine the dietary patterns among the adult population. The study mainly identified the intake of three dietary patterns. The first *dietary pattern* was *western* which is characterized by the consumption of meat/organ meat, biscuits/sweets, chicken stew, pasta-macaroni recipes, butter, white wheat bread, egg recipe, and *shiro-wet.* Urban residence, obesity, hypertension, blood glucose, and total cholesterol levels were positively associated with the *western dietary pattern*. Adherence to the consumption of tubers, whole-grain maize products, coffee leaves-and-herbs beverage, legumes, and sweet potatoes featured the *traditional dietary pattern*. It showed a positive relationship with rural residence, physical activity, and obesity, while it had a negative relationship with hypertension. The *healthy dietary pattern* was characterized by the intake of green leafy vegetables, green pepper, and whole-grain maize products, and negatively related to urban residence, obesity, and hypertension.

In this study, we found a more significant association of the *western, and healthy dietary patterns* with the urban part of the population, compared to the rural population after controlling for potential confounders. The observed relationship between the *western dietary pattern* and the urban environment is in agreement with the findings reported elsewhere in the LMICs (20, 39, 47). This might be due to lifestyle changes related to the rapidly growing urbanization in Wolaita (20, 48). However, this study also indicated the presence of a *healthy dietary pattern* in the urban environment. This might indicate the emergence of transition to new diets in the study area. Furthermore, some individuals might have the awareness of the importance of healthy dietary choices. In contrast, we recorded a significant relationship between the *traditional dietary pattern* and the rural populations. This is consistent with the findings of other studies (20, 49). This might be the reason that traditional dishes are commonly consumed by the rural population. Further, adherence to the *traditional dietary pattern* was associated with physical activity. This is consistent with the finding reported elsewhere in West Africa (49). In rural areas, moderate or vigorous activities like farming may account for the observed relationship.

Consumption of *western and traditional dietary patterns* was associated with obesity. The finding regarding the relationship between *western dietary pattern* and obesity was supported by various studies (2, 13, 47). Compliance with the western diet is associated with higher energy intake, which accounts for weight gain and increased risk of obesity (50, 51). With a further look at the positive association between the *traditional dietary pattern* and obesity, we noted that the proportion of obesity was not higher for the third tertile (23%), but it was for the second tertile (51%), both compared to the first tertile (26%) showing this association was not straight forward. This might also be due to the limitation of a single 24-h dietary intake assessment not representing a long-term dietary habit, even though population-level usual dietary intake can be measured using a single-day dietary assessment provided that the study participants were selected randomly, and all days of the week are represented in the sample (36). But there exists some evidence from Asian countries that indicate a positive relationship between *traditional dietary pattern* and obesity (52–54). However, we suggest further investigation to ascertain this relationship in the context of the study area. Meanwhile, a *healthy dietary pattern* appears to be inversely associated with obesity, which is supported by the findings of other studies (13, 55).

Hypertension was positively associated with the *western dietary pattern* (2), as it was negatively associated with the *traditional and healthy dietary patterns* (21, 56). There has been little understanding of the mechanisms involved. However, the mechanisms linked to the etiology of arterial hypertension brought on by the western diet are complex, and include several factors. High salt intake is one of the best-known risk factors for hypertension (57), but a number of different antioxidants are also associated with the development of hypertension (58). There was a positive linear association between blood glucose levels and a *western dietary pattern*. Similar findings were reported elsewhere (2, 15, 52). As a pro-inflammatory diet, the western diet can trigger inflammatory markers and cytokines and increase oxidative stress, which in combination lead to cell and DNA damage, reducing insulin receptors, and lowering insulin production (59). Similarly, total cholesterol levels increased with adherence to the *western dietary pattern*. Increases in plasma cholesterol may occur if the sources of cholesterol are consumed along with saturated and trans fats, as is the case with the *western dietary pattern* (60). Other community-based studies have also revealed similar results (2, 61, 62). Unlike *Western dietary pattern*, cardio-metabolic risk factors such as total cholesterol, triglyceride, and blood glucose levels were not associated with *traditional and healthy dietary patterns*. This is supported by the findings from other studies (63–66). The mechanisms by which *traditional and healthy dietary patterns* are linked to cardio-metabolic risk factors are not fully understood. However, the absence of association may be due to the high fiber and low glycemic load of plant-based food items such as whole grains, legumes, and vegetables in *traditional and healthy dietary patterns* (67). The population had very few smokers, and this factor is very unlikely to have influenced the results. This is not the situation in most other studies, and this makes the present study quite unique.

TABLE 3 Multivariable log-linear regression analysis of socio-demographic, behavioral, and biochemical factors with dietary patterns among adults in Wolaita, southern Ethiopia.

Variables (n =2,483)	Dietary patterns					
	Western		Traditional		Healthy	
	Crude β (95% CI)	Adjusted β (95% CI)	Crude β (95% CI)	Adjusted β (95% CI)	Crude β (95% CI)	Adjusted β (95% CI)
Age (year)	0.03 (0.01, 0.04)	0.01 (−0.001, 0.02)	0.007 (−0.013, 0.001)	0.002 (−0.003, 0.01)	0.002 (−0.005, 0.01)	0.004 (−0.001, 0.01)
Gender						
Female	0	0	0	0	0	0
Male	−0.1 (−0.4, 0.2)	−0.1 (−0.3, 0.2)	−0.07 (−0.26, 0.12)	−0.1 (−0.3, 0.04)	0.15 (−0.02, 0.32)	0.13 (−0.03, 0.29)
Residence						
Rural	0	0	0	0	0	0
Urban	1.6 (1.4, 1.8)	0.6 (0.3, 0.9)	−1.31 (−1.58, −1.03)	−0.9 (−1.6, −0.3)	0.59 (0.42, 0.75)	0.8 (0.5, 1.0)
Education						
≤ Primary	0	0	0	0	0	0
High school	0.7 (0.4, 1.1)	0.2 (−0.2, 0.6)	−0.23 (−0.51, 0.06)	−0.1 (−0.2, 0.1)	0.24 (0.09, 0.39)	0.02 (−0.16, 0.20)
College+	0.8 (0.4, 1.2)	0.1 (−0.1, 0.3)	−0.65 (−1.13, −0.16)	0.02 (−0.16, 0.21)	0.30 (0.05, 0.55)	−0.1 (−0.3, 0.1)
Wealth index						
Poor	0	0	0	0	0	0
Medium	0. 1 (−0.2, 0.4)	−0. 01 (−0.2, 0.2)	0.03 (−0.16, 0.21)	0.01 (−0.12, 0.13)	0.05 (−0.15, 0.25)	0.1 (−0.1, 0.3)
Rich	0.3 (−0.1, 0.7)	0.2 (−0.05, 0.4)	0.04 (−0.16, 0.24)	0.1 (−0.1, 0.2)	−0.0004 (−0.24, 0.23)	−0.01 (−0.20, 0.19)
Physical activity						
No	0	0	0	0	0	0
Yes	−0.8 (−1.1, −0.4)	−0.03 (−0.3, 0.2)	0.6 (0.3, 0.8)	0.4 (0.2, 0.6)	−0.24 (−0.35, −0.14)	0.1 (−0.1, 0.2)
Obesity						
No	0	0	0	0	0	0
Yes	1.5 (1.2, 1.9)	1.2 (0.9, 1.5)	−0.6 (−0.9, −0.4)	1.1 (0.5, 1.6)	−0.54 (−0.82, −0.25)	−0.5 (−0.9, −0.02)
Hypertension						
No	0	0	0	0	0	0
Yes	1.4 (1.1, 1.8)	1.02 (0.7, 1.3)	−1.3 (−1.5, −1.2)	−1.2 (−1.4, −0.9)	−0.58 (−0.84, −0.32)	−0.7 (−1.0, −0.5)
BG (mmol/L)	0.23 (0.19, 0.26)	0.15 (0.12, 0.18)	−0.07 (−0.11, −0.04)	0.03 (−0.06, 0.12)	−0.04 (−0.08, 0.002)	−0.003 (−0.05, 0.04)
TC (mmol/L)	0.3 (0.2, 0.5)	0.15 (0.07, 0.23)	−0.22 (−0.31, 0.12)	−0.03 (−0.12, 0.06)	−0.04 (−0.12, 0.04)	−0.08 (−0.16, 0.001)
TG (mmol/L)	0.5 (0.2, 0.7)	0.12 (−0.03, 0.27)	−0.34 (−0.50, −0.18)	−0.15 (−0.32, 0.01)	−0.07 (−0.19, 0.06)	−0.03 (−0.16, 0.09)

β, Beta coefficient; BG, Blood glucose; TC, Total cholesterol; TG, Triglyceride.

The findings of this study may have public health significance through promotion of *healthy and traditional dietary patterns* along with physical activity. Interventions related to the findings, if initiated early in life, may benefit the public in preventing cardiovascular risk factors such as obesity, hypertension, and type-2 diabetes (68–72). Furthermore, this study has policy implications in terms of the importance of focusing on nutrition-related non-communicable diseases and provides latest data on dietary patterns for policies related to nutrition.

Strengths and limitations

The design of the present study was cross-sectional. A cross-sectional study with a 24-h dietary intake assessment is a single-day experience and does not guarantee the understanding of the usual dietary pattern, and lacks temporal relations. The causality between the diets and the risk factors cannot be interpreted, but still, findings from this study bring forward new information that might be useful in the understanding of diet and other factors.

The response rate in this study was very high, as 2,483 people participated, out of a population of 2,486. The reason for the high response is likely to be the provision of information including the objective of the study and schedules before the data collection, and repeated visits to the homes in their absence.

Interviews were used for obtaining information from the participants. This is a feasible method in a population where some individuals might be illiterate and others are not used to writing at all. Also, many do not have much knowledge about nutrition and health (73).

Information was obtained for all interviewed individuals using a 24-h recall method and this has been used in low-income settings for many years. The method has been debated whether it can serve as a substitute

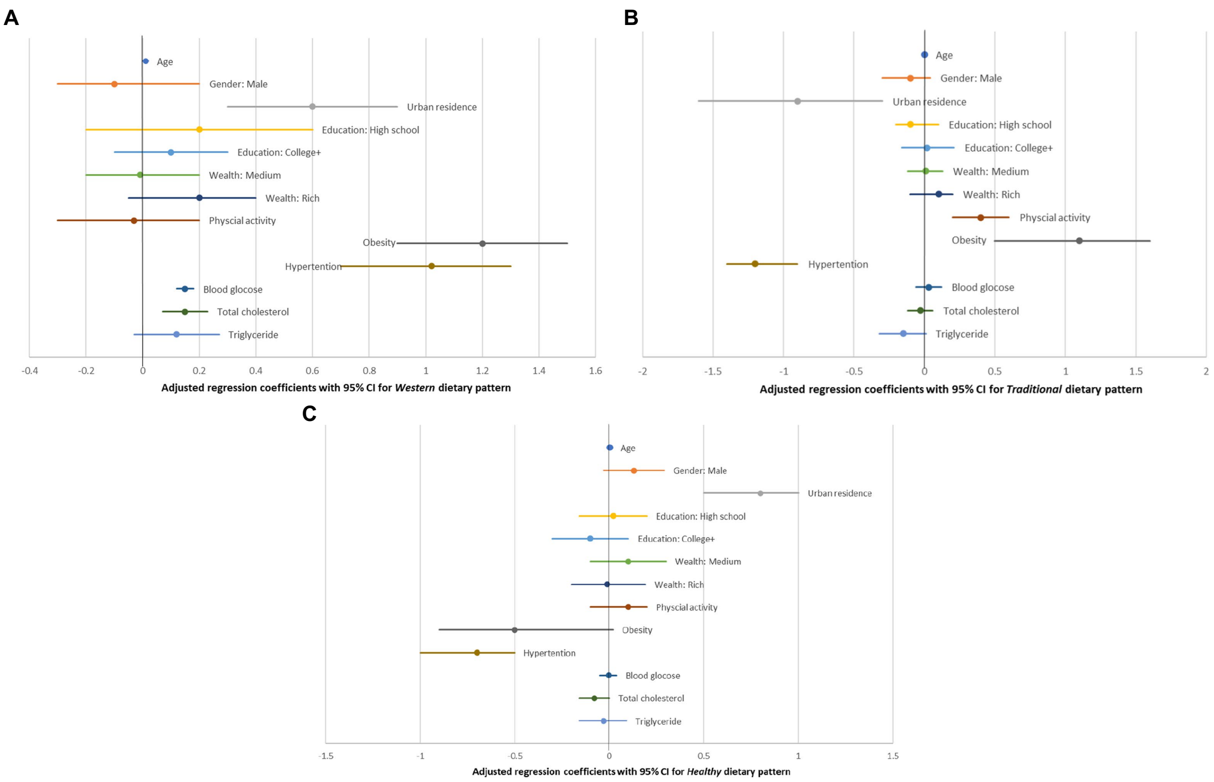

FIGURE 1
(A–C) Show the adjusted regression coefficients in relation to their position to the null value of zero for each of the three dietary patterns. The numerical values of the coefficients along with their crude estimates were depicted in Table 3.

for the weighted food records to assess the absolute nutrient intake (74, 75). Nevertheless, in this study, the amount of food and type of nutrient intake was not required as we aimed at identifying the dietary patterns.

It is very difficult to register food consumption in a population. Recall bias might be present, and the answers received in the present study might not be accurate. Another method that could have been used is weighted records. However, this requires more resources, and may also have uncertainties due to the workload put on the people who must weigh their food and record it.

Smoking is one of the major modifiable CVD risk factors. However, in our study, we did not investigate the relationship between smoking and dietary patterns because of the smaller number of smoking participants.

There might be misclassifications in our study related to the blood glucose and lipid profile. Although the study participants were told to have been fasting before the blood test, we cannot be confident that this was the situation for everyone. We tried to reduce this weakness of the study by giving the participants the required information before the examination day.

The study had several research assistants and which may increase inter-observer bias, and to minimize this, all were trained together and a common understanding of the tools was assured as much as possible.

The findings of this study might be valid for Ethiopian populations. However, dietary issues might not be similar in other cultures and countries, and the results may not be generalized outside of the country.

For even more certain conclusions, future studies should have a longitudinal design with repeated measurements of the diet. It would also be of interest to study the presence of cardiovascular diseases in a longitudinal setting, but this must be done with caution due to the ethical considerations needed.

Conclusion

The coexistence of western, *traditional, and healthy dietary patterns* in the present study may indicate the transition to a new dietary pattern among people in the study area. All dietary patterns were associated with one or more cardiovascular risk factors, but the *western dietary pattern* was associated with most of these. The *traditional diet* showed fewer such associations. Therefore, it might be useful to promote *healthy and traditional dietary patterns* along with physical activity. Interventions related to the current findings, if initiated early in life, may benefit the public in preventing cardiovascular risk factors such as obesity, hypertension, and type 2-diabetes. Furthermore, this study may have policy implications in terms of the importance of focusing on nutrition-related non-communicable diseases, and provides recent data on dietary patterns for policies related to nutrition. Hence, this information should be disseminated and discussed both at local and national levels in Ethiopia.

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 both the Institutional Review Board at Hawassa University in Ethiopia (IRB/005/10) and the Regional Committee for Medical Research Ethics Northern Norway, REK North (2017/2248/REK nord). The patients/participants provided their written informed consent to participate in this study.

Author contributions

WK and EL conceptualized and planned the study, performed the formal analysis of the data, review, editorial activities, and approved the final version of the manuscript. WK carried the protocol development, data collection, supervision activities, and prepared the original manuscript. 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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The relationship between vitamin K and metabolic dysfunction-associated fatty liver disease among the United States population: National Health and Nutrition Examination Survey 2017–2018

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Background: The effect of vitamin K is associated with several pathological processes in fatty liver. However, the association between vitamin K levels and metabolic dysfunction-associated fatty liver disease (MAFLD) remains unclear.

Objective: Here, we investigated the relationship between vitamin K intake and MAFLD risk by employing the American National Health and Nutrition Examination Surveys (NHANES) including 3,571 participants.

Methods: MAFLD was defined as hepatic steatosis with one or more of the following: overweight or obesity, type 2 diabetes, or>2 other metabolic risk abnormalities. The total vitamin K was the sum of dietary and supplement dietary intake. The relationship of between $\log_{10}$(vitamin K) and MAFLD was investigated using survey-weighted logistic regression and stratified analysis, with or without dietary supplementation.

Results: The MAFLD population had a lower vitamin K intake than the non-MAFLD population (p=0.024). Vitamin K levels were inversely associated with MAFLD in the fully adjusted model (OR=0.488, 95% CI: 0.302–0.787, p=0.006). Consistent results were seen in the group without dietary supplements (OR=0.373, 95% CI: 0.186–0.751, p=0.009) but not in the group consuming dietary supplements (OR=0.489, 95% CI: 0.238–1.001, p=0.050).

Conclusion: Vitamin K intake may be a protective factor for MAFLD, especially for individual not using dietary supplements. Nevertheless, more high-quality prospective studies are needed to clarify the causal relationship between them.

KEYWORDS

vitamin K, metabolic dysfunction-associated fatty liver disease, dietary supplements, NHANES, cross-sectional analysis

1. Introduction

Fatty liver is a rapidly progressive chronic liver disease with an estimated global prevalence of 24% (1) and approximately 1/3 of adults in the United States have a fatty liver (2). Nonalcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome, a spectrum of diseases ranging from benign hepatic steatosis to nonalcoholic steatohepatitis that may progress to cirrhosis and liver cancer (3). In 2020, a classification of metabolic dysfunction-associated fatty liver disease (MAFLD) was proposed based on the diagnosis of hepatic steatosis, while incorporating other markers of metabolic abnormalities such as insulin-resistance, high-sensitivity reactive protein, and other metabolic risk factors for pathological progression (4, 5). Ultrasound is a pragmatic and widely accepted first-line examination that has good sensitivity (85%) and specificity (95%) in identifying moderate and severe steatosis. Since liver fibrosis may increase liver echogenicity, the presence of underlying chronic liver disease reduces the accuracy of liver fat assessment. To overcome the limitations of ultrasound in assessing low hepatic steatosis levels, more advanced ultrasound techniques have been developed. Controlled Attenuation Parameter (CAP), available on the FibroScan system (Echosens, France), measures the attenuation of the United States beam (6). CAP uses ultrasound and vibration-controlled elastography to measure the ultrasound attenuation degree due to liver fat (7). Meanwhile, owing to the lack of effective therapeutics and efficient policies to evaluate the prevalence of MAFLD, the economic burden of healthcare in the United States is expected to increase. It is important to define appropriate interventions and prevent serious complications regarding MAFLD.

In recent years, studies have shown that the formation and development of fatty liver are a combination of multiple factors that ultimately lead to liver damage, including insulin resistance, adipokines secretion, oxidative stress, lipid peroxidation, mitochondrial damage, endoplasmic reticulum stress, gut microbiota, innate immunity, and genetic and epigenetic mechanisms (8–10). On the other hand, vitamins are micronutrients vital to health and they have previously been identified as new potential targets for indirect therapy for MAFLD (11). Several studies have linked liver disease to vitamin deficiencies, and vitamin supplementation may protect liver tissue by reducing insulin resistance, lipid peroxidation, and fatty acid synthesis, and improving hepatic steatosis (12–14). Studies have shown that vitamin K deficiency occurs in many pathological conditions (e.g., liver disease, cholestasis, cystic fibrosis, alcoholism, malabsorptive states, and bariatric surgical interventions) (15, 16), and that vitamin K supplementation affects the immune system, anti-inflammation, gut microbes and their metabolites, antioxidants and coagulation, and epithelial development (17, 18). These effects are associated with several pathological processes in fatty liver; therefore, vitamin K may have a protective effect against the occurrence and progression of MAFLD.

Little is known about the role of vitamin K in lipid metabolism. Although one study described a positive association between concentrations in adipose tissue (19), studies exploring the association between vitamin K and MAFLD are lacking, and the association between vitamin K and MAFLD remains unexplored. Therefore, in the current study, we investigated the relationship between vitamin K levels and the risk of MAFLD by employing the American National Health and Nutrition Examination Surveys (NHANES).

2. Materials and methods

2.1. Data source and study sample

The data were collected by the United States Centers for Disease Control and Prevention (CDC) using a stratified, multistage, and probability-cluster design. The Ethics Review Board of the National Center for Health Statistics approved the NHANES protocol and informed consent was obtained from all participants (20). All data can be freely downloaded from the NHANES website.[1] NHANES 2017–2018 is the only publicly available survey database for liver fibrosis assessment by FibroScan® and it has been used in studies of MAFLD (21). The initial sample size was 9,254 people from 2017 to 2018. We excluded subjects younger than 20 years old ($n = 3,685$), viral hepatitis ($n = 50$), pregnancy ($n = 54$), the incomplete diagnostic indicators for MAFLD ($n = 489$), and missing a mean of two 24 h recall dietary data for vitamin K ($n = 1,040$), body mass index (BMI, $n = 51$), alanine aminotransferase (ALT, $n = 260$), aspartate aminotransferase (AST, $n = 13$), C-reactive protein (CRP, $n = 12$), and minutes sedentary activity ($n = 29$). Finally, 3,571 participants were included in this study. The details are shown in Supplementary Figure S1.

2.2. Dietary intake data and supplement dietary intake date

Dietary intake data and dietary supplement intake were obtained from two 24 h-recall interviews with NHANES. The first interview was arranged face-to-face at the Mobile Examination Center (MEC) and the second interview was conducted by telephone 3–10 days later. Energy and nutrient intake for each food or beverage were calculated using the Food and Nutrient Database for Dietary Studies (FNDDS). In this study, dietary intake and dietary supplement intake were estimated using the mean of two 24-recall data points, and the total energy and nutrients were the sum of the dietary intake and dietary supplement intake. Another, study showed that was no significant difference in the energy intake reported in the first and second interviews (22), therefore, this was considered a good intake dataset to determine the average dietary intake for each individual.

Abbreviations: MFALD, Metabolic dysfunction-associated fatty liver disease; NAFLD, Non-alcoholic fatty liver disease; NHANES, National Health and Nutrition Examination Surveys; DGA, Dietary Guidelines for Americans; CDC, Centers for Disease Control and Prevention; HEI, Healthy Eating index; CRP, C-reactive protein; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; SE, Standard error; BMI, body mass index; LSM, Liver stiffness measurements; DM, Diabetes mellitus; TC, Cholesterol; TG, Triglycerides; LDL, Low-density lipoprotein; HDL, High-density lipoprotein.

1 https://www.cdc.gov/nchs/nhanes/index.htm

2.3. Diagnosis of MAFLD

MAFLD was defined as hepatic steatosis with one or more of the following: (1) overweight or obesity (body mass index ≥ 25 kg/m^2); (2) type 2 diabetes; or (3) two or more other metabolic risk abnormalities: (1) blood pressure ≥ 130/85 mmHg or specific drug treatment; (2) overweight or obesity (body mass index ≥ 25 kg/m^2); (3) plasma high-density lipoprotein-cholesterol < 40 mg/dL for men and <50 mg/dL for women or specific drug treatment; (4) plasma triglycerides ≥ 150 mg/dL or specific drug; (5) homeostasis model assessment of insulin resistance score ≥ 2.5; (6) prediabetes (fasting glucose 100–125 mg/dL or hemoglobin A1c (HA1c) 5.7%–6.4%); and (7) plasma CPR level > 2 mg/L (21).

Hepatic steatosis was defined by CAP, and the steatosis was stratified as S0-S3. The thresholds of CAP for S1-S3 were 248, 268, and 280, respectively (6). Hepatic fibrosis was defined by liver stiffness measurements, and the stiffness was stratified as F1-4, and the LSM for F1-4 were 6.3, 8.3, 10.5, and 12.5, respectively (23). Significant steatosis and stiffness were diagnosed as a grade greater than S1 and F1. In this study, participants with a fasting time of <3 h, less than 10 complete LSM readings, or a liver stiffness interquartile (IQR) range/median LSM of more than 30% were deemed to have failed FibroScan® measurements and were excluded (21).

2.4. Other covariates

2.4.1. Demographic characteristics

Self-reported demographic variables included age (years), sex (men/women), race (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, non-Hispanic Asian, or other races, including multiracial), and educational level (<11th grade, high school graduate, some college education, college graduate, or above).

2.4.2. Body measurement

Trained health technicians obtained various body measurements including height, and weight at the MEC. BMI was calculated using weight and height information. The formula used is as follows:

$$BMI\left(\frac{kg}{m^2}\right) = weight(kg) \div height^2(m)$$

2.4.3. Biochemical indicators

Serum samples were processed, stored, and shipped to the University of Minnesota Advanced Research Diagnostic Laboratory (ARDL) in Minneapolis for analysis (24). Regarding biochemical indicators, AST, ALT, TC (cholesterol), TG (triglycerides), LDL (low-density lipoprotein), HDL (high-density lipoprotein), and CRP were included in the regression analysis as covariates.

2.4.4. Lifestyle

The Smoking-Cigarette Use (variable name prefix SMQ) dataset provides a history of cigarette use. Smoking status was classified as never smoker (never smoked 100 cigarettes in a lifetime), some days, or every day Alcohol use was assessed using the dietary interview Total Nutrient Intakes, First Day (DR1TOT_J). Minute sedentary activity was assessed using the Physical Activity Questionnaire (variable name prefix PAQ). The Global Physical Activity Questionnaire (GPAQ) provides respondent-level interview data on physical activity.

2.5. Diseases and medications

The diagnostic criteria for hypertension included: the patient being informed by a doctor that they have hypertension, SBP ≥ 140 mmHg or DBP ≥ 90 mmHg. The diagnostic criteria for diabetes mellitus (DM) are: doctor told you have diabetes, or fasting glucose (mmol/l) ≥7.0, or random blood glucose (mmol/L) ≥ 11.1, or glycohemoglobin HbA1c (%) > 6.5, or 2-h OGTT blood glucose (mmol/L) ≥ 11.1, or use of diabetes medication or insulin. Drug information (told to take prescription for cholesterol) used the Blood Pressure/Cholesterol section (variable name prefix BPQ) in the NHANES.

2.6. Healthy eating index-2015

Healthy eating index (HEI)-2015 was designed and scored from 0 to 100, which was derived from the sum of 13 components: total fruits, whole fruits, total vegetables, greens and beans, total protein foods, seafood and plant proteins (each 0–5 points); whole grains, dairy, fatty acids, sodium, refined grains, added sugars, and saturated fats (each 0–10 points) (25). A higher HEI-2015 score indicated better diet quality. In this study, the HEI-2015 was calculated using the mean of two 24 h recalls.

2.7. Statistical methods

The sampling weights recommended by NHANES for the planned oversampling of specific groups were used in this study. All analyses were sample-weighted and accounted for the complex stratified, multistage, cluster sampling design of NHANES (26, 27). For continuous variables, the survey-weighted median ± standard error was used, and the p-value was calculated by the survey-weighted linear regression. For categorical variables, the survey-weighted percentage (standard error) was used, and the p-value was calculated by the survey-weighted Chi-square test.

Because vitamin K values had a negatively skewed distribution, they were converted to base 10 log values to conform to a normal distribution. The relationship between log vitamin K and MAFLD was explored using the survey-weighted logistic regression. Model 1 was unadjusted. Model 2 was adjusted for age, sex, and race. Model 3 was adjusted for age, sex, race, education, smoking, alcohol, BMI, CRP, AST, ALT, minutes of sedentary activity, drug, energy, HEI-2015, dietary supplements, drugs, hypertension, and DM. To better explore the association between log vitamin K and MAFLD, multivariable logistic regression was conducted with log vitamin K as the categorical variable, and we divided log vitamin K quartiles. Then, we fitted a linear relationship between log vitamin K and MAFLD by smoothing the curve. Finally, we used stratified logistic regression models for interaction analysis and used restricted cubic splines to estimate the dose–response relationship between log vitamin K intake and MAFLD.

Additional sensitivity analyses were performed to determine: (1) collinearity between vitamin K and other variables by the variance inflation factor (VIF) and (2) extreme vitamin K values that were lower 1% and greater than 99%.

All analyses were performed using R software, version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). A two-sided p-value < 0.05 was considered significant for all analyses.

3. Results

3.1. Characteristics in a sex-specific MAFLD population

The general characteristics of the participants are shown in Table 1. The MAFLD population had lower vitamin K intake than the non-MAFLD population (123.113 ± 4.635 vs. 145.064 ± 7.759, $p = 0.024$). Compared with the non-MAFLD population, age, BMI, ALT, CRP, TC, TG, LDL, HDL, energy, the proportion of Mexican Americans, DM, and hypertension were higher in the MAFLD population, while the proportion of higher education was lower.

3.2. The relationship of log vitamin K and MAFLD

We used restricted cubic splines to estimate the dose–response relationship between log vitamin K intake and MAFLD. The results showed a nonlinear relationship between log vitamin K and MAFLD (Figure 1). The log vitamin K inflection point was approximately 2.00 (equal to vitamin K intake of 100 μg), and log vitamin K was a protective factor for MAFLD after the inflection. In a continuous log vitamin K variable, log vitamin K was negatively correlated with MAFLD (OR = 0.462, 95% CI: 0.277–0.796, $p = 0.006$) after adjusting for other control factors in the multivariate regression analysis. Compared with the Q1 quartile of the log vitamin K population, the Q4 quartile had a significantly different MAFLD prevalence in model 3 (OR = 0.488, 95% CI: 0.302–0.787, p = 0.006). p-values for trends were significant in all three models (Table 2). Furthermore, as shown in Supplementary Tables S1, S2, TG was negatively correlated with log vitamin K (β = −0.027, 95% CI: −0.041– −0.013, $p < 0.001$) and positively correlated with MAFLD (OR = 2.384, 95%CI: 2.392–3.367, $p < 0.001$); conversely, HDL was positively correlated with log vitamin K (β = 0.132, 95% CI: 0.082–0.182, $p < 0.001$) and negative correlated with MAFLD (OR = 0.164, 95%CI: 0.115–0.232, $p < 0.001$).

3.3. Subgroup analysis

We divided the population into those with and without dietary supplements. In those who did not take dietary supplements, we found that log vitamin K was a protective factor for MAFLD, regardless of the adjustment for other covariates ($p < 0.05$). In model 3, compared with the Q1 (≤1.75, equal to <56 μg), the Q4 (>2.21, equal to >162 μg) population had a lower risk of MAFLD (OR = 0.373, 95% CI: 0.186–0.751, $p = 0.009$). Among those who consumed dietary supplements, although log vitamin K was found to be a protective factor for MAFLD in model 1 and model 2 (p < 0.05), the relationship between log vitamin K and MAFLD was not statistically significant in model 3 (OR = 0.489, 95% CI: 0.238–1.001, $p = 0.050$). The details are given in Table 3.

We also analyzed other subgroups and found that in the population aged <40 or 40–65 years, men and women, BMI < 30 kg/m^2, HEI-2015 ≤ 50 or > 58, energy ≤1,641 or > 2,358 kcal, non-DM or DM, and non-hypertension, the log vitamin K was negatively associated with the prevalence of MAFLD ($p < 0.05$). These potential factors did not interact with log vitamin K values in the prevalence of MAFLD. The details are provided in Supplementary Figure S2.

3.4. Sensitivity analysis

We performed a series of sensitivity analyses to assess the robustness of the findings. Collinearity diagnostics showed that vitamin K did not have collinearity with other variables (all VIF < 10) in multivariate regression models, regardless of sex (Supplementary Table S3). After replacing the extremes of vitamin K intake, the correlation between log vitamin K and MAFLD in models 1, 2, and 3 was consistent with the findings in Table 2, showing good model stability in this study (Supplementary Table S4).

4. Discussion

Studies have shown that vitamins are essential trace elements involved in various biological functions, and the processes of their metabolism, storage, and activation occur in the liver (28); therefore, liver diseases are usually associated with vitamin disorders. Metabolic fatty liver disease is characterized by hepatic steatosis combined with one or more metabolic abnormalities. In this study, fatty liver degeneration was defined by CAP. Compared with conventional ultrasound, CAP can detect a milder degree of steatosis and has a good correlation with liver biopsy being used for steatosis detection (6). Although the number of patients diagnosed with MAFLD is similar to the number of patients with NAFLD (29), the risk of cirrhosis and liver cancer is more likely to be higher in people with MAFLD because they have higher liver enzyme levels and more diseases—related to glucose and lipid metabolism (30). To the best of our knowledge, this is the first study to explore the relationship between vitamin K intake from diet and dietary supplements and MAFLD. The results showed that compared with the non-MAFLD population, vitamin K intake was lower in the MAFLD population (123.113 ± 4.635 μg vs. 145.064 ± 7.759 μg). Higher vitamin K intake could lower the risk of MAFLD, compared to the population with a vitamin K intake of ≤56.23 μg (equal to log vitamin K ≤ 1.75), there was a 50% risk of MAFLD in the population with a vitamin K intake of >162.18 μg (equal to log vitamin K > 2.21), which was 40% in the population without dietary supplements intake.

Vitamin K is a lipophilic micronutrient divided into phylloquinone and menaquinone (31). In humans, the dietary intake of phylloquinone comes mainly from vegetables and vegetable oils, while menaquinone comes from animal products and bacterially fermented foods (32). Vitamin K was first known for its role in blood clotting, but it can also regulate inflammation, and reduce oxidation (33, 34). Ferroptosis is regulated by multiple cellular metabolic events, including redox homeostasis, iron handling, mitochondrial

 10.3389/fnut.2023.1086477

activity, and metabolism of amino acids, lipids, and sugars (35). Recent studies have found that the vitamin K cycle can protect cells against detrimental lipid peroxidation and ferroptosis (36). In animal experiments, it was found that vitamin K supplementation can downregulate the glycosylated blood glucose protein and insulin resistance in diabetic rats (37), and reduce body fat accumulation and serum triglyceride (TRG) levels in obese rats (38). Population trials have found that increased dietary intake of vitamin K appears to be associated with a lower incidence of DM (39, 40). Vitamin K administration in mice fed a high-fat diet can significantly increase Gla-Gas6 protein levels, upregulate AMPK phosphorylation state, and reduce SREBP1 and PPARα expression. Therefore, it is speculated that the mechanism of vitamin K on lipid metabolism may be mediated by the activation of Gas6 protein (41), then regulating AMPK SREBP1/FAS and PPARα/CPT1A/UCP2 signaling cascades in hepatic lipid metabolism, thereby maintaining

TABLE 1 Characteristics of participants by categories of MAFLD: NHANES 2017–2018.

Characteristics (weighted)	Total	Non-MAFLD	MAFLD	*p*-value
N	3,571	1,479	2,052	
Vitamin K (μg)	132.939 ± 4.519	145.064 ± 7.759	123.113 ± 4.635	0.024
Log vitamin K	1.975 ± 0.014	2.003 ± 0.017	1.952 ± 0.017	0.017
Energy (kcal)	2091.696 ± 21.263	2030.246 ± 25.442	2141.495 ± 27.386	0.004
Age (years)	48.500 ± 0.681	44.229 ± 0.831	51.962 ± 0.699	<0.0001
BMI (kg/m^2)	29.896 ± 0.294	25.845 ± 0.310	33.178 ± 0.330	<0.0001
ALT (u/L)	23.170 ± 0.410	19.414 ± 0.615	26.214 ± 0.692	<0.0001
AST (u/L)	22.252 ± 0.285	21.506 ± 0.598	22.857 ± 0.503	0.176
CRP	3.819 ± 0.188	2.515 ± 0.215	4.875 ± 0.226	<0.0001
Minutes sedentary activity	356.817 ± 7.438	350.947 ± 8.859	361.574 ± 8.533	0.259
Alcohol (g)	11.471 ± 0.551	11.315 ± 0.887	11.597 ± 0.816	0.831
HEI-2015	52.543 ± 0.687	54.099 ± 0.938	51.282 ± 0.631	0.002
TC (mmol/L)	4.931 ± 0.045	4.856 ± 0.050	4.991 ± 0.053	0.028
TG (mmol/L)	1.609 ± 0.037	1.222 ± 0.028	1.922 ± 0.042	<0.0001
LDL (mmol/L)	2.897 ± 0.046	2.792 ± 0.046	2.984 ± 0.063	0.01
HDL (mmol/L)	1.388 ± 0.012	1.524 ± 0.013	1.277 ± 0.014	<0.0001
Race (%)				0.002
Mexican American	7.940(0.014)	5.536(1.118)	9.889(1.832)	
Other Hispanic	3.085(0.004)	2.762(0.568)	3.347(0.383)	
Non-Hispanic white	65.371(0.037)	66.687(2.956)	64.305(2.639)	
Non-Hispanic black	14.304(0.016)	16.208(1.849)	12.761(1.629)	
Other races	9.300(0.010)	8.807(1.191)	9.698(1.241)	
Education (%)				0.003
<11th grade	9.006(0.007)	7.990(0.908)	9.830(0.718)	
High school graduate	26.781(0.019)	25.440(2.502)	27.869(1.177)	
Some college	31.165(0.015)	28.381(2.258)	33.421(1.546)	
College graduate or above	33.048(0.030)	38.189(3.656)	28.881(2.408)	
Smoking (%)				0.063
No	84.430(0.026)	83.783(1.570)	84.954(1.112)	
Some day	3.582(0.004)	2.873(0.610)	4.156(0.536)	
Every day	11.989(0.010)	13.344(1.264)	10.890(0.950)	
Dietary supplements				0.156
Yes	58.598(0.025)	57.190(2.121)	59.760(1.722)	
Hypertension (%)				< 0.0001
Yes	39.692(0.023)	23.361(1.839)	52.927(1.984)	
Diabetes (%)				< 0.0001
Yes	15.414(0.008)	4.390(0.669)	24.347(1.228)	

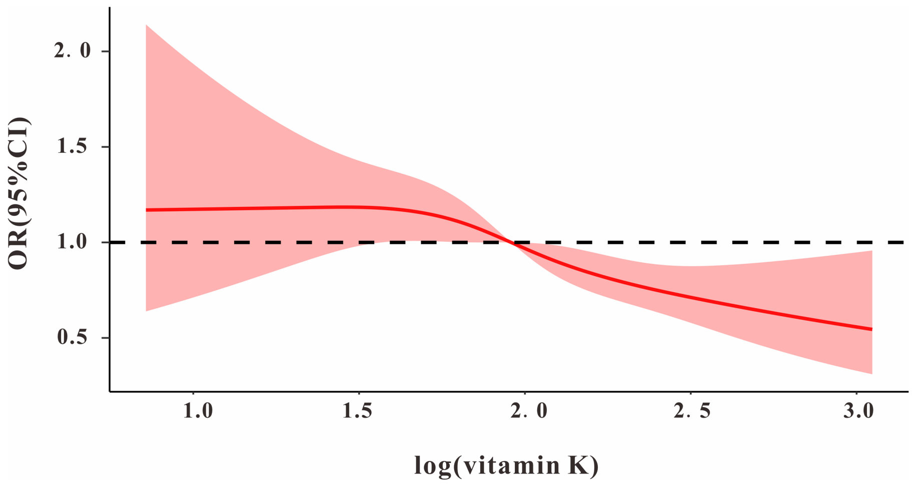

FIGURE 1
The dose–response relationship between log vitamin K and MAFLD. Model adjusted for age, sex, race, body mass index, education, smoking, alcohol, aspartate aminotransferase, alanine Aminotransferase, minutes sedentary activity, energy, HEI-2015; diabetes mellitus; hypertension, and drug.

TABLE 2 The relationship of log vitamin K and MAFLD.

Exposure	Model 1[a]	Model 2[b]	Model 3[c]
Log vitamin K (continuous)	0.663(0.481,0.915) 0.016	0.559(0.410,0.762) 0.003	0.462(0.277,0.769) 0.006
Quartile of log vitamin K			
Q1(≤1.75)	1.000	1.000	1.000
Q2(1.75–1.97)	1.052(0.828,1.337) 0.650	0.978(0.739,1.295) 0.853	0.692(0.515,0.928) 0.017
Q3(1.97–2.21)	0.863(0.665,1.120) 0.243	0.698(0.517,0.941) 0.026	0.592(0.407,0.861) 0.009
Q4(>2.21)	0.708(0.490,1.022) 0.063	0.601(0.415,0.870) 0.015	0.488(0.302,0.787) 0.006
P-trend	0.024	0.004	0.008

[a]Non adjusted model.
[b]Minimally adjusted model: adjusted for age, sex, and race.
[c]Fully adjusted model: age, sex, race, body mass index, education, smoking, alcohol, aspartate aminotransferase, alanine Aminotransferase, minutes sedentary activity, energy, HEI-2015; diabetes mellitus; hypertension, and drug.

whole-body lipid homeostasis (42). Vitamin K is involved in the activation of the AMP-activated protein kinase/sirtuin 1 pathway in the liver, which, in turn, upregulates phosphoinositide 3-kinase and glucose transporter 2 to reduce insulin resistance and fasting blood glucose (37). In this study, we also found that higher vitamin K intake could reduce the risk of MAFLD in the DM population, and individuals with a higher intake of vitamin K had lower TG and higher HDL levels. These results suggest that the anti-inflammatory, anti-ferroptosis, and antioxidant effects of vitamin K regulate glucose and lipid metabolism disorders (43). The pathogenesis of MAFLD is mainly related to metabolic disorders and altered glucose-insulin homeostasis; therefore, vitamin K may reduce the risk of MAFLD by stabilizing glucose-lipid metabolism.

Interestingly, in the stratified analysis, when adjusting for no other variables or adjusting for sex, age, and race, vitamin K significantly reduced the risk of MAFLD among those who consumed dietary supplements, but when the model continued to add moderator variables (activity, other nutrients, and disease), this protective effect was not statistically significant. This result may indicate that the effect of vitamin K on MAFLD may not be significant in the population consuming dietary supplements, which may be affected by other dietary nutrients. In this study, we also observed inconsistency regarding the relationship between vitamin K and MAFLD based on the HEI-2015 and energy subgroup analyses. In another study, a diet rich in vitamin K was associated with a lower prevalence of decreased high-density lipoprotein cholesterol, high serum triglycerides, and hyperglycemia. However, after adjusting for dietary confounders, the effect remained significant only for hyperglycemia (44). Studies have shown that vitamin K function is also related to age, sex, and/or menopause. Additionally, since vitamin K is a fat-soluble vitamin, obesity increases the storage of some fat-soluble nutrients (19). Therefore, vitamin K intake may not play a role in obesity. Age, sex, and menopause have been shown to affect vitamin K metabolism (31). Plasma phylloquinone levels were found to be significantly higher in the elderly (>60 years) than in the young (<40 years) independent of dietary intake (45, 46). This finding indicates that there may be a protective effect of vitamin K against MAFLD in young men, but not in older age. In this study, it was also found that vitamin K did not reduce the risk of MAFLD in the elderly and obese groups. In short, although it is unclear how vitamin K exerts its antioxidant and anti-inflammatory effects, this study suggests that increasing vitamin K

TABLE 3 The relationship of log vitamin K and MAFLD in with or without dietary supplements.

Population	Exposure	Model 1[a]	Model 2[b]	Model 3[c]
Without dietary supplements	Log vitamin K (continuous)	0.709(0.496,1.012) 0.057	0.632(0.425,0.938) 0.028	0.346(0.159,0.750) 0.011
	Quartile of log vitamin K			
	Q1(≤1.75)	1.000	1.000	1.000
	Q2(1.75–1.97)	1.273(0.802,2.022) 0.277	1.192(0.652,2.182) 0.503	0.962(0.504,1.837) 0.901
	Q3(1.97–2.21)	0.938(0.648,1.356) 0.710	0.812(0.511,1.289) 0.302	0.721(0.420,1.239) 0.217
	Q4(>2.21)	0.714(0.508,1.004) 0.052	0.658(0.434,0.996) 0.048	0.373(0.186,0.751) 0.009
	P-trend	0.083	0.035	0.018
With dietary supplements	Log vitamin K (continuous)	0.569(0.329,0.986) 0.045	0.509(0.286,0.906) 0.027	0.555(0.247,1.247) 0.142
	Quartile of log vitamin K			
	Q1(≤1.75)	1.000	1.000	1.000
	Q2(1.75–1.97)	0.816(0.598,1.115) 0.181	0.770(0.526,1.127) 0.144	0.500(0.300,0.833) 0.011
	Q3(1.97–2.21)	0.717(0.467,1.101) 0.117	0.585(0.357,0.959) 0.038	0.519(0.286,0.940) 0.033
	Q4(>2.21)	0.587(0.348,0.989) 0.046	0.517(0.297,0.902) 0.027	0.489(0.238,1.001) 0.050
	P-trend	0.059	0.030	0.142

[a]Non adjusted model.
[b]Minimally adjusted model: adjusted for age, sex, and race.
[c]Fully adjusted model: age, sex, race, body mass index, education, smoking, alcohol, aspartate aminotransferase, alanine Aminotransferase, minutes sedentary activity, energy, HEI-2015; diabetes mellitus; hypertension, and drug.

intake may reduce the risk of MAFLD, especially in populations that do not consume any dietary supplements.

Our study has several strengths. First, the source of vitamin K is not only food but also dietary supplements. The relationship between vitamin K and MAFLD was analyzed between those who used dietary supplements and those who did not. Second, the NHANES database contains a large and nationally representative sample of the adult population in the United States. Third, the study used the weight in the analysis method to adjust for covariates in different models and considered the association of vitamin K and MAFLD in different populations, thus performing a sensitivity analysis. However, our study also had a few limitations. First, the cross-sectional design of our study could not accurately reflect the causal relationship between vitamin K levels and the risk of MAFLD. Second, 24 h dietary interviews do not necessarily reflect long-term dietary consumption habits. Third, although the study was adjusted for many covariates, the effect of unmeasured confounders cannot be ruled out. Fourth, dietary supplements are not intended for people who only take vitamin K supplements and do not distinguish between different food sources of vitamins and different vitamin subtypes (K1 and K2).

5. Conclusion

Vitamin K is inversely associated with the prevalence of MAFLD, suggesting that a higher vitamin K intake is associated with a lower risk of MAFLD. This negative relationship is more significant in the individuals who were not taking dietary supplements. Nevertheless, this study was an observational study, and the plasma concentration of vitamin K was not detected to confirm the effect of the vitamin K increase in the body. Therefore, the relationship between vitamin K intake and plasma vitamin K levels and the relationship between plasma vitamin K and glucose and lipid metabolism should be continued explored in population studies, and their possible mechanisms should be explored in animal experiments.

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 authors.

Ethics statement

The Ethics Review Board of the National Center for Health Statistics approved the NHANES protocol and informed consent was obtained from all participants (18). All data can be freely downloaded from NHANES website (https://www.cdc.gov/nchs/nhanes/index.htm).

Author contributions

XW, WZ, JH, HL, and JG contributed to the study conception and design. XW, HL, and JG designed the study. WZ and JH organized and analyzed the data and wrote the manuscript. XW and HL contributed materials/analysis tools. 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.2023.1086477/full#supplementary-material

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Alternate-day modified fasting diet improves weight loss, subjective sleep quality and daytime dysfunction in women with obesity or overweight: a randomized, controlled trial

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Background: Both sleep time and quality can be associated with overweight or obesity. In obese people, visceral fat tissue develops, which results in an increment in the production of cytokines. The increased production of inflammatory cytokines can disturb the sleep/wake cycle. Therefore, weight loss by reducing fat tissue can improve sleep disorders. Intermittent fasting diets are popular and effective diets that can decrease body weight and improve anthropometric data and body composition. The present study aimed to evaluate the effect of Alternate-day Modified Fasting (ADMF) on sleep quality, body weight, and daytime sleepiness.

Methods: Classification of 56 obese or overweight women, based on age and body mass index (BMI), was done using stratified randomization. Then individuals were assigned to the ADMF group (intervention) or Daily Calorie Restriction (CR) group (control) using the random numbers table for 8 weeks. We measured the Pittsburgh sleep quality Index (PSQI), weight, BMI, and the Epworth sleepiness scale (ESS) as primary outcomes and assessed subjective sleep quality (SSQ), sleep latency, sleep disturbances, habitual sleep efficiency, daytime dysfunction, and sleep duration as secondary outcomes at baseline and after the study.

Results: Following an ADMF diet resulted in a greater decrease in weight (kg) [−5.23 (1.73) vs. −3.15 (0.88); P <0.001] and BMI (kg/m^2) [−2.05 (0.66) vs. −1.17 (0.34); P <0.001] compared to CR. No significant differences were found in the changes of PSQI [−0.39 (1.43) vs. −0.45 (1.88); P =0.73] and ESS [−0.22 (1.24) vs. −0.54 (1.67); P =0.43] between two groups. Also, following the ADMF diet led to significant changes in SSQ [−0.69 (0.47) vs. −0.08 (0.40); P =<0.001], and daytime dysfunction [−0.65 (0.57) vs. 0.04 (0.75); P: 0.001] in compare with CR diet.

Conclusion: These results suggested that an ADMF could be a beneficial diet for controlling body weight and BMI. The ADMF diet didn't affect PSQI and ESS in women with overweight or obesity but significantly improved SSQ and daytime dysfunction.

Clinical Trial Registration: The Iranian Registry of Clinical Trials (IRCT20220522054958N3), https://www.irct.ir/trial/64510.

KEYWORDS

alternate-day modified fasting, intermittent fasting, calorie restriction, sleep quality, Pittsburgh

Background

Sleep disorders affect nearly one-third of adults. The association between sleep quality and food intake has been shown in studies (1). Both people with severe and moderate obesity are affected by low sleep quality (2, 3). The increment of visceral adipose tissue results in the release of inflammatory cytokines that may lead to a disturbance of the sleep-wake cycle (3). Current studies have shown a bidirectional relation between sleep and oxidative stress and inflammation. It has been shown that extremely long sleep duration and sleep disturbances could be related to increased levels of IL-6 and c-reactive protein, while insufficient sleep duration with IL-6 (4). Therefore, both duration and sleep quality can ameliorate with a decrease in weight (5). Gangwisch et al. showed that higher BMI is related to lower sleep duration (6). The first-line therapy for the reduction of weight in individuals with obesity or overweight is calorie restriction (7). Adherence to conventional diets for weight loss is low because of daily energy restriction (8). In recent years, the fasting diet has been proposed as an unconventional diet for losing weight [17]. In addition to losing body weight, it improves metabolic health (9). Among the different fasting methods that have been investigated, the ADMF diet is known to be an effective diet to lose weight. ADMF comprises intermittent periods of feasting and fasting, on alternate days. Some studies have shown a 3–7% decrease in weight under an ADMF diet during 8–12 weeks (10). Compared to CR, intermittent fasting diets have exhibited greater participant compliance over longer periods (11). What is not clear is whether calorie restriction or a fasting diet will further ameliorate body weight (7) and, after that, affect the quality of sleep. Several studies suggest that alternate-day fasting compared with CR could preserve muscle mass and reduce visceral adipose area (12). A systematic review reported that alternate-day fasting diets reduced body weight similar to CR (7, 13). Hutchison et al. have shown that alternate-day fasting leads to greater weight loss compared with CR (14). Recent evidence has reported that calorie restriction increases the quality of sleep (15–17), but studies on the effect of intermittent fasting diets on the quality of sleep are limited. Teong et al. showed that a significant change wasn't found between CR and intermittent fasting diet on the sleep quality of women with overweight or obesity (18). Therefore, to achieve more definitive results in this field, the effect of a method of fasting "ADMF" on body weight, sleep quality, and daytime sleepiness was investigated in this trial.

Abbreviations: BMI, Body mass index; ADMF, Alternate-day Modified Fasting; CR, Daily calorie restriction; ESS, Epworth sleepiness scale; PSQI, Pittsburgh sleep quality index; SSQ, Subjective sleep quality; MET, Metabolic equivalents.

Methods

Design of study

This study was a randomized, controlled, trial to investigate the effect of an ADMF diet and CR diet on body weight, daytime sleepiness, and sleep quality in overweight/obese women for 8 weeks. Individuals were recruited from several health centers located in Kashan, Iran by simple random sampling. Then participants were randomly assigned into groups control (CR) and intervention (ADMF). The study protocol was registered at the Iranian Registry of Clinical Trials (IRCT20220522054958N3) and was approved by the Ethics Committee of Kashan University of Medical Sciences (IR. KAUMS.MEDNT.REC.1401.046). All patients gave written consent to participate in the study. Inclusion criteria included women between 18 and 50 years old and 40 >BMI ≥25. Exclusion criteria included pregnancy, breastfeeding, having a chronic disease such as hypertension, diabetes, gastrointestinal disorders, and heart disease, losing 1–2 kg of weight in the past month, the habit of smoking, alcohol abuse, taking specific medication or following a specific diet, taking dietary supplements for weight loss, overnight shifts, having psychological and mental disorders.

In this study, 56 women were recruited by Simple Random Sampling from some health centers located in Kashan considering the inclusion and exclusion criteria. Eligible individuals were stratified based on age and BMI to make sure homogeneity of between-group. Individuals per stratum were placed into an ADMF group or CR group after baseline investigations. The allocation sequences were generated by using random numbers table by an independent statistician. The statistician was blinded throughout the entire trial.

Diet protocol

The flow diagram of the study has been presented in Figure 1. Individuals per stratum were placed into an ADMF group or CR group after baseline investigations. All individuals were needed to follow diets that were given to them based on daily energy requirements and their group for 8 weeks. The daily energy requirements of the participants were estimated by using the Mifflin equation (19). ADMF involved intermittent periods of fasting and feeding, every-other-day (on the fasting days, participants consumed only 25% of the daily recommended calorie and then on feeding days they consumed 100% of the estimated daily energy requirements). The fast and feed days started at midnight. All meals of the fast days were eaten as lunch between 12.00 p.m. to 2.00 p.m. to make sure that each participant was sustaining the same time of fasting. The consumption of non-starchy veggies (green leaf, cucumber, tomato, and lettuce) as well as energy-free beverages like water, tea, and coffee without sugar (less than 400 mg caffeine daily) was permitted. Participants were encouraged to drink plenty of water. The individuals consumed 100%

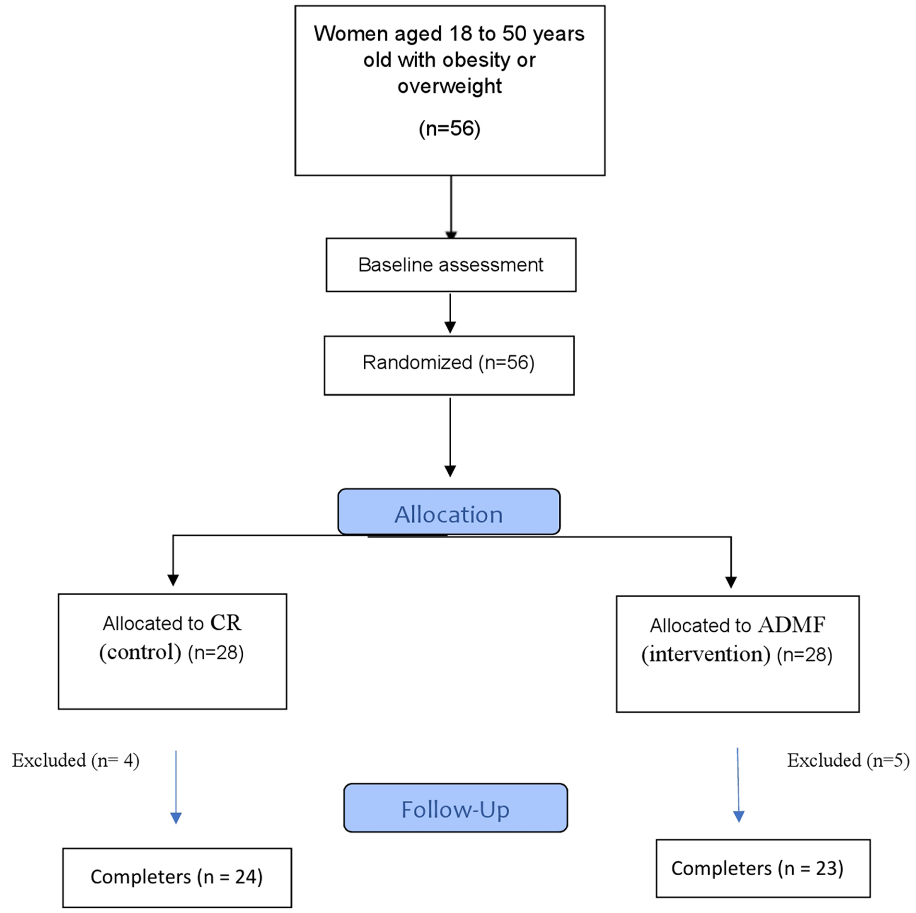

FIGURE 1
Flow chart of the study.

of their daily energy needs (three main meals and three snacks) on feeding days and were asked to intake breakfast at 8:00 a.m., lunch at 13:00 and dinner at 8:00 p.m. Also, they were asked to eat their snacks at 10:00, 16:00, and 22:00. Participants of the CR group daily consumed 63% of their calculated calories (three main meals and three snacks) and were asked to take their main meals at 8:00, 13:00, and 20:00, respectively. Also, they were asked to take their snacks at 10:00, 16:00, and 22:00. The duration of the diet for both groups was 8 weeks. All participants in intervention and control groups needed to cook all their meals in their houses. Daily dietary protein, fat, and carbohydrates, accounted for 15, 30, and 55% of energy needs, respectively. Intervention and control group patients were required to keep their regular physical activity over the trial. All participants had the same number of calls to the dietician.

Adherence to the diet

Participants' adherence to the regimen was monitored every two weeks by completing food record questionnaires three times a week (two normal days and one day off) (20) The questionnaires were compared to the prescribed diet to assess adherence. To ensure accurate completion of the questionnaires, all participants received instructions on how to complete the forms, which included selecting appropriate days and units of measurement. The information obtained from the questionnaires was converted to grams using a home scale guide and analyzed with N4 software (First Databank Inc.; Hearst Corporation), which was adapted for Iranian foods. The software calculated the amount of energy and macronutrients received. Adequate adherence was defined as a total caloric intake between 80 and 110% of the prescribed amount (21). Participants whose daily calorie or macronutrient intake was less than 80% or more than 110% of the recommended amount were excluded from the study. Additionally, participants were contacted by phone every two weeks and monitored to answer any questions and encourage adherence to the diet and study protocol.

Assessment of variables

The impact of ADMF and CR on primary outcomes (body weight, BMI, PSQI, and ESS) and secondary outcomes (SSQ, sleep latency, sleep duration, sleep efficiency, daytime dysfunctions, sleep disturbances) was assessed by change from baseline to end of the intervention.

Weight and BMI

Participants' weight, without shoes and with light clothes, was evaluated using the scale with an accuracy of 0.1 kg. A stadiometer with an accuracy of 0.5 cm was used to measure the height of patients.

BMI was computed as weight (in kilograms) divided by height (in meters squared).

Sleep quality

The sleep quality in the previous 4 weeks is evaluated self-reportedly by the PSQI. The PSQI questionnaire has 19 items and assesses 7 components of sleep: SSQ, sleep latency, sleep duration, use of sleeping medication, sleep disturbance, habitual sleep efficiency, and daytime dysfunction (22). The items are rated on a 4-point Likert scale in terms of the severity of the problem or frequency. The score range of each item is from 0 to 3. The total PSQI score has a range between 0 and 21. Higher scores express lower sleep quality (22).

Daytime sleepiness

In this study, daytime sleepiness was measured by the ESS, a self-reported questionnaire. Participants answered items based on how likely they were to fall asleep or doze off during sedentary activities. ESS is an eight-item questionnaire and includes a respondent format "high chance of dozing" = 3, "moderate chance of dozing" = 2, "slight chance of dozing" = 1, and "would never doze" = 0. The total ESS score was calculated by summing the total scores of eight items. The total score range was from 0 to 24. Higher values indicate higher levels of sleepiness. The change scores were evaluated as the changes between the total ESS scores at the beginning and the end of the study (23).

Physical activity record questionnaire

The physical activity questionnaire, based on metabolic equivalents (MET), was used to evaluate physical activity in this trial. It consisted of nine activity levels, ranging from rest and sleep with a metabolic equivalent of 0.9 to intense activity with a metabolic equivalent of ≥6 (24). Each level (A: 0.9 MET, such as sleep and rest; B: 1.0 MET, such as sitting quietly; C: 1.5 METs, such as working at a computer; D: 2.0 METs, such as standing or washing dishes; E: 3.0 METs, such as light cleaning; F: 4.0 METs, such as bicycling; G: 5.0 METs, such as gardening; H: 6.0 METs, such as aerobics; and I: >6 METs, such as running) was described with examples of activities of that particular MET level, and by a drawing. The physical activity scale was created so that the amount of time spent on each MET activity level (15, 30, or 45 min, and 1–10 h) on an average 24-h weekday could be recorded. The participants indicated the number of times per day they participated in each of the nine levels of activities, numbered 1–9. The Physical Activity score was evaluated by multiplying the MET level of the activity by the number of times per day and summing all the activity scores together (24).

Statistical assessment

The Kolmogorov-Smirnov test was applied to investigate the normality of data distribution. A Chi-square test was used to compare qualitative data between the two groups. An independent *t*-test was used to evaluate between-group differences in quantitative data. To compare the mean of the quantitative data (within the group) at the beginning and end of the study, the paired *t*-test was used in parametric conditions and the Wilcoxon test was used in non-parametric conditions. To compare the mean between the two groups, a *t*-test was used in parametric conditions and the Mann-Whitney test was used in non-parametric conditions.

Results

As shown in the study diagram (Figure 1), 56 women were eligible. Participants were randomly allocated to the intervention and control groups. During the study, 5 women from intervention [pregnancy ($n = 1$), discontinued intervention ($n = 3$), and migration ($n = 1$)] and 4 patients from the control group [discontinued intervention ($n = 4$)] were excluded. Finally, 47 participants completed the study and were consisted in the final analysis. The baseline characteristics of the participants in the present analysis were shown in Table 1. No significant changes were found between groups in demographic characteristics, age, BMI, and physical activity.

Primary outcomes

After an 8-week follow-up, a higher, significant, decrease in body weight (kg) [−5.23 (1.73) vs. −3.15 (0.88); P <0.001] and BMI (kg/m^2) [−2.05 (0.66) vs. −1.17 (0.34); P< 0.001] was observed in ADMF group in comparison to CR. The significant differences weren't observed in the change of PSQI [−0.39 (1.43) vs. −0.45 (1.88); P= 0.73] and ESS [−0.22 (1.24) vs. −0.54 (1.67); P= 0.43] between 2 groups. Also, no significant differences were found in physical activity before and after the intervention, in the ADMF group [26.65 (8.19) vs. 26.91 (8.60); P = 0.309] and CR group [28.67 (6.17) vs. 28.83 (6.24); P = 0.553] (Table 2). The adverse effects related to following the diets weren't reported among the intervention or control group all over the study.

Secondary outcomes

Following the ADMF diet led to significant positive changes in SSQ [−0.69 (0.47) vs. −0.08 (0.40); P= <0.001] and daytime dysfunction [−0.65 (0.57) vs. 0.04 (0.75); P: 0.001] in compare with CR diet. However, significant negative changes were seen in sleep latency [0.87 (0.69) vs. −0.17 (1.01); P= <0.001] and sleep duration [0.52 (0.66) vs. −0.12 (0.68); P= 0.002] and in parameters of habitual sleep efficiency [0.04 (0.63) vs. 0.00 (0.72); P= 0.83] and sleep disturbances [−0.13 (0.75) vs. −0.21 (0.77); P= 0.71] no significant changes were observed between groups (Table 3).

Discussion

The present study indicated that following the ADMF diet for 8 weeks among women with obesity or overweight improved BMI, body weight, SSQ, and daytime dysfunction compared to CR, however, had no effect on PSQI, ESS, habitual sleep efficiency, and sleep disturbances and harmed sleep duration and sleep latency. Our research indicated that although both diets (ADMF and CR) could result in weight loss after 8 weeks; the effect of the ADMF diet on body weight and BMI loss was higher than the CR. This finding was consistent with several

TABLE 1 General characteristics of study participants.

Variables	Intervention n=23	Control n =24	p-value
Age [Mean (SD)]	35.09 (8.38)	36.08 (8.58)	0.689[1]
Marital [n (%)]			0.529[2]
Single	4 (17.4)	5 (20.8)	
Married	19 (82.6)	19 (79.2)	
Child [n (%)]			0.462[2]
0	5 (21.7)	6 (26.1)	
1	6 (26.1)	2 (8.7)	
2	10 (43.5)	11 (47.8)	
3	2 (8.7)	4 (17.4)	
Job [n (%)]			0.995[2]
Student	2 (8.7)	2 (8.3)	
Employee	5 (21.7)	5 (20.8)	
Housewife	16 (69.6)	17 (70.8)	
Economic [n (%)]			0.712[2]
Poor	2 (8.7)	1 (4.2)	
Average	14 (60.9)	17 (70.8)	
Good	7 (30.4)	6 (25)	
Education [n (%)]			0.924[2]
Below diploma	4 (17.4)	4 (16.7)	
Diploma	12 (52.2)	11 (45.8)	
Bachelor and above	7 (30.4)	9 (37.5)	
Physical activity	26.91 (8.60)	28.83 (6.24)	0.385[1]
[Mean (SD)]			
(METs.hr/day)			
BMI [Mean (SD)]	31.63 (3.11)	31.58 (3.66)	0.617[3]
(kg/m^2)			

[1]p-value: Independent samples t-test. [2]p-value: Fisher's Exact test. [3]p-value: Mann-Whitney U-test.

previous studies, for example, Johnson et al. (25) and Razavi et al. (26). In contrast, the study by Trepanowski et al., which lasted for 6 months, did not show any significant beneficial effect on weight loss. While short-term fasting may promote greater weight loss than traditional diets, this effect may not be significant in longer interventions. In fact, other studies have suggested that longer intermittent fasting diets can lead to greater weight loss, and researchers have proposed that intermittent fasting may be a useful dietary method for obese individuals (27, 28). However, the reasons for the discrepancies in anthropometric indices observed in these studies are not well understood, and more long- and short-term trials are needed to fully evaluate the effectiveness of the intermittent fasting. ADMF has exhibited greater participant compliance compared to daily calorie restriction, for longer periods (29). In conventional CR diets, calorie intake must be restricted every day (30), however, the ADMF diet requires food restriction every other day which increases compliance with the diet (29). A decrease in body weight is directly associated with the degree of adherence to the diet (27) and the high adherence rate to the ADMF diet leads to significant weight loss. It has been reported that fasting-induced weight loss is mainly from body fat tissue reduction, while muscle mass is usually preserved during a fasting diet (31). As subjects in ADMF require to fast 3–4 days a week, more decrease in weight is often observed in such diets in comparison with CR (32). The energy balance manages body weight changes (33). During fasting hours, less glucose is available to the body, hence, fat and ketones are considered the main source of energy, and therefore, a decrease in weight and fat tissue will occur (34, 35). The result of several studies indicated that participants in the fasting group reported lower appetite after the intervention. The changes in appetite-regulating hormones may have changed people's appetite. In animal trials, treatment with alternate-day fasting increased adiponectin concentrations, while decreasing leptin and resistin (36).

Sleep disturbances are common findings in obese people (37), which affects not only people with extreme obesity but also people with medium obesity. Excessive fat tissue especially visceral adipose plays a main role in this relation (38). Indeed, visceral adipose tissue increases the secretion of inflammatory cytokines (TNF-α, IL-1, and IL-6). These cytokines cause low-grade chronic inflammation (39). The studies reported that some pro-inflammatory cytokines could have a role in sleep regulation (40). Especially, IL-1β and TNF-α have circadian secretion, with the maximum TNF-α and IL-6 secretion between 01:00 to 02:00 a.m., thus they can regulate the physiology of sleep in both humans and animals (41). In individuals with obesity, IL-6 and TNF-α have more secretion in the morning instead of the night and are related to BMI and sleep disorder (42). Therefore, these findings propose a hypothetical vicious circle including pro-inflammatory cytokines, obesity, and sleep disorder (3). Although our intervention did not affect the total PSQI score and ESS score, subscales of PSQI such as daytime dysfunction and SSQ improved significantly in the ADMF group. While the subscales of sleep latency and duration significantly worsened in the intervention group. It can be said that ADMF has increased sleep quality and daily function. However, the findings of various studies are inconsistent and further research is needed to better understand the possibility of a relationship between adherence to the ADMF diet and sleep quality. There are some limitations in our study. The sample size and duration of the study were relatively short. Nevertheless, in this short follow-up, we showed the effects of ADMF on body weight and some sleep indices. Another limitation of our study is that the assessment of sleep quality and daytime sleepiness was based only on self-reported questionnaires, which might have resulted in misstatements. We determined compliance with the prescribed diet using a food record questionnaire. Forms were completed by the patients three days a week, once every two weeks. Also, we monitored individuals via phone interviews all over the study.

Conclusion

Since obesity has been suggested as one of the main causes of sleep disorders, weight loss diets may play a role in sleep quality. The present study suggests that ADMF is an efficacious dietary method for decreasing body weight and managing BMI in women with obesity or overweight. In addition, we indicated that an ADMF diet can increase SSQ and improve daytime dysfunction, in comparison with a CR diet. These results give us better insight into the ability of ADMF versus CR for the management of weight and BMI. However, more studies are required to address the direction of causality and generalize the results to other diverse population groups with different health statuses.

TABLE 2 Weight, BMI, sleep indexes, and physical activity at baseline and after the 8-week.

Characteristics	Group	Baseline	After 8 weeks	Change	*p*-value[1]	*p*-value[2]
Weight (kg)	ADMF	80.31 (12.64)	75.07 (12.26)	−5.23 (1.73)	<0.001	<0.001
	CR	82.17 (13.43)	79.02 (12.96)	−3.15 (0.88)	<0.001	
BMI (kg/m^2)	ADMF	31.63 (3.11)	29.58 (3.17)	−2.05 (0.66)	<0.001	<0.001
	CR	31.58 (3.66)	30.42 (3.58)	−1.17 (0.34)	<0.001	
PSQI (score)	ADMF	4.08 (1.50)	3.69 (1.32)	−0.39 (1.43)	0.247	0.735
	CR	3.45 (2.22)	3.00 (1.21)	−0.45 (1.88)	0.220	
ESS (score)	ADMF	5.65 (2.80)	5.43 (2.84)	−0.22 (1.24)	0.393	0.431
	CR	7.08 (2.91)	6.54 (2.58)	−0.54 (1.67)	0.155	
Physical activity (METs.hr/day)	ADMF	26.91 (8.60)	26.65 (8.19)	0.26 (1.21)	0.309[3]	0.775[4]
	CR	28.83 (6.24)	28.67 (6.17)	0.15 (1.28)	0.553[3]	

Values reported as Mean (SD). [1]*p*-value: Wilcoxon. [2]*p*-value: Mann- Whitney *U*-test. [3]*p*-value: Paired *t*-test. [4]*p*-value: Independent samples *t*-test. ADMF, Alternate-day Modified Fasting; CR, Daily Calorie Restriction; BMI, Body mass index; PSQI, Pittsburgh Sleep Quality Index; ESS, Epworth sleepiness scale.

TABLE 3 Effects of ADMF and CR on PSQI parameters.

Characteristics	Group	Baseline (score*)	After 8 weeks (score)	Change	*p*-value**	*p*-value***
SSQ	ADMF	0.96 (0.63)	0.26 (0.44)	−0.69 (0.47)	<0.001	<0.001
	CR	0.46 (0.58)	0.37 (0.57)	−0.08 (0.40)	0.317	
Sleep latency	ADMF	0.35 (0.57)	1.22 (0.67)	0.87 (0.69)	<0.001	<0.001
	CR	0.75 (0.84)	0.58 (0.50)	−0.17 (1.01)	0.475	
Sleep duration	ADMF	0.26 (0.61)	0.78 (0.73)	0.52 (0.66)	0.003	0.002
	CR	0.63 (0.71)	0.50 (0.59)	−0.12 (0.68)	0.366	
Habitual sleep efficiency	ADMF	0.35 (0.57)	0.39 (0.49)	0.04 (0.63)	0.739	0.831
	CR	0.33 (0.48)	0.33 (0.48)	0.00 (0.72)	0.900	
Sleep disturbances	ADMF	0.78 (0.79)	0.65 (0.64)	−0.13 (0.75)	0.405	0.706
	CR	0.63 (0.71)	0.42 (0.50)	−0.21 (0.77)	0.197	
Daytime dysfunction	ADMF	0.96 (0.56)	0.30 (0.47)	−0.65 (0.57)	<0.001	0.001
	CR	0.42 (0.58)	0.46 (0.58)	0.04 (0.75)	0.782	

*Values reported as Mean (SD). ***p*-value: Wilcoxon. ****p*-value: Mann-Whitney *U*-test. ADMF, Alternate-day Modified Fasting; CR, Daily Calorie Restriction; SSQ, Subjective sleep quality.

Moreover, more research is required to study the long-term effects of ADMF on holistic and metabolic health. The findings of this study will increase our information on fasting diets, which can be applied to ameliorate dietary recommendations. The addressing of the public health effect of sleep behaviors in the prevention of chronic diseases corroborates this call for further research.

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 Ethics Committee of Kashan University of Medical Sciences (IR.KAUMS.MEDNT.REC.1401.046). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SJ conceived the trial, designed the experiment, and was the Chief Investigator. AY analyzed the data and was responsible for the statistical design of the study. SH assisted with the conduction of the study and wrote the manuscript. All study authors 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.

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Effects of early introduction of solid foods on nutrient intake in preterm infants during their 1st year of life: a secondary outcome analysis of a prospective, randomized intervention study

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Very low birth weight (VLBW) infants have higher nutritional needs even after hospital discharge. However, data concerning current nutrient intakes at different time points after the introduction of solid foods and whether dietary reference values are being met are scarce. To adress this issue, this secondary analysis of a prospective, two-arm interventional study in 177 VLBW infants 21 investigates dietary intake comparing early and late (early: 10–12 weeks corrected for gestational age, late: 16–18 weeks corrected for gestational age) introduction of standardized complementary food during the first year of life. Nutritional intake was assessed using self-reported monthly 3-day dietary records from 3 until 12 months, corrected for gestational age. The time point of the introduction of solid foods did not influence nutrient intake, but the early introduction of solids tended toward a higher proportional intake of protein and carbohydrates and a lower intake of fat as a percentage of total energy) during the 1st year of life, corrected for gestational age. The results of this study indicate that this standardized feeding concept was sufficient for zinc, calcium, and phosphorus intake. However, dietary iron and vitamin D intakes did not meet the recommendations. Thus, prolonged iron supplementation should be considered beyond the introduction of meat and vitamin D supplementation at least until 12 months, corrected for gestational age.

Trial registration number: ClinicalTrials.gov: NCT01809548.

KEYWORDS

complementary feeding, preterm infants, nutrient intake, iron intake, vitamin D intakes

1. Introduction

The first 1,000 days of life, also known as "the window of opportunity," represent a critical period of not only immense potential but also an immense vulnerability that can affect an infant's development and metabolic programming (1–3). The nutritional intake of very low birth weight (VLBW) infants requires specific attention, as preterm infants are assumed to have higher nutritional requirements even after being discharged from the hospital (4). After a certain period, breastmilk or formula alone is no longer sufficient to cover their nutritional needs. Thus, solid foods have to be gradually introduced into the infants' diet (5). Current evidence indicates that preterm infants are introduced to solid foods earlier than term infants (6, 7). However, dietary intakes at different time points after introducing solid foods and whether dietary intake reference values are met during the period of complementary feeding (CF) are unknown. Especially at the beginning of CF, there is a higher risk of nutritional imbalances due to changes in macronutrients and micronutrients (8). Notably, protein, iron, vitamin D, zinc, calcium, and phosphorus rank among the critical nutrients in preterm infants, as suboptimal intakes are associated with impaired growth (9), poor short- and long-term health outcomes, impaired neurodevelopment (10), and poor bone health (11). To ensure optimal growth in preterm infants, it is necessary to determine whether the time point of the introduction of solids is safe in terms of macronutrient intake. Protein intake plays a major role, as low protein intake is associated with undernutrition (12, 13), and high protein intake might increase the risk of obesity in later life (14, 15). Moreover, micronutrient supply during CF in preterm infants is an intensively debated topic due to the essential role of CF in physical growth and neuromotor development (16). However, it is unclear to what extent the timing of the introduction of solid foods affects micronutrient intake throughout the 1st year.

This study aimed to investigate the dietary macronutrient and micronutrient intake at two different time points after the introduction of solid foods in VLBW infants fed a standardized diet during the 1st year of life and whether the dietary intake meets current dietary reference values.

2. Methods

2.1. Study design

This is a secondary analysis of nutritional data collected during a prospective, randomized, two-arm intervention trial of VLBW infants with a birthweight below 1,500 g that were followed in the outpatient clinic of the Division of Neonatology, Department of Pediatrics, Medical University of Vienna from October 2013 until February 2020. Infants were randomized to an early CF group (introduction of complementary food between 10–12 weeks corrected for gestational age) or a late CF group (introduction of complementary food between 16–18 weeks corrected for gestational age) and fed a standardized feeding concept throughout the 1st year of life. Infants with a birth weight of <1,500 g were eligible to participate in the study. The exclusion criteria were any diseases that affect stable growth (i.e., Hirschsprung disease (17), chronic inflammatory bowel disease (18), bronchopulmonary dysplasia (19), necrotizing enterocolitis (NEC) with short bowel syndrome (20), any chromosomal aberrations, congenital heart disease (21), or major congenital birth defects). Information on the study design, sample size planning, and randomization process can be found in the primary outcome report of this trial (22).

The trial was approved by the ethics committee of the Medical University of Vienna (EK: 1744/2012) and registered on clinicaltrials.gov (NCT01809548). Written informed consent was obtained from at least one parent.

2.2. Study visits and diet

During the intervention period, study visits were scheduled in the neonatal outpatient clinic at the expected due dates of 6 weeks, 12 weeks, 6 months, and 12 months, corrected for gestational age. According to a standard operating procedure, anthropometric data (body weight, length, and head circumference) were measured at the respective dates; these primary outcome data were published previously (22). In addition to formula or mother's milk, infants were fed a standardized age-dependent step-up CF concept consisting of five different boxes with manifold, preprepared complementary foods according to the infants' ability to tolerate textures and pieces. Beforehand, a nutritionist calculated a diet rich in vitamin D, iron, calcium, phosphorus, omega-3 fatty acids, zinc, and folic acid and compiled the food boxes, offering a varied range of flavors. Feeding boxes ranged from finely pureed fruits and vegetables in the scoop familiarization phase to coarser-textured menus extended by grains, meat, fish, and milk products in the later phases of CF. The commercially available, ready-to-use baby jar food was provided for free by Nestle? company (Vienna, Austria). Parents were able to pick up the food boxes at any time and had to adhere to the diet for more than 80% of the day during the infants' 1st year of life, corrected for gestational age. To verify adherence to the diet, parents had to complete self-reported 3-day dietary records in each of the study months (23, 24).

2.3. Data collection and evaluation

This secondary analysis aimed to evaluate the dietary intake of critical nutrients in VLBW infants during the 1st year of life. Dietary intake was estimated using 3-day dietary records for 3 consecutive days, including 1 weekend day. Parents of the infants enrolled in the trial were instructed and trained by a nutritionist to make a log of a detailed food report listing each enteral intake once a month from 3 to 12 months, corrected for gestational age (M3-M12). Diet records were analyzed using a nutritionist using nutrient software (nut.s nutritional. software, Vienna, Austria) based on the German Nutrient Database and the Austrian Nutrient Table (Version II.3.1). In infants who were breastfed, the exact milk intake was unknown. Hence, the estimated average values of consumed mother's milk, published by Dewey KG et al. (25), were used. Infant formula was not provided by the study team. However, detailed information on the formula used had to be documented in each protocol. To ensure accurate nutrient analysis, recipes for all infant formulas were requested by the distinct manufacturers, and changes in formulations were considered for calculation. Body weight at the respective date of the dietary record was used to calculate protein, vitamin D, and iron intake based on the amount per kg of body weight. For the remaining months in which no measurements were conducted, body weight was calculated based on the daily increase in body weight between the closest two measurements. Infants received 650 IU/d of vitamin D3 supplementation until 1 year, corrected for gestational age, and 2–3 mg/kg/d iron (Ferrum Hausmann, iron oxide polymaltose complex, Vifor? France, Paris, France) until meat was fed regularly.

Furthermore, a multivitamin preparation (vitamins A, E, D3, B1, B2, B6, C, niacin, and pantothenic acid; Multibionta?Merck Selbstmedikation GmbH, Darmstadt, Germany) was given until 1 year, corrected for gestational age. The exact dosage and the respective start and end dates of all supplements were documented and used for daily intake calculation. Dietary intake is defined as oral intake solely from foods, whereas total intake combines dietary and supplemental intake.

2.4. Outcome parameters

The primary outcome of this analysis was protein intake (g/kg/d) throughout the 1st year of life, corrected for gestational age. Secondary outcomes included macronutrient distribution, i.e., protein, fat, and carbohydrates as a percentage of total energy, energy, vitamin D, iron, zinc, calcium, and phosphorus intake. If possible, nutrient intake was further compared with currently available intake recommendations for preterm infants [protein (8), vitamin D, and iron (26)]. If there were no specific reference values for preterm infants, those of term infants were used for comparison (energy, macronutrient distribution, zinc, calcium, and phosphorus) (27).

2.5. Statistical analysis

Nutrient intake was compared between the early and late introduction of solid foods. Data were analyzed according to the per-protocol principle. Patients with less than 80% adherence to the study food were excluded from the analysis. Furthermore, subjects who moved, were lost to follow-up, withdrew informed consent, or had no dietary record were also removed from data analysis. However, dietary protocols for subjects who moved or withdrew informed consent before being excluded from the analysis were included. Per-protocol statistical analysis of all primary and secondary outcomes was assessed using the linear mixed-effects models, accounting for randomization group, sex, gestational age, and nutrition at discharge as covariates, with a random intercept to account for possible correlations between siblings of multiple births. Marginal means (i.e., averaged across covariates) for the two groups were calculated from the linear mixed models according to standard errors and p-values for the null hypothesis of no between-group difference. Graphical analyses represent estimated marginal means and standard errors (error bars). P-values of <0.05 were considered statistically significant. As an additional analysis, the p-values for between-group comparisons of the same nutrient at different time points were adjusted using the Bonferroni method for results that were statistically significantly unadjusted, and the adjusted p-values (p-adj) are given in the text. Statistical analysis was conducted using RStudio Core Team (2022) (28).

TABLE 1 Dietary Protocols valid for per-protocol analysis.

Months of life corrected for gestational age	Early group ($n = 83$)	Late group ($n = 82$)	Total ($n = 165$)
3	69	56	125
4	58	60	118
5	66	57	123
6	64	62	126
7	56	57	113
8	58	63	121
9	60	55	115
10	58	54	112
11	55	54	109
12	58	53	111

3. Results

3.1. Participants and baseline characteristics

In total, 177 infants were included in the study, of whom 89 of them were randomized to the early feeding group and 88 to the late feeding group. The per-protocol cohort included 83 infants in the early group and 82 infants in the late group. Six infants (three in each group) were excluded from the analysis as they did not adhere to the study protocol. The parents of two infants withdrew consent (one in each group). Two infants in the late group moved prior to CF introduction; hence, no dietary protocols were available. Moreover, two infants in the early group were excluded from the analysis because of missing data on the variable "nutrition at discharge," which was integrated into the statistical model.

Demographic parameters, as well as neonatal, obstetric, and parental parameters, were previously published (22, 29). The mean gestational age at birth was 27 + 1/7 in the early group and 27 + 2/7 in the late group, whereas the mean birthweight was 929 (SD ± 248) g and 932 (SD ± 256) g, respectively.

3.2. Dietary records

Dietary records valid for per-protocol analysis varied among groups and the respective time points, ranging from 66% (55/83) to 83% (69/83) in the early group and from 64% (53/82) to 77% (63/82) in the late group (Table 1).

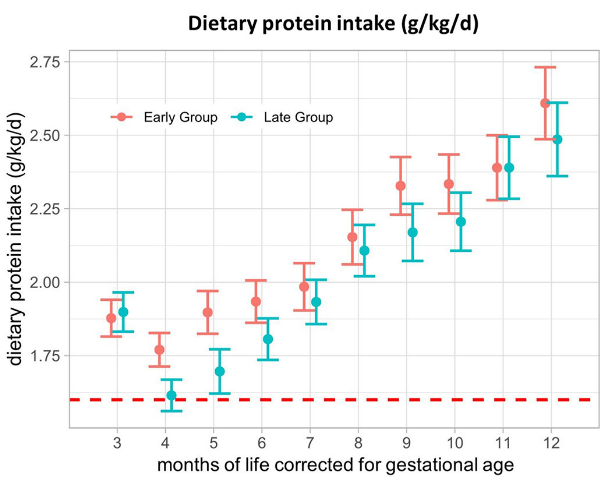

FIGURE 1
Dietary protein intake (g/kg/d) from 3–12 months of life, corrected for gestational age. The red dotted line represents the reference value for protein intake in preterm infants (1.6 g/kg/d). Presented as the estimated marginal mean and standard error of the linear mixed-effects models.

3.3. Protein

The results of the primary outcome of dietary protein intake (g/kg/d) are shown in Figure 1. We observed no significant difference in protein intake between the early and late groups throughout the 1st year of life, corrected for gestational age. In M3, protein intake was 1.88 (SE ± 0.06) g/kg/d in the early group and 1.90 (SE ± 0.07) g/kg/d in the late group and increased up to 2.61 (SE ± 0.12) g/kg/d and 2.49 (SE ± 0.13) g/kg/d in M12, respectively. Immediately after CF introduction, protein intake dropped in both groups, with a greater decline in the late group and a rebound in M5. Participants generally had a mean protein intake above the recommendations (6–12 months uncorrected age: 1.6 g/kg/d) (8) at any of the investigated time points, with an excessive intake of up to 2.61 g/kg/d in the second half of the intervention period (Table 2).

3.4. Energy intake and macronutrient distribution

There was no significant difference in energy intake (kcal/d) between the early and late groups at any of the investigated time points (Table 2). At M3, mean energy intake was 500 (SE ± 11) kcal/d in the early group and 497 (SE ± 11) kcal/d in the late group, increasing to 814 (SE ± 27) kcal/d and 827 (SE ± 27) kcal/d in M12, respectively. Macronutrient distribution varied in the 1st months after the introduction of solid foods between the groups. The percentage of protein intake from total energy was significantly higher in M5 [early: 8.2% (SE ± 0.2), late: 7.7% (SE ± 0.2); $p = 0.03$; p-adj = 0.27] and M8 (early: 9.7% (SE ± 0.2), late: 9.1% (SE ± 0.2); $p = 0.03$; p-adj = 0.27) in the early feeding group, with a persisting trend toward higher intake when compared to the late group (Figure 2A). Fat intake in the percentage of total energy was significantly higher in the late group in M3 (early: 46.6% (SE ± 0.4), late: 48.3% (SE ± 0.4); $p = 0.002$; p-adj = 0.02) and M5 (early: 40.4% (SE ± 0.7), late: 43.1% (SE ± 0.8), $p = 0.01$; p-adj = 0.12) (Figure 2B), whereas the percentage of carbohydrate intake from total energy was higher in the early group in M3 (early: 45.7% (SE ± 0.4), late: 43.6% (SE ± 0.4); $p = 0.0002$; p-adj = 0.002) and M5 (early: 51.4% (SE ± 0.7), late: 49.2% (SE ± 0.7); $p = 0.03$; p-adj = 0.28) (Figure 2C). Upon correction for multiple testing, proportional fat intake and carbohydrate intake at M3 remained significant. A comparison of the fat intake data from this study with term infant recommendations for energy distribution (0–3 months: 45–50%, 4–12 months: 35–45%) (27) showed that fat intake (% of energy) was below the recommendations from M7 in the early group and M8 in the late group onwards (Table 2).

3.5. Iron

We found no difference in mean dietary iron intake between the early and late groups during the intervention period (Figure 3A). In M3, mean dietary iron intake was 0.77 (SE ± 0.05) mg/kg/d in the early group and 0.73 (SE ± 0.05) mg/kg/d in the late group. After an initial decrease, iron intake increased again, with levels reaching 0.98 (SE ± 0.05) mg/kg/d and 0.92 (SE ± 0.06) mg/kg/d in M12, respectively (Figure 3A), At M3, total iron intake was 4.0 (SE ± 0.2) mg/kg/d in the early group and 3.6 (SE ± 0.2) mg/kg/d in the late group and decreased to 1.5 (SE ± 0.2) mg/kg/d and 1.6 (SE ± 0.2) mg/kg/d at M12, respectively (Figure 3B). From 8 months, corrected for gestational age onwards, total iron intake dropped below the intake recommended by ESPGHAN (2–3 mg/kg/d until 6–12 months of age, depending on the diet) (26). This was mainly caused by the termination of additional iron supplementation for most of the infants. Dietary iron intake by CF could not compensate for iron intake administered by iron supplementation, resulting in a very low intake in the second half of the intervention period (Table 2).

3.6. Vitamin D

The mean dietary vitamin D intake did not differ between the early and late groups, ranging from a minimum of 272 (SE ± 21) IU/d to a maximum of 322 (SE ± 21) IU/d (Figure 4A) and was not influenced by CF introduction. Dietary intake together with supplemental vitamin D intake was not different between the groups and remained within the range of the recommendations (800–1,000 IU/d during the 1st year of life (26) until 10 months, corrected for gestational age (M10- early: 865 (SE ± 49) IU/d, late: 857 (SE ± 48) IU/d) (Figure 4B). From M11, total vitamin D intake fell below recommendations in the late group and from M12 in the early group, mainly related to the termination of vitamin D supplements (Table 2).

TABLE 2 Nutrient intake—Early vs. late introduction of complementary feeding.

Months corrected age		3		Intake Recommendation		4		5	
Group		Early (*n* = 69)	Late (*n* = 56)	0-3 months		Early (*n* = 58)	Late (*n* = 60)	Early (*n* = 66)	Late (*n* = 57)
Protein	g/kg/d	1.88 (0.06)	1.90 (0.07)	1.6 g/kg/d		1.77 (0.06)	1.61 (0.05)	1.90 (0.07)	1.70 (0.08)
Energy	kcal/d	500 (11)	497 (11)	500–550 kcal/d		534 (14)	508 (13)	576 (17)	557 (18)
Protein	% of energy	7.7 (0.2)	8.2 (0.2)	Not available		7.6 (0.1)	7.6 (0.1)	**8.2 (0.2)***	**7.7 (0.2)***
Fat	% of energy	**46.6 (0.4)***	**48.3 (0.4)***	45–50% of energy		44.6 (0.5)	45.9 (0.5)	**40.4 (0.7)***	**43.1 (0.8)***
Carbohydrates	% of energy	**45.7 (0.4)***	**43.6 (0.4)***	Not available		47.9 (0.5)	46.5 (0.5)	**51.4 (0.7)***	**49.2 (0.7)***
Iron	mg/kg/d	0.77 (0.05)	0.73 (0.05)	2–3 mg/kg/d		0.73 (0.04)	0.69 (0.04)	0.80 (0.04)	0.72 (0.04)
Iron total	mg/kg/d	4.0 (0.2)	3.6 (0.2)			3.9 (0.2)	3.4 (0.2)	3.5 (0.2)	3.2 (0.2)
Vit D diet	IU/d	279 (23)	297 (25)	800–1000 IU/d		272 (21)	296 (19)	306 (21)	303 (22)
Vit D total	IU/d	948 (28)	960 (30)			923 (28)	955 (26)	967 (28)	978 (29)
Calcium	mg/d	342 (15)	371 (16)	220 mg/d		350 (15)	348 (14)	388 (19)	376 (19)
Phosphorus	mg/d	201 (9)	210 (10)	120 mg/d		220 (10)	212 (9)	251 (13)	241 (13)
Zinc	mg/d	3.7 (0.2)	3.9 (0.2)	1.5 mg/d		3.9 (0.2)	4.0 (0.2)	4.3 (0.2)	4.1 (0.2)
Months corrected age		6		7		8		9	
Group		Early (*n* = 64)	Late (*n* = 62)	Early (*n* = 56)	Late (*n* = 57)	Early (*n* = 58)	Late (*n* = 63)	Early (*n* = 60)	Late (*n* = 55)
Protein	g/kg/d	1.93 (0.07)	1.81 (0.07)	1.98 (0.08)	1.93 (0.08)	2.15 (0.09)	2.11 (0.09)	2.33 (0.10)	2.17 (0.10)
Energy	kcal/d	602 (16)	605 (16)	633 (19)	638 (18)	672 (21)	689 (20)	737 (24)	710 (24)
Protein	% of energy	8.7 (0.2)	8.3 (0.2)	9.2 (0.2)	8.7 (0.2)	**9.7 (0.2)***	**9.1 (0.2)***	10.0 (0.2)	9.5 (0.2)
Fat	% of energy	37.7 (0.8)	38.1 (0.8)	33.7 (0.9)	35.5 (0.9)	32.4 (0.9)	34.0 (0.9)	32.2 (1.1)	32.4 (1.1)
Carbohydrates	% of energy	53.6 (0.7)	53.6 (0.7)	57.1 (0.9)	55.8 (0.8)	57.9 (0.8)	56.9 (0.8)	57.7 (1.0)	58.0 (1.1)
Iron	mg/kg/d	0.83 (0.04)	0.79 (0.04)	0.81 (0.05)	0.88 (0.05)	0.89 (0.05)	0.91 (0.05)	0.95 (0.05)	0.90 (0.05)
Iron total	mg/kg/d	3.2 (0.2)	2.8 (0.2)	2.2 (0.2)	2.1 (0.2)	1.8 (0.2)	1.9 (0.2)	1.8 (0.2)	1.8 (0.2)
Vit D diet	IU/d	299 (19)	308 (18)	276 (21)	304 (19)	304 (20)	308 (19)	322 (21)	305 (21)
Vit D total	IU/d	963 (26)	975 (25)	946 (34)	952 (32)	972 (32)	958 (30)	960 (37)	930 (37)
Calcium	mg/d	408 (17)	405 (16)	424 (19)	424 (18)	473 (23)	451 (21)	520 (24)	483 (24)
Phosphorus	mg/d	269 (11)	268 (11)	302 (12)	279 (11)	329 (14)	322 (13)	371 (13)	355 (13)
Zinc	mg/d	4.6 (0.2)	4.5 (0.2)	4.4 (0.2)	4.8 (0.2)	4.9 (0.2)	5.1 (0.2)	5.5 (0.2)	5.2 (0.2)
Months corrected age		10		11		12		Intake Recommendations	
Group		Early (*n* = 58)	Late (*n* = 54)	Early (*n* = 55)	Late (*n* = 54)	Early (*n* = 58)	Late (*n* = 53)	4–12 months	
Protein	g/kg/d	2.33 (0.10)	2.21 (0.10)	2.39 (0.11)	2.39 (0.11)	2.61 (0.12)	2.49 (0.13)	1.6 g/kg/d	
Energy	kcal/d	755 (24)	732 (24)	759 (26)	776 (25)	814 (27)	827 (27)	600–700 kcal/d	
Protein	% of energy	10.2 (0.2)	9.8 (0.2)	10.7 (0.2)	10.4 (0.2)	11.4 (0.3)	10.7 (0.3)	Not available	
Fat	% of energy	32.1 (0.9)	31.5 (0.9)	30.3 (0.9)	30.8 (0.9)	30.4 (0.8)	29.5 (0.9)	35–45 % of energy	
Carbohydrates	% of energy	57.8 (0.8)	58.7 (0.8)	59.0 (0.8)	58.8 (0.8)	58.2 (0.8)	59.8 (0.8)	Not available	
Iron	mg/kg/d	0.95 (0.05)	0.95 (0.05)	0.96 (0.05)	0.92 (0.05)	0.98 (0.05)	0.92 (0.06)	2–3 mg/kg/d	
Iron total	mg/kg/d	1.4 (0.2)	1.7 (0.2)	1.5 (0.2)	1.5 (0.2)	1.5 (0.2)	1.6 (0.2)		
Vit D diet	IU/d	305 (19)	310 (19)	302 (20)	276 (19)	318 (21)	275 (21)	800–1,000 IU/d	
Vit D total	IU/d	865 (49)	857 (48)	813 (54)	785 (51)	694 (56)	660 (58)		
Calcium	mg/d	**577 (26*)**	**501 (26*)**	601 (30)	569 (28)	656 (31)	613 (31)	330 mg/d	
Phosphorus	mg/d	**419 (16*)**	**361 (16*)**	446 (21)	430 (20)	511 (23)	472 (23)	300 mg/d	
Zinc	mg/d	5.7 (0.2)	5.4 (0.2)	5.7 (0.2)	5.7 (0.2)	5.9 (0.2)	6.0 (0.2)	2.5 mg/d	

The data are presented as the estimated marginal mean and standard error of the linear mixed-effects models of the per-protocol study population. Significant differences before correction for multiple testing (Bonferroni) were marked with*.

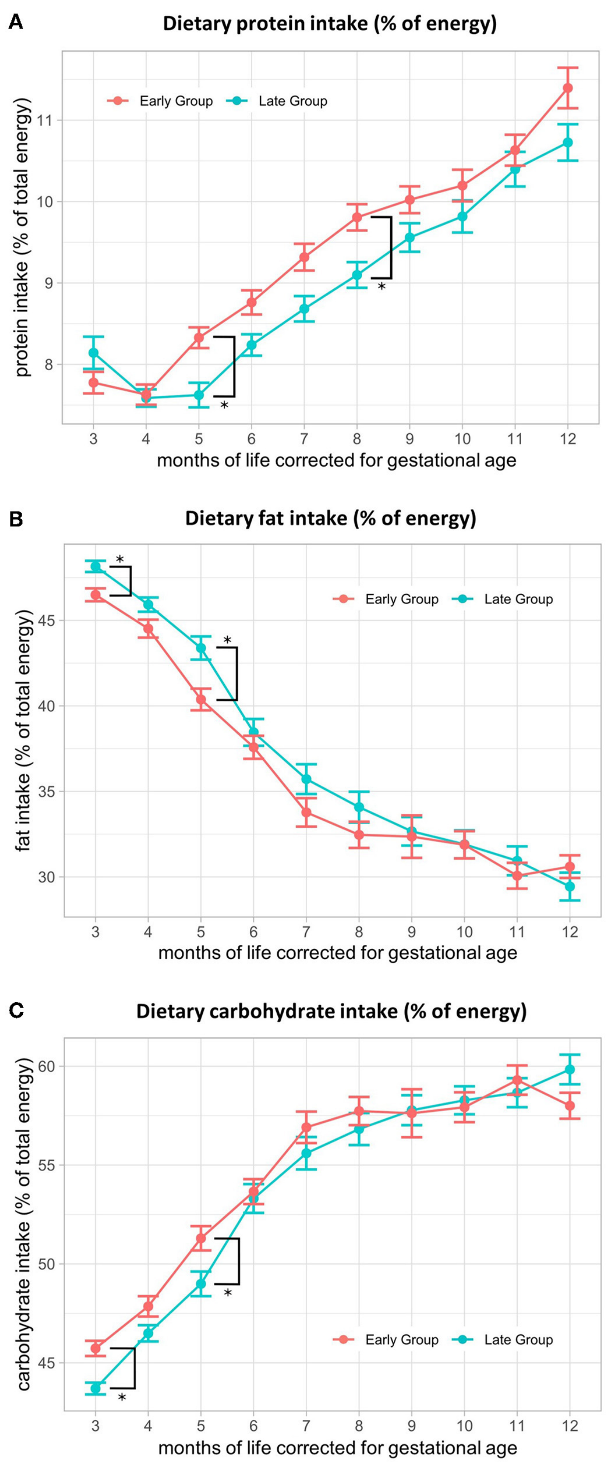

FIGURE 2
Macronutrient distribution (% of energy) from 3–12 months of life, corrected for gestational age. Comparison of **(A)** protein intake in % of energy, **(B)** fat intake in % of energy, and **(C)** carbohydrate intake in % of energy between early and late CF introduction from 3 to 12 months, corrected for gestational age. Presented as the estimated marginal mean and standard error of the linear mixed-effects models.

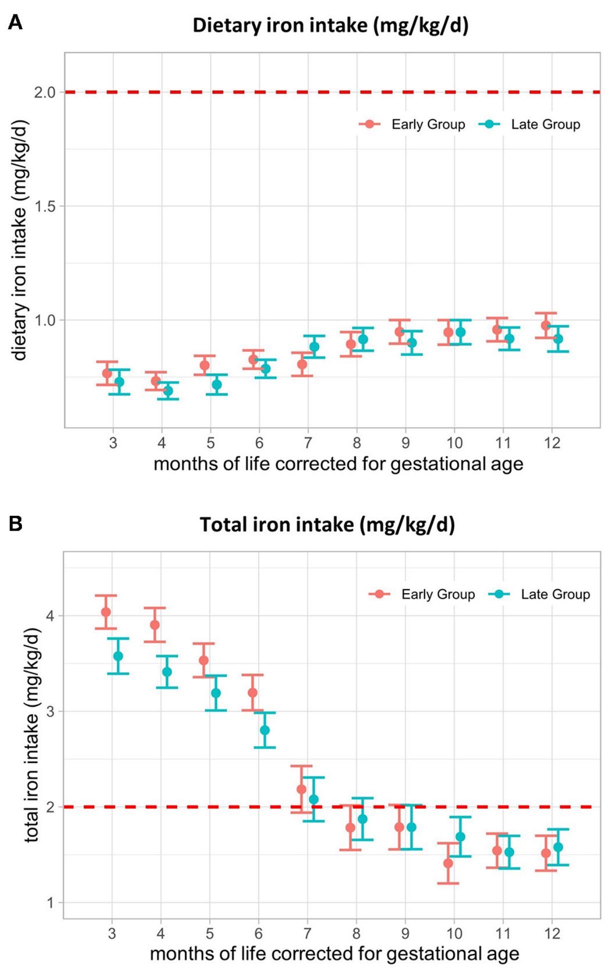

FIGURE 3
Iron intake (mg/kg/d) from 3–12 months of life, corrected for gestational age. **(A)** Dietary iron intake and **(B)** total iron intake from 3–12 months, corrected for gestational age in mg/kg/d. The red line represents the lower limit of ESPGHAN iron intake recommendations for VLBW infants (2–3 mg/kg/d until 6–12 months, depending on the diet). Presented as the estimated marginal mean and standard error of the linear mixed-effects models. Total = supplemental + dietary intake.

3.7. Calcium

Dietary calcium intake did not differ between the groups throughout the 1st year, corrected for gestational age, except for M10 (early: 577 (SE ± 26) mg/d, late: 501 (SE ± 26) mg/d; $p = 0.04$; p-adj = 0.45). After multiple testing adjustments, no significance was detected anymore. Mean dietary calcium intakes ranged from 342 (SE ± 15) mg/d in M3 to 656 (SE ± 31) mg/d in M12 in the early group and from 371 (SE ± 16) mg/d in M3 up to 613 (SE ± 31) mg/d in M12 in the late group. Plotting our data against the current recommendations for calcium intake for term infants (220 mg/d for infants from 0–3 months and 330 mg/d for 4–12 months) (27), we found that both groups met these recommendations at all of the investigated time points (Table 2).

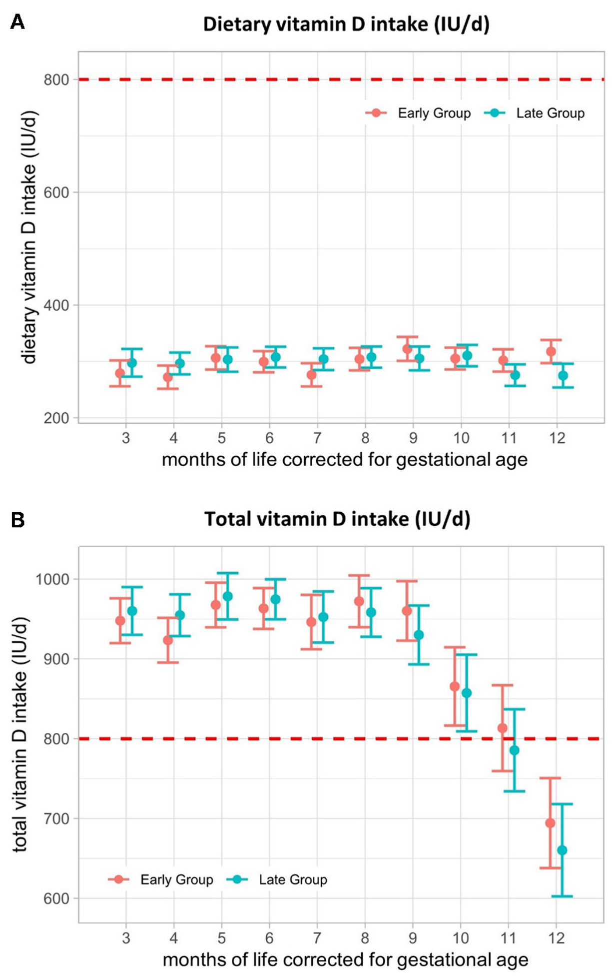

FIGURE 4
Vitamin D intake (IU/d) from 3–12 months of life, corrected for gestational age. **(A)** Dietary vitamin D intake and **(B)** total vitamin D intake from 3–12 months, corrected for gestational age in IU/d. The red line represents the lower limit of ESPGHAN vitamin D recommendations for VLBW infants during the 1st year of life. Presented as the estimated marginal mean and standard error of the linear mixed-effects models. Total = supplemental + dietary intake.

3.8. Phosphorus

Dietary phosphorus intake only differed significantly in M10 between the early and the late groups [early: 419 (SE ± 16) mg/d, late: 361 (SE ± 16) mg/d; $p = 0.01$; p-adj = 0.14]. After multiple testing adjustments, no significance was detectable. Mean dietary phosphorus intake increased from 201 (SE ± 9) mg/d in the early group and 210 (SE ± 10) mg/d in the late group at the beginning to 511 (SE ± 23) mg/d and 472 (SE ± 23) mg/d at the end of the intervention period. Dietary reference values (0–3 months: 120 mg/d; 4–12 months: 300 mg/d) (27) were met only at the very beginning of weaning (M3) and from M8 onwards. Dietary phosphorus intake was below 300 mg/d in both groups in M4-M6 and M7 in the late group (Table 2).

3.9. Zinc

The time point of the introduction of solid foods did not influence dietary zinc intakes, which were above the reference values (0–3 months: 1.5 mg/d; 4–12 months: 2.5 mg/d) of term infants at all investigated time points in both groups (Table 2). Mean dietary intakes ranged from 3.7 (SE ± 0.2) mg/d in the early group and 4.0 (SE ± 0.2) mg/d in the late group to 5.9 (SE ± 0.2) mg/d and 6.0 (SE ± 0.2) mg/d, respectively (Table 2).

4. Discussion

This is a secondary outcome analysis of a randomized intervention trial investigating the nutritional intake after the introduction of standardized CF in VLBW infants at two different time points. This study showed that the time point of the introduction of solid foods had no impact on protein intake or any other investigated nutrients. The early introduction of CF was associated with a higher proportion of protein and carbohydrate intake and a lower fat intake at the beginning of CF. Dietary intake recommendations were met for zinc, calcium, and phosphorus but not for iron and vitamin D.

4.1. Protein intake and macronutrient distribution

The time point of the introduction of solid foods had no impact on dietary protein intake (g/kg/d) during the 1st year of life, corrected for gestational age. To date, only little is known about protein intake during CF in preterm infants and its relation to the time point of CF introduction. In the study by Mariott et al., published in 2003, preterm infants were randomized to a "preterm weaning strategy" (PWS) group or a control group. Infants in the PWS group started eating semisolid foods at 13 weeks of postnatal age, whereas infants in the control group were introduced to solid foods after 17 weeks of postnatal age, provided that they weighed 3.5 and 5 kg, respectively. The authors reported a significantly higher mean growth rate of length/week in the PWS group and a significantly higher intake of total energy (PWS: 822 kcal/d, control: 728 kcal/d) and protein at 6 months, corrected for gestational age (PWS: 26.7 g/d, control: 23 g/d) but no differences at 12 months, corrected for gestational age. (30) In the study by Mariott et al., protein and energy intake were significantly higher, with a protein intake that was twice as high as ours. It is unclear how such a high intake could be achieved. However, the results are no longer universally applicable because the study was performed when post-discharge formula and breastmilk fortification were not available. Hence, solids were used for enhanced nutritional intake as other options were lacking. Optimal protein intake during CF in preterm infants is important, as insufficient protein intake can contribute to undernutrition (12, 13). The beginning of CF is especially vulnerable to the risk of nutritional imbalances due to the changes in macronutrients and micronutrients (8). Although protein intake immediately decreased after the introduction of CF, mean protein intake (g/kg/d) did not drop below the recommendations (1.6 g/kg/d) in both groups at any of the investigated time points, assuming that early and late introduction is adequate for the prevention of undernutrition in former preterm infants. However, protein intake exceeded the recommendations, with levels up to 2.61 (SE ± 0.1) g/kg/d in the second half of the 1st year, corrected for gestational age. There is a growing body of evidence supporting the hypothesis that excessive protein intake during the period of CF is associated with overweight and obesity during childhood in healthy-term infants (14, 15). Günther et al. (31) showed that a consistently high protein intake at the age of 12 months was related to a higher mean BMI standard deviation score and percentage of body fat at 7 years of age To prevent overweight and obesity later in life, it is suggested that protein intake not exceed 15% of energy (32). In our study, protein intake (% of energy) increased from 7.7% (SE ± 0.2) in the early group and 8.2% (SE ± 0.2) in the late group at M3 to 11.4% (SE ± 0.3) and 10.7% (SE ± 0.3) at M12, respectively. Thus, protein intake (% of energy) was within the safety range in both groups, avoiding the potential risk of wrong metabolic programming and adiposity in later life. Moreover, a higher protein intake may be advantageous for former preterm infants, especially those who have not reached catch-up growth by the time of the CF introduction. (33) The primary outcome of this study assessed length at 12 months, corrected for gestational age, which did not differ between the early and late groups. At 6 months, corrected for gestational age, the early group had significantly higher weight z-scores (22). A recent study demonstrated an association between high proportional protein intake and rapid weight gain in a dose-dependent manner during the CF period (34). Thus, a higher proportional protein intake (% of total energy) in the early group at the beginning of the weaning period might have contributed to higher weight z-scores at 6 months, corrected for gestational age. The results must be interpreted cautiously, as no significance was detected after correction for multiple testing. Furthermore, the study was not powered to detect a significant difference in nutrient intake between groups, and dietary records were not available for all patients enrolled in this study.

Furthermore, a proportional fat intake of 35–45% during the period of CF is suggested (27). The late group had a significantly higher fat intake compared to the early group at the beginning of CF. To date, no data on the proportional fat intake during CF and later health outcomes in former preterm infants is available. Concern has been raised that a higher fat intake is associated with obesity in adults (32). However, there is increasing evidence that, in term infants, a higher fat intake during CF is not associated with overweight later in life. Indeed, the results of the study by Rolland–Cacher et al. demonstrated that a low fat intake at 2 years might lead to an increased risk of obesity and leptin resistance in adulthood (35). With respect to this, a late CF introduction might be more favorable to preventing obesity during childhood. However, mean proportional fat intake was below the reference values in both groups in the second half of the intervention period. Thus, there is a general need to improve proportional fat intake to optimize growth and minimize adverse health outcomes later in life. It must be considered that not only fat quantity but also quality is of critical importance. A high intake of trans fatty acids is associated with increased inflammation and adverse effects on somatic development and should be avoided during CF (36). Polyunsaturated fatty acids, i.e., n-3 and n-6 fatty acids, are important for growth, neurodevelopment, and immune function (37). Thus, solid foods that are rich in polyunsaturated fatty acids, such as fish and vegetable oils, e.g., soybean and rapeseed oil, should be offered more frequently (38). However, further studies on proportional quantities of macronutrients during CF with respect to growth parameters and later health outcomes are needed.

4.2. Iron intake

In this study, the mean dietary iron intake did not differ between the early and late groups. In contrast, Kattelman et al. (39) reported that mean iron intake was greater when complementary foods were introduced early. The study authors concluded that the higher iron intake was likely due to the greater consumption of iron-fortified cereals as the first complementary food. In our study, infants mainly received pureed fruits and vegetables as their first solids, which provide only small amounts of iron and might explain the deficiency in dietary iron intake in both groups. Finn et al. showed that infants that consumed iron-fortified cereals from 6 to 18 months had significantly higher levels of iron compared to non-users, suggesting that this could be a strategy to reduce iron deficiency (40). Generally, dietary iron intake was low, with levels remaining below 1 mg/kg/d throughout the intervention period. Thus, CF failed to adequately improve dietary iron intake. This is in line with current literature that shows that up to 60% of infants have iron intake levels below the estimated average requirements from 6 to 36 months in some European countries, including Austria. (41). VLBW infants are even more prone to iron deficiency than term infants (42). Iron requirements cannot be met solely by dietary sources, emphasizing the need for iron supplementation in this vulnerable cohort. Although standard iron supplementation was given until meat was fed regularly, as suggested by ESPGHAN (43, 44), the mean total iron intake fell below the recommendations in the second half of the 1st year, corrected for gestational age. Data on iron status from this study had already been published previously (29). At 6 months, corrected for gestational age, 6% of infants in the early group and 8% in the late group developed iron deficiency (defined as ferritin <12 μg/L). The incidence of iron deficiency was significantly higher in the early feeding group at 12 months, corrected for gestational age (early: 13%, late: 2%). Because dietary iron intake was not statistically different and total iron intake was even higher in the early group at the beginning of CF, we assumed that the difference in iron deficiency between the groups may result from other factors, such as heme and none-heme iron composition, host-related factors, and potential individually different iron requirements, rather than overall iron intake. (45) Regardless of the timepoint of CF introduction, there is a need to improve iron intake and status in VLBW infants during the 1st year of life. This could be achieved by prolonged iron supplementation, nutrition counseling, and parental education on dietary sources of iron, as well as by improved compliance with iron supplementation and consequent monitoring of iron status.

4.3. Vitamin D intake

The results of this study showed that the timepoint of the introduction of solids had no influence on dietary vitamin D intake and that vitamin D intake did not change and improved throughout the 1st year of life, corrected for a term with constantly low intake levels ranging from 269 (SE ± 21) IU/d to 325 (SE ± 21) IU/d (see Table 2). These findings are consistent with previous literature that indicates that vitamin D requirements cannot be met solely by dietary sources during the 1st year of life (46). Infants received 650 IU/d of vitamin D3 supplementation, and the total vitamin D intake was within the recommended range of 800–1000 IU/d (ESPGHAN) until 10 months, corrected for gestational age. Although the recommendations for vitamin D intake were met, 67% in the early group and 49% in the late group developed vitamin D deficiency (serum 25-OH-vitamin D levels <50 nmol/L) at 6 months, corrected for gestational age (47). The incidence of vitamin D deficiency was even higher at 12 months, corrected for gestational age (early: 89%, late: 81%) (47), suggesting that vitamin D requirements in preterm infants might be higher than previously assumed. Hence, vitamin D supplementation dose should be reconsidered, and factors affecting vitamin D bioavailability and absorption efficiency [i.e., food matrix, its interaction with other fat-soluble compounds, age, genetic variation, and disease (48)] should be further addressed to optimize vitamin D intake during CF in former VLBW infants. In the present study, the parents often stopped supplementation before 12 months, corrected for gestational age, further explaining low vitamin D serum levels at the end of the 1st year as dietary intake was insufficient. We suggest continuing vitamin D supplementation at least until 12 months after correction for gestational age and monitoring vitamin D status to adapt therapy if necessary.

4.4. Calcium and phosphorus intake

Dietary calcium and phosphorus intake were not influenced by the time point of the introduction of solid foods. Calcium intake was found to be within the reference values of term infants throughout the whole study period. However, whether these reference values are appropriate for use in former preterm infants is questionable because calcium absorption is compromised due to poor gastrointestinal tolerance and motility in preterm infants (49). Serum calcium levels at 6 weeks, 6 months, and 12 months, corrected for gestational age, were within the normal range, and no case of deficiency was reported (47), assuming that calcium intake was sufficient. Dietary phosphorus intake was below the recommendations, mainly in the transition phase from exclusive breastfeeding or formula feeding to solid foods from 5 to 7 months, corrected for gestational age. Phosphorus intake mainly derives from meat, fish, seafood, dairy products, seeds, whole grains, and nuts, all foods that are rather introduced later in the period of CF, thus explaining the shortfall during the respective months (50). Low dietary intakes of phosphorus may affect the optimal growth of infants and the deposition of lean body mass, as phosphorus is an important component of body tissue (51). Furthermore, inadequate intakes of phosphorus and calcium contribute to the etiology of metabolic bone disease in premature infants. Biomarkers suggestive of the diagnosis of metabolic bone disease are hypophosphatemia and high levels of alkaline phosphatase (52). Phosphorus and alkaline phosphate serum levels were within the reference range (47), assuming that intake levels were adequate to prevent metabolic disease.

4.5. Zinc intake

Zinc is a critical nutrient, especially in the early stages of life, as it serves many cellular processes (53). In our study, zinc intake was not influenced by the timepoint of introduction, and intake recommendations for term infants were met throughout the intervention period. Still, it is unclear whether the needs of premature infants with corrected ages correspond to those of mature infants. Preterm infants have higher zinc requirements in the early postnatal phases (54). However, no data regarding zinc requirements during the weaning period in former preterm infants exist. Because we did not measure serum zinc concentration, it was impossible to assess whether dietary zinc intakes efficiently prevent deficiency. Thus, the actual zinc requirements of former VLBW infants during the 1st year of life must be addressed further.

4.6. Study strengths and limitations

The standardized CF concept represents the major strength of this study, as it enabled exact calculations of nutritional intake in VLBW infants for the first time. The food provided in this study was commercially available baby jar food. Thus, the results of this study are generally applicable to preterm infants that are fed preprepared complementary food in Austria. Another strength of this study is the precise calculation of intakes from infant formula, as changes in formulations were considered for calculation. However, as this is a secondary outcome analysis of a randomized controlled trial, it was not powered to detect a difference in nutrient intake between study groups. A baseline imbalance in birthweight and gestational age occurred during study recruitment after an interim analysis in July 2017. Infants in the early group had a significantly lower birthweight and a significantly lower gestational age compared to the late group (22). Therefore, the randomization process was switched to a baseline adaptive randomization design with additional stratification according to birth weight. However, the products of the standardized solid food, their nutritional content, and the infant's nutritional intake remained the same over the whole study period. Hence, it is very unlikely that the baseline adaptive randomized design influenced any outcome parameter of this analysis. Another limitation of this study was that mothers' milk intakes were calculated as mean intakes based on previous studies because exact intake data were not available. Previous reports reported that the levels of some nutrients, including vitamin A, B6, vitamin B12, fatty acids, zinc, vitamin D, and iron, are associated with maternal factors such as maternal diet or supplementation (55–57). Because this study did not investigate maternal dietary intakes, variabilities in human milk were not considered. Furthermore, it was previously described that various factors, such as medication, maternal endocrine disorders, and temporarily blocked ducts, may pose a potential risk for low milk production, affecting milk volume intakes (58). Since these factors were not considered, the data must be interpreted cautiously. To inform readers about the lack of confounding that might be due to differences in breast lactation, maternal baseline characteristics from the per-protocol population are provided in the Supplemental material section.

5. Conclusions

The time point of the introduction of solid foods did not have an impact on nutrient intake. However, early introduction leads to a higher proportional intake of protein and carbohydrates and a lower fat intake (percentage of total energy) at the beginning of weaning. Further studies on macronutrient distribution during CF with respect to growth parameters and later health outcomes are needed to ensure optimal growth without the risk of obesity or wrong metabolic programming later in the lives of former preterm infants. The results of this study indicate that this standardized feeding regime provided sufficient zinc, calcium, and phosphorus intake. However, dietary iron intake was low even after introducing iron-rich foods, and recommendations were not met in the second half of the 1st year, corrected for gestational age. Therefore, prolonged iron supplementation should be considered, as iron intake solely from dietary sources is insufficient. Furthermore, dietary intake of vitamin D was insufficient to meet the recommendations throughout the 1styear of life, highlighting the importance of vitamin D supplementation until at least 12 months, corrected for gestational age. This study adds to our understanding of the dietary intake of critical nutrients during the complementary feeding period in VLBW infants, which is crucial in preventing both over- and under-supply and thus optimizing post-discharge nutritional management in this vulnerable cohort.

Data availability statement

The datasets presented in this article are not readily available because data requestors will need to sign a data access agreement and in keeping with patient consent for secondary use, obtain ethical approval for any new analyses. The study protocol and the individual participant data that underlie the results reported in this article, after deidentification, are available upon request from the corresponding author 6 months after publication. Researchers will need to state the aims of any analyses and provide a methodologically sound proposal. Requests to access the datasets should be directed to nadja.haiden@meduniwien.ac.at.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Medical University of Vienna (EK:1744/2012, date of approval 10 January 2013) and registered on clinicaltrials.gov (https://clinicaltrials.gov, NCT01809548). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

NH conceptualized the study. MG researched and analyzed the literature and wrote the manuscript, including interpretations. MG and MT contributed to the data acquisition for the study. MG and FE contributed to the formal analysis and visualization. NH, MT, FE, AB, RR, and BJ revised and edited the manuscript critically for intellectual content. All authors agree to be accountable for all aspects of the study, ensuring that questions related to the accuracy or integrity of any part of the study are appropriately investigated and resolved.

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Conflict of interest

NH reports consulting fees from Medis, MAM, Baxter, and Nestle and honoraria for lectures from Nestle, Baxter, Danone, and Hipp outside the submitted work.

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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1124544/full#supplementary-material

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Dietary glycemic index, glycemic load, and renal cancer risk: findings from prostate, lung, colorectal, and ovarian cancer trial

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Background: Dietary glycemic index (GI) or glycemic load (GL) has been associated with the development of many cancers, but the evidence for renal cancer is still limited. The aim of the present study was to investigate the association between GI or GL and renal cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer (PLCO) Screening Trial.

Methods: The cohort for our analysis consisted of 101,190 participants. GI and GL were calculated from the FFQ data using previously published reference values. Multivariate-adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox regression model after adjusting for most known renal cancer risk factors.

Results: During a median of 12.2 years of follow-up, 443 incident renal cancer cases occurred. Higher dietary GI was significantly associated with a higher risk of renal cancer (HR_{Q3vsQ1}: 1.38; 95% CI: 1.09–1.74; p for trend=0.008). There was no significant association between dietary GL and renal cancer risk (HR_{Q3vsQ1}=1.12, 95% CI=0.79–1.59, p for trend=0.591). Spline regression plot revealed a higher risk of renal cancer with a higher GI but not GL. There was no statistical evidence for nonlinearity (p for nonlinearity >0.05).

Conclusion: In summary, findings of this large-scale prospective cohort study suggested that dietary GI may be associated with the risk of renal cancer. If confirmed in other populations and settings, dietary GI could be considered as a modifiable risk factor for renal cancer prevention.

KEYWORDS

glycemic index, glycemic load, renal cancer, cohort, PLCO

1. Introduction

Renal cancer currently ranks seventh among the most frequently diagnosed cancer in males and ninth in females (1). In 2020, more than 431,000 cases of renal cancer were projected to occur worldwide (2). The geographic distribution of renal cancer is highest in the Baltic countries and in Eastern European countries and lowest in most parts of Asia and Latin America (3). There is a consistent male to female excess of renal cancer observed in both low- and high-incidence regions (4). The main established risk factors of renal cancer include tobacco smoking, body size, history of hypertension and chronic kidney disease (5, 6). However, they do not sufficiently explain these geographical and ethnic differences. Additional investigation is required to identify other suspected risk factors, which can improve prevention of renal cancer.

Emerging evidence have suggested a potential relationship between cancer development and diets associated with glucose and insulin metabolism. Hyperglycemia is associated with greater cancer risk and progression (7). This cancer-promoting effect may be mediated by systemic effects of insulin/insulin-related growth factor-1 (IGF-1) and inflammatory signaling. Direct uptake of glucose by cancer cells may also lead to epigenetic and biosynthetic changes (8). Glycemic index (GI) is a scale of zero to 100 for ranking carbohydrate-rich foods based on how quickly and how much they raise blood sugar levels after eating. Glycemic load (GL), a related measure, is a ranking system that takes into account the GI of a food and the carbohydrate content in a serving. High GI or GL has been associated with an increased risk of cardiovascular disease incidence and mortality (9–11) and some types of cancer (e.g., breast cancer) (12). A few prospective studies also have examined the potential association between dietary GI or GL and risk of renal cancer, with controversial results (13–15). A previous meta-analysis reported a significant positive association between GI and the risk of renal cancer (16). Given the potential impact of glucose and insulin on cancer, and limited evidence with mixed findings regarding GI and GL in relation to renal cancer risk, we investigated the associations of GI and GL with renal cancer risk within the large prospective Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial.

2. Methods

2.1. Study population

PLCO screening trial was initially designed to evaluate whether screening tests might decrease mortality from prostate, lung, colorectal, and ovarian cancers. PLCO trial included 154,897 individuals aged 55 to 74 years at the inception of the study, recruited from 10 medical centers throughout the US from 1993 to 2001 (17). At enrollment, participants answered self-administered baseline questionnaire (BQ) and provided information on demographic information, medical history, anthropometric factors (i.e., height and weight), health behaviors, sex-associated exposures and other relevant factors. For the current analysis, 4,918 participants were excluded because of a lack of BQ data. We further excluded participants who did not complete a valid questionnaire or had history of cancer ($n = 48,237$), those had the highest or lowest 1 percentile of calorie intake ($n = 546$), and follow-up time was missing ($n = 6$). Ultimately, this resulted in the inclusion of 101,190 participants. The study protocol was approved by the institutional review boards of all participating centers, and all participants provided written informed consent (18). Our study was approved by the NCI with the project number of PLCO-1020.

2.2. Dietary assessment

The Diet History Questionnaire (DHQ) version 1.0 (National Cancer Institute, 2007) is a food frequency questionnaire that was added in 1998 and was administered to both arms of the trial. The DHQ collected a list of foods eaten in the past 12 months. The frequency and quantity of intake of 124 food items and supplement use were recorded (19). The DHQ has been found to do as well as or better than two widely used FFQs when the PLCO trial was conducted (19). The complete list of the GI and GL values for each food were based on the recent international tables (20) to find the optimal match as previously described (21). Weighted mean method was used to calculate the sex and serving size specific GLs for 225 food groups (19, 22). Each food's GL was multiplied by the frequency of consumption of the food per day to calculate dietary GL for each participant. Daily GI was then calculated by dividing GL by total available carbohydrate intake and multiplying the result by 100. Total carbohydrates were classified as total available carbohydrates minus total dietary fiber.

2.3. Case ascertainment

Individuals were followed until cancer diagnosis or death, or end of follow-up (December 31, 2009). Participants were asked to update information about their health periodically with a self-administrated questionnaire. Participants were asked to identify whether and what type of cancer they had been diagnosed with in the previous year. The information of diagnosis date and location were also collected. Cancer registries, death certificates and physician reports also have been used as to provide additional data in cancer incidence. Medical record abstraction was performed by trained personnel to pathologically confirm all cancers. In this study, renal cancer case was defined as malignant neoplasm of renal parenchyma and renal pelvis coded using ICD-O-2 codes (C649 and C659).

2.4. Statistical analysis

The baseline information of the participants was presented by tertiles of dietary GI and GL. Hazard ratios (HR) with 95% confidence intervals (CIs) were evaluated using Cox proportional hazards regression models adjusted for the following potential confounders including age, sex, race, body mass index (BMI), education level, smoking status, drinking status, marital status, and total energy intake. We used Schoenfeld residuals to examine the proportionality of hazards (PH) assumption and no violation was found (23). To examine potential nonlinear associations, a restricted cubic spline model (24) with three knots (placed at the 10th, 50th, and 90th percentiles) was used to model a smooth curve. In sensitivity analysis, cancer cases occurring in the first two years of follow-up were excluded to minimize reverse causality. All statistical analyses were conducted using the software STATA version 15 (Stata Corp, College Station, TX, USA), with $p < 0.05$ considered statistically significant.

3. Results

3.1. Study characteristics

After a median of 12.2 years of follow-up, 443 incident renal cancer cases were identified. GI and GL from diet ranged from 32.97 to 79.16 (median value: 53.62) and from 9.97 to 494.89 (median value: 101.645), respectively. Participants with the highest GI or GL (i.e., tertile 3), compared with those with the lowest GI or GL (i.e., tertile 1), were more often men and current or former smokers. They also had higher total energy intake, were more likely to be black race, and had a higher BMI on average at baseline (Tables 1, 2).

3.2. Dietary GI or GL and renal cancer risk

As shown in Table 3, in categorical analysis with a maximally adjusted model, GI was significantly positively associated with

TABLE 1 Main characteristic of participants in the PLCO cancer screening trial by GI.

Variables	Q1 (*n*=33,763)	Q2 (*n*=33,702)	Q3 (*n*=33,725)	*p* value
Age (y), mean ± SD	62.4 (5.3)	62.5 (5.3)	62.3 (5.2)	< 0.001
Male (*n*, %)	14,529 (43.0%)	16,641 (49.4%)	17,909 (53.1%)	< 0.001
Arm (*n*, %)				< 0.001
Screen	17,469 (51.7%)	17,138 (50.9%)	16,924 (50.2%)	
Control	16,294 (48.3%)	16,564 (49.1%)	16,801 (49.8%)	
Smoking (*n*, %)				< 0.001
Never	16,626 (49.2%)	16,537 (49.1%)	15,202 (45.1%)	< 0.001
Current	2,745 (8.1%)	2,835 (8.4%)	3,741 (11.1%)	
Former	14,388 (42.6%)	14,323 (42.5%)	14,780 (43.8%)	
Missing	4 (<1%)	7 (<1%)	2 (<1%)	
Education (*n*, %)				< 0.001
≤High school	12,735 (37.7%)	13,716 (40.7%)	16,177 (48.0%)	
≥Some college	20,959 (62.1%)	19,923 (59.1%)	17,484 (51.8%)	
Missing	69 (0.2%)	63 (0.2%)	64 (0.2%)	
BMI (*n*, %)				< 0.001
<25.0 kg/m^2	12,175 (36.1%)	11,250 (33.4%)	10,878 (32.3%)	
≥25.0 kg/m^2	21,140 (62.6%)	22,021 (65.3%)	22,392 (66.4%)	
Missing	448 (1.3%)	431 (1.3%)	455 (1.3%)	
Race (*n*, %)				< 0.001
White, Non-Hispanic	31,532 (93.4%)	30,908 (91.7%)	29,611 (87.8%)	
Other	2,220 (6.6%)	2,782 (8.3%)	4,100 (12.2%)	
Missing	11 (<1%)	12 (<1%)	14 (<1%)	
Drinking (*n*, %)				<0.001
Never	3,430 (10.2%)	3,242 (9.6%)	3,413 (10.1%)	
Former	4,275 (12.7%)	4,542 (13.5%)	5,862 (17.4%)	
Current	25,194 (74.6%)	25,009 (74.2%)	23,362 (69.3%)	
Missing	864 (2.6%)	909 (2.7%)	1,088 (3.2%)	
Total energy intake (kcal/d), mean ± SD	1692.8 (704.3)	1736.0 (691.5)	1751.2 (714.8)	<0.001

GI, glycemic index; PLCO, prostate, lung, colorectal and ovarian; y, year; SD, Standard deviation; BMI, body mass index.

renal cancer risk (HR_{Q3vsQ1}: 1.38; 95% CI: 1.09–1.74; *p* for trend = 0.008). When GI was treated as a continuous variable, the HR (95% CI) of one-SD increment in the GI for renal cancer risk was 1.15 (1.04–1.26). HRs for renal cancer risk across GL tertiles are also presented in Table 3. After adjusting for various potential confounders, there was no significant association between GL and renal cancer risk (HR_{Q3vsQ1} = 1.12, 95% CI = 0.79–1.59, *p* for trend = 0.591). The results did not differ by continuous analysis. The HR (95% CI) of one-SD increment in the GL for renal cancer risk was 1.06 (0.89–1.28).

3.3. Additional analyses

The results of subgroup analyses based on several potential effect modifiers have been summarized in Tables 4, 5. The association between GI and renal cancer was more significant for studies conducted in male (HR_{Q3vsQ1} = 1.40, 95% CI = 1.04–1.88), in white non-Hispanic population (HR_{Q3vsQ1} = 1.51, 95% CI = 1.18–1.93) and in participants with BMI ≥ 25.0 kg/m^2 (HR_{Q3vsQ1} = 1.45, 95% CI = 1.10–1.90). No significant associations were observed in any subgroups of the association between GL and renal cancer. Spline regression plots of renal cancer risk in relation to GI or GL are shown in Figure 1, which revealed a higher risk of renal cancer with a higher GI but not GL. There was no statistical evidence for nonlinearity (*p* for nonlinearity >0.05). In sensitivity analysis, there was little change in the findings after excluding cases who were diagnosed within the first two years of follow-up (GI: HR_{Q3vsQ1}: 1.38; 95% CI: 1.09–1.76; GL: HR_{Q3vsQ1} = 1.11, 95% CI = 0.78–1.58).

4. Discussion

In this large prospective PLCO cohort, there was a statistically significant association between dietary GI and renal cancer risk. No obvious association between GL and renal cancer risk was observed. Similar results were obtained when excluding cases diagnosed within

TABLE 2 Main characteristic of participants in the PLCO cancer screening trial by GL.

Variables	Q1 (*n*=33,732)	Q2 (*n*=33,735)	Q3 (*n*=33,723)	*p* value
Age (y), mean ± SD	62.5 (5.3)	62.6 (5.3)	62.2 (5.3)	< 0.001
Male (*n*, %)	11,540 (34.2%)	15,654 (46.4%)	21,885 (64.9%)	< 0.001
Arm (*n*, %)				
Screen	17,263 (51.2%)	17,144 (50.8%)	17,124 (50.8%)	0.52
Control	16,469 (48.8%)	16,591 (49.2%)	16,599 (49.2%)	
Smoking (*n*, %)				< 0.001
Never	16,132 (47.8%)	16,398 (48.6%)	15,835 (47.0%)	
Current	3,187 (9.4%)	2,906 (8.6%)	3,228 (9.6%)	
Former	14,411 (42.7%)	14,424 (42.8%)	14,656 (43.5%)	
Missing	2 (<1%)	7 (<1%)	4 (<1%)	
Education (*n*, %)				< 0.001
≤ High school	14,507 (43.0%)	13,970 (41.4%)	14,151 (42.0%)	
≥ Some college	19,155 (56.8%)	19,694 (58.4%)	19,517 (57.9%)	
Missing	70 (0.2%)	71 (0.2%)	55 (0.2%)	
BMI (*n*, %)				< 0.001
< 25.0 kg/m^2	11,843 (35.1%)	11,863 (35.2%)	10,597 (31.4%)	
≥ 25.0 kg/m^2	21,436 (63.5%)	21,449 (63.6%)	22,668 (67.2%)	
Missing	453 (1.3%)	423 (1.3%)	458 (1.4%)	
Race (*n*, %)				< 0.001
White, Non-Hispanic	30,713 (91.1%)	30,959 (91.8%)	30,379 (90.1%)	
Other	3,005 (8.9%)	2,766 (8.2%)	3,331 (9.9%)	
Missing	14 (<1%)	10 (<1%)	13 (<1%)	
Drinking (*n*, %)				< 0.001
Never	3,445 (10.2%)	3,374 (10.0%)	3,266 (9.7%)	
Former	4,510 (13.4%)	4,764 (14.1%)	5,405 (16.0%)	
Current	24,756 (73.4%)	24,669 (73.1%)	24,140 (71.6%)	
Missing	1,021 (3.0%)	928 (2.8%)	912 (2.7%)	
Total energy intake (kcal/d), mean ± SD	1123.3 (332.0)	1633.5 (348.0)	2423.3 (630.8)	< 0.001

GL, glycemic load; PLCO, prostate, lung, colorectal and ovarian; y, year; SD, Standard deviation; BMI, body mass index.

TABLE 3 Associations between GI/GL and renal cancer risk in the PLCO cancer screening trial.

Variables	Median	Cohort (*n*)	Cases (*n*)	Crude HR (95% CI)	Adjusted HR (95% CI)*
GI					
Q1 (< 52.29)	50.51	33,763	118	Reference group	Reference group
Q2 (≥ 52.29 to <54.94)	53.62	33,702	148	1.25 (0.98–1.59)	1.19 (0.93–1.51)
Q3 (≥ 54.94)	56.59	33,725	177	1.51 (1.20–1.91)	1.38 (1.09–1.74)
				p for trend <0.001	*p* for trend = 0.008
GL					
Q1 (< 85.06)	67.46	33,732	124	Reference group	Reference group
Q2 (≥ 85.06 to <120.26)	101.51	33,735	157	1.24 (0.98–1.57)	1.17 (0.91–1.51)
Q3 (≥ 120.26)	148.19	33,723	162	1.29 (1.02–1.63)	1.12 (0.79–1.59)
				p for trend = 0.045	*p* for trend = 0.591

*Adjusted for age (categorical), sex (male vs. female), race (non-Hispanic white vs. Other), body mass index (< 25 kg/m^2 vs. ≥ 25 kg/m^2), education (≤ high school vs. ≥ some college), smoking status (never vs. former vs. current), drinking status (never vs. former vs. current) and total energy intake (continuous). GI, glycemic index; GL, glycemic load.

TABLE 4 Subgroup analyses between GI and renal cancer risk.

Variables	Q1	Q2	Q3	*p* for interaction
Sex				> 0.05
Male	Reference	1.19 (0.87–1.61)	1.40 (1.04–1.88)	
Female	Reference	1.18 (0.80–1.75)	1.32 (0.89–1.95)	
Race				< 0.05
White, Non-Hispanic	Reference	1.20 (0.93–1.54)	1.51 (1.18–1.93)	
Other	Reference	1.09 (0.50–2.38)	0.48 (0.20–1.14)	
BMI (*n*, %)				> 0.05
<25.0 kg/m^2	Reference	0.97 (0.60–1.58)	1.19 (0.75–1.89)	
≥25.0 kg/m^2	Reference	1.26 (0.95–1.67)	1.45 (1.10–1.90)	

GI, glycemic index; BMI, body mass index.

TABLE 5 Subgroup analyses between GL and renal cancer risk.

Variables	Q1	Q2	Q3	*p* for interaction
Sex				> 0.05
Male	Reference	1.18 (0.84–1.66)	1.14 (0.74–1.76)	
Female	Reference	1.11 (0.73–1.70)	1.00 (0.51–1.95)	
Race				> 0.05
White, Non-Hispanic	Reference	1.19 (0.91–1.56)	1.22 (0.84–1.76)	
Other	Reference	0.98 (0.42–2.25)	0.37 (0.10–1.42)	
BMI (*n*, %)				> 0.05
<25.0 kg/m^2	Reference	1.14 (0.68–1.89)	0.78 (0.37–1.62)	
≥25.0 kg/m^2	Reference	1.17 (0.87–1.59)	1.25 (0.84–1.88)	

GL, glycemic load; BMI, body mass index.

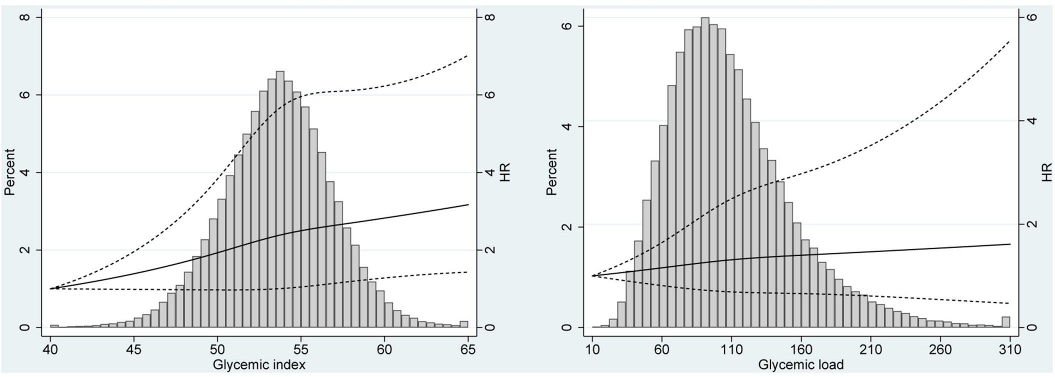

FIGURE 1
Spline regression plots of renal cancer risk in relation to **(A)** glycemic index (GI) and **(B)** glycemic load (GL). Hazard ratios (HRs) were calculated after adjusting for age (categorical), race (White, Non-Hispanic vs. Other), education (≤ high school vs. ≥ some college), smoking status (never vs. former vs. current), drinking status (never vs. former vs. current), body size (<25kg/m^2 vs. ≥25kg/m^2), and dietary energy intake (continuous). The histograms show the percentage of participants (left y axis) in each level of GI or GL.

the first two years of follow-up. Findings from continuous analyses and spline regression plots were comparable with the results in main analyses.

These results, which were based on a large prospective cohort study, were consistent with our previous findings based on a meta-analysis (16). A significant positive association was observed between GI and the risk of renal cancer (poled RR 1.16, 95% CI 1.02–1.32). GL was not significantly associated with renal cancer risk (pooled RR 1.14, 95% CI 0.81–1.60). However, only five studies (three case–control and two cohort studies) were eligible in this meta-analysis. Case–control studies were more likely to be prone to recall bias and select bias. The evidence on the association between GI or GL and renal cancer risk may be still limited and not robust. Therefore, we further performed an analysis based on a large US cohort. As a result, we also found a significant association between GI and renal cancer risk and no association was observed for GL in PLCO cohort, which further enhanced the current evidence.

In subgroup analyses for GI, a more significant association was observed in male, in white non-Hispanic population and in overweight/obese participants, which suggested a potential differential susceptibility. The incidence of renal cancer has obvious ethnic and sex differences. In addition. A high BMI is also a well-established risk factor for renal cancer.

Several mechanisms have been proposed to explain a potential association between dietary GI and human cancers. Foods with a high GI will increase the concentration of glucose and insulin in blood and thus induce hyperinsulinemia (25, 26). Hyperinsulinemia can increase the insulin-like growth factor-1 (IGF-1) expression (27). Higher IGF-1 has been reported to be modestly associated with increased risk of overall cancer risk, including kidney cancer based on a cohort study analysis from the UK Biobank (28). Previous studies have demonstrated that IGF-1 pathway plays an important role in cell proliferation and apoptosis resistance in renal cell carcinoma (29). In addition, long-term exposure of tubular cells to hyperglycemia can lead to disturbances in DNA repair mechanisms, which may drive and promote renal cancer development (30).

Several limitations of the current study should be acknowledged. First, the outcome of this study was renal cancer incidence. Although we have excluded the renal pelvis cancer, we were not able to further classify the types of renal cancer because of the limited data on original questionnaire. Second, the majority of GI values (32.97 to 79.16, median value: 53.62) centered around the middle of the theoretical range for GI (i.e., 0–100) in PLCO trial. Therefore, it was hard to evaluate the effects of different levels of GI unless it is a strong cancer risk determinant at middle values (31). Third, dietary questionnaire used in PLCO cohort was self-administrated, which may cause some measurement error (32). In addition, dietary intake was assessed only once at baseline and any changes in diet during follow-up could not be examined. Finally, although the statistical models were adjusted for various important confounders, a certain degree of residual confounding may be unavoidable because of collinearity from other nutrients, particularly macronutrients.

This study had some unique strengths. As a prospective study, the chance of reverse causality from subclinical disease-causing changes in diet was small and the recall bias was avoided as the exposure was preceding the onset of cancer. This study included almost 100,000 participants with a median of 12.2 years of follow-up, which provided strong power to detect differences in renal cancer incidence if they truly existed. Additionally, the rate of participants lost to follow up was very low in PLCO study. The large study population was recruited from institutions across the United States, which improved the generalizability. The availability of data on various potential confounders made comprehensive adjustment possible. Lastly the methods used to assign GI and GL values to foods was rigorous, which was based on American data wherever possible (21).

In summary, analysis of the PLCO cohort suggested that diets high in GI was associated with greater renal cancer risk. If confirmed in other populations and settings, dietary GI could be considered as a modifiable risk factor for renal cancer prevention.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://cdas.cancer.gov/datasets/plco/.

Ethics statement

The studies involving human participants were reviewed and approved by National Cancer Institute. The patients/participants provided their written informed consent to participate in this study.

Author contributions

XX and DX contributed to the conception or design of the work. XX, HQ, and DX contributed to the acquisition, analysis, or interpretation of data for the work. HQ and DX drafted the manuscript. XX critically revised the manuscript. All authors gave final approval and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

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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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The big food view and human health from the prospect of bio-manufacturing and future food

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The "big food view" has attracted widespread attention due to the view of sustainable nutrition and human health as part of sustainable development. The "big food view" starts from better meeting the people's needs for a better life. While ensuring the supply of grain, the effective supply of meat, vegetables, fruits, aquatic products and other foods also should be guaranteed. Using cell factories to replace the traditional food acquisition methods, establishing a new model of sustainable food manufacturing, will greatly reduce the demand for resources in food production, and improve the controllability of food production and manufacturing, and effectively avoid potential food safety and health risks. Cell factories can provide key technologies and supporting methods for the biological manufacturing of important food components, functional food ingredients and important functional nutritional factors, realizing a safer, nutritious, healthy and sustainable way of food acquisition. The combination of cell factory technology and other technologies meets the people's new dietary demand, and also supports that sustainable nutrition and human health as part of sustainable development. This paper focuses on the big food view and human health from the prospect of bio-manufacturing and future food, which aims to better meet people's dietary needs for increasingly diversified, refined, nutritious and ecological food through diversified food manufacturing.

KEYWORDS

big food view, bio-manufacturing, cell factory, future food, sustainable nutrition

Introduction

Recently, the "big food view" has attracted widespread attention due to the view of sustainable nutrition and human health as part of sustainable development (1). The "big food view" is to "start from better meeting the people's needs for a better life." While ensuring the supply of grain, the effective supply of meat, vegetables, fruits, aquatic products and other foods also should be guaranteed. It includes the structure adjusting and regional layout of the food production, enrich and expand food sources, and actively promote agricultural supply-side reforms based on demand, which fully embodies the concept of "innovation, coordination, green, openness and sharing."

Healthy, safe, and sustainable food manufacturing is a key element of human health and sustainable social development. The active application of cell factories in the field of food manufacturing has vigorously promoted the rapid development of new foods such as artificial meat, milk, and eggs, becoming an effective way to relieve agricultural pressure and meet the growing food demand (2). As an emerging technology, the use of cell factories to replace traditional food acquisition methods and establish a new sustainable food manufacturing model will greatly reduce the demand for resources in food production, and improve the controllability of food production and manufacturing (3). In the future, this technology is expected to end the use of pesticides and fertilizers, reduce land dependence and pollution, promote the development of agricultural industrialization, and truly realize a new model of efficient, low-carbon and green food production, and expected to become an important guarantee for the "big food view" (4).

This concept is to comply with the changing trend of the people's food consumption structure and ensure that the people eat safe, healthy and nutritionally balanced. In the new era, food consumption has shifted from quantity to quality, and residents are more inclined to buy low-fat, high-protein animal food (5, 6). The increase in the consumption demand of processed food by urban and rural residents is more reflected in the increase of the demand for safe, green and nutritious processed food (7). According to the changing trend of people's consumption, improving the quality and diversity of agricultural products and processed food needs to meet people's demand for food from the supply side and the demand side. On the one hand, the "big food view" requires the government and enterprises to promote the high-quality and differentiated development of agricultural products and food industries, and make efforts from the supply side to meet the people's needs for food diversification, refinement, and nutrition. On the other hand, it requires efforts to promote the renewal of food consumption methods from the demand side (8).

Establishing a "big food view" will subvert the production model of traditional planting and aquaculture, and lead the development of the future food industry (9, 10). Building a "big food view" is inseparable from the innovation of future food technology, which is also a key area for future food production to tackle key problems (11). It requires the use of future food technology, especially food synthetic biotechnology, through factory fermentation production to replace traditional planting and breeding production methods, to make breakthroughs in large-scale, low-cost, sustainable high-efficiency manufacturing of food raw materials such as protein, starch and oil, to achieve "industrialization of agricultural production" (12). For instance, an emerging agricultural method, fish-vegetable symbiosis, uses the symbiosis between fish and plants to uniquely combine hydroponics in a circular aquaculture system with hydroponics in a closed-loop system, in fish-vegetable symbiosis, water is recirculated in a closed loop around the system (13). It also requires future food technology to ensure the high-quality supply of food. For important food ingredients and food functional factors that affect food texture, flavor and nutrition, bio-manufacturing technology is needed to help food precision nutrition and intelligent manufacturing (14). The fourth industrial revolution (Industry 4.0) has revolutionized the way in which food is produced, transported, stored, perceived, and consumed worldwide, leading to the emergence of new food trends (15). The Food Industry 4.0 era has been characterized by new challenges, opportunities, and trends that have reshaped current strategies and prospects for food production and consumption patterns (16).

The necessity of the concept of "big food view"

The "big food view" needs to be oriented to the "big system," which includes traditional crops and livestock and poultry resources, as well as special new foods such as artificial milk and artificial meat. To meet this huge demand for food consumption in the world, it is only possible to develop food resources in multiple ways and to develop diverse food varieties.

Quantitative security is always the basis and premise of food security. As people's needs for a better life continue to grow and their expectations for safe and high-quality food grow stronger, food security requires not only a stable increase in the quantity of food, but also a simultaneous improvement in food quality. This requires promoting the transformation of food supply from "quantity-based" to "combining quality and quantity, improving quality and ensuring quantity," and accelerating the construction of a consumption-driven, rationally structured, and effective food supply guarantee system to better meet the growing consumer demand. On the premise of fully assessing the carrying capacity of resources and the environment and ensuring ecological security in an orderly and reasonable manner, it is necessary to promote the formation of a modern agricultural production structure and regional layout that is in line with market demand and with the carrying capacity of resources and the environment.

The "big food view" is a continuation of the new era of ecological civilization ideas such as "lucid waters and lush mountains are invaluable assets." Its essence is to achieve the balanced development of ecological protection and agricultural and rural modernization, and to ask for food from mountains, rivers, forests, fields, lakes, grass, and sand on the premise of ensuring ecological security in a reasonable and orderly manner. It is necessary to further broaden the horizons of agricultural talents, and deeply understand the rich connotation of the life community of "landscapes, forests, fields, lakes, grass, and sand" from the perspective of "big food view." Not just asking for food, but also paying attention to the protection of the ecological environment, unswervingly taking the road of ecological priority and green development, and promoting the integrated protection and systematic management of mountains, rivers, forests, fields, lakes, grass, and sand. Agricultural talents should not only study how to improve water quality, soil quality, and agricultural product quality, but also conduct in-depth research on the living space, habitat space and migration system of animals and plants, so as to achieve a win–win situation in meeting the diverse food needs of the people and protecting the ecological environment, so as to promote more sustainable development.

The application of "big food view" in emerging technology

Building a "big food view" is inseparable from the innovation of future food technology, which is also a key area for future food production to tackle key problems. Through the supporting role of science and technology, we can search for food from forests and rivers, lakes and seas. At the same time, we can expand biological resources, develop biotechnology and bio-industry, and obtain energy from plant, animal and microorganisms (17).

Cell factories

In the face of environmental pollution, climate change and increasingly depleted non-renewable resources, how to ensure a healthy, safe and sustainable food supply faces enormous challenges (18). Using cell factory manufacturing to replace traditional food acquisition methods and establishing a new sustainable food manufacturing model will greatly reduce the demand for resources in food production, reduce greenhouse gas emissions, improve the controllability of food production and manufacturing, and effectively avoid potential food production (19). Using cell factories to improve the synthesis efficiency of important food components, functional food additives and nutritional chemicals is an important research direction to solve the current problems faced by food manufacturing.

Cell factory-related technologies provide important technical support for solving the challenges faced by food manufacturing, and are an important research direction in the field of food (20). Remarkable progress has been made in the bio-manufacturing of typical food components represented by key components of plant protein meat and artificial milk (21). The range of target products to be synthesized is to be further expanded to create an "intelligent cell factory" that will significantly improve the efficiency of synthesis of food ingredients and functional foods. Important directions for future research include the realization of whole-cell utilization and industrial-scale preparation.

The cell factory can individually design and adjust products according to requirements, which will be the future development trend of green, safe and high quality food (22). The cell factory will revolutionize the production and supply chain of agricultural products, provide sustainable and healthier food for the growing population, and provide irreplaceable support for the world's agricultural carbon peaking and carbon neutrality goals.

Artificial milk

Milk proteins are important component in animal milk, which have various biological activities including easy uptake and digestion, high nutrition and immunity enhancement (23). Faced with issues such as sustainability, public health, and animal welfare in dairy production, the research and development of plant- or animal-sourced milk protein replacement technologies has gradually attracted widespread attention (24). Although substantial progress has been made, there are still many problems to be solved in terms of flavor, taste, and functional properties.

Important progress has been made in recent years by using innovative technologies such as synthetic biotechnology to build cell factories with specific synthetic capabilities to produce various agricultural products such as starch, protein, oil, sugar, milk, and meat that humans need (25). The nutrition and flavor of artificial milk are equivalent to natural milk, but it does not contain adverse factors such as lactose, cholesterol, antibiotics and allergens. The production process does not require breeding animals, which can save resources and energy effectively, and will lead the future development of food industry.

TABLE 1 Commercialized artificial food currently developed.

Time (year)	Event
2009	Beyond Meat Inc. was established in the United States, which provided vegetable meat products
2011	Impossible Foods Inc. was established in the United States, which sold meat free hamburgers and sauces
2019	Mycorena in Sweden produced Fungi-based alternative protein for the food industry
2019	Nature's Fynd in the United States produced microbial protein meat and dairy substitutes
2019	The beef flavor vegetable meat was produced by Jinzi Ham Co., Ltd in P. R. China and Dupont in the United States
2020	KFC launched vegetable meat burgers and chicken nuggets

Artificial meat

The overall technical route of artificial food is to build a cell factory to synthesize artificial meat, eggs and milk, etc. by workshop production, so as to relieve agricultural pressure and meet the growing demand for food. Among them, artificial meat is an emerging and breakthrough technology in the food field (Table 1). The breakthrough in large-scale manufacturing technology of artificial meat is expected to reduce the consumption of resources and energy in traditional agriculture (26). In recent years, artificial meat has attracted widespread attention due to its traceability, high food safety and sustainable advantages. Artificial meat can be divided into plant protein meat and cell-cultured meat (27). The plant protein meat has relatively low cost, low technical requirements, and high market acceptance. Therefore, it has the advantages of mature technology and the potential for priority development. A number of companies have developed plant protein products using plant protein as raw materials and have achieved commercial production. Cell-cultured meat is similar to natural meat, but the cost is relatively high in the current stage, and although the market potential is great, it still needs to be fully developed.

Cell-cultured meat

Cell-cultured meat, also called cell-cultivated meat, refers to meat that was produced by livestock and poultry stem cells using tissue engineering technology to produce meat from cell culture (28). In contrast to conventional meat, cell-cultured meat promises to address animal welfare ethics, nutritional properties, and public health issues (29).

At present, cell-cultured meat related products are still mainly in the laboratory research stage, and more comprehensive research and application promotion are needed for widespread market recognition (30). Both plant protein meat and cell-cultured meat need to use key ingredients such as enzymes, vitamins and lipids produced by microbial cell factories (31). The obtained food raw materials and functional ingredients are organically integrated, and finally a recombinant food with a harmonious flavor, stable texture and high simulation is obtained (32).

Cell-cultured meat is commonly expected to alleviate the environmental issues related to the agricultural sector. Nowadays, the livestock operations has used most agricultural land and closely related to the global climate change as well as land and water pollution (33). The production of cell-cultured meat has also been considered to enhance food security, and benefit for the unstable climate conditions (34). The production inside sterile laboratories or factory conditions reduces the concerns of food contamination. Meanwhile, the production of cell-cultured meat prevents the livestock-based infection of epidemics (35). To attract consumers in comparison with conventional meats, the continuous development of cell-cultured meat in sensory and nutritional characteristics is demanded (36).

Regulation of cell-cultured meat

The regulations on cell-cultured meat are intended to facilitate consumption and investment with a certain sense of safety and assurance under scientific uncertainty. Constraints are possible with naming regarding cell-based meats (37). Laws in the US and EU limit the words including "milk" and "steak" for plant-based alternatives, which will prevent the use of the word "meat" for cell-cultured meat, and it reflects the pressure from traditional livestock organizations.

Keeping the word "meat" to sell cell-cultured meat, make the concept of meat more acceptable to ordinary people. The production of cell-cultured meat demands funds for large-scale production and governments are likely to lead in technical innovation for alternative meats. Governmental policy for R&D can also direct corporations to be greener in developing their products. The spread of alternative meats production can also solve the issues of the work environment in meat processing industries (38).

Although the production of cell-cultured meat may contribute to the resilience of food systems, developing countries without technologies and financial resources for investment may not promote alternative meat production (39). The alternative meat production raises the positive prospect to solve the environmental issues related to current livestock production (40). The urban population in these countries can reasonably depend on alternative meats once the product becomes more common. The attitudes of vegetarians toward cell-cultured meat are another issue. They can eat cell-based meats because they solve the ethical problems about meat production (41).

Recent studies have discussed the impact of cell-cultured meat production on environmental factors, such as greenhouse gases emissions, land use, energy use, and water use (42). The production of cell-cultured meat demands much fewer resources than European beef, and the greenhouse gases emission was more than 78% less (21). The technological development of cell-cultured meat can target the entire process of production to consumption including texture refinement and composition improvement (43). Furthermore, the policies must integrate social, ethical, and environmental concerns with energy-efficient production and connect with current efforts on carbon peaking and carbon neutrality goals and biodiversity conservation. The production of cell-based meats continuously demands sound science-policy discussion to resolve the contestation over cell-based meats, which will form the basis for current R&D activities and future industrialization.

However, there is still a big gap between the quality of artificial meat and real meat, and breakthroughs in texture simulation, nutritional optimization, flavor adjustment, and product customization are urgently needed. Using enzymes with specific functions, can achieve structural strength modification, structural hydrophilicity and hydrophobicity modification, allergen degradation, protein utilization improvement, and glycolipid protein integration, degradation of odor components and enhancement of flavor substances. The synergistic utilization of enzymes and flavor substances with specific functions will improve the quality of artificial meat, and lay the foundation for the customized process of finished products based on 3D printing food.

3D printing

Three-dimensional (3D) food printing technology combines 3D printing and food manufacturing. The potential of delivering personalized products tailored to meet the taste preferences and specific dietary needs is one of the reasons for increasing researches in this technology (44). With the increased living standards, people have higher demand on healthy functional food and even personalized food (45).

Food 3D printing can be blended with various raw materials according to individual's physical and nutritional status, so that the functional factors such as protein, fat, dietary fiber, vitamins, and minerals, are balanced according to the demand (46). In food processing, and realize digital nutrition and complex food design, which cannot be achieved by traditional food processing methods (47).

The 3D food printing combines 3D printing technology and food manufacturing and uses edible materials such as fruit and vegetable juice and powder, starch, meat, chocolate, and algae etc. as printing materials (48). As we know, the most important feature/advantage of 3D printing is the creation of complex 3D structures. But in food field, the potential of delivering personalized nutrition and personalized food choice may be the main reasons that the 3D food printing technology is advancing so rapidly. 3D food printing technology can enable formulation of food to meet the need of people having different preferences for taste, dietary needs and physical condition such as dysphagia (49). Specifically, tailored foods by adding specific nutrients and functional compounds or eliminating/replacing certain ingredients in the formulation can help promote health and prevent diseases (50).

The 3D printing technology can seamlessly integrate nutrition, enable manufacturing of personalized foods that satisfy the requirements of consumers according to their occupation, gender, age, and lifestyle (51). Food with desirable texture has been produced using 3D printing by developing different nozzles and filling modes (52). The 3D printed foods can be popular and more appealingly designed to cater for specific needs of the children (53). 3D printing technology helps to create healthy snacks with novel shapes and rich in vitamins and minerals, attracting children and becoming a model of personalized food. In fact, 3D printing enables manufacturing of personalized foods that have both health promoting and enjoyment elements (54). 3D printing is an innovation that promises to revolutionize food formulation and manufacturing processes. Preparing foods with customized sensory attributes from different ingredients and additives has always been a need (55).

The future task of the food industry is to focus on developing robust 3D food printers, understand material printability, identify unique food sources for printing, and simplify the technology for convenient use by all. An added aspect is to focus on the effect of pre- and post-printing operations on the quality and consumer acceptance of 3D-printed foods, and overall process control. While pre-processing involves alterations in material supply ingredients to make a recipe compatible for printing, post-processing of fabricated structures focuses on improving the palatability of 3D-printed foods.

The significance and value of future food

The manufacturing mode of the traditional food industry may be changed in the future. It is mainly through the combination of food and biotechnology to change the traditional methods of cultivation and reproduction and production. The typical representative is artificial meat, including plant protein meat made from soybean and other plant protein as raw materials, and cell-culture meat made from cells extracted from animals.

Food will make people and the earth healthier. The chronic diseases are caused by the way people eat and drink. A large number of medical studies showed that adding certain plant protein to animal protein can significantly reduce the risk of death (56, 57). Now, the way of livestock and poultry breeding to obtain animal protein is much higher than that of plants, microorganisms to obtain protein, in terms of resource occupation and environmental impact. Substitute protein not only has the above resource and environmental benefits, but also has obvious advantages over traditional livestock and poultry breeding in terms of protein production efficiency.

The core content of future food includes plant-based food, alternative protein, food perception, etc. The development of food in the future will highlight six "new": breakthroughs in food nutrition and health will become the new engine of food development; the progress of physical properties of foods will become a new source of food manufacturing; the results of the detection and control of food hazards will become a new support for active security; the innovation of green manufacturing technology will become a new driving force for the sustainable development of the food industry; the revolution of intelligent equipment for food processing will become a new driving force for the upgrading of the food industry; the integration of the whole food chain technology will become a new model of the food industry.

Conclusion

It is the starting point of the "big of food view" to improve food production, improve quality and diversity through scientific and technological innovation, at the same time open up new food access so that people can have enough, good and healthy food and better satisfy people who yearn for a better life. Since the beginning of the new century, the emerging science such as synthetic biology, bioinformatics, bionics and artificial intelligence have developed rapidly, which is promoting the transformation of agricultural production mode from traditional agriculture and industrial agriculture to cell agriculture. Cell factory is one of the main methods to solve the major challenges of food in the future, including the development and high-value utilization of new food resources and the transformation of diversified food production mode. Cell factories can personalized design and adjust products according to requirements, which will be the future development trend of green, safe and high-quality food. We should strengthen the research and development of cell factory-related technologies, and meet people's new dietary needs through diversified food manufacturing ways.

Author contributions

JW: conceptualization and writing original draft. XZ: supervision and writing review and editing. Both 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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Vitamin D deficiency and risk of recurrent aphthous stomatitis: updated meta-analysis with trial sequential analysis

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Background: Growing evidence suggests a significant association between vitamin D deficiency and RAS. Hence, the present meta-analysis and trial sequential analysis sought to investigate the potential association between low serum vitamin D levels and RAS.

Methods: PubMed, Scopus, Embase, and Web of Science were comprehensively searched on December 1st, 2022 to retrieve all relevant studies. The grey literature was also searched via ProQuest. All case-control studies on the association between vitamin D and RAS were considered. The quality appraisal of the included studies was done using Newcastle-Ottawa scale. RevMan 5.0 and trial sequential analysis (TSA) programs were used for analyses.

Results: A total of 14 case-control studies with 1468 subjects (721 RAS patients and 747 controls) were included. The pooled data revealed a significant association between low serum levels of vitamin D and the risk of RAS (mean difference = − 8.73, 95% CI: − 12.02 to − 5.44, $I^2 = 94\%$, $P < 0.00001$). Additionally, TSA findings indicated that the current studies surpassed the required information size, confirming that the differences were reliable.

Conclusion: The available evidence suggests that Vitamin D deficiency may have a role in the pathogenesis of RAS. Therefore, evaluation of vitamin D should be considered in RAS patients. Additionally, the results support the possibility of using vitamin D supplements in the management of RAS patients with inadequate serum levels of vitamin D. Future interventional studies are required to evaluate the benefits of vitamin D replacement in prevention and treatment of RAS.

KEYWORDS

aphthous stomatitis, vitamin D, association, risk factor, meta-analyses

Introduction

Recurrent aphthous stomatitis (RAS) - also known as recurrent aphthous ulcers or canker sores - is the commonest cause of oral mucosal ulceration (1–3). RAS is a highly prevalent condition affecting up to 25% of the general population, mainly adolescent and young adults, although it can occur at any age (3, 4). It is characterized by recurring, painful, ovoid or round, single or multiple ulcers of the oral mucosa, and primarily affect the non-keratinized mucosa (3, 4). The RAS-associated pain and discomfort might be severe and impact the patients' quality of life adversely by interfering with routine oral functions such as eating, swallowing, and speaking (1, 3, 5). Clinically, there are three variants of RAS: minor (less than 1 cm in diameter), major (more than 1 cm), and herpetiform (2–3 mm across) (2–4). Minor RAS is the most common form accounting for 90% of all RAS cases (1, 3).

Despite the extensive research done on the topic, the exact etiopathogenesis of RAS remains unclear (1). An immunological reaction to an unknown trigger is considered the most plausible mechanism involved in the development of RAS (6, 7). Several systemic and local factors increase predisposition to RAS including psychological stress, genetic makeup, immunological dysfunction, mucosal trauma, gastrointestinal disorders, hematological factors, and nutritional and hematinic deficiencies (1, 3, 6–10). The potential role of nutritional deficiencies of certain vitamins and minerals has been explored extensively in the literature (10). In this regard, numerous studies assessed hematinic and vitamins deficiencies (such as, B-complex vitamins and Folic acid) in RAS patients. However, the results remain inconclusive (11–16).

In recent years, the role of vitamin D in pathogenesis of several oral diseases including RAS has generated a significant level of interest (12, 14, 17, 18). Vitamin D, a lipid soluble secosteroid, plays key biological roles in calcium-phosphorus homeostasis and bone metabolism (17, 19). Recent evidence supports the role of vitamin D in inhibition of inflammatory process: Vitamin D is believed to modulate the immune system through inhibition of maturation of dendritic cells, and establishing a balance between different components of the immune system (17, 20, 21). In regard to systemic health, vitamin D deficiency has been linked to many disorders including hypertension, musculoskeletal disorders, obesity, cancers and autoimmune diseases (17, 22–26). In relation to oral health, a growing body of evidence links vitamin D deficiency to several oral mucosal diseases such as oral lichen planus and RAS (27, 28). In context of the latter, many recent studies investigated the potential association between vitamin D and the risk of RAS but showed inconsistent results (11–14, 28–30). Our previous meta-analysis, which involved all relevant studies published up to June 2019 (n = 5) revealed a significant association between vitamin D deficiency and RAS (31). Since then, numerous case-control studies have investigated the role of vitamin D in RAS, and appeared to report variable results (11–14, 32–35). Hence, we sought to update the available evidence regarding the potential association between low serum levels of vitamin D and RAS, supported by a trial sequential analysis (TSA). TSA is a novel approach used in systematic reviews and meta-analysis to control the random errors in the conventional meta-analysis and identifies the information size and weather further studies are still required or not (36).

The focused research question for this study was: "Is low serum level of vitamin D associated with RAS?

Methods

The present meta-analysis followed PRISMA 2020 guidelines and PICO/PECO principles (37), and the protocol was registered in PROSPERO (ID: CRD42022365428).

Eligibility criteria

All case-control and cohort studies that investigated the association between serum levels of vitamin D (25-hydroxycholecalciferol) and RAS, and fulfilled the following criteria were considered eligible: (1) comprised systemically healthy RAS subjects who were compared with systematically healthy control subjects, (2) the outcome measures reported serum levels of vitamin D quantitatively (mean ± SD).

The exclusion criteria were: (1) Lack of control group, (2) experimental studies, (3) case reports (4) case series, (5) missing or inadequate quantitative data (means of vitamin D), (6) editorials, and (7) review papers.

Search strategy

Two investigators independently conducted extensive online searches on December 1, 2022 in PubMed, Scopus, Embase, and Web of Science databases for all relevant studies from date of inception till and including November 2022. The grey literature was also searched via Proquest. The following Mesh terms and free keywords were used for the electronic searches: "Stomatitis, Aphthous"[Mesh] (for PubMed) OR "recurrent aphthous ulcers" OR "aphthous ulcers" OR "recurrent aphthous stomatitis" OR "recurrent aphthosis" OR "recurrent oral ulcer" Or "aphthous stomatitis" AND "vitamin D" OR "25-hydroxycholecalciferol" (Detailed search strategy is presented in Supplementary Table 1). The online searches were supplemented with a manual search of the references of the included studies. The retrieved studies were then exported to EndNote program, and duplicates were removed.

Data extraction

Two investigators (NA, GA) independently extracted all relevant data using a pre-designed form. The following data were extracted: authors, year of publication, country, study design, sample size, age of participants, the means and SD of serum levels of Vitamin D (ng/mL).

Assessment of quality of evidence

Two investigators (NA, GA) independently assessed the quality of the included studies using the Newcastle-Ottawa Scale (NOS). The quality of each study was evaluated based on the following

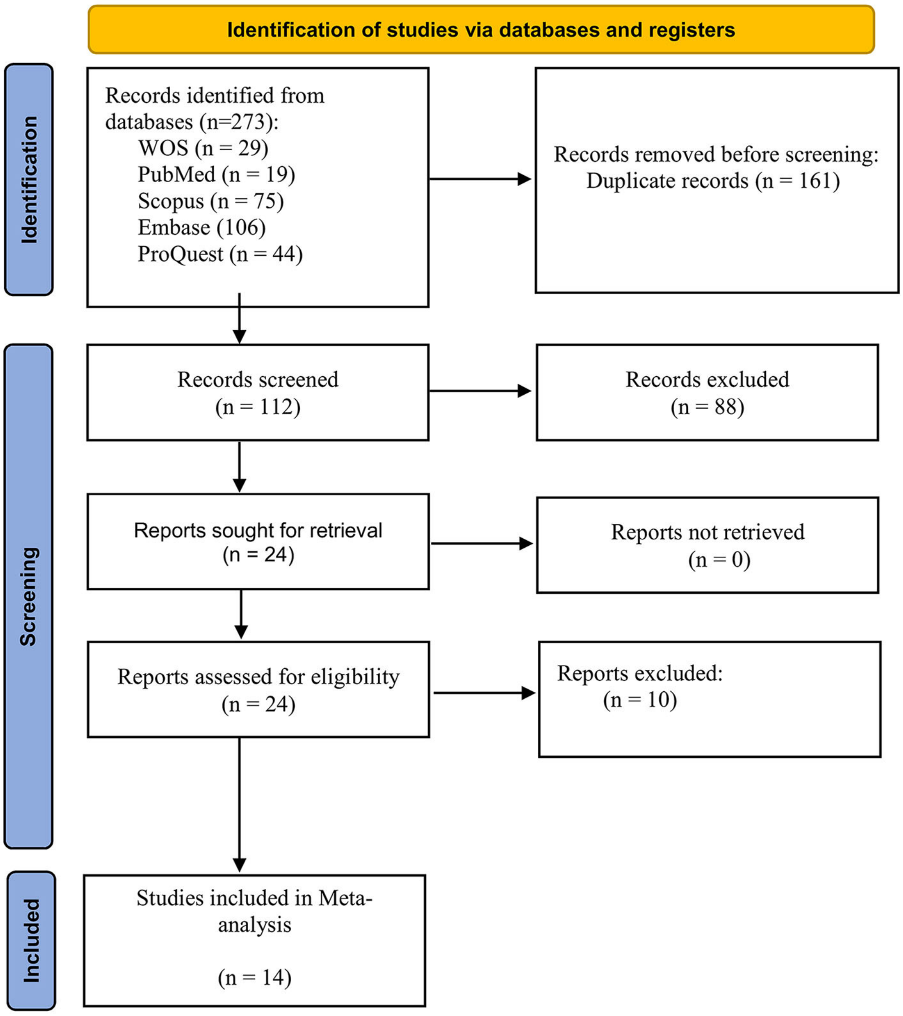

FIGURE 1
Flowchart of the study search strategy.

three domains: selection of cases and controls; comparability of cases and controls; and assessment of the exposure. Subsequently, each study was judged as either high quality (at least 7 stars); moderate quality (4–6 stars); or poor quality (0–3 stars).

Data synthesis

Statistical analyses were conducted using Review Manager (RevMan) Version 5.3 (Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration, 2014). The mean difference (MD) in vitamin D between the two groups along with 95% confidence interval (CI) were calculated. The heterogeneity across the included studies was evaluated using the Cochrane Q test ($\chi 2$ test) and I-squared index (I^2). A P-value of < 0.05 was considered statistically significant.

Trial sequential analysis (TSA)

TSA software version 0.9.5.10 beta was used for TSA (www.ctu.dk/tsa). In brief, we used two-sided trial sequential monitoring boundary type, and the required information size (RIS) was estimated (36, 38).

Publication bias

Publication bias was assessed using funnel plot and Egger's test.

Results

Study selection

A total of 273 records were retrieved from online searches, 161 of which were duplicates and were thus removed (Figure 1). The titles and abstracts of the remaining 112 articles were screened by two independent investigators (SA, GA) for inclusion. Of these, 88 articles were found to be irrelevant and were excluded. The full text of the potentially eligible 24 articles were read by the two investigators, and 10 were excluded for various reasons (Supplementary Table 2). Eventually, 14 studies were included in the present meta-analysis.

General characteristics of the included studies

A total of 14 case-controlled studies comprising 1468 subjects (721 RAS patients and 747 controls) were included in this meta-analysis (11–14, 18, 28–30, 32–35, 39, 40). Six studies were conducted in Turkey (12–14, 34, 39, 40), three in Iran (28, 30, 35), one in Poland (18), one in India (33), one in Saudi Arabia (32), one in the United Arab Emirates (11) and one in Iraq (29). The mean age of study participants ranged from 29.26 to 40.60 years, and from 27.44 to 40.80 years in the control groups. Two studies were conducted in children with mean age ranging from 7.6 to 8.7 years (34, 40). Of 721 RAS cases, around 56% ($n = 404$) were females (Table 1). Eight studies (28–30, 32, 34, 35, 39, 40) included patients with minor RAS, two studies (18, 33) included patients with minor, major, and herpetiform RAS, and one study (12) included patients with minor and major RAS, while three studies (11, 13, 14) did not mention the type of RAS. All the included studies assessed the

TABLE 1 General characteristics of the included studies.

Author and year	country	Study design	Participants No/gender/mean age (years)		Type of RAS	Diagnostic criteria of RAS	Assay method
			RAS	Controls			
Koparal et al. (12)	Turkey	Case-control	N:70 F:37, M:33 Age: 40.60	N:70 F:34, M:36 Age:40.31	Minor, Major	Clinical, history of recurrence RAS within ≥ 2-year	NS
Mustafi et al. (33)	India	Case-control	N: 40 F: 18, M:22 Age: 34.32	N:40 F:18, M:22 Age: 33.43	Minor, Major, Herpetiform	Clinical, history of RAS minimum 3 episodes within last 3 months	ELISA
Oner et al. (41)	Turkey	Case-control	N: 60 F:34, M:26 Age: 31	N: 70 F: 41, M: 29 Age: 36.1	NS	Clinical, history of RAS > 3 times/year	NS
Zakeri et al. (35)	Iran	Case-control	N:43 F:32, M:11 Age:32.56	N:43 F:35, M:8 Age: 33.74	Minor	Clinical, history of RAS at least 3 periods/year	ELISA
Al-Amad and Hasan (11)	UAE	Case-control	N:52 F:20, M:32 Age:34	N:52 F:20, M:32 Age:31	NS	Clinical, History of recurrence of similar ulcers	ECLIA
Hussein et al. (32)	KSA	Case-control	N:70 F:39, M:31 Age:29.26	N:70 F:33, M:37 Age:32.59	Minor	Clinical, history of minimum 3 ulcers/year	ELISA
Nalbantoglu and Nalbantoglu (34)	Turkey	Case-control	N:72 F:39, M:33 Age: 8.7	N:70 F:34, M:36 Age: 7.6	Minor	Clinical, history of RAS minimum 3 episodes within last year	EIA
Tamer and Avci (14)	Turkey	Case-control	N:20 F:15, M:5 Age:34	N:20 F:14, M:6 Age:33.9	NS	Medical records	NS
Ali (29)	Iraq	Case-control	N: 30 F:30, M:0 Age: 36.4	N: 30 F: 30,M: 0 Age: 33.6	Minor	Clinical, history of RAS at least 3 times/year	ELISA
Bahramian et al. (28)	Iran	Case-control	N: 26 F10, M:16 Age:38.8	N: 26 F:9, M:17 Age: 40.80	Minor	Clinical, history of RAS at least 3 times/year	ECLIA
Oztekin and Oztekin (39)	Turkey	Case-control	N: 40 F:25, M:15 Age: 31.2	N: 70 F: 38, M:32 Age: 27.44	Minor	Clinical, history of RAS at least 3 times/year	ECLIA
Krawiecka et al. (18)	Poland	Case-control	N: 66 F:42, M: 24 Age: 34.15	N: 66 F:50, M: 16 Age: 32.05	Minor, Major, herpetiform	Clinical, history of regular recurrence of ulcers	ECLIA
Khabbazi et al. (30)	Iran	Case-control	N: 46 F:18, M: 28 Age: 33.4	N: 49 F: 19, M: 30 Age: 34.1	Minor	Clinical, history of at least 3 episodes per year.	ELISA
Başarslan and Kaba (40)	Turkey	Case-control	N: 86 F:45, M: 41 Age: 8.61	N: 71 F:31, M: 40 Age: 8.06	Minor	Clinically and history	ELISA

RAS, recurrent aphthous stomatitis; M, male; F, female; NS, Not Specified; ECLIA, electro-chemiluminescence binding assay; ELISA, enzyme-linked immunosorbent assay; EIA, Enzyme immunoassay.

TABLE 2 Summary of the main outcomes.

	Vitamin D levels (ng/ml)		Conclusion
	RAS	Controls	
Koparal et al. (12)	22.16 ± 9.55	26.15 ± 11.01	Vitamin D levels were significantly lower in RAS patients compared to controls ($P = 0.019$)
Mustafi et al. (33)	14.34 ± 6.73	26.23 ± 3.99	Vitamin D levels were significantly lower in RAS patients compared to control ($P < 0.0001$)
Oner et al. (41)	12.42 ± 2.8	16.95 ± 4.10	Although RAS had lower serum vitamin D levels than controls, no statistically significant difference was found between the groups ($P = 0.056$). Also, there was no significant association between vitamin D levels and duration or frequency of RAS (P>0.05).
Zakeri et al. (35)	13.89 ± 8.19	22.59 ± 16.06	Vitamin D level in control group was significantly higher than in the case group ($P = 0.002$)
Al-Amad and Hasan (11)	53.6 ± 24.6	51.5 ± 26.9	No significant difference was found between RAS patients and healthy controls ($P = 0.68$). However, binary logistic regression showed a significant association between vitamin D deficiency and number of RAS ($P = 0.027$)
Hussein et al. (32)	20.25 ± 6.01	29.92 ± 6.80	The mean level of vitamin D in RAS group was significantly lower than the control group ($P < 0.001$). The results also showed a significant correlation between vitamin D deficiency and number, frequency and severity of RAS ($P < 0.0001$)
Nalbantoglu and Nalbantoglu (34)	16.4 ± 8.6	23.1 ± 11.5	Vitamin D levels were significantly lower in RAS patients compared to control group ($P = 0.002$). There was no significant correlation between serum vitamin D levels and number, frequency, healing time and severity of RAS
Tamer and Avci (14)	13.6 ± 6.5	20.9 ± 10	The mean serum vitamin D level was significantly lower in RAS patients compared to healthy individuals ($P = 0.01$)
Ali (29)	13.90 ± 12.72	22.08 ± 17.77	Vitamin D levels were significantly lower in RAS group ($P = 0.045$). There was a significant correlation between the serum levels of 25(OH) D and the number RAS in each attack ($r = 0.435$; $P = 0.016$). However, no significant correlation was found between serum Vitamin D levels and duration and frequency of RAS
Bahramian et al. (28)	33.07 ± 12.41	50.89 ± 9.30	RAS group showed significantly lower vitamin D levels than control group ($P = 0.001$)
Oztekin and Oztekin (39)	11.00 ± 7.03	16.4 ± 10.19	RAS group showed significantly lower vitamin D levels ($P = 0.004$). Yet, no significant association was observed between vitamin D levels and RAS size, healing time and frequency
Krawiecka et al. (18)	16.81 ± 8.45	19.22 ± 10.44	Although vitamin D levels were lower in RAS patients, the results were not statistically significant ($P = 0.207$). The lowest vitamin D level was observed in the most severe form of RAS (based on frequency of RAS), but the results did not reach statistical significance ($P = 0.074$)
Khabbazi et al. (30)	12.10 ± 7.70	27.40 ± 9.70	RAS group showed significantly lower vitamin D levels than control group ($P = 0.001$). Yet, no correlation was found between vitamin D levels, duration, number of ulcers and frequency of RAS
Başarslan and Kaba (40)	12 ± 4.53	31 ± 7	Individuals with RAS revealed significantly lower vitamin D levels than healthy control group ($P = 0.001$)

RAS, recurrent aphthous stomatitis.

serum levels of vitamin D, seven of which used ELISA (enzyme-linked immunosorbent assay) (29, 30, 32–35, 40), while four studies (11, 18, 28, 39) used ECLIA (electro-chemiluminescence binding assay). Three studies (12–14) did not mention the type of the assay. With respect to diagnostic criteria of RAS, all studies relied on clinical presentation and history of recurrence of RAS, while one study did not provide sufficient information (14) (Table 1).

Concerning the outcome measures, all studies assessed and compared serum levels of vitamin D in RAS and controls, and eight studies also (11, 13, 18, 29, 30, 32, 34, 39) assessed the association between serum levels of vitamin D and RAS variables such as duration, severity, and frequency (Table 2).

Quality of the included studies

A summary of the quality assessment of the included studies is presented in Table 3. Of the included studies, 12 (12–14, 18, 28–30, 32–35, 40) were of moderate quality, while two studies (11, 39) were of high quality.

Qualitative results

Of the 14 included studies, 11 studies (12, 14, 28–30, 32–35, 39, 40) found significantly lower serum levels of vitamin D in RAS patients compared to the controls (Table 2); one study found insignificant ($P = 0.056$) lower serum levels of vitamin D in RAS patients (13); while two studies did not find any differences between the two groups (11, 18).

Eight studies (11, 13, 18, 29, 30, 32, 34, 39) assessed the association between serum levels of vitamin D and RAS variables such as duration, severity, and frequency. Five studies (13, 18, 30, 34, 39) found no significant association between vitamin D and duration, frequency, and severity of RAS, while three studies showed a significant association between serum levels of vitamin D and number of RAS (11, 29, 32) (Table 2).

TABLE 3 Quality of studies assessed by Newcastle Ottawa Scale (case-control studies).

Study	Selection	Comparability	Exposure	Total score	Quality
Koparal et al. (12)	**	**	*	5	Moderate
Mustafi et al. (33)	***		**	5	Moderate
Oner et al. (41)	**	**	**	6	Moderate
Zakeri et al. (35)	*	**	**	5	Moderate
Al-Amad and Hasan (11)	****	**	**	8	High
Hussein et al. (32)	**	**	**	6	Moderate
Nalbantoglu and Nalbantoglu (34)	**	*	**	5	Moderate
Tamer and Avci (14)	**		**	4	Moderate
Ali (29)	***	*	**	6	Moderate
Bahramian et al. (28)	***	*	**	6	Moderate
Oztekin and Oztekin (39)	**	**	**	7	High
Krawiecka et al. (18)	***	*	**	6	Moderate
Khabbazi et al. (30)	***	*	**	6	Moderate
Başarslan and Kaba (40)	***	*	**	6	Moderate

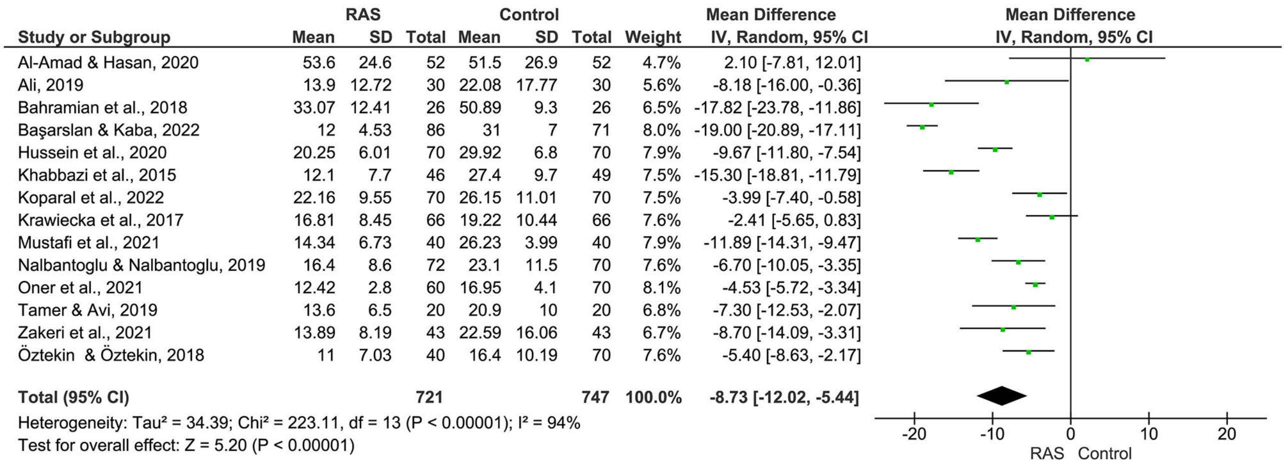

FIGURE 2
Meta analysis of vitamin D levels between RAS and controls.

Meta-analysis results

The pooled results of the 14 studies revealed a significant association between low serum levels of vitamin D and the risk of RAS (MD = – 8.73, 95% CI: – 12.02to – 5.44, I^2 = 94%, $P <$ 0.00001 (Figure 2).

TSA results

Figure 3 depicts the TSA: The cumulative Z curves crossed the conventional boundary and the trial sequential monitoring boundary and surpassed the required information size ($n = 443$) as well. As such, the evidence is reliable and confirmatory, and further trials are no longer needed.

Publication bias

The funnel plot (Figure 4) reveals symmetric distribution of the included studies, indicating no publication bias.

Discussion

As discussed earlier, RAS is a common mucosal disease that may impact adversely on the patients' quality of life (5). Despite a large body of research on the topic, the exact etiopathogenesis of RAS remains unclear (1, 6, 10). Given the lack of a specific etiology, RAS management is challenging (2, 42–44). In light of the mounting evidence regarding the potential role of vitamin D in the pathogenesis of certain oral

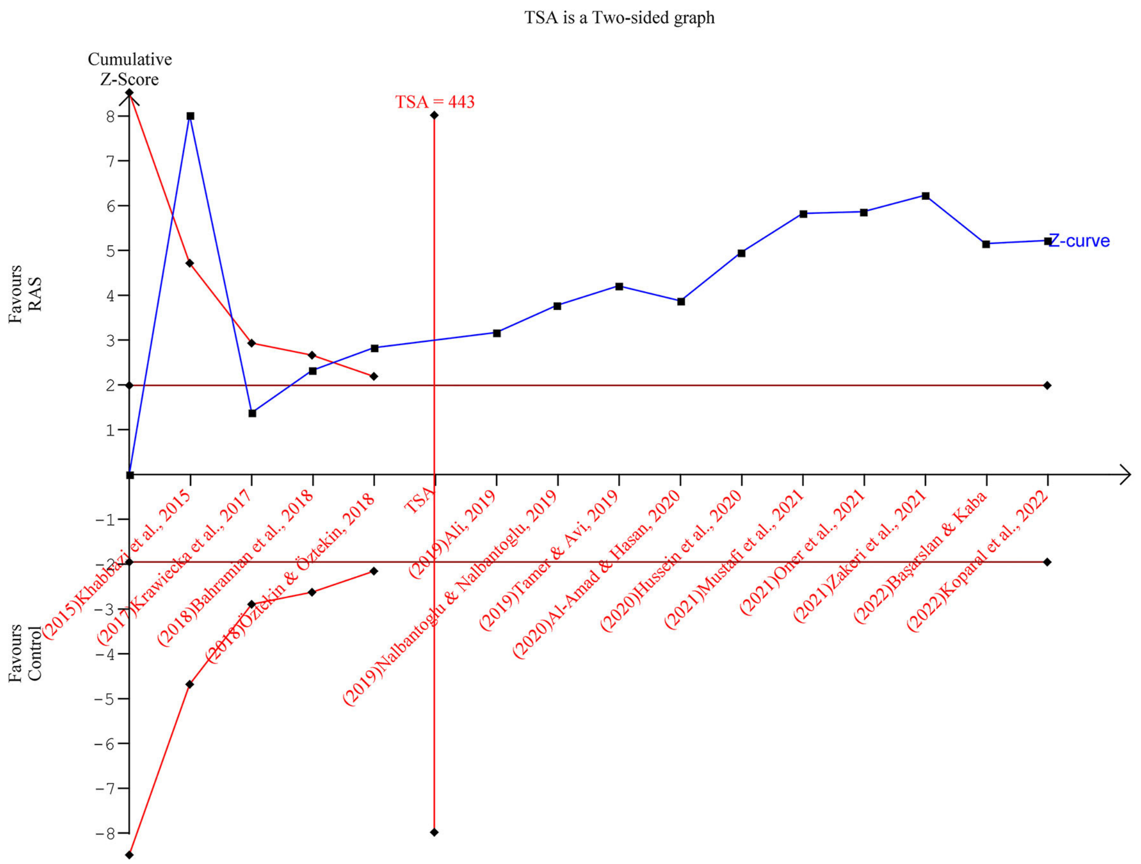

FIGURE 3
Trial sequential analysis results.

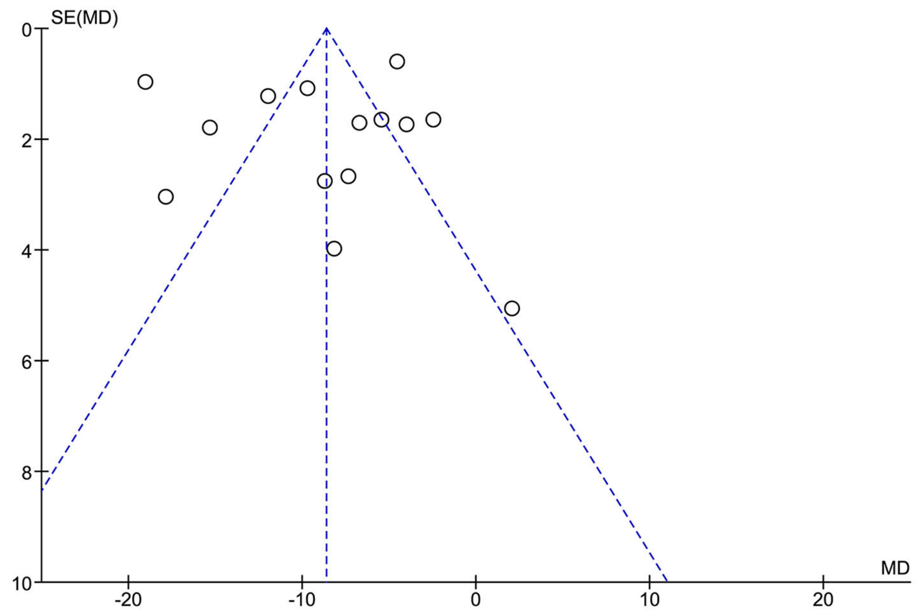

FIGURE 4
Funnel plots.

mucosal diseases including RAS (13, 29, 31, 35), the present meta-analysis was conducted to answer the following focused question: Is low serum levels of vitamin D associated with higher risk of RAS? Overall, the results of the pooled 14 studies revealed a significant association between low serum levels of vitamin D and the risk of RAS. Additionally, the result of the meta-analysis was supported by the TSA findings, which showed that the current studies surpassed the required information size, confirming that the differences were reliable. However, the qualitative analysis revealed conflicting results regarding the association between serum levels of vitamin D and severity and frequency of RAS.

The results of the present updated meta-analysis confirm our original meta-analysis (31), and substantiate many previous systematic reviews and meta-analyses that found significant associations between vitamin D deficiency and several autoimmune diseases and dermatological conditions such as lupus erythematosus, vitiligo, autoimmune bullous dermatoses, rheumatoid arthritis, and primary Sjögren's syndrome (22–26). Interestingly, our results are in accord with a recent clinical trial which investigated the efficacy of vitamin D supplementations in RAS patients with vitamin D deficiency, and reported a significant reduction in the frequency of RAS episodes, number of ulcers, and duration of healing time after one year (45). The exact mechanism behind the effects of vitamin D on RAS is still unclear yet, but may be explained by its immunomodulatory effects. Studies confirmed that vitamin D has strong immunomodulatory effects on both innate and acquired immunity responses, as well as on cytokines levels (20, 46, 47), all of which are thought to be involved in the pathogenesis of RAS (6, 7, 10).

It is recognized that the level of any evidence obtained from each meta-analysis is largely dependent on the quality of the included studies. Hence, we meticulously scrutinized the quality of all included studies using NOS, a reliable and validated appraisal tool. The results showed that two studies were of high quality (low risk of bias), and 11 were of moderate quality, and no study was with low quality, suggesting fair evidence. Additionally, the result of the meta-analysis was further substantiated by TSA results, which further confirmed the reliability and conclusiveness of the results (36).

The present updated meta-analysis has some limitations that should be considered. The main limitation is the marked heterogeneity across the included studies in terms of geography, age of the participants, methods of vitamin D ascertainment, types of RAS included, among others. This in turn may have biased the results. Additionally, although the included studies were conducted in different parts of the world and involved large samples (721 RAS cases and 747 controls), five studies (around 40% of the included studies) came from one country, Turkey, and thus the generalization of the results may not be appropriate.

In conclusion, the present updated meta-analysis confirms the association between low serum levels of vitamin D and the risk of RAS. Hence, vitamin D assessment may be considered in RAS patients. The results also support the use of vitamin D supplementations in RAS patients with inadequate serum levels of vitamin D. However, future interventional studies (for prevention and/or treatment purposes) investigating the effect of vitamin D supplements on RAS patients are required.

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.

Author contributions

SA: study concept, search strategy, and drafting the manuscript. GA-Q: data extraction, quality appraisal, and drafting the manuscript. EH: concept of the study and critically revised and edited the paper. NA: data extraction, quality appraisal, and drafting the manuscript. AA: concept of the study, data analysis, and critically revised and edited the paper. KA: concept of the study and critically revised and edited the paper. SO: data curation and critically revised and edited the paper. All authors approved the final 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.2023.1132191/full#supplementary-material

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Association of dietary total antioxidant capacity and its distribution across three meals with all-cause, cancer, and non-cancer mortality among cancer survivors: the US National Health and Nutrition Examination Survey, 1999–2018

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The effect of the antioxidant capacity of diet and its distribution across three meals on mortality risk among cancer patients remains unexplored. We aimed to prospectively investigate the association of dietary total antioxidant capacity (DAC) and its distribution across three meals with all-cause, cancer, and noncancer mortality among cancer survivors. We included 5,009 patients with cancer from the National Health and Nutrition Examination Survey conducted between 1999 and 2018. The adjusted hazard ratio (aHR) was estimated using the survey-weighted Cox proportional hazards model. During a median follow-up of 7.9 years, 1811 deaths, including 575 cancer-related deaths, were recorded. Among cancer survivors, compared with participants in the lowest quartile of total DAC from three meals, those in the highest quartile had a 24% decreased risk of noncancer mortality (aHR = 0.76, 95% confidence interval [CI]: 0.60–0.92), but not of all-cause and cancer mortality (each p trend >0.1). However, this association became insignificant for total DAC after excluding dinner DAC. In addition, higher dinner DAC rather than breakfast or lunch DAC was associated with a 21% lower risk of all-cause mortality (aHR = 0.79, 95% CI: 0.65–0.98) and 28% lower risk of noncancer mortality (aHR = 0.72, 95% CI: 0.57–0.90). Similar associations were found for ΔDAC (dinner DAC − breakfast DAC) with noncancer mortality (aHR = 0.56, 95% CI: 0.38–0.83), but DAC was not associated with cancer mortality (p trend >0.3). Among cancer survivors, total DAC from three meals was associated with reduced noncancer mortality, with the primary effect attributable to increased DAC intake from dinner. Our findings emphasize that DAC consumption from dinner should be advocated to reduce mortality risk in cancer survivors.

KEYWORDS

DAC, dinner, noncancer mortality, NHANES, nutrition

1. Introduction

There are approximately 33 million cancer survivors worldwide; this number is projected to increase due to population aging and improvements in the early detection and treatment of cancer (1). However, despite these advancements, people with cancer still have a shorter life expectancy than those without the disease (2). Cancer survival is defined as the time between cancer diagnosis and mortality (1, 3, 4). Diet is an important concern after cancer diagnosis for cancer survivors. Therefore, nutrition guidelines during and after cancer treatment have been introduced to improve the quality of life and mortality of patients with cancer, such as recommending the intake of abundant fruits and vegetables rich in antioxidant capacity (5–8). However, whether dietary antioxidants improve cancer survival is unknown.

Nevertheless, the association of dietary antioxidants with cancer mortality and prognosis has been widely reported in human and animal studies, but the findings have been largely diverse (9–15). Although some studies revealed a significant inverse association (10, 11, 13), others reported a null association (9, 12, 14), and a study reported the pro-tumorigenic role of dietary antioxidants (15). Emerging evidence has recently shown that in addition to the level and type of food, nutrition intake distribution across three meals can influence overall health (16–19). Therefore, we speculated that these inconsistent findings can be modified by monitoring dietary antioxidant distribution across three meals.

To date, only one study has investigated individual antioxidant distribution across three meals. It reported that antioxidants (i.e., vitamin C and E) in dinner were associated with lower cardiovascular disease (CVD) risks and all-cause mortality in the general population (20). However, whether dietary antioxidants across three meals would also impact mortality among cancer survivors was not clarified.

Given the importance of cumulative and/or synergistic effects of individual antioxidants from diets, we used an index of dietary total antioxidant capacity (DAC), a total estimate of the antioxidant capacity of all dietary antioxidants, to recapture an individual's overall consumption of antioxidants in this study (21). We examined the associations of daily DAC distribution with the risk of all-cause, cancer, and noncancer mortality among cancer survivors in the US National Health and Nutrition Examination Survey (NHANES) cohort from 1999 to 2018.

Abbreviations: aHR, Adjusted hazard ratio; CVD, Cardiovascular disease; DAC, Dietary total antioxidant capacity; HEI, Healthy Eating Index; NHANES, National Health and Nutrition Examination Survey; BMI, Body mass index; METs, Metabolic equivalent score.

2. Materials and methods

2.1. Study population

Participants were selected from the NHANES Cohort 1999–2018, a prospective study of health and nutrition established by the US National Center for Health Statistics (NCHS). For more details on the cohort, see https://www.cdc.gov/nchs/nhanes/index.htm. The project was approved by the NCHS review board, and all participants provided written informed consent before enrollment.

2.2. Inclusion/exclusion criteria

The inclusion criteria in this analysis were that participants had at least one valid dietary recall interview and were diagnosed with cancer at baseline. We excluded participants who had total energy intake >5,000 kcal/d or < 500 kcal/d ($n = 46$), were pregnant ($n = 30$), and had missing dietary intake and/or mortality events ($n = 87$), leaving 5,009 participants for final analysis during a median follow-up of 7.9 years (1999–2018) (Figure 1).

2.3. Exposure assessment

Two non-consecutive 24-h dietary recalls were used to investigate the quantity, quality, and time of food intake; details have been described on the NHANES official website. Further, dietary supplement data were further collected by using a dietary supplement questionnaire. Strict, standardized protocols were performed to ensure the quantity of the interview quality. Nutrient compositions were acquired from the national nutrient database of the United States Department of Agriculture (USDA).

The antioxidant capacity assigned to each food item was expressed as the ferric-reducing ability of plasma (FRAP) value based on the single-electron transfer method. It was calculated with energy adjustment using the Antioxidant Food Database and the USDA's Food Patterns Equivalents Database 2015–2016 (FPED 2015–2016) (22). The dietary antioxidant capacity per equivalent serving of the 30 categories of foods defined by the FPED 2015–2016 was presented (Supplementary Table S1). DAC was further calculated as the summation of the serving size of each food item multiplied by the FRAP value (mmol/serving) of each food item. The total DAC (the sum of three meals a day), DAC distribution (breakfast, lunch, and dinner), and the difference between dinner and breakfast (ΔDAC = dinner DAC − breakfast DAC) were further calculated. DAC from coffee and dietary supplements was excluded because of the inconclusive antioxidant ability of coffee with high content, which may weaken the correlation between DAC from other foods and mortality (23). The intake time of supplements was also missing.

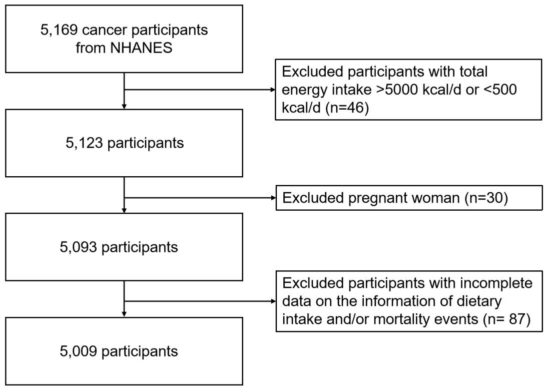

FIGURE 1
Cohort flow diagram of cancer survivors enrolled in NHANES.

2.4. Defining outcomes

All deaths (time and cause) were ascertained by linkage with the National Death Index through 31 December 2019. The outcome of interest was mortality: cancer mortality, defined as deaths due to cancer coded as the main cause of death (10th revision of International Classification of Disease, C00-C97), noncancer mortality, and all-cause mortality. The numbers of cases by cancer code in 5009 cancer cases are presented in Supplementary Table S2.

2.5. Statistical analysis

The analytic guidelines released by NHANES were adapted for all analyses incorporating complex sampling design methods of sample weights, stratification, and clustering. Descriptive statistics were used to summarize the baseline characteristics, expressed as mean or median with standard deviation (SD) or standard error (SE) for continuous variables and percentages for categorical variables. Data were weight-adjusted as appropriate. We performed one-way ANOVA for continuous variables and used the Chi-square test for categorical variables to examine baseline characteristics.

The survey-weighted Cox proportional hazards model is an officially recommended method in NHANES data[1] and has been widely applied in previous studies (24, 25). Therefore, we also used survey-weighted Cox proportional hazards models to assess the associations of DAC (total, breakfast, lunch, and dinner; by quartiles) with all-cause, cancer, and noncancer mortality, showing adjusted hazard ratio (aHR) and 95% confidence interval (CI). To eliminate the difference between breakfast and dinner food types, we also evaluated the associations of ΔDAC (by quartiles) with all-cause, cancer, and noncancer mortality. Additionally, the linear or non-linear relationship between total and dinner DAC and mortality risk was analyzed using a restricted cubic spline model. Subgroup analysis was further performed, categorized by age (<60 or ≥ 60 years), sex (male or female), and body mass index (BMI) (<25, 25–30, or > 30 kg/m^2) in survey-weighted Cox proportional hazards models.

Covariates were adjusted in three models. Model 1 was adjusted for age, sex (male; female), and race/ethnicity (Mexican American; non-Hispanic black; non-Hispanic white; other Hispanic; other). Model 2 was additionally adjusted for the following: education (below 9th grade; 9th–11th grade; college graduate or above; high school graduate/GED or equivalent; some college or associate of arts degree), family income ($ 0–$ 19,999; $20,000–$44,999; $45,000–$74,999; $75,000–$99,999; ≥$100,000), BMI, alcohol intake per day, dietary energy intake, smoking now or not, and physical activity per week (metabolic equivalent score, METs). Model 3 was further adjusted for serum high-density lipoprotein (HDL)-cholesterol, serum triglycerides, serum glycohemoglobin, diabetes, hypertension, CVD, dietary antioxidant supplement intake (vitamin C or vitamin E), and adherence to Healthy Eating Index 2015 (HEI-2015) score. Models for DAC intake at breakfast, lunch, and dinner were further adjusted, except the one defining the group. Diabetes was defined as having self-reported or diagnosed diabetes, hemoglobin A1c (HbA1c) ≥6.5%, or fasting plasma glucose ≥7.0 mmol/L. Hypertension was defined as diagnosed hypertension reported in NHANES. CVD was defined as diagnosed arthritis, congestive heart failure, coronary heart disease, angina, heart attack, or stroke.

To further validate the association between DAC from breakfast or dinner and mortality risk, we performed substitution analysis to partition one dietary item's risk into another to calculate the relative risk for a fixed amount of intake (18, 26). In substitution analyses, we reassessed the associations of DAC with all-cause and noncancer mortality by replacing 10% DAC at breakfast with the equivalent amount of DAC or DAC from specific food at dinner.

In sensitivity analyses, we explored whether the associations persisted using the median value of DAC in survey-weighted Cox proportional hazards models. We further reassessed the weighted Cox proportional hazards models after including DAC from snack after dinner (food intake after 9 pm) data.

All statistical analyses were performed using R software (version 4.2.0). Missing data are described in Supplementary Table S3 and were imputed using multivariate imputation with chained equations. A two-sided p-value of <0.05 was deemed statistically significant.

3. Results

3.1. Baseline characteristics

During a median follow-up of 7.9 years, there were 5,009 cancer cases. At baseline, the mean age of participants was 61.67 years, and 52.3% were women. Among them, 39.9% consumed dietary antioxidant supplements. The average contents of total, breakfast, lunch, dinner, and snack after dinner DAC were 4.17 mmol, 1.11 mmol, 1.88 mmol, 1.18 mmol, and 0.31 mmol, respectively. Approximately 54.6, 24.5, and 58.1% of the participants had a history of hypertension, diabetes, and CVD, respectively (Table 1). Compared with participants in the lowest quartiles, those in higher total and dinner DAC quartiles were more likely to be non-Hispanic white, leaner, have a college graduate degree or above, and adhere to the Healthy Eating Index (HEI)-2015 score. In addition, they had higher physical activity and serum HDL-cholesterol levels but lower

1 https://wwwn.cdc.gov/nchs/nhanes/tutorials/Weighting.aspx

TABLE 1 Baseline descriptive characteristics of 5,009 cancer survivors.

Characteristics	Mean ± SE or *n* (%)
Patients, n	5,009
Age (years)	61.67 ± 0.40
Female	2,622 (52.3)
Race/ethnicity	
Mexican American	292 (6.2)
Non-Hispanic Black	604 (12.9)
Non-Hispanic White	3,405 (72.5)
Other Hispanic	214 (4.6)
Other	179 (3.8)
Education	
Less than 9th grade	464 (9.3)
9th–11th grade	602 (12.0)
College graduate or above	1,317 (26.3)
High school graduate/GED or equivalent	1,141 (22.8)
Some college or Associate of Arts degree	1,481 (29.6)
Income	
$ 0–$ 19,999	1,080 (21.6)
$20,000–$44,999	1,513 (30.2)
$45,000–$74,999	909 (18.1)
$75,000–$99,999	639 (12.8)
$100,000 and over	868 (17.3)
BMI (kg/m²)	28.47 ± 0.14
Alcohol intake (g/day)	7.62 ± 0.44
Smoke status	
Never smoked	2,209 (44.1)
Past smoker	2049 (40.9)
Current smoker	751 (15.0)
Physical activity (METs-h/week)	6.91 ± 0.10
Dietary energy intake (kcal)	1940.63 ± 18.83
Adherence to HEI-2015 score	53.00 ± 0.34
Dietary antioxidant supplement intake (yes)	2000 (39.9)
Total DAC intake (mmol)	4.17 ± 0.06
Breakfast DAC intake (mmol)	1.11 ± 0.03
Lunch DAC intake (mmol)	1.88 ± 0.04
Dinner DAC intake (mmol)	1.18 ± 0.04
DAC in snack after dinner (mmol)	0.31 ± 0.04
Serum HDL-Cholesterol (mg/dL)	58.23 ± 0.47
Serum triglycerides (mmol/L)	5.84 ± 0.03
Glycohemoglobin (%)	2.25 ± 0.05
Hypertension	2,735 (54.6)
Diabetes	1,227 (24.5)
CVD	2,908 (58.1)

BMI, body mass index; HEI-2015, Healthy Eating Index 2015; DAC, dietary total antioxidant capacity; METs, metabolic equivalent score; CVD, cardiovascular disease. Continuous variables were adjusted for survey weights of NHANES. Categorical variables were unweighted.

glycohemoglobin levels. They were less likely to be current smokers or have a history of CVD (Supplementary Tables S4, S5). Moreover, participants with higher ΔDAC consumption, relative to lowest consumption, were more likely to be women, be Mexican American, have a college or associate of arts degree, and have higher physical activity and higher serum HDL-cholesterol levels. In addition, they were less likely to be current smokers, adhere to HEI-2015 scores, have a history of CVD, and have a lower dietary antioxidant supplement intake (Supplementary Table S6).

3.2. DAC and its distribution across three meals and mortality risk

The associations of DAC and its distribution across three meals with mortality were evaluated using survey-weighted Cox proportional hazards models (Figure 2 and Supplementary Tables S7–S9). During the follow-up period, 1811 all-cause deaths occurred in the cohort, of which 575 were attributed to cancer and 1,236 to other noncancer causes. In adjusted Model 1 (age, sex, and race), a higher intake of total, breakfast, lunch, and dinner DACs and ΔDAC was progressively associated with lower all-cause and noncancer mortality risk (each *p* trend <0.05), with cancer mortality risk varying by distinct DAC groups. In Model 2, which was further adjusted for demographic and dietary factors, total DAC was still associated with lower all-cause and noncancer mortality (*P* trend <0.001 and *p* trend <0.001, respectively). However, there was no significant association between breakfast and lunch DACs and all-cause and noncancer mortality (*p* trend >0.1). The adjusted association with reduced all-cause and noncancer mortality risk for dinner DAC remained significant (*p* trend = 0.002 and *p* trend <0.001, respectively). For ΔDAC, the aHR for reduced noncancer mortality risk remained significant, while that for all-cause mortality became insignificant (all-cause mortality: *p* trend = 0.107; noncancer mortality: *p* trend = 0.029). However, further inclusion of disease and related markers in Model 3 attenuated these associations. Compared with the lowest quartiles, aHRs (95% CI) for all-cause and noncancer mortality associated with the highest quartiles of total DAC consumption were 0.86 (95% CI: 0.67–1.10) and 0.76 (95% CI: 0.60–0.92), respectively (all-cause mortality: *p* trend = 0.143; noncancer mortality: *p* trend = 0.009). However, compared with the highest quartiles of dinner DAC, they were 0.79 (95% CI: 0.65–0.98) and 0.72 (95% CI: 0.57–0.90), respectively (all-cause mortality: *p* trend =0.017; noncancer mortality: *p* trend = 0.003). Likewise, similar associations were found for ΔDAC with reduced all-cause mortality (all-cause mortality: 0.77 [95% CI: 0.56–1.06], *p* trend = 0.138; noncancer mortality: 0.56 (95% CI: 0.38–0.83), *p* trend = 0.022), but no significant associations existed between breakfast and lunch DACs and all-cause and noncancer mortality (each *p* trend >0.3). Notably, in multivariable-adjusted Models 2 and 3, total, breakfast, lunch, and dinner and ΔDACs were not associated with cancer mortality (each *p* trend >0.1).

Further, restricted cubic spline analysis showed a linear or non-linear relationship between total and dinner DAC and mortality risk (Supplementary Figure S1). There was non-linearity between total DAC and all-cause, cancer, and noncancer mortality. Meanwhile, dinner DAC and all-cause, cancer, and noncancer mortality had a linear inverse association.

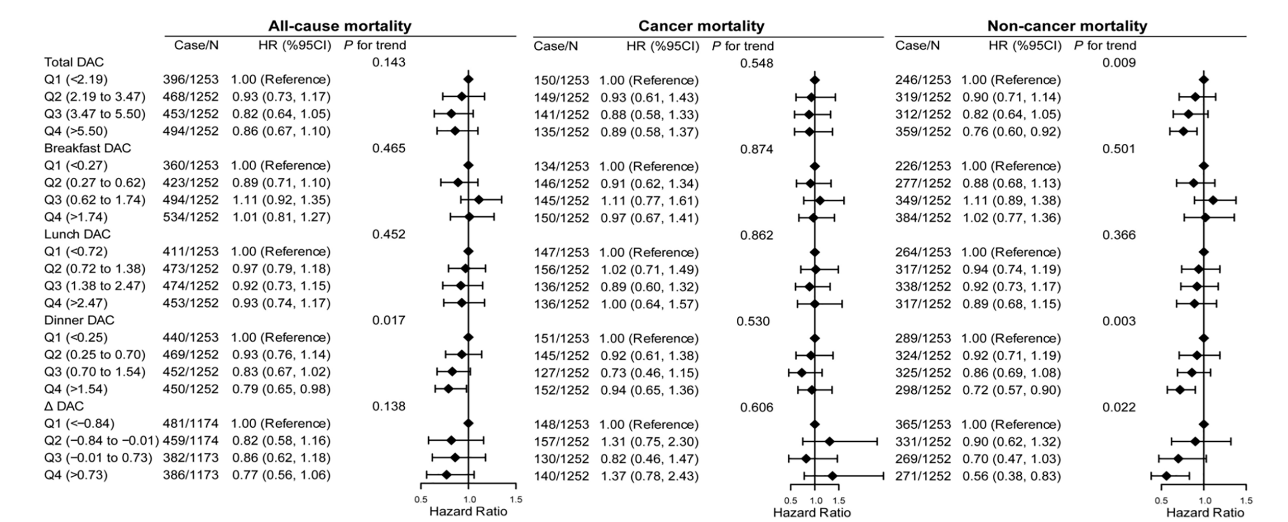

FIGURE 2
Association of all-cause, cancer, and noncancer mortality with DAC and its distribution across three meals in cancer survivors. HR, hazard ratio; CI, confidence intervals; DAC, dietary total antioxidant capacity; HEI-2015, Healthy Eating Index 2015; BMI, body mass index; METs, metabolic equivalent score; CVD, cardiovascular disease. **p* for trend across the quartile of DAC. HR with 95% CI was assessed using weighted Cox regression analyses. Δ equals dinner DAC minus breakfast DAC. Models were adjusted for age, sex, race, education, family income, dietary energy intake, alcohol consumption per day, smoke status, METs, BMI, serum HDL-cholesterol, serum triglycerides, serum glycohemoglobin, diabetes, hypertension, CVD, dietary antioxidant supplement intake (vitamin C or vitamin E), and adherence to HEI-2015 score. Models for breakfast DAC intake, lunch DAC intake, and dinner DAC intake were further adjusted except the one that defined the group.

Since noncancer mortality was inversely associated with dinner DAC, we further analyzed the associations of dinner DAC with the detailed causes of noncancer mortality (Supplementary Table S10). After adjusting for covariates, dinner DAC was associated with lower mortality risk due to chronic lower respiratory disease, nephritis, nephrotic syndrome and nephrosis, influenza and pneumonia, accidents (unintentional injuries), and heart diseases. Notably, a higher intake of dinner DAC was progressively associated with lower death due to Alzheimer's disease and all other causes (each p trend <0.05). However, this association was not observed between dinner DAC and mortality due to diabetes mellitus and cerebrovascular diseases.

3.3. Associations of DAC stratified by food sources with mortality risk

Figure 3 and Supplementary Figure S2 show the associations of dinner and ΔDAC stratified by food sources with all-cause, cancer, and noncancer mortality. Compared with patients in the lowest quintile, those in the highest quintile of dinner fruit DAC had a lower risk of all-cause (aHR: 0.71, 95% CI: 0.56–0.91, p trend = 0.008) and noncancer mortality (aHR: 0.67, 95% CI: 0.50–0.91, p trend = 0.008). However, dinner DAC from other food sources, including vegetables, grains, dairy products, meat, oil, added sugars, alcohol, and solid fats, showed no association with all-cause, CVD, or cancer mortality. Likewise, ΔDAC stratified by food sources also showed no association with the risk of all-cause, CVD, and cancer mortality.

3.4. Subgroup analysis

Furthermore, subgroup analysis revealed that age, sex, and BMI did not impact the association between dinner DAC with noncancer mortality (Supplementary Table S11). A reverse association between dinner DAC with noncancer mortality was significantly observed only in females and persons with BMI >30 kg/m^2. However, higher dinner DAC was still related to a lower risk trend of noncancer mortality in males, persons with BMI ≤30.0 kg/m^2, and those aged <60 or ≥ 60 years.

3.5. Substitution analysis

Figure 4 shows the reassessed association between DAC consumption and the risk of all-cause and noncancer mortality after replacing DAC consumption at breakfast with dinner. A hypothetical replacement of 10% DAC intake at breakfast with an equivalent proportion at lunch was not significantly associated with a lower risk of all-cause and noncancer mortality (each $p > 0.1$). Likewise, all-cause and noncancer mortality did not significantly decrease in models substituting 10% breakfast DAC with 10% dinner DAC from different foods, including vegetables, grains, dairy products, meats, oils, added sugars, alcohol, and solid fats (each $p > 0.05$). However, the replacement of 10% DAC intake at breakfast with an equivalent proportion of dinner DAC merely from fruits was associated with a decrease of 6% in all-cause mortality risk (aHR = 0.94, 95% CI: 0.90–1.00) and 10% in noncancer mortality risk (aHR = 0.90, 95% CI: 0.85–0.97).

3.6. Sensitivity analyses

In sensitivity analyses, the inverse association between total DAC and noncancer mortality became insignificant when we excluded dinner DAC from total DAC. No significant associations existed between total DAC and all-cause and cancer mortality (Supplementary Table S12). Further, the additional inclusion of DAC

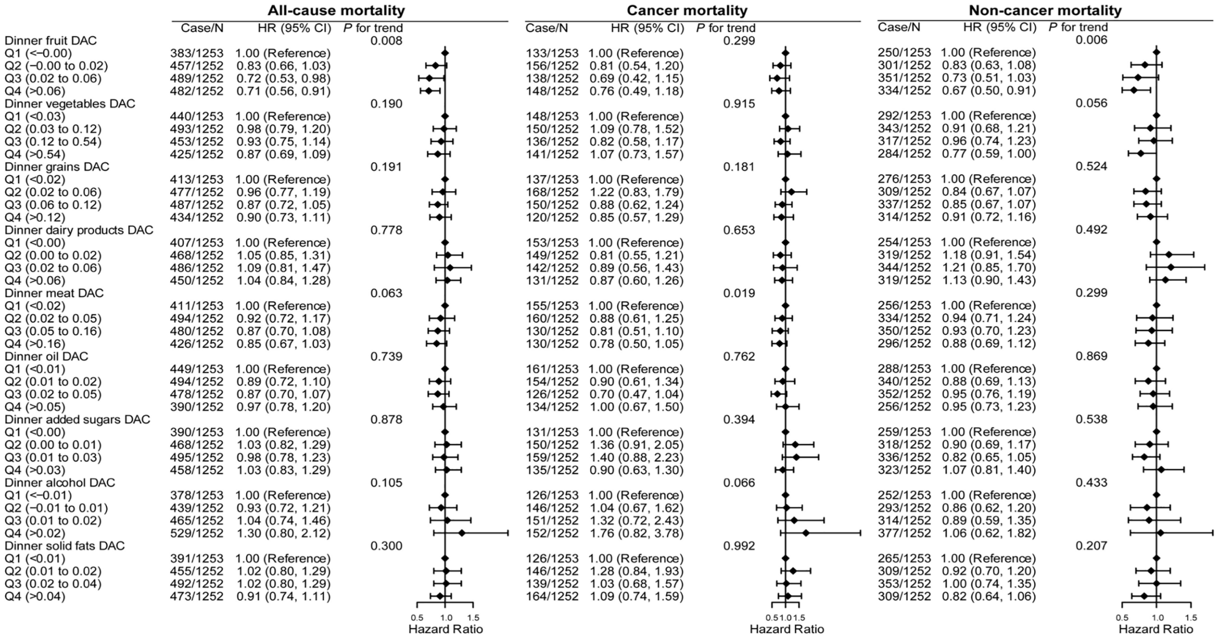

FIGURE 3
Association of all-cause, cancer, and noncancer mortality with dinner DAC, stratified by food sources in cancer survivors. HR, hazard ratio; CI, confidence intervals; DAC, dietary total antioxidant capacity; HEI-2015, Healthy Eating Index 2015; BMI, body mass index; METs, metabolic equivalent score; CVD, cardiovascular disease. *p for trend across the quartile of DAC. HR with 95% CI was assessed using weighted Cox regression analyses. Models were adjusted for age, sex, race, education, family income, dietary energy intake, alcohol consumption per day, smoke status, METs, BMI, serum HDL-Cholesterol, serum triglycerides, serum glycohemoglobin, diabetes, hypertension, CVD, dietary antioxidant supplement intake (vitamin C or vitamin E), adherence to HEI-2015 score, breakfast DAC intake, and lunch DAC intake.

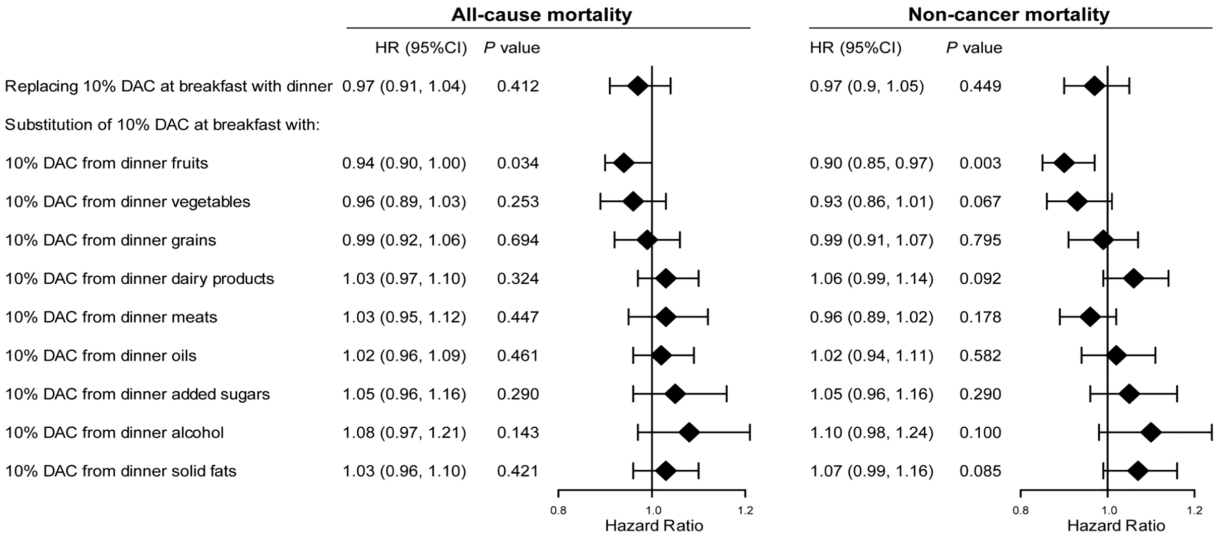

FIGURE 4
Substitution of DAC at breakfast with dinner and its association with all-cause and noncancer mortality in cancer survivors. HR, hazard ratio; CI, confidence intervals; DAC, dietary total antioxidant capacity; HEI-2015, Healthy Eating Index 2015; BMI, body mass index; METs, metabolic equivalent score; CVD, cardiovascular disease. *p for trend across the quartile of DAC. HR with 95% CI was assessed using weighted Cox regression analyses. Models were adjusted for age, sex, race, education, family income, dietary energy intake, alcohol consumption per day, smoke status, METs, BMI, serum HDL-Cholesterol, serum triglycerides, serum glycohemoglobin, diabetes, hypertension, CVD, dietary antioxidant supplement intake (vitamin C or vitamin E), adherence to HEI-2015 score, and lunch DAC (for substitution model of DAC at breakfast with dinner).

from snack after dinner to total or dinner DAC partially influenced the results; however, the trend was unchanged. Compared with the lowest quartiles, the total DAC obtained from including snacks after dinner showed aHRs for all-cause, cancer, and noncancer mortality of 0.88 (95% CI: 0.70–1.09), 0.95 (95% CI: 0.64–1.40), and 0.82 (95% CI: 0.63–0.1.06), respectively. Similarly, in the dinner DAC, including snacks after dinner group, the aHRs for all-cause, cancer, and noncancer mortality were 0.91 (95% CI: 0.75–1.10), 1.04 (95% CI: 0.76–1.43), and 0.84 (95% CI: 0.66–1.07), respectively. Therefore, DAC from snacks after dinner showed no association with all-cause, cancer, or noncancer mortality risks (Supplementary Table S13).

4. Discussion

To the best of our knowledge, this is the first study to investigate the association between DAC and its daily distribution and all-cause, cancer, and noncancer mortality in cancer survivors. We observed that among participants consuming high amounts of DAC from all three meals, the noncancer mortality risk decreased by 24%. In addition, among participants consuming DAC from dinner rather than breakfast or lunch, all-cause, and noncancer mortality risks robustly decreased by 21 and 28%, respectively. Furthermore, a higher ΔDAC was associated with a lower risk of noncancer mortality. This association was independent of multiple traditional risk factors, such as age, sex, BMI, and dietary and lifestyle factors. However, no association was observed between daily DAC intake distribution and cancer mortality in patients with cancer.

The relationship between individual dietary antioxidants and cancer death and incidence has been extensively explored with partially conflicting results (27–29). However, a single antioxidant may not reflect an individual's overall consumption of antioxidants; therefore, we considered measuring the DAC to fill this gap. Many observational studies have investigated the association between DAC and the risk of cancer and mortality in the general population and yielded mixed findings (30–33). Some studies have revealed an inverse association (32, 33), while others have reported a null relationship (30, 31). Moreover, our prior findings showed that total DAC was negatively correlated with all-cause mortality in the general population but not with cancer and CVD mortality (unpublished data). Although cancer mortality has declined, the absolute mortality of patients with cancer has been increasing due to the high cancer incidence (1, 34). Thus, studies targeting cancer survivors have important public and clinical implications for improved cancer treatment. To date, only one study has investigated the association between DAC and cancer mortality among breast cancer survivors. The study found that total DAC was not associated with total mortality among breast cancer survivors (10). Following the prior study, our results showed that among people with cancer, no association existed between total DAC and all-cause or cancer mortality; however, total DAC was negatively associated with noncancer mortality.

More importantly, the findings of this present study are consistent with those of previous studies showing that a higher consumption of DAC from dinner than from breakfast was linearly associated with a lower risk of noncancer mortality in cancer survivors. Notably, we found that the inverse association between dinner DAC (but not total DAC) and mortality was attenuated if we included DAC intake from the snacks after dinner. This result suggests that if the meal timing is disrupted, the health benefit may be instantly compromised; thus, strict adherence to meal timing is needed in chrono-nutrition intervention strategies, as previously reported (35, 36). Moreover, this finding highlights the importance of meal timing of DAC on noncancer mortality risk in cancer survivors. Consistent with our findings, a previous study showed that dietary antioxidants, vitamin C, and vitamin E intake at dinner rather than at breakfast were related to reduced CVD and all-cause mortality in the general population (20). Although data supporting an association between meal timing of DAC and mortality in cancer survivors are scarce, other extensive studies that have examined the relationship between meal timing of nutrients and mortality similarly suggest the vital role of chrono-nutrition for survival. Previous bulk studies have shown that the optimal timing of meals differs when different nutrients are considered; some nutrients are best consumed at breakfast, whereas others are at dinner (16, 18, 37, 38). Further, studies have shown that dietary potassium, calcium, and magnesium intake at dinner was significantly associated with a lower risk of all-cause and cancer mortality (38). Meanwhile, a higher intake of energy, fat, and protein at dinner rather than breakfast increased all-cause, diabetes, and CVD mortality among people with diabetes (18).

Additionally, we found that DAC exclusively from dinner fruits significantly lowered the risk of all-cause and noncancer mortality. Generally, a healthy diet containing high vegetables and fruits plays a vital role in primary cancer prevention, but specific dietary recommendations for cancer survivors are lacking (39). Notably, a previous study reported that a high intake of prediagnosed fruit was associated with a lower risk of all-cause mortality in patients with ovarian cancer (39). However, whether fruit consumption reduces mortality risk in cancer survivors needs to be further investigated in other population studies. Furthermore, we found that ΔDAC stratified by food source was not associated with the risk of all-cause and cancer mortality. Therefore, this result suggests a minor effect of individual antioxidants; thus, combining individual antioxidants from different foods is required.

It is also worth noting that noncancer mortality (particularly death due to Alzheimer's disease and all other causes), not cancer mortality, robustly reduced in association with total and dinner and ΔDACs among cancer survivors in this study. Notably, the three highest proportions of cancers recorded, i.e., skin cancer (nonmelanoma), breast cancer, and prostate cancer, accounting for 46% of patients with cancer in this study. Nonmelanoma skin cancer has a very low fatality rate, and its mortality tends to be related to poorer survival from causes unrelated to cancer in the affected patients (40–42). In addition, patients with breast and prostate cancers are also less likely to die of cancer but are more likely to die of noncancer causes, such as heart disease, infection, and suicide (43, 44). Thus, it is reasonable to conclude that targeting the lowering of noncancer mortality by increasing the consumption of total and dinner DACs is a promising strategy for survival improvement in patients with cancer. In particular, we considered that high consumption of total and dinner DACs might be more effective in lowering mortality risk among people with skin (nonmelanoma), breast, and prostate cancers; however, more research is warranted to confirm this suggestion.

The reasons for the association between total DAC, particularly high DAC from dinner rather than from breakfast, and reduced mortality in patients with cancer are complex. However, several biological processes may partially be used to explain this association. For instance, heightened oxidative and inflammatory stresses are commonly observed in cancer (45). Although some recent studies have demonstrated the anti-tumorigenic role of reactive oxygen species (ROS), others have shown that antioxidants possibly act in a pro-tumorigenic manner (15, 46). Nevertheless, many studies have recently suggested that antioxidants protect tumor cells from ROS- and DNA-induced damage that could lead to the proliferation of tumor cells (47, 48). Additionally, antioxidants play an important role as anti-inflammatory factors in the tumor process (49–52).

Most importantly, an intertwined relationship between the circadian rhythm and cancer has been extensively addressed, and targeting the circadian rhythm has been shown to inhibit cancer progression (53, 54). In particular, time-restricted feeding/eating has been shown to have a tumor suppression effect by synchronizing it with the circadian rhythm (55). Likewise, synchronizing dinner DAC with the rhythm may be related to reduced mortality, as exemplified by tumor necrosis factor-alpha (TNF-α) and interleukin-1β mRNA, with a night-time or afternoon peak rhythm (56, 57), consistent with the high consumption of dinner DAC. Moreover, this is further supported by the fact that the circadian clock regulates these inflammatory factors, which respond to meal timing (58). However, further studies are required to clarify these underlying mechanisms.

This study has several strengths. First, this is the first study to examine the association of daily DAC distribution with mortality in cancer survivors. Second, multivariable adjustment and a set of sensitivity analyses facilitated the representation of the association reported in this study. Third, this study used high-quality data with long-term follow-up from a well-designed cohort of the NHANES, which provided great statistical precision to assess the associations between DAC and the risk of mortality in cancer survivors.

However, this study has some potential limitations. First, although the repeatability and effectiveness of the dietary interviews were validated, long-term eating habits should be considered. Second, the external validity of our findings is lacking due to the missing information regarding meal timing and food intake in many datasets. Third, cooking methods that may alter the nutrient content and affect the antioxidant content of food are also lacking. Fourth, detailed information on cancer type, stage, or treatment is lacking, which would probably affect the association of DAC with cancer and noncancer mortality. Fifth, with the inconclusive antioxidant ability of coffee, excluding DAC from coffee and dietary supplements in this study may have weakened the association of DAC with mortality in cancer survivors. Finally, although traditional risk factors were comprehensively adjusted, we could not completely exclude the effect of unmeasured confounding factors. However, we believe that the findings of this study will facilitate evidence-based nutrient guidelines for cancer-directed care and improve survival and quality of life in patients with cancer.

In conclusion, among cancer survivors, the consumption of total DAC was inversely associated with the risk of noncancer mortality. This benefit was completely recaptured by higher DAC from dinner rather than breakfast or lunch, showing that a higher intake of dinner DAC was more favorable to the lower risk of all-cause noncancer mortality. High DAC consumption from dinner might be advocated for and incorporated into a healthy dietary pattern to reduce mortality risk in cancer survivors.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: The data that support the findings of this study are available in [National Health and Nutrition Examination Survey] at [https://wwwn.cdc.gov/nchs/nhanes/default.aspx], [Food Patterns Equivalents Database] at [https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/fped-databases/], [Mortality data] at [https://ftp.cdc.gov/pub/Health_Statistics/NCHS/datalinkage/linked_mortality/]. These data were derived from the following resources available in the public domain: [National Center for Health Statistics, https://www.cdc.gov/nchs/index.htm; Agricultural Research Service, https://www.ars.usda.gov/].

Author contributions

PW: conceptualization and formal analysis. YT: data curation. QT: methodology. DS: project administration, resources, and supervision. PW and QT: software. XH: validation. SZ and DS: writing—original draft. PW and DS: writing—review & editing. 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.

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1141380/full#supplementary-material

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Double burden of malnutrition and its associated factors among adolescents in Debre Berhan Regiopolitan City, Ethiopia: a multinomial regression model analysis

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Background: The double burden of malnutrition (DBM), contained both undernutrition and overnutrition, is a growing public health concern that presents a significant challenge to the food and nutrition policies of developing nations such as Ethiopia. However, the prevalence and contributing factors of DBM among adolescents in the study area have not been adequately investigated by Ethiopian researchers. Therefore, this study aims to determine the prevalence of DBM and contributing factors among secondary school students in Debre Berhan City, Ethiopia.

Methods: A school-based cross-sectional study was conducted among 742 adolescents aged 10–19 years from October 13, 2022, to November 14, 2022, using a multi-stage sampling method. Data were collected using the online Kobo toolbox tool. A multinomial logistic regression model was used to analyze the data. The data were cleaned and analyzed in R software 4.2.2. Adolescents who had body mass index for age Z score (BAZ) < −2 SD, > +1 SD, and > +2 from the median value were considered thin, overweight, and obese, respectively.

Results: The overall prevalence of DBM was 21.5% (14.8% thinness and 6.7% overweight/obesity). In the multivariable multinomial logistic regression analysis models factors such as age [AOR=0.79, 95% CL: (0.67, 0.93)], sex [AOR=3.86, 95% CL: (2.35, 6.32)], school type [AOR 5.03, 95% CL: (2.30, 10.99)], minimum dietary diversity score [AOR=2.29, 95% CL: (1.27, 4.14)], frequency of meals [AOR=2.09, 95% CL: (1.13, 3.89)], home gardening practice [AOR=2.31, 95% CL: (1.44, 3.67)], history of illness [AOR=0.57, 95% CL: (0.36, 0.93)], and knowledge of nutrition [AOR=4.96, 95% CL: (1.61, 15.33)] were the significant predictors of either thinness or overweight/obesity (DBM).

Conclusion: More than one-fifth of adolescents were affected by DBM in the study area. This prevalence is higher compared with the national and regional prevalence that found to be a public health concern. Thus, interventions like double-duty interventions should consider the age, sex, school type, minimum dietary diversity score, frequency of meals, home gardening practice, history of illness, and nutritional knowledge of adolescents.

Clinical Trial Registration: clinicaltrial.gov, identifier NCT05574842.

KEYWORDS

double burden of malnutrition, school adolescents, overnutrition, undernutrition, Ethiopia

1. Introduction

Undernutrition and overweight or obesity coexisting in the same individuals, households, and populations at the national and international levels throughout the life course are known as the Double Burden of Malnutrition (DBM). For instance, when DBM is present at the individual level, it may manifest as obesity combined with a vitamin or mineral deficit, or as overweight in an adult who was stunted as a child. Moreover, it can be coexist as stunted mother with stunted child at the household level and underweight children and overweight adolescents in one community or city at the population level (1–3). The Food and Agriculture Organization (FAO) and World Health Organization (WHO) initially introduced the DBM concept at the International Conference on Nutrition (ICN) in 1992 (4).

In the past, undernutrition and overnutrition were seen as distinct problems affecting various groups with various risk factors. However, it is becoming more common for these two types of malnutrition to co-occur in the same groups of people, such as overweight and stunted individuals, families, and even towns (5–8). Evidence suggests that early nutrition, diet quality, biology-related factors, epigenetics, food environments, food systems, socioeconomic issues, and weak governance were the common predictors and drivers of DBM (5, 9–11). Of these, four of them are the most modifiable and fundamental causes of various forms of malnutrition. These include early nutrition, socioeconomic determinants, dietary quality, and food environment (5, 8).

For the reason that dramatically accelerated growth and development, adolescence, like that of infancy, is a critical time of life (12). According to the WHO, it is a period between 10 and 19 years that marks the transition from childhood to adulthood (13). It is also a time when higher food intake is necessary for their rapid growth, as a result of developmental changes connected to puberty and brain development, which result in needing appropriate and adequate diets (14, 15).

The DBM is one of the main sources of morbidity and mortality worldwide, notably in low- and middle-income countries (LMICs) among these populations. It threatens and bargains adolescents' future health and puts their young lives at high risk. Globally, around 820 million people (1 in 9) and one-third of the world population (1 in 3) are hungry and overweight or obese, respectively (3, 11, 16–18). Furthermore, 117 million boys and 75 million girls aged 5–19 years were either moderately or severely underweight in 2016 worldwide. In the same year, there were 74 million more boys and 50 million more girls who were obese (19).

According to several researches done on adolescents in various nations throughout the world indicates, the global overall prevalence of DBM (both thinness and overweight/obesity) ranges from 22.5% to 55.6%. More particularly, the prevalence of thinness ranges from 10.1 to 30.5%, and overweight/obesity ranges from 8.6% to 12.4% (20–23). Likewise, in Ethiopia, the overall prevalence of DBM (both thinness and overweight/obesity) ranges from 9.7% to 48.9%. More specifically, the prevalence of thinness ranges from 4.7% to 46.2%, and overweight/obesity ranges from 2.7% to 27.2% (24–34).

The cause of DBM can be categorized as immediate causes (inadequate/excess dietary intake and diseases), underlying causes (inadequate access to food, inadequate healthcare practice, inadequate health services, and unhealthy environment), and basic causes (poor knowledge of nutrition, lack of education, poor socioeconomic status, cultural beliefs, poor agricultural practices, poverty, poor governance, and disparities in households) (3, 35–37).

The DBM has enormous consequences on adolescents' health which may affect the life course of adolescence in different ways. The greater risk of chronic disorders such as diabetes, hypertension, and some cancers is linked with being overweight in adolescence. Moreover, adolescents are more vulnerable to food marketing, which exposes them to obesogenic foods. Poor educational accomplishment and adverse psychosocial difficulties are also the consequences of adolescent obesity and undernutrition. On the other hand, thinness in adolescents is highly associated with a higher risk of infectious diseases. Especially, childbearing age female adolescents are adversely exposed to unwanted prenatal period outcomes such as intrauterine growth retardation, maternal mortality, stillbirth, preterm birth, and delivery complications (37–39).

Although adolescence is a period of vulnerability for DBM, most countries' food and nutrition policies and programs in many of the LMICs and some affluent countries give little focus to the effect of DBM on the well-being and health of adolescents (9). Currently, addressing these two sorts of apparently contrasting forms of malnutrition (underweight and overweight) simultaneously brings an enormous challenge for the food and nutrition policies of developing countries like Ethiopia. Consequently, there has been a paradigm shift in thinking to reduce its effect on the health of adolescents and avert the nutritional vulnerability due to increasing linear growth, bone growth, and neurodevelopmental issue (3).

As a research gap, the prevalence and potential predictors of DBM among adolescents enrolled in secondary schools in the study area have not been examined by Ethiopian researchers. Furthermore, the association between DBM and factors such as home gardening practice, knowledge of nutrition, sex, age, type of school, and the minimum dietary diversity score of adolescents were not studied through a multinomial regression model, which is another element that was not taken into consideration in past studies. Therefore, this study aimed to determine the prevalence and associated factors of DBM in Debre Berhan City, Ethiopia.

2. Materials and methods

2.1. Study area, period, and setting

This study was conducted in Debre Berhan Regiopolitan City (DBRPC), North Shoa Zone, Amhara Region, Central Ethiopia. Debre Berhan Regiopolitan city is located 130 km away from Addis Ababa (the capital city of Ethiopia) and 690 km from Bahir Dar (the capital city of the Amhara region). It was founded by Emperor Zara Yakoob and it is the capital city of the North Shoa Zone of the Amhara region. The city has coordinated with 9°41′N 39°32′E latitude and longitude, respectively. It is found at 2,840 m above sea level, which makes it the highest city of this size in Africa. The city is subclassified into five sub-cities with an estimated number of populations of 310,254 according to the 2021/2022 municipal administrative office report of the city. There are 13,595 secondary school adolescents with 5,295 males and 8, 300 females in 10 secondary schools according to the 2022 report of the city education bureau. The city has 36 kebeles (the smallest administrative unit in Ethiopia), one comprehensive specialized hospital, 5 health centers, and about 16 health posts. This study was conducted from October 13, 2022, to November 14, 2022, in a school-based setting.

2.2. Study design

This study used a school-based cross-sectional study design among school adolescents in the city.

2.3. Population

The source population of the study was all secondary school adolescents in the city during the study period. Whereas the study population was all secondary school adolescents in the selected schools of the city during the study period. Moreover, the sample population of the study was all randomly selected sections of secondary school adolescents in the selected schools.

2.4. Inclusion and exclusion criteria

2.4.1. Inclusion criteria

The study targeted secondary school adolescents aged 10–19 years in the study area. Secondary school adolescents who have followed their teaching-learning process in the selected schools and who had no intention of leaving that school until the end of the study were included in this study.

2.4.2. Exclusion criteria

Secondary school adolescents who were not able to respond to an interview and who have a physical disability including deformities such as kyphosis, scoliosis, and limb deformity which prevents standing erect for height measurement were excluded. Moreover, participants who had confirmed diseases such as diabetes mellitus, hypertension, etc., were excluded from this study to improve the precision of anthropometric measurements and dietary practice.

2.5. Sample size determination

The sample size was calculated using a single proportion formula. During calculation, the following assumptions were considered: 95% confidence level, 46.2% proportion of underweight based on a previous study (40), 5% non-response rate, 1.5 design effect, and 5% marginal error, to get the maximum sample size. Accordingly, the calculated sample size was 602. However, due to the nature of cluster sampling, the final sample size of the study was included 745 study participants.

2.6. Sampling techniques and procedures

A multi-stage cluster sampling technique was applied to select secondary school adolescents from respective schools in DPRPC which coordinated in five sub-cities and 37 kebeles. There are 10 secondary schools in the city. Of these, eight are government and two are private schools. Six secondary schools were chosen from a pool of 10 schools using a simple random sampling technique. The sample size was distributed proportionally to these chosen secondary schools using proportional allocation to the population. The number of secondary school students in each chosen school was compiled from all of these secondary school records. The final sample students in the schools were chosen from the chosen section of selected schools. A revisit was done up to three times if an eligible study participant missed the first visit. Participants in the study were deemed to have not responded if they missed three appointments.

2.7. Data collection methods, procedures, and measurements

2.7.1. Data collection methods and procedures

An interviewer-administered, pretested, structured questionnaire was utilized to gather the necessary data for this study. It was initially set up in English and administered face-to-face to respondents in their homes. Language experts translated this questionnaire into the Amharic spoken in the study area to make the data collection procedure operate more smoothly. The study's data were gathered by 12 health professionals. The survey asked questions were about sociodemographic factors, adolescent dietary practice, and knowledge of nutrition.

2.7.2. Measurements

With their shoes off and wearing light clothing, participants were asked to weigh themselves using a calibrated portable electronic digital scale (Seca, Germany model) to the nearest 0.1 kg. Additionally, using a portable hardwood height-measuring board with a sliding head bar and traditional anthropometric methods, the height was measured to the nearest 0.1 cm. A position for the participants was on the Frankfurt Plane. As well, the vertical stand was touched at the four locations (shoulder, calf, heel, and buttocks), and their shoes were removed. Before beginning the measurement, the stadiometer was checked using a calibration rod. All anthropometric measurements were taken twice, and if there were any differences in the measurements, the average results were chosen for analysis.

Double burden of malnutrition was the primary outcome of the study. It was measured using body mass index (BMI) of adolescents. Adolescents who had BMI for age Z score (BAZ) < -2 SD from the median value were considered as thinness. On the other hand, overweight those with BAZ $> +1$ SD and obese adolescents with a BAZ $> +2$ SD from the median value of the WHO's 2006 reference data (41, 42). The outcome variable (DBM) which has three categories (thinness, normal, and overweight/obesity) was created by the groupings of the two indicators.

Dietary diversity score of adolescents was measured using a Minimum Dietary Diversity Score (MDDS) indicator. The dietary data was evaluated foods groups taken by adolescents during the previous 24 h. Then adolescent's dietary diversity score was classified as adequate (≥ 5 food groups) or not adequate (<5 food groups) from 10 food groups. During analysis, it was coded as "1" for adequate and "0" for inadequate.

Based on six questions, knowledge of adolescents about nutrition was computed using a mean score. The questions were including about the awareness of adolescents about nutrition, dietary diversity practice on taking varieties of food groups and types of varieties of food groups they take, and understanding about cause and consequences of double burden of malnutrition. During analysis, it was coded as "1" for good knowledge and "0" for poor knowledge.

2.8. Data quality control

Pretest, retranslation, and contextualization of the questionnaires were done. Translations of the English language version into the Amharic language version were carried out by an Amharic language speaker who has earned a Master of Arts in the Amharic language to maintain the questionnaire's quality and reliability. Once more, the retranslation into English was completed by a person with a Master of Arts in English. A comparison was done to preserve the consistency between the two versions. Then the questionnaire was translated and tested in Amharic before being used to collect data. The translation was compared by putting the two versions side by side by other experts other than the translators. Discrepancies were solved by discussion between the translators and comparators. The questionnaire was further reformed after the pretest. The interviewers explained every aspect of the study to the subjects while collecting the data. A practical session and a real demonstration were also included.

The data collectors and supervisors participated in a two-day intensive training session that included fieldwork and role-playing exercises before the real data gathering. Using a training document created by the research team, the training was concentrated on the methods, tools, study goals, and ethical concerns.

A standardization of anthropometric measures was conducted to lower inter-observer error. To reduce random anthropometric measurement error, the relative technical error of measurement (% TEM) was calculated. To verify the accuracy of the scale between measurements, a known object weight was employed every morning. The standardization of the anthropometrists was carried out with a coefficient of variation of less than 0.03 (3%). During training and pretesting, the precision of the data collectors' anthropometric measures was standardized with their trainer. Adolescents had their height and weight measured twice, and the results were retaken when there was a difference of more than 0.1 kg in weight or 0.1 cm in height between the two measurements.

Every morning before data collection started, the accuracy of digital weight scales was tested using known weight, and before every weight measurement, the data collectors were confident that the scale was reading exactly at zero. Teenagers in school were urged to be themselves during the interview. Teenagers in high school who were willing to participate and who had their parents or guardians sign an assent form and an informed consent form were then questioned.

2.9. Data management and analysis methods

The principal investigator checked all the interviewed questionnaires visually for completeness, missing, and consistency after the data collection has been completed. To collect the data, Kobo toolbox online tool was used. The data was exported to R software version 4.2.2, and cleaned for missing values and outliers before analysis. Simple descriptive statistics such as simple frequency distribution, measures of central tendency, measures of variability, and percentages were performed to describe the demographic and socioeconomic characteristics of the respondents. Then the information was presented using tables and figures.

Descriptive statistics such as frequency tables, summary measures, and measures of dispersion were used to summarize the baseline data like sociodemographic characteristics of the adolescents. For continuous data, normality was checked using different methods such as graphical (histogram, Q-Q plot) and numerical methods (mean, median, mode, skewness, kurtosis), and statistical tests (Kolmogorov Smirnov test and Shapiro–Wilk test). Using the variance inflation factor, other assumptions multi-collinearity was checked.

To examine the data, a multinomial logistic regression model was used. To determine the relationship between each independent variable and the outcome variable in the bivariable multinomial logistic regression model, a bivariable analysis with crude odds ratio (COR) and a 95% confidence interval (CI) was performed. For independent variables that passed the bivariable test, a p-value cutoff less than or equal to 0.25 was employed in the model to find significant associations and control confounding effects. Using this model, it was possible to determine the variables that significantly association with DBM and obtain an adjusted odds ratio (AOR) with a 95% confidence interval. The Hosmer Lemeshaw test was used to evaluate the model's fitness ($p > 0.999$), and standard error less than 2.0 was used to assess multicollinearity. p-value ≤ 0.05 was used to declare the statistical significance of the study.

3. Results

3.1. Sociodemographic characteristics of respondents

In this study, from 745 total respondents, 742 responded to the survey making a response rate of 99.6%. The mean (±SD) age and family size of the respondents were 16.74 (±1.43) and 5.19 (±1.58), respectively. Of the respondents, 31.8% were grade 9th students, 70.1% were from government schools, 69.7% were urban residents, 53.3% were female, and 94.5% were Orthodox Christian religion followers (Table 1).

3.2. Dietary and related-practice of adolescents

Adolescents who had inadequate dietary diversity score were exposed to 12.1% and 6.1% for thinness and overweight/obesity (overnutrition), respectively. Similarly, adolescents who had poor knowledge of nutrition were exposed to 12.7% and 6.2% for thinness and overweight/obesity (overnutrition), respectively (Table 2).

3.3. Prevalence of double burden of malnutrition

The summarized prevalence of DBM (both thinness and overweight/obesity) among school adolescents was 21.5%. More specifically, the prevalence of thinness among school adolescents in the study area was 14.8% [95% CL: 12.3, 17.6%] and overweight/obesity was 6.7% [95% CL: 5.0, 8.8%], respectively (Figure 1).

TABLE 1 The sociodemographic characteristics of the respondents in the study area.

Variables	Categories	Frequency (%)
Grade level of students	Grade 9th	236 (31.8)
	Grade 10th	212 (28.6)
	Grade 11th	149 (20.1)
	Grade 12th	145 (19.5)
Type of school	Government	520 (70.1)
	Private	222 (29.9)
Age	Mean (±SD)	16.74 (±1.43)
Family size	Mean (±SD)	5.19 (±1.58)
Residence	Urban	517 (69.7)
	Rural	225 (30.3)
Sex	Male	354 (47.7)
	Female	388 (53.3)
Religion	Orthodox Tewahido	700 (94.3)
	Protestant	27 (3.6)
	Muslim	14 (1.9)
	Others	1 (0.1)
Ethnicity	Amhara	738 (99.5)
	Oromo	1 (0.1)
	Others	3 (0.4)
Mother education	Unable to read and write	226 (30.5)
	Read and write	48 (6.5)
	Primary education	163 (22.0)
	Secondary education	140 (18.9)
	More than secondary education	165 (22.2)
Mother occupation	Housewife	465 (62.7)
	Merchant	66 (8.9)
	Daily laborer	3 (0.4)
	Government employee	145 (19.5)
	Self-employed	48 (6.5)
	Others	15 (2.0)
Father education	Unable to read and write	209 (28.2)
	Read and write	162 (21.8)
	Primary education	86 (11.6)
	Secondary education	148 (19.9)
	More than secondary education	137 (18.5)
Father occupation	Farmer	280 (37.7)
	Merchant	92 (12.4)
	Daily laborer	6 (0.8)
	Government employee	171 (23.0)
	Self-employed	90 (12.1)
	Others	103 (3.9)

The mean (± SD) of the overall height and weight of the participants were 160.1 ± 7.8 cm and 49.9 (± 8.1 kg), respectively. The mean body mass index (BMI) for the age by Z-score of the participants was −0.72 (± 0.1).

The percentage of all adolescents with Z-score was displayed in Figure 2. The percentage of adolescents with Z-score by sex of the school adolescents for both male ($n = 354$) and female ($n = 388$) was displayed in Figure 3. The mean Z-score of respondents with age in months of adolescents was displayed in Figure 4. The highest numbers of respondents by months were found in the age group 192–203 ($n = 161$) and 204–215 ($n = 161$).

The status of DBM was compared using the level of grade of the school adolescents. A high prevalence of undernutrition was observed in grade 9th students whereas a high level of overnutrition was observed among grade 12th students (Figure 5).

3.4. Factors associated with the double burden of malnutrition

In the multivariable multinomial logistic regression analysis model, factors such as age, sex, school type, minimum dietary diversity score, frequency of meals, home gardening practice, history of illness in the last 2 weeks, and knowledge of nutrition were the significant and independent predictors of DBM (either thinness or overweight/obesity) in-school adolescents (Table 3).

The age of adolescents had a significant association with thinness compared to adolescents who had normal nutritional status. Every one-year increase in the age of school adolescents, the odds of thinness was increased by 0.79-point considering other factors constant [AOR = 0.79, 95% CL: (0.67, 0.93)] (Table 3).

Sex of school adolescents had a noteworthy association with thinness and overweight/obesity compared to the normal category. Male adolescents had 3.86 times [AOR = 3.86, 95% CL: (2.35, 6.32)] more likely to have thinness compared to female adolescents. Similarly, for adolescents who are in the overweight/obesity than normal category, male adolescents had 88% less likely [AOR = 0.12, 95% CL: (0.05, 0.33)] to have overweight/obese compared to female adolescents (Table 3).

School type had a significant association with thinness and overweight/obesity compared to the normal category. Private school adolescents were 2.24 times [AOR = 2.24, 95% CL: (1.33, 3.77)] more likely to have a significant association with thinness compared to government school adolescents. In the same way, private school adolescents were 5.03 times [AOR 5.03, 95% CL: (2.30, 10.99)] more likely to have a significant association with overweight/obesity compared to government school adolescents (Table 3).

The home gardening practice had a significant association with thinness and overweight/obesity compared to the normal category. School adolescents who had no home gardening practice were 2.31 times [AOR = 2.31, 95% CL: (1.44, 3.67)] more likely to have thinness compared to adolescents who had home gardening practice. Likewise, school adolescents who had no home gardening practice were 3.96 times [AOR = 3.96, 95% CL: (1.92, 8.21)] more likely to have overweight/ obesity compared to adolescents who had home gardening practice in their garden (Table 3).

The minimum dietary diversity score of adolescents had a significant association with thinness and overweight/obesity compared to the normal category. Adolescents who had inadequate minimum dietary diversity score were 2.29 times [AOR = 2.29, 95% CL: (1.27, 4.14)] more likely to have thinness compared to adolescents who had adequate minimum dietary diversity score. Correspondingly, adolescents who had inadequate minimum dietary diversity score were 5.26 times [AOR = 5.26, 95% CL: (1.88, 14.67)] more likely to

TABLE 2 The crosstab distribution between the DBM and dietary and related-practice of adolescents in Debre Berhan City, Ethiopia (*n*=742).

Variables	Categories	Double burden of malnutrition			
		Thinness (%)	Normal (%)	Overnutrition (%)	Total (%)
Dietary diversity score					
	Adequate	20 (2.7)	208 (28.0)	5 (0.6)	233 (31.4)
	Inadequate	90 (12.1)	374 (50.4)	45 (6.1)	509 (68.6)
	Total	110 (14.8)	582 (78.4)	50 (6.7)	742 (100)
Frequency of meals per day					
	<3 meals/day	24 (3.2)	56 (7.5)	22 (3.0)	102 (13.7)
	≥3 meals/day	86 (11.6)	526 (70.9)	28 (3.8)	640 (86.3)
	Total	110 (14.8)	582 (78.4)	50 (6.7)	742 (100)
Home garden practice					
	Yes	51 (6.9)	413 (55.7)	16 (2.2)	480 (64.7)
	No	59 (8.0)	169 (22.8)	34 (4.6)	262 (35.3)
	Total	110 (14.8)	582 (78.4)	50 (6.7)	742 (100)
History of illness					
	Yes	75 (10.1)	321 (43.3)	35 (4.7)	431 (58.1)
	No	35 (4.7)	261 (35.2)	15 (2.0)	311 (41.9)
	Total	110 (14.8)	582 (78.4)	50 (6.7)	742 (100)
Knowledge of nutrition					
	Good	16 (2.2)	213 (28.7)	4 (0.5)	233 (31.4)
	Poor	94 (12.7)	369 (49.7)	46 (6.2)	509 (68.6)
	Total	110 (14.8)	582 (78.4)	50 (6.7)	742 (100)

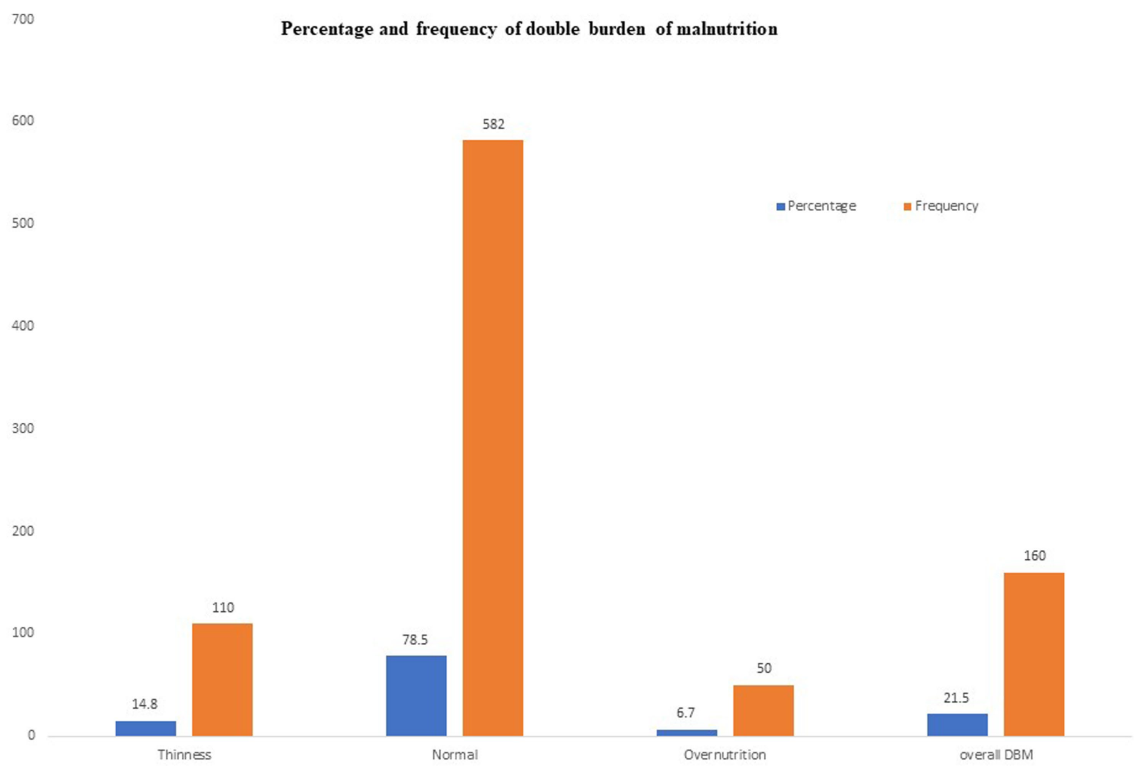

FIGURE 1
Prevalence of DBM (thinness and overweight/obesity) among school adolescents in Debre Berhan City, Ethiopia.

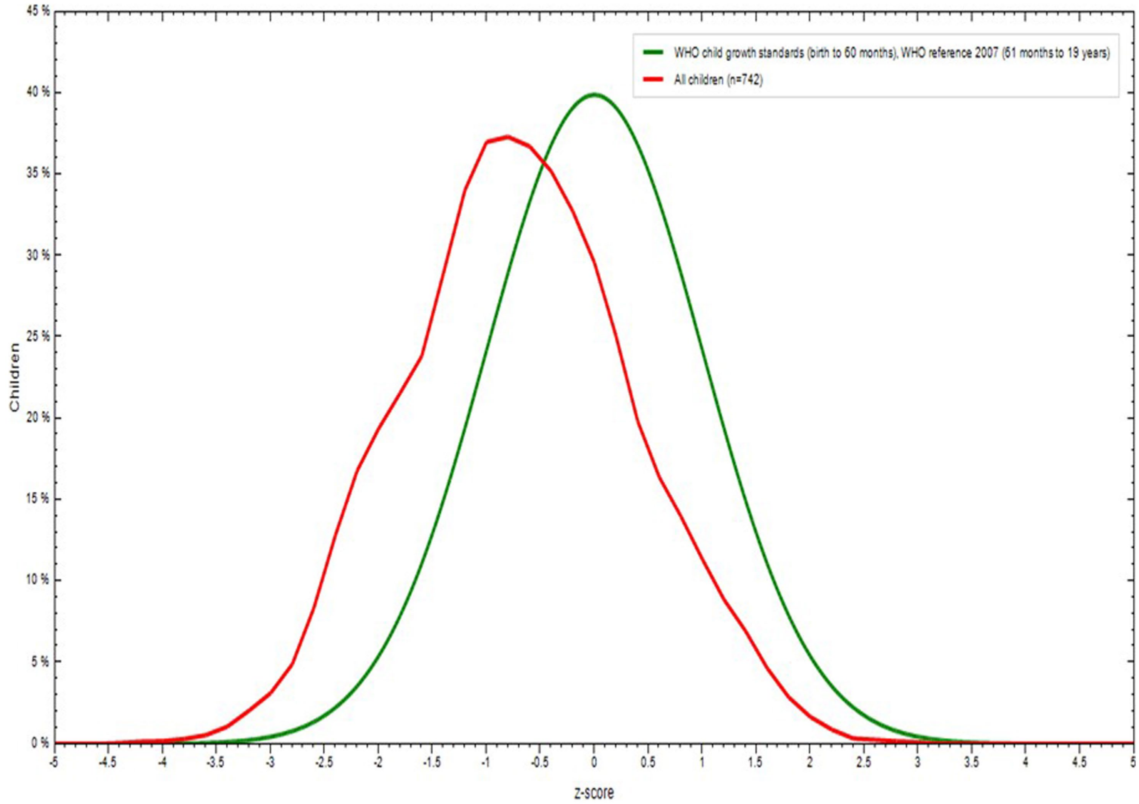

FIGURE 2
Body mass index (BMI) for Age Z score (BAZ) among school adolescents in Debre Berhan City, Ethiopia.

have overweight/obesity compared to adolescents who had adequate minimum dietary diversity score (Table 3).

The frequency of meals had a significant association with thinness and overweight/obesity compared to the normal category. School adolescents who had less than three frequencies of meals were 2.09 times [AOR = 2.09, 95% CL: (1.13, 3.89)] more likely to have a significant association with thinness compared to adolescents who had three and greater than three frequencies of meals. Similarly, school adolescents who had less than three frequencies of meals were 4.58 times [AOR = 4.58, 95% CL: (2.15, 9.76)] more likely to have overweight/obese compared to adolescents who had three and greater than three frequencies of meals (Table 3).

History of illness in the last month had a significant association with thinness compared to the normal category. School adolescents who had no history of illness in the last month were 43% [AOR = 0.57, 95% CL: (0.36, 0.93)] less likely to have a significant association with thinness compared to school adolescents who had a history of illness in the last month (Table 3).

Knowledge of nutrition among adolescents had a significant association with thinness and overweight/obesity compared to the normal category. School adolescents who had poor knowledge of nutrition were 2.58 times [AOR = 2.58, 95% CL: (1.41, 4.74)] more likely to have thinness compared to school adolescents who had good knowledge of nutrition. Correspondingly, School adolescents who had poor knowledge of nutrition were 4.96 times [AOR = 4.96, 95% CL: (1.61, 15.33)] more likely to have overweight/obese compared to school adolescents who had good knowledge of nutrition (Table 3).

4. Discussion

The prevalence and predictors of DBM were examined in this study. In the research area, the overall prevalence of DBM was 21.5%. More particular, thinness 14.8% (95% CL: 12.3, 17.6%) and overweight/obesity 6.7% (95% CL: 5.0, 8.8%) were detected in school-aged adolescents. The significant and independent determinants of DBM in school-aged teenagers included age, sex, school type, minimum dietary variety score, frequency of meals, home gardening practice, history of illness in the previous months, and nutritional knowledge.

The overall prevalence of DBM in this study was higher than the previous studies conducted in Dessie town where the percentage was 14.5% (underweight 6.3% and overweight/obese 8.2%) (25), in Jimma Zone where the percentage was 18.7% (11.6% thinness and 7.1% overweight/ obesity) (32), in Southern Ethiopia where the percentage was 9.7% (4.7% thinness and 5% overweight/obesity) (30), in Addis Ababa where the percentage was 14.7% (6.2% underweight and 8.5% overweight/obesity) (31) and in Greece 22.5% (12.4% overweight/ obese and 10.1 underweight) (20). However, it was lower than studies done in southwest Ethiopia where the percentage was 33.2% (28% underweight and 5.2% overweigh/obese) (34), in Gondar city where the percentage was 40.1% (undernourished 12.9%, overweight 21.3% and obese 5.9%) (26), in Bahir Dar city where the percentage was 28.1% (underweight 15%, overweight 8.4%, and obesity 4.7%) (28), in Finote Selam Town where the percentage was 48.9% (underweight 46.2% and overweight 2.7%) (40), in Indonesia where the percentage

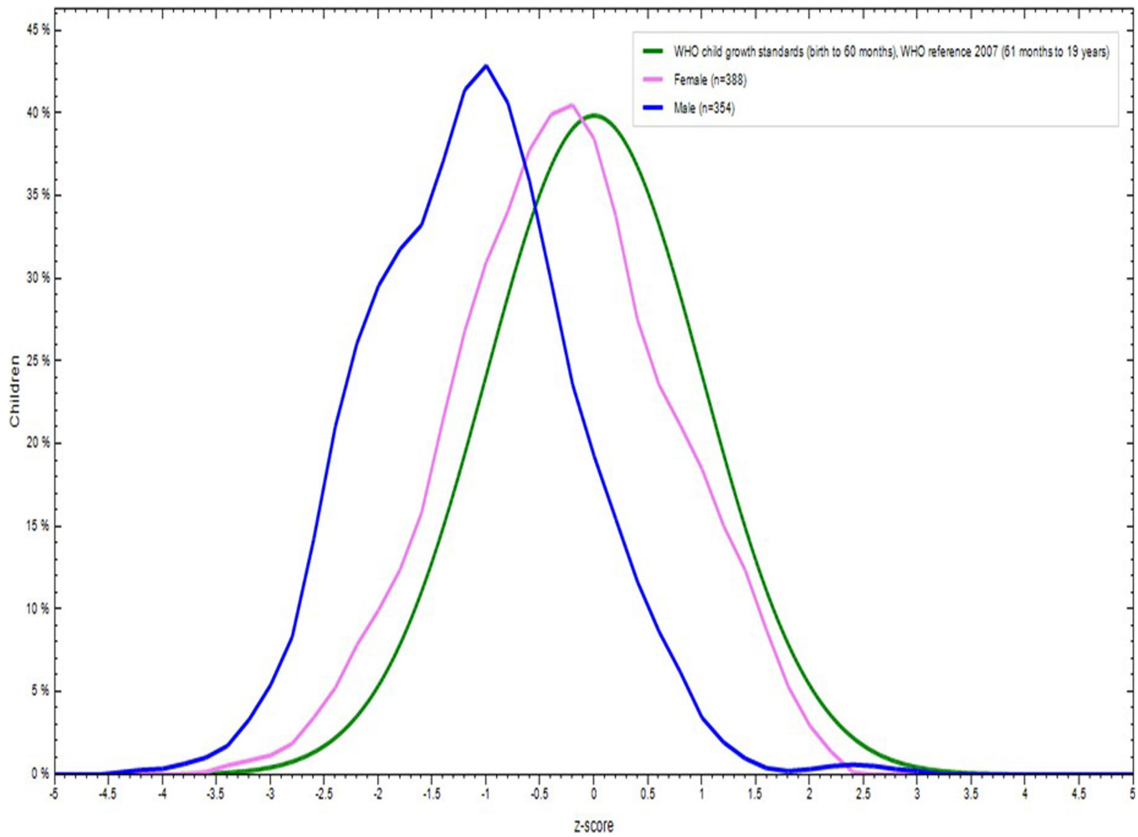

FIGURE 3
Body mass index (BMI) for Age Z score (BAZ) by sexes among school adolescents in Debre Berhan City, Ethiopia.

was 27% (16% thinness and 11% overweight/obese) (21), in India where the percentage was 55.6% (47% underweight, 5.9% overweight, and 2.7% obese) (23), in south India where the percentage was 39.2% (14.7% severely thin, 15.8% thin, 6.9% overweight and 2.2% obese) (22), in southern Ethiopia where the percentage was 30.9% (underweight 19.7% and overweigh/obesity 11.2%) (27), and in Adama city where the percentage was 25.6% (21.3% underweight, 3.3% overweight, and 1.0% obese) (24). This mismatch may be brought on by differences in sociodemographic characteristics such as family educational and occupational diversity, access to information on nutrition and feeding practice, and nutrition knowledge of adolescent. Furthermore, for this perceived variation, other factors like outcome classification methods (since this study classify the outcome into three classifications), sample size, and analysis methodology may be taken into account.

Compared to school adolescents with normal nutritional status, age demonstrated a significant association with thinness. When all conditions are held constant, there is an approximately 0.79-point rise in the odds of thinness for every one-year increase in age. As age increases, the probability of getting thin increases. This finding is similar to the study conducted previously (28, 43). This may be related to the fact that some adolescents think that our shape will be disfigured and disturbed when we take much amount of food (44, 45). Due to this assumption, they will reduce food intake and exposed to thinness.

Sex among school-aged adolescents was significantly associated with both thinness and overweight or obesity, in contrast to the normal group. Male adolescents were approximately four times as likely to be thin as compared to female teenagers. This result was in line with earlier research (23, 25, 30, 40). On the other hand, male adolescents had an 88% lower likelihood of being overweight or obese. This outcome is consistent with the available data (46–48). The probable reason may be that, in contrast to females, males lead an active lifestyle that involves energy-demanding activities. Moreover, since girls tend to stay at home, they might have better access to food than males do. This might make males more prone to being thin.

School type had a substantial connection with thinness and overweight/obesity compared to the normal category. Equated to government school adolescents, adolescents in private schools were more than two times more likely to have a significant connection with thinness. This result was not supported by the previous studies (30, 31). This dissimilarity may due to variation in a model of analysis since this study uses a multinomial regression analysis model instead of a binary logistic regression analysis model. In a similar vein, private adolescents were more than five times more likely to have a significant association with overweight/obesity than those in government schools. This finding was consistent with the previous studies (30, 31, 47, 49–51). The greater financial access of student households may be the possible reason. Additionally, they might receive specific assistance and special care from their family, such as access to transportation.

Compared to the normal category, the odd of home gardening demonstrated a strong correlation with thinness and overweight/

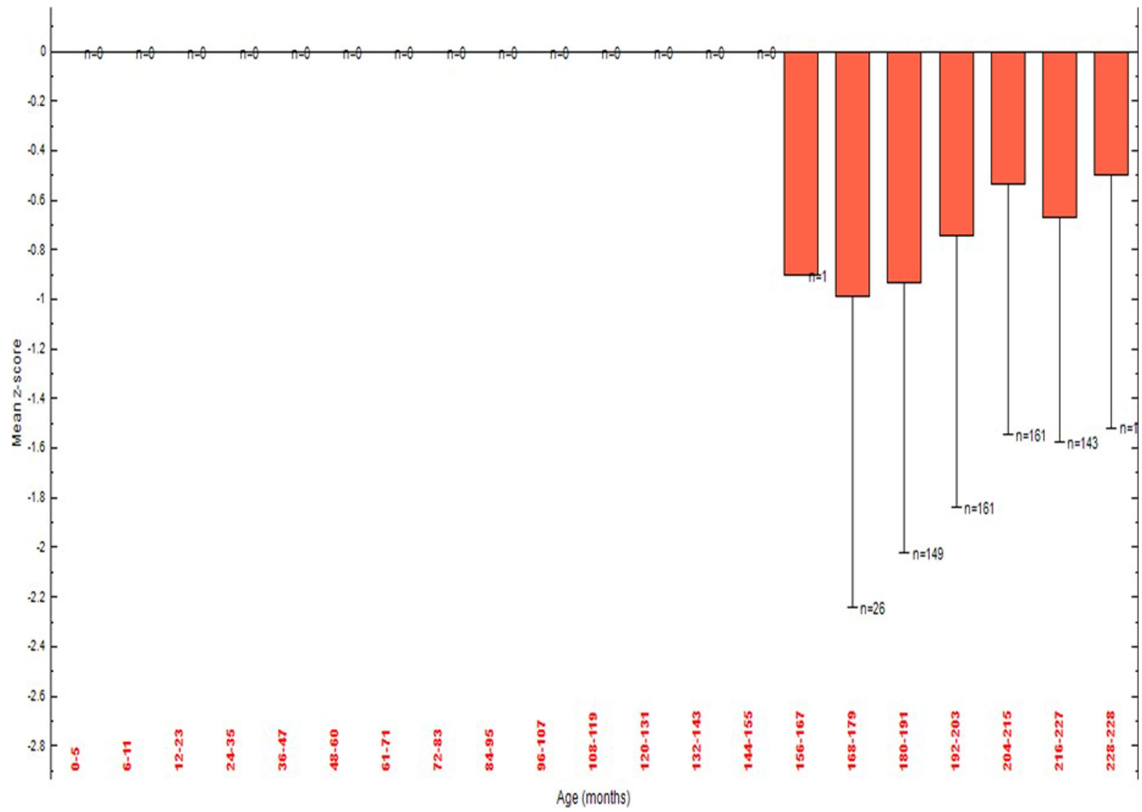

FIGURE 4
Body mass index (BMI) for Age Z score (BAZ) by age among school adolescents in Debre Berhan City, Ethiopia.

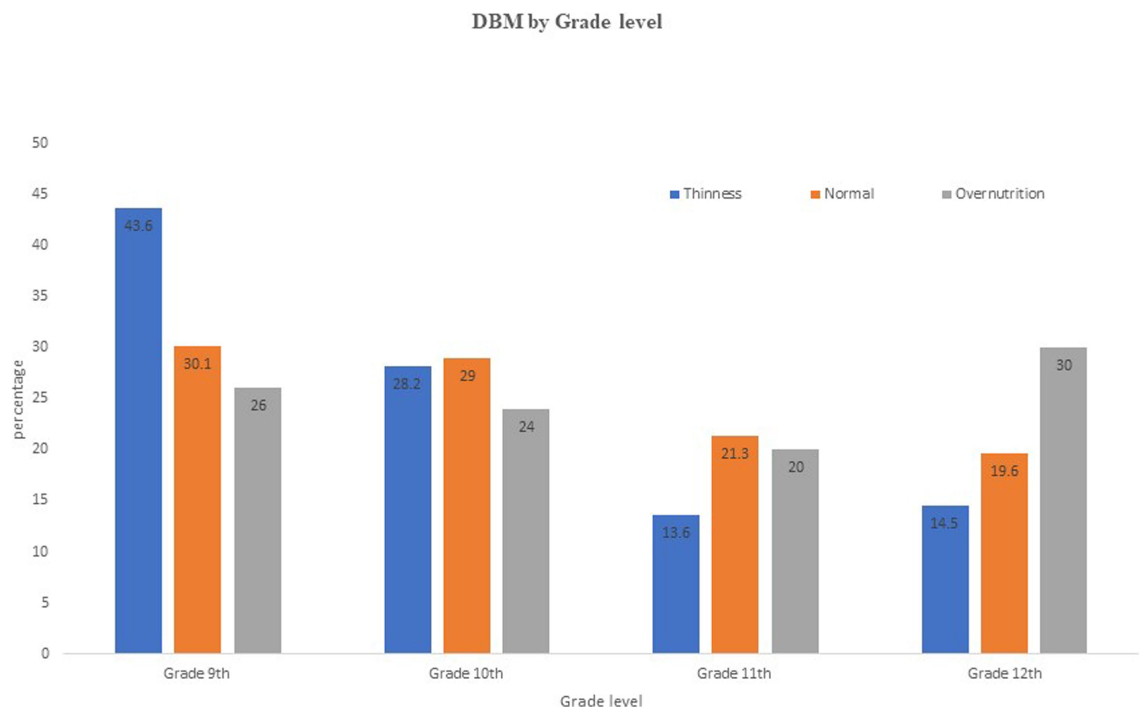

FIGURE 5
The DBM status of school adolescents by grade level in Debre Berhan City, Ethiopia.

TABLE 3 Modeling the bivariable and multivariable multinomial logistic regression[1] analysis model for factors associated with DBM among school adolescents in Debre Berhan City, Ethiopia, 2023 (*n*=742).

Variables	Double burden of malnutrition (DBM)$			
	Thinness		Overweight/obesity	
	COR (95% CI)	AOR (95% CI)	COR (95% CI)	AOR (95% CI)
Age	0.77 (0.66, 0.89)**	**0.79 (0.67, 0.93)****	1.11 (0.90, 1.36)	1.27 (0.99, 1.63)
Sex				
Male	3.65 (2.29, 5.81)*	**3.86 (2.35, 6.32)****	0.13 (0.05, 0.340)*	**0.12 (0.05, 0.33)****
Female	1.00	1.00	1.00	1.00
Type of school				
Private	1.55 (1.02, 2.38)*	**2.24 (1.33, 3.77)****	1.74 (0.96, 3.16)	**5.03 (2.30, 10.99)**
Gov't	1.00	1.00	1.00	1.00
Dietary diversity score				
Adequate	1.00	1.00	1.00	1.00
Inadequate	2.51 (1.49, 4.18)**	**2.29 (1.27, 4.14)****	5.01 (1.96, 12.81)**	**5.26 (1.88, 14.67)****
Frequency of meal				
<3	2.62 (1.54, 4.45)**	**2.09 (1.13, 3.89)**	7.38 (3.96, 13.76)**	**4.58 (2.15, 9.76)****
≥3	1.00	1.00	1.00	1.00
Home garden practice				
Yes	1.00	1.00	1.00	1.00
No	2.83 (1.87, 4.28)**	**2.31 (1.44, 3.67)****	5.19 (2.79, 9.66)**	**3.96 (1.92, 8.21)****
History of illness				
Yes	1.00	1.00	1.00	1.00
No	0.57 (0.37, 0.88)*	**0.57 (0.36, 0.93)****	0.53 (0.28, 0.98)*	0.61 (0.29, 1.26)
Knowledge of nutrition				
Good	1.00	1.00	1.00	1.00
Poor	3.39 (1.94, 5.92)**	**2.58 (1.41, 4.74)****	6.64 (2.36, 18.69)**	**4.96 (1.61, 15.33)****

$Normal is the reference group; **P*-value < 0.005; ***P*-value < 0.001; AOR, Adjusted odds ratio; COR, Crude odds ratio; CI, Confidence interval; 1.00, reference category; Gov't, government;
[1]Hosmer and Lemeshow's test (*p*-value = 0.999). Bold values indicates the significant associated factors to give in focus.

obesity. School teenagers who did not engage in home gardening had nearly two and half times higher likelihood of becoming thin than those who did. This finding is similar to a study conducted in Rwanda (52). Participants in home gardening have much higher results for child nutrition and nutrition security than non-participating households. Likewise, school-aged children and adolescents who did not engage in home gardening were nearly four times more likely to be overweight or obese than those who did home gardening practice. This result is similar from the previous studies. Home gardening had a positive impact on anthropometric measures and more generally on children's health (53, 54). This indicates that the access to many nutrients can be increased by growing our own produce at home, including fruits, vegetables, and other significant plant sources. Teenagers may be exposed to both sides of malnutrition in the absence of such situations. This suggests that not engage in home gardening practice can acts as the driver factor of both side of malnutrition.

Adolescents' minimum dietary diversity scores significantly predicted thinness and overweight or obesity as compared to the normal group. Adolescents with inadequate minimum dietary variety scores had nearly two and half times greater likelihood of being thinner than those with adequate minimum dietary diversity scores. This result was in line with earlier research (29, 43, 55, 56). Respondents who had adequate minimum dietary diversity scores had a high probability to be prevented from developing thinness. Also, adolescents with inadequate minimum dietary variety scores were more than five times more likely to be overweight or obese than those with adequate minimum dietary diversity scores. Higher dietary diversity had a strong association with reductions in overweight or obese according to the research done in the former time (56–59). This could be as a result of the association between dietary diversification and decrease in the total calories from carbohydrate, fat, saturated fat, and high lipodensity cholesterol. This will result in a reduction in the percentage of the food groups that are consumed and is linked to little weight gain. This shows that both sides of malnutrition may be caused by poor dietary diversity scores.

Compared to the normal group, frequency of meals had a significant correlation with the odds of thinness and overweight/obesity. When compared to school teenagers who ate three or more times per day, those who ate less frequently had a more than two times larger likelihood of having a meaningful link with thinness. This finding was consistent with the study conducted in different parts of Ethiopia (25, 30, 34). Corresponding to this, adolescents who ate less frequently were nearly five times more likely to have a meaningful link with overweight/obesity than adolescents who had three and larger frequency of meals. This finding is consistent with the existing studies (60–62). According to these studies, eating out less than three meals frequently had a significantly positive effect on overweight and obesity. Eating timely and taking adequate amounts of food may decrease excessive eating at one time and avoid over-accumulation of fat in the body. Furthermore, low meal frequency is also associated with inadequate nutritional intake in terms of quantity, variety, and quality. Because adolescent growth is the fastest and they have greater nutritional needs at this time, eating infrequently will result in underweight or thinness. On the other side, people run the risk of becoming overweight or obese if they follow fewer frequency each day but consume more of foods at a time. This indicates that both sides of malnutrition may be caused by low meal frequency.

When compared to the normal group, the odd of thinness was significantly correlated with no illness history during the previous month. When compared to school adolescents who had a history of illness in the previous month, those with no history of illness were 43% less likely to have an association with thinness. According to the findings of this study per the available information, respondents who had a history of illness in the past were exposed to thinness (30, 34). This may be connected to the idea that being ill will reduce intake and exposure to certain diseases.

Teenagers' nutritional knowledge was significantly correlated with thinness and overweight/obesity when compared to the normal category. When compared to school adolescents with strong good nutrition awareness, those with poor nutrition knowledge were more than two and half times more likely to be skinny. The previous studies reported that knowledge of nutrition improves the nutritional status of respondents (63–65). Corresponding to this, school teenagers with poor nutrition knowledge were nearly five times more likely to be overweight or obese than those with strong nutrition knowledge. In the same way, the knowledge of nutrition decreases the overweight and obesity of respondents in the preceding studies (66, 67). The likelihood of preventing both undernutrition and overnutrition will rise as our knowledge of nutrition grows. This shows that poor understanding nutrition may be a driving force for both sides of malnutrition.

In a nutshell, the results of this study have advanced support for the goals of the national food and nutrition strategy, which aims to increase the intake and use of a varied and nutritious diet to guarantee adolescents optimal health over the course of their lives in the nation. Its primary goal is to lessen the prevalence of DBM across all population groups, including school-aged adolescents nationally. Lastly, the implementation of double-duty interventions will have a double impact on DBM prevention.

Regarding the strength and limitations of the study, it has the strength of using an advanced model of analysis, which is using a multinomial regression model of analysis compared to the dominant binary logistic regression model in the existing body of research. On the other hand, this study was limited by recall bias and reporting for some of the variables like dietary practice and history of illness. However, these limitations were minimized by detail probing and remembering the issues using different techniques such as introducing what were pertinent events when they experienced such issues.

5. Conclusion

The status and DBM predictors were examined in this study. The overall prevalence of DBM was 21.5% (14.8% thinness and 6.7% overweight/obesity). This prevalence is higher compared with the national and regional prevalence that found to be a public health concern. The significant and independent determinants of DBM in school-aged teenagers were age, sex, school type, minimum dietary variety score, frequency of meals, home gardening practice, history of illness in the previous 2 weeks, and nutritional awareness. Thus, the federal minister of health, particularly the Debre Berhan City health office in collaboration with education office should design double duty interventions that aimed at reducing the two sides of DBM which comprise both thinness and overweigh/ obesity among adolescents. Especially, the intervention packages like double-duty interventions should focus on how to improve minimum dietary variety score, follow appropriate frequency of meals, grow home garden vegetables and fruits, get treatment on illness, and increase knowledge of nutrition.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Ethics statement

The guidelines of the Helsinki Declaration and good clinical practice (GCP) were followed in the conduct of the study (68). The Jimma University Institutional Review Board (IRB) accepted the research protocol and ethical approval was obtained. The study objectives were communicated to Debre Berhan city administrators, the zonal education office, kebele administrators, and other pertinent authorities through a letter produced by the Jimma University IRB office in order to improve and facilitate support and cooperation. All data collection procedures followed the rules and regulations of the Ethiopian National Research Ethics and Jimma University Research Ethics. Written informed consent to participate in this study was provided by the participants' legal guardian/ next of kin.

Author contributions

LG, BA, and TB agreed on the journal to which the article was submitted, gave final approval of the version to be published, contributed to data analysis, drafting, or revising the paper, and agreed to be responsible for all parts of the work. 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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Effect of COVID-19 pandemic on eating habits and lifestyle of college students in Tabriz, Iran: a cross-sectional study

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Background: The pandemic of coronavirus disease (COVID-19) has influenced lifestyle behaviors and the health of populations worldwide. The purpose of this study was to determine the effects of the COVID-19 pandemic on the eating habits and lifestyle behaviors of Tabriz University of Medical Sciences students in Tabriz, Iran.

Methods: This cross-sectional study was conducted on 220 college students selected using a convenience sampling method in May–June 2022. Data were collected by the questionnaire, which included information on eating habits, physical activity, smoking, watching television, social media use, sleep, anxiety and stress, and smoking before and during the pandemic. The Chi-square test was used to analyze the association of COVID-19 with lifestyle behaviors.

Results: The median age of participants was 22.00 (IQR: 3.00) years old. The median BMI was 21.69 (IQR: 3.82) kg/m^2, and 74.5% of participants had a BMI of 18.5 to 25 kg/m^2. Around 34.5% of participants reported a weight gain during the pandemic. During the pandemic, students' eating habits improved by maintaining a regular meal pattern, eating a balanced diet, consuming 2–3 servings of milk or its products, consuming one or more servings of pulses, eggs, or meat per day, decreasing consumption of fast food, fried, and junk foods, adding less sugar to meals and beverages, and consuming fewer sugar-sweetened beverages and foods with high sugar (all $p = 0.000$). They also reported less physical activity and more sitting and screen time. Sleep time and poorer quality of sleep increased during the pandemic ($p = 0.000$). Feeling stress or anxiety in a day increased, and 2.2% of our participants decided to smoke. The biggest reasons for eating habits changes were less eating out, fear of coronavirus spreading through food, preferring home-cooked food, and improved knowledge about nutrition.

Conclusion: The results indicated that the eating habits of university students improved; however, participants stated increased weight gain, screen, sitting, and sleep time, declined physical activity, worse sleep quality, and feeling stress or anxiety during the COVID-19 pandemic. The findings can help to develop nutritional and behavioral recommendations for maintaining adults' health during and after the pandemic.

KEYWORDS

coronavirus, COVID-19, eating habits, lifestyle, college students

Introduction

Coronavirus disease 2019 (COVID-19) is an acute respiratory syndrome that its outbreak has been the most serious health threat globally in recent years (1). The World Health Organization (WHO) on March 11, 2020, stated that COVID-19 is a pandemic and declared it a "health emergency" due to the widespread massively in almost every country in the world (2). It is demonstrated that eating habits and lifestyle of individuals could be influenced by various diseases such as obesity. Worries about the one's health contribute to greater healthcare and healthier lifestyle, on the other hand, emotional and physical consequence of disease might lead to the poor lifestyle patterns (3, 4). The COVID-19 pandemic also leads to unprecedented health, environmental, and economic crises (5). It has negatively impacted mental health, education, social life, and physical activity and overwhelmed the healthcare systems (6–8).

During the COVID pandemic, countries developed several strategies such as lockdowns for the avoidance of mass gatherings that forced the total population into home confinement and the academic community promptly switched to teleworking. Numerous changes in lifestyle habits such as eating habits and physical activity have been induced by the lockdowns (9). These changes may contribute to unhealthy behaviors, including poor diet, sedentariness or less physical activity, disturbed sleep patterns, and distress and anxiety that can potentially lead to obesity and its related cardiometabolic risks (10). Evidence of past outbreaks indicated that as a pandemic evolves, it substantially impacts lifestyle-related behaviors and poses a challenge to maintaining health and nutritional status (11). Thus, this pandemic has extensive medical, behavioral, and social implications. Understanding the extent of lifestyle-related changes and their contributing COVID-19-specific reasons is important to prevent these changes and maintain individual and community health (12).

Recent studies have assessed how the eating behaviors and lifestyles of individuals have altered during the lockdowns, some of them indicating general trends toward weaker lifestyles and the adaption of unhealthy eating behaviors (13–16). Despite this, some others reported higher general population adherence to the Mediterranean diet as a healthy eating pattern during the lockdown (17–19). However, some of these studies had methodological limitations such as non-validated data collection tools and less representative samples. Moreover, the influences of COVID-19 infection on lifestyle-related behaviors may vary from country to country because of different cultural, social, and economic constructs (20). Some of recent studies reported that women more affected by the restrictions because of COVID-19 pandemic (21–23). In Iran, there is a lack of research examining the COVID-19 pandemic's impacts on lifestyle habits. It is required to explore the dietary habits, and lifestyle behaviors among university students in the Iranian context. Addressing the information gaps will provide the government, public health stakeholders, and higher education sector baseline data needed to properly plan and implement lifestyle related policies for young adults during quarantine and future outbreaks.

To the best of our knowledge, no study has assessed the eating habits and lifestyle changes of Iranian college students during the COVID-19 lockdown. This study aimed to assess the COVID-19 pandemic's impacts on the eating habits and lifestyle behaviors of college students by interview method and using a validated questionnaire in Tabriz, Iran.

Materials and methods

Study design and participants

The present cross-sectional study was carried out among female students at Tabriz University of Medical Sciences from May to June 2022. The inclusion criteria were females in the age of more than 18 years old, being student in Tabriz University of Medical Sciences, and willingness to participate in the study. All female medical sciences students from Tabriz University of Medical Sciences who agreed to participate in this study met the inclusion criteria. There was no restriction on the socioeconomic level, nationality, or occupation of the participants. The sample size was calculated based on the prevalence of the increasing in the consumption of chocolate and salty snacks (28%) reported by Celorio-Sardà et al. (24) and considering a 95% confidence level, a power of 90% in two tailed tests using G-Power software was calculated to be 211 subjects. Finally, 220 college students were chosen using the convenience sampling method. All participants, before completing the questionnaire, were informed of the study procedure and signed a written informed consent. This study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (ethical code: IR.TBZMED.REC.1401.597).

Data collection

We used the questionnaire developed and validated by Chopra et al. among adults (12). They developed a questionnaire to assess the effects of COVID-19 on lifestyle behaviors, such as eating habits. We only removed one item from their questionnaire about drinking alcohol because our participants were females, and in our country, females do not drink or smoke. The items of the questionnaire are presented in Tables 1–5. Four sections are included in the questionnaire. Section A consisted of socio-demographic and anthropometric parameters, and Sections B and C had twenty-three items for evaluating the changes in eating, physical activity, and sleep behaviors before and during COVID-19, respectively. In addition, Section D involved six items for investigating COVID-19-specific reasons for the change of lifestyle. The questionnaire was in paper form. Body mass index value (BMI) was obtained by self-reported weight and height, and participants with BMI = 18.5 to 25 kg/m^2, BMI < 18.5 kg/m^2, BMI = 25 to 30 kg/m^2, and BMI > 30 kg/m^2 were categorized as normal weight, underweight, overweight, and obese, respectively.

Statistical analysis

Data analysis was done using IBM SPSS version 26 software. The normality of continuous data was checked by the distribution of the data and the Kolmogorov–Smirnov test. Based on these methods, the distribution of our data was non-normal; hence, the median and interquartile range (IQR) was reported for continuous data. Categorical data were reported as frequency and percentage. The chi-square test the Wilcoxon Signed-Rank test were used to compare two scores before and during the COVID-19 pandemic for each item. A value of $p < 0.05$ was set as the significance level.

TABLE 1 Baseline characteristics of participants (n = 220).

Characteristics	Median	IQR
Age (year)	22	3
Weight (kg)	59	12.15
Height (cm)	164	9
BMI (kg/m²)	21.69	3.82
	n	%
BMI (kg/m²)		
BMI < 18.5	22	10
BMI = 18.5 to 25	164	74.5
BMI = 25 to 30	28	12.7
BMI > 30	6	2.7
Marital status		
Married	16	7.3
Single	204	92.7
Field of study		
Medicine	48	21.8
Pharmacology	26	11.8
Nutrition	21	9.5
Laboratory	8	3.6
Nursing	23	10.5
Midwifery	7	3.2
Health	25	11.4
Anesthesiology	3	1.4
Information technology	8	3.6
Occupational therapy	4	1.8
Surgical technologist	3	1.4
Management of healthcare services	6	2.7
Informatics	4	1.8
Speech therapy	5	2.3
Nanomedicine	6	2.7
Physiotherapy	4	1.8
Radiology	2	0.9
Food sciences	2	0.9
Food Industry Engineering	3	1.4
Audiology	2	0.9
Dentistry	2	0.9
Bacteriology	2	0.9
Anatomy	4	1.8
Physiology	4	1.8
Grade		
BSc	115	52.3
MSc	18	8.2
PhD	82	37.3
Prof	5	2.3

(Continued)

TABLE 1 (Continued)

Characteristics	Median	IQR
Weight gain during the pandemic		
No, my weight is stable	88	40
No, I think I lost weight	46	20.9
Yes, I think I gained some weight	76	34.5
I do not know	10	4.5

BMI, body mass index; IQR, interquartile range.

Results

A total of 220 female students from a medical university participated in the current study. The general characteristics of the participants are presented in Table 1. The median age of the students was 22.00 (IQR: 3.00) years old. The majority were unmarried (92.7%), and 21.8% of them were medical students. Most respondents were BSc students (52%), and 37% were PhD students. In terms of BMI, the median was 21.69 (IQR: 3.82) kg/m², and 10.0, 74.5, 12.7, and 2.7% of participants had BMI < 18.5, BMI = 18.5 to 25, BMI = 25 to 30, and BMI > 30, respectively. In addition, 40% of respondents reported that their weight was stable during the COVID-19 pandemic, while 34.5% of participants stated that they had gained weight during this time.

Effects of the COVID-19 pandemic on the eating habits of participants

As shown in Table 2, the students' eating habits significantly changed during the COVID-19 pandemic compared to before the pandemic. Having a regular meal pattern (Chi-square = 191.609, df = 16, p = 0.000), a balanced diet with healthy ingredients (Chi-square = 151.763, df = 16, p = 0.000), 2–3 servings of dairy products (Chi-square = 233.074, df = 16, p = 0.000), and one or more servings of pulses, meat, or egg daily five to six times a week (Chi-square = 224.817, df = 16, p = 0.000) all increased during the COVID-19 pandemic. Consuming fast food as snacks or meals (like pasta, pizza, noodle, or burgers; Chi-square = 57.301, df = 12, p = 0.000), consuming fried food (Chi-square = 125.678, df = 12, p = 0.000), consuming junk foods (like chips, popcorn, etc.) as snacks (Chi-square = 289.273, df = 16, p = 0.000), not adding sugar in meals/beverages (Chi-square = 257.785, df = 16, p = 0.000), consuming sugar-sweetened beverages (Chi-square = 105.825, df = 16, p = 0.000), and consuming foods with high sugar (Chi-square = 193.373, df = 16, p = 0.000) and eating junk food/fast food one to two times a week (Chi-square = 114.037, df = 16, p = 0.000) decreased during the pandemic. Based on the Wilcoxon Signed-Rank test results, not routinely consumption of junk foods (like popcorn, chips etc.) as snacks was increased during the COVID-19 (44.5%) compared to the before of the pandemic (26.4%; p = 0.025). Also, not routinely consumption of foods with high sugar (such as sweet porridges, pastries, sweets and chocolate etc.) also increased during the COVID-19 pandemic (18.6% vs. 16.8%, p = 0.049) (Table 2).

TABLE 2 Eating habits changes in before and during COVID-19.

Items	Pre-COVID-19 *n* (%)	During COVID-19 *n* (%)	Chi-square, df	*p*-value*	*p*-value**
How often did you maintain a regular meal pattern?			191.609, 16	**0.000**	0.85
a. Not routinely	38 (17.3)	24 (10.9)			
b. One to two times a week	29 (13.2)	44 (20.0)			
c. Three to four times a week	50 (22.7)	49 (22.3)			
d. Five to six times a week	19 (8.6)	39 (17.7)			
e. Almost daily	84 (38.2)	64 (29.1)			
How often did you consume fast food like pizza, burger, pasta or noodle as snacks or meals?			57.301, 12	**0.000**	0.795
a. Not routinely	82 (37.3)	100 (45.5)			
b. One to two times a week	119 (54.1)	82 (37.3)			
c. Three to four times a week	18 (8.2)	32 (14.5)			
d. Five to six times a week	1 (0.5)	5 (2.3)			
e. Almost daily	0 (0.0)	1 (0.5)			
How often did you consume fried food (fried bread/poori, fried snack such as fries)?	22 (10.0)	42 (19.1)	125.678, 12	**0.000**	0.113
a. Not routinely	119 (54.1)	103 (46.8)			
b. One to two times a week	66 (30.0)	58 (26.4)			
c. Three to four times a week	13 (5.9)	15 (6.8)			
d. Five to six times a week	0 (0.0)	2 (0.9)			
e. Almost daily					
How often did you consume junk foods (like popcorn, chips etc.) as snacks?			289.273, 16	**0.000**	**0.025**
a. Not routinely	58 (26.4)	98 (44.5)			
b. One to two times a week	117 (53.2)	72 (32.7)			
c. Three to four times a week	36 (16.4)	35 (15.9)			
d. Five to six times a week	6 (2.7)	12 (5.5)			
e. Almost daily	3 (1.4)	3 (1.4)			
What was the frequency of your fruits and vegetables intake?			225.964, 16	**0.000**	0.385
a. Not routinely	3 (1.4)	8 (3.6)			
b. One to two times a week	50 (22.7)	56 (25.5)			
c. Three to four times a week	80 (36.4)	66 (30.0)			
d. Five to six times a week	36 (16.4)	40 (18.2)			
e. Almost daily	51 (23.2)	50 (22.7)			
How often did you have a balanced diet by including healthy ingredients (whole wheat, pulses, legumes, eggs, nuts, fruits and vegetables) in your meals?			151.763, 16	**0.000**	0.058
a. Not routinely	15 (6.8)	10 (4.5)			
b. One to two times a week	60 (27.3)	59 (26.8)			
c. Three to four times a week	75 (34.1)	70 (31.8)			
d. Five to six times a week	35 (15.9)	39 (17.7)			
e. Almost daily	35 (15.9)	42 (19.1)			
How often did you have 2–3 servings of milk or its products (curd, buttermilk, cheese, paneer etc.) in a day?			233.074, 16	**0.000**	0.765
a. Not routinely	24 (10.9)	19 (8.6)			
b. One to two times a week	58 (26.4)	70 (31.8)			
c. Three to four times a week	74 (33.6)	65 (29.5)			
d. Five to six times a week	31 (14.1)	31 (14.1)			
e. Almost daily	33 (15.0)	35 (15.9)			

(Continued)

TABLE 2 (Continued)

Items	Pre-COVID-19 *n* (%)	During COVID-19 *n* (%)	Chi-square, df	*p*-value*	*p*-value**
How often did you have one or more servings of pulses, egg or meat in a day?			224.817, 16	**0.000**	0.15
a. Not routinely	4 (1.8)	4 (1.8)			
b. One to two times a week	31 (14.1)	35 (15.9)			
c. Three to four times a week	106 (48.2)	79 (35.9)			
d. Five to six times a week	34 (15.5)	60 (27.3)			
e. Almost daily	45 (20.5)	42 (19.1)			
How many teaspoons of sugar/honey/jaggery did you consume in a day?			257.785, 16	**0.000**	0.786
a. Zero teaspoons per day, I do not add sugar in my meals/ beverages	64 (29.1)	72 (32.7)			
b. One to two teaspoons per day	87 (39.5)	74 (33.6)			
c. Three to four teaspoons per day	49 (22.3)	49 (22.3)			
d. Five to six times teaspoons per day	14 (6.4)	19 (8.6)			
e. More than 6 teaspoons per day	6 (2.7)	6 (2.7)			
	0 (0.0)	0 (0.0)			
How often did you consume sugar-sweetened beverages (juice, soft drinks, flavored soda etc.)?			105.825, 16	**0.000**	0.387
a. Not routinely	69 (31.4)	77 (35.0)			
b. One to two times a week	101 (45.9)	93 (42.3)			
c. Three to four times a week	32 (14.5)	33 (15.0)			
d. Five to six times a week	14 (6.4)	15 (6.8)			
e. Almost daily	4 (1.8)	2 (0.9)			
How often did you consume foods with high sugar (such as sweet porridges, pastries, sweets and chocolate etc.)?			193.373, 16	**0.000**	**0.049**
a. Not routinely	37 (16.8)	41 (18.6)			
b. One to two times a week	97 (44.1)	103 (46.8)			
c. Three to four times a week	52 (23.6)	50 (22.7)			
d. Five to six times a week	22 (10.0)	18 (8.2)			
e. Almost daily	12 (5.5)	8 (3.6)			
How often did you eat junk food/fast food due to boredom/distress/ disappointment?			114.037, 16	**0.000**	0.063
a. Not routinely	137 (62.3)	135 (61.4)			
b. One to two times a week	65 (29.5)	53 (24.1)			
c. Three to four times a week	14 (6.4)	24 (10.9)			
d. Five to six times a week	2 (0.9)	5 (2.3)			
e. Almost daily	2 (0.9)	3 (1.4)			

Data are presented as frequency [percent (%)].
*Chi-square test. **Wilcoxon Signed-Rank test, $p < 0.05$ is significant (the bold values).

Effects of the COVID-19 pandemic on the lifestyle of participants

Table 3 shows that participants' physical activity changed significantly before and during COVID-19. In this way, participation in moderate-intensity aerobic exercises for 30 min (Chi-square = 116.725, df = 16, $p = 0.000$) and leisure-related activities (grocery shopping, walking in the park, and gardening; Chi-square = 122.501, df = 16, $p = 0.000$) decreased. While performing household chores (cleaning, cooking, and laundry; Chi-square = 188.215, df = 16, $p = 0.000$), sitting time at work of less than 2 h per day (Chi-square = 61.961, df = 16, $p = 0.000$), not breaking from sitting (for example, stretching, taking a short walk, or standing up) during office hours (Chi-square = 87.560, df = 16, $p = 0.000$), and spending more than 5 h per day on social media, mobile phones, watching television, and playing video games (Chi-square = 71.715, df = 16, $p = 0.000$) were increased. The Wilcoxon Signed-Rank test results showed that not participating in household chores (cooking, laundry, cleaning) was decreased (14.1% vs. 62.3%, $p = 0.046$), while,

TABLE 3 Physical activity changes in before and during COVID-19.

Items	Pre-COVID-19 *n* (%)	During COVID-19 *n* (%)	Chi-square, df	*p*-value*	*p*-value**
How often did you participate in 30 min of moderate intensity aerobic exercises/sports?			116.725, 16	**0.000**	0.216
a. Not routinely	37 (16.8)	80 (36.4)			
b. One to two times a week	97 (44.1)	67 (30.5)			
c. Three to four times a week	52 (23.6)	45 (20.5)			
d. Five to six times a week	22 (10.0)	16 (7.3)			
e. Almost daily	12 (5.5)	12 (5.5)			
How often did you participate in household chores (cooking, laundry, cleaning)?			188.215, 16	**0.000**	**0.046**
a. Not routinely	137 (62.3)	31 (14.1)			
b. One to two times a week	65 (29.5)	75 (34.1)			
c. Three to four times a week	14 (6.4)	56 (25.5)			
d. Five to six times a week	2 (0.9)	26 (11.8)			
e. Almost daily	2 (0.9)	32 (14.5)			
How often did you participate in leisure related activities (grocery shopping, walking in park, gardening)?			122.501, 16	**0.000**	**0.002**
a. Not routinely	28 (12.7)	49 (22.3)			
b. One to two times a week	97 (44.1)	101 (45.9)			
c. Three to four times a week	59 (26.8)	39 (17.7)			
d. Five to six times a week	23 (10.5)	20 (9.1)			
e. Almost daily	13 (5.9)	11 (5.0)			
How much was your daily sitting time at work?			61.961, 16	**0.000**	0.408
a. Less than 2 h	30 (13.6)	50 (22.7)			
b. 2–4 h	43 (19.5)	47 (21.4)			
c. 4–6 h	76 (34.5)	42 (19.1)			
d. 6–8 h	47 (21.4)	39 (17.7)			
e. More than 8 h	24 (10.9)	42 (19.1)			
How many breaks from sitting (such as standing up, or stretching or taking a short walk) during your office hours did you typically take at work?			87.560, 16	**0.000**	0.555
a. 0	41 (18.6)	48 (21.8)			
b. 1–2	92 (41.8)	95 (43.2)			
c. 3–4	55 (25.0)	43 (19.5)			
d. 5–6	21 (9.5)	15 (6.8)			
e. More than 6	11 (5.0)	19 (8.6)			
How much screen time did you spend daily for watching television, using social media, mobile phones and playing video games?			71.715, 16	**0.000**	**0.000**
a. 0–1 h	22 (10.0)	15 (6.8)			
b. 1–2 h	55 (25.0)	35 (15.9)			
c. 2–4 h	104 (47.3)	65 (29.5)			
d. >5 h	39 (17.8)	105 (47.8)			

Data are presented as frequency [percent (%)].
*Chi-square test. **Wilcoxon Signed-Rank test, $p < 0.05$ is significant (the bold values).

not participating in leisure related activities (grocery shopping, walking in park, gardening) was increased (22.3% vs. 12.7%, $p = 0.002$) during the COVID-19 compare to the before. In addition, >5 h screen time spend daily for watching television, using social media, mobile phones and playing video games was significantly increased during the pandemic (47.8% vs. 17.8%, $p = 0.000$; Table 3).

Sleep changes and anxiety before and during COVID-19 are indicated in Table 4. Their sleep time and quality significantly changed during the pandemic (all $p = 0.000$). Having >8 h of sleep daily was enhanced during the COVID-19 pandemic (Chi-square = 76.569, df = 4, $p = 0.000$). The students that have a very good quality of sleep decreased during the pandemic, whereas those who have a very bad quality of sleep increased during the pandemic compared to before (Chi-square = 165.795, df = 16, $p = 0.000$). Feeling very much stress or anxiety in a day was increased (Chi-square = 99.360, df = 16, $p = 0.000$), and no smoking was decreased during the COVID-19 pandemic, and 2.2% of our participants decided to smoke (Chi-square = 81.239, df = 16, $p = 0.000$). Family and friends' support for maintaining a healthy lifestyle has increased during the COVID-19 pandemic compared to before the pandemic (Chi-square = 232.322, df = 16, $p = 0.000$). According to the Wilcoxon Signed-Rank test, except for the support of family and friends for maintaining a healthy lifestyle, sleep quality and stress and anxiety adversely influenced by the pandemic (all $p < 0.05$; Table 4).

The lifestyle changes' reasons during the COVID-19 pandemic

Reasons for lifestyle changes during the pandemic of COVID-19 are shown in Table 5. The biggest reasons for dietary pattern changes compared to pre-COVID-19 times were less eating out and improved knowledge about nutrition, and the most reasons for junk food and fast-food consumption changes in comparison to pre-pandemic times

TABLE 4 Sleep changes and anxiety in before and during COVID-19.

Items	Pre-COVID-19 *n* (%)	During COVID-19 *n* (%)	Chi-square, df	*p*-value*	*p*-value**
How many hours did you sleep daily?			76.569, 4	**0.000**	**0.000**
a. <6 h	20 (9.1)	14 (6.4)			
b. 6–8 h	156 (70.9)	105 (47.7)			
c. >8 h	44 (20.0)	101 (45.9)			
How would you rate your quality of sleep?			165.795, 16	**0.000**	**0.000**
a. Excellent	35 (15.9)	30 (13.6)			
b. Very good	67 (30.5)	42 (19.1)			
c. Good	109 (49.5)	101 (45.9)			
d. Bad	7 (3.2)	39 (17.7)			
e. Very bad	2 (0.9)	8 (3.6)			
How much stress or anxiety did you feel in a day?			99.360, 16	**0.000**	**0.000**
a. Not at all	19 (8.6)	12 (5.5)			
b. A little	133 (60.5)	70 (31.8)			
c. Much	49 (22.3)	94 (42.7)			
d. Very much	14 (6.4)	32 (14.5)			
e. Extremely	5 (2.3)	12 (5.5)			
Did you smoke?			81.239, 2	**0.000**	**0.034**
a. No	218 (99.1)	214 (97.3)			
b. Yes, 1–3 cigarettes per day	2 (0.9)	4 (1.8)			
c. Yes, 4–6 cigarettes per day	0 (0.0)	0 (0.0)			
d. Yes, 7–9 cigarettes per day	0 (0.0)	0 (0.0)			
e. Yes, >10 cigarettes per day	0 (0.0)	0 (0.0)			
Did your family and friends support you to maintain a healthy lifestyle?			232.322, 16	**0.000**	0.243
a. Always (more than 90% times)	76 (34.5)	85 (38.6)			
b. Most of the time (approx. 75% times)	80 (36.4)	79 (35.9)			
c. Sometimes (approx. 50% times)	36 (16.4)	26 (11.8)			
d. Occasionally (approx. 25% times)	19 (8.6)	24 (10.9)			
e. Rarely (less than equal to10% times)	9 (4.1)	6 (2.7)			

Data are presented as frequency [percent (%)].
*Chi-square test. **Wilcoxon Signed-Rank test, $p < 0.05$ is significant (the bold values).

TABLE 5 Reasons for lifestyle changes during COVID-19 pandemic.

Items	*n*	%
What are the reasons for changes in dietary pattern in comparison to pre-COVID-19 times?		
a. Improved knowledge about nutrition	30	13.6
b. Lack of access to fresh fruits and vegetables	7	3.2
c. Higher cost of ingredients	18	8.2
d. More available cooking time	18	8.2
e. Better family support	16	7.3
f. Less eating out	63	28.6
g. Lack of family support	0	0
h. Stress and anxiety	19	8.6
i. Relaxed mind	6	2.7
j. No change	39	17.7
k. Any other, please specify_	4	1.8
What are the reasons for changes in junk food/fast food consumption pattern in comparison to pre-COVID-19 times?		
a. Fear of coronavirus spread through food	57	25.9
b. Non-availability of cook	7	3.2
c. Less eating out/socializing	45	20.5
d. Availability of cooking time	13	5.9
e. Preferring home cooked food	42	19.1
f. Focus on eating healthy to build immunity	26	11.8
g. Managing food craving using different techniques such as listening to songs, taking awake	1	0.5
h. Lack of family support	1	0.5
i. Stress and/or anxiety	12	5.5
j. Any other, please specify	16	7.3
In order to increase your physical activity, which activities have you included?		
a. At-home aerobics	15	6.8
b. Yoga	2	0.9
c. At-home workout videos	46	20.9
d. Gyming (treadmill, cycle and weights)	26	11.8
e. Walks	60	27.3
f. At-home dancing and stretching	38	17.3
g. Not doing any activities	29	13.2
h. Any other, Please specify	4	1.8
What are the reasons for your change in physical activity regime during COVID-19?		
a. Lack of motivation	59	26.8
b. Lack of knowledge of exercises	4	1.8
c. Lack of access to sport facilities and gym	57	25.9
d. Social restrictions to parks and public places	37	16.8
e. Lack of social support	2	0.9
f. Lack of time	28	12.7
g. Any other, Please specify	33	15
What are the reasons for a change in sleeping pattern during COVID-19?		
a. Daytime sleeping	79	35.9
b. Stress and anxiety	60	27.3
c. Long working hours	18	8.2

(Continued)

TABLE 5 (Continued)

Items	*n*	%
d. Environmental factors such as noise and lighting	8	3.6
e. Shortness of breath during sleep	3	1.4
f. Flexibility in days' time	13	5.9
g. Any other, please specify	39	17.8
What are the reasons for a change in stress and anxiety levels during COVID-19?		
a. Fear of COVID infection	45	20.5
b. Worrying about family and friends	99	45
c. Stigma or discrimination from other people (e.g., people treating you differently because of your identity, having symptoms, or other factors related to COVID-19)	4	1.8
d. Frustration/boredom/loneliness	37	16.8
e. Financial loss	3	1.4
f. Confusion about what COVID-19 is, how to prevent it, or why social	11	5
distancing/isolation/quarantines are needed	3	1.4
g. Lack of support from family and friends	18	8.2
h. Any other, please specify	0	0

Data are presented as frequency ± percent (%). *$p < 0.05$, Chi-square test.

were fear of coronavirus spread via food, less eating out and socializing, and preferring home-cooked food. To increase physical activity, students included walks and at-home workout video activities, and the reasons for their physical activity levels changing during COVID-19 were a lack of motivation and access to sports facilities and a gym. The reasons for changes in sleeping patterns during COVID-19 were daytime sleeping and stress and anxiety. The reasons for changes in anxiety and stress levels during COVID-19 were worrying about family and friends and fear of COVID-19 infection.

Discussion

The COVID-19 pandemic brought about significant changes in people's lifestyles, diets, and psycho-emotional behaviors (25). Diet, exercise, physical activities, and even harmful habits (e.g., alcohol, caffeine-rich drinks, sugar-rich drinks, and smoking) are all components of an individual's lifestyle (26, 27). The current study sought to assess the effects of the COVID-19 pandemic on college students' eating habits, physical activities, sleep quality, anxiety, and stress. Majority of the included participants had a normal BMI; however, 34.5% of them reported that they gained weight during the COVID-19 pandemic. Similar to our findings, the study by Cheikh Ismail et al. indicated that 32.8% of the adults gained weight during the lockdown (28). Chen et al. also found that the college students reported a ≈ 3.4 kg weight gain after the first month of lockdown due to the pandemic (29). Rafique et al. determined that 48% of the young adults gained weight during the pandemic (30). Similarly, two meta-analyses assessing nearly 100 studies revealed that lockdowns during the COVID-19 pandemic increased the body weight among a high number of young adults (11.1–72.4%) (31, 32). The primary cause of enhanced BMI among young adults was the shift to online education which increased screen time, sitting hours, sleeping hours, food consumption, snacking, and cooking (29–32). Robinson et al. also demonstrated that higher BMI was associated with lower physical activity levels, poor diet quality, and frequent overeating (33).

According to the results of this study, the eating habits of students were characterized by an increase in having a regular meal pattern and balanced diet, milk or its products, pulses, and egg or meat in the diet, as well as by a decrease in consuming fast food, fried food, junk foods (e.g., potato chips, popcorn, etc.), sugar-sweetened beverages, and foods high in sugar. The findings of this study were consistent with global data suggesting that the COVID-19 pandemic had a significant effect on lifestyle including eating habits. Monteiro et al. showed that healthier eating habits were adopted by university students during the lockdown period compared to a typical semester period. Their self-reported healthier eating habits included limited application of meal delivery platforms, less consumption of fast food, pre-cooked meals, foods high in sugar and salt, and sugar-sweetened beverages, more consumption of fruits, vegetables, and legumes, as well as more serious engagement in physical activities (34). Enriquez-Martinez et al. also conducted a study on 6,325 participants aged over 18 years from five countries, and demonstrated that more favorable patterns of eating choice were observed among those who changed their eating habits compared to those who did not change their eating habits (35). However, our study findings were inconsistent with the results from some other studies in this regard. In the study by Robinson et al., a higher number of the participants reported more negative changes in their eating habits (e.g., more frequent snacking) after the lockdown compared to the pre-lockdown period (33). A study by Cheikh Ismail et al. also found that majority of the adults did not eat fruits and vegetables on a daily basis; similar to our study, however, their study showed that the number of consumed meals per day as well as the daily consumption of homemade meals significantly increased during the lockdown compared to the pre-pandemic period (28). A

Portuguese study showed that the majority of the participants consumed more foods rich in fat, salt, and/or sugar (e.g., fast food, snacks, sweets, and sugar-sweetened beverages) during the confinement period (7). Another Portuguese study, however, found that 58.2% of the participants increased the consumption of fruits and vegetables but reduced the consumption of pre-cooked meals and sugar-sweetened beverages (36). Similar studies carried out in other countries highlighted the improvement in nutritional habits including increased intake of fruits, vegetables, yogurt, legumes, and eggs during the lockdown (13, 15, 24, 25, 34, 37–41).

Based on our study results, the main causes of the changes in eating habits during the COVID-19 pandemic compared to pre-pandemic period were associated with eating out less frequently, having fear of coronavirus spread by food, preferring home-cooked food, and improving the knowledge about nutrition. These findings may have been attributed to spending more time indoors, working from home, receiving tele-education, and engaging in restricted outdoor physical activities due to the COVID-19 pandemic (41–43). Since people stayed homes for longer time, they may have spent more time to cook homemade meals as well as paid more attention to their diet and, particularly, to the nutritional recommendations proposed for this time period (44). Another factor is concerned with nutrition because most people are aware of the fact that a healthy lifestyle considerably improves the immune system which, in turn, could enhance the likelihood of surviving the virus (45). Sedentary habits mean replacing activity with sleeping, which substantially changes the lifestyle (46), and sleep could be a risk factor for obesity (47). Furthermore, disruption of routine activities due to quarantine could lead to boredom which is connected with higher energy intake (42). Exposure to stressful news about COVID-19 might be associated with overeating and, especially, high-sugar food intake (i.e., "food cravings") (48).

The physical activities of our participants changed during COVID-19, so that participation in moderate-intensity aerobic exercises for 30 min and leisure-related activities decreased, while participation in household chores, sitting at work for less than 2 h every day, keeping sitting during office hours, watching television for >5 h every day, using mobile phones and social media, as well as playing video games increased. Generally, our university students reported that their participation in physical activities decreased, and their screen and sitting time increased during the pandemic. Our study findings were confirmed by most observational studies reporting that physical activity levels decreased, but screen/sitting time and household activity levels increased (20, 30, 40, 41, 49). For example, Abouzid et al. investigated 5,896 participants aged ≥18 years and demonstrated that nearly 38.4% of the individuals stopped physical activities and exercising during the COVID-19 confinement, and 57.1% of them spent more than 2 h on following social media (40). Chopra et al. also reported that the physical activity levels decreased while the daily screen time increased (20). Our study participants spent more time on walking, working out at home, and engaging in video-based activities in order to increase their physical activity levels during the pandemic. They also reported that the lack of motivation as well as the lack of access to sport facilities and gym were the causes of changes in their physical activities during COVID-19 pandemic. Similarly, Sekulic et al. indicated that the most common physical activities among medical sciences students were walking and exercising at home before and during the COVID-19 pandemic, respectively. Moreover, they found that sitting time increased during the pandemic, but the daily time spent on physical activities during the pandemic was not different from that recorded before the pandemic period (41). Enriquez-Martinez et al. conducted a study on 6,325 individuals aged >18 years from five countries and demonstrated that majority of them performed physical activities at homes (35). However, some studies indicated the increase in self-reported physical exercises during the lockdown (7, 15, 34). The longer time spent at home and on online education may increase the screen time and, consequently, decrease the physical activity level. The negative effects of screen time on weight are usually mediated when screen time displaces the time for physical activity. Therefore, if screen time for education is not replaced with important activities, these negative effects can be avoided during the pandemic (30, 50).

The university students participated in this study stated that oversleeping (>8 h) and poor quality of sleep increased during COVID-19 pandemic. The changes in sleeping patterns during COVID-19 were associated with daytime sleeping, stress, and anxiety. Similar to our study, recent studies showed that a poor sleep quality during COVID-19 confinement period may have been associated with higher stress and anxiety (18, 46, 51, 52). Daily stress and anxiety levels were increased among our participants during COVID-19 pandemic, which were associated with worries about family/friends and fear of COVID infection. Chopra et al. demonstrated that stress and anxiety in nearly one-fourth of the participants were related to quarantine, and that mental health was adversely affected by it (20) Moreover, Enriquez-Martinez et al. revealed that the participants over 18 years of age were affected by anxiety (35). Stress and anxiety induced by the COVID-19 lockdown may cause changes in eating habits (7). Another factor resulting in stress and anxiety among students is fear and concern over their health and loved ones (53). In addition, the lack of social interaction, particularly among active or young individuals, can induce anxiety, stress, and depression. The isolation feelings may stimulate emotional eating for relieving negative feelings (42). Familiarity with these factors facilitates developing effective interventions for mitigating the negative lifestyle behaviors during the COVID-19 pandemic. As for smoking, 2.2% of our participants started smoking, and it did not decrease during COVID-19 pandemic. Berlin et al. have shown that social isolation and mental distress might increase the false need for smoking (40). In the study by Di Renzo et al., however, 3.3% of the smoker respondents decided to quit smoking (15).

It is generally believed that healthy eating and physical activities, particularly during the immune system's challenging times, are the key factors for improving health and well-being (54). A healthy diet and eating habits together with weekly exercise can help the individuals to fight against the viral diseases such as COVID-19 (54). Worries about the one's health or the loved ones' health contribute to greater monitoring of one's health and personal hygiene as well as to paying more attention to the nutritional value and quality of foods (25). In this study and earlier studies, it was shown that the healthy eating was widely practiced during the pandemic. However, low physical activity levels, stress and anxiety, and tediousness due to social isolation may have negatively affected the lifestyle patterns (55).

The strengths and limitations

The current research is the first study that evaluated the effects of the COVID-19 pandemic on the lifestyle of college students, however, this study had some limitations. First, the sample size was relatively small and the design was cross-sectional. Second, simultaneous collection of before and after pandemic data. Third, the sample came from various study fields and grades. Fourth, the questionnaire used for collecting data was validated for the population of another country, and the collected data were self-reported.

Conclusion

In sum, university students stated that their eating habits improved during the COVID-19 pandemic; however, they reported increased weight gain, screen, sitting, and sleeping time, declined physical activities, poor sleep quality, and daily stress or anxiety during the pandemic. Therefore, although the COVID-19 pandemic led to some adverse effects on participants' lifestyles, the eating habits of university students improved likely due to concerns about their health and efforts to contain COVID-19. Our study's findings may have had some implications for public health policy. We can use the reported healthy eating habits influencing factors reported by university students during the COVID-19 lockdown to promote better nutrition behaviors outside of the lockdowns. It was recommended that future longer follow-up studies on both genders should be conducted to more examine the relationship between the COVID-19 pandemic and eating habits.

Data availability statement

The original contributions presented in this study are included in the article, 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 Tabriz university of medical sciences (ethical cod: IR.TBZMED.REC.1401.597). The patients/participants provided their written informed consent to participate in this study.

Author contributions

RM-G and MR wrote the main manuscript text. MS contributed to data collection. 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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Effects of oral intake fruit or fruit extract on skin aging in healthy adults: a systematic review and Meta-analysis of randomized controlled trials

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Background: In recent years, oral various fruits or supplements of fruits natural extracts have been reported to have significant anti-aging effects on the skin (1, 2), However, despite many studies on this topic, there is often no clear evidence to support their efficacy and safety. In this paper, we present a comprehensive review and Meta-analysis of the evidence for the safety and efficacy of oral fruits and fruits extracts in improving skin aging.

Methods: Four databases, Pubmed, Embase, Web of Science, and Cochrane Library (CENTRAL), were searched for relevant literature from 2000–01 to 2023–03. Seven randomized controlled trials (RCTs) of fruit intake or fruit extracts associated with anti-skin aging were screened for Meta-analysis.

Results: Compared to placebo, oral intake of fruit or fruit extracts showed significant statistical differences in skin hydration and transepidermal water loss (TEWL), with a significant improvement in skin hydration and a significant decrease in TEWL. No significant statistical difference was observed in minimal erythema dose (MED), overall skin elasticity (R2), or wrinkle depth, and no evidence of significant improvement in skin condition was observed.

Conclusion: Meta-analysis results suggest that consume administration of fruits or fruit extracts significantly enhances skin hydration and reduces transcutaneous water loss, but there is insufficient evidence to support other outcome recommendations, including minimal erythema dose (MED), overall skin elasticity(R2), and wrinkle depth.

Systematic Review Registration: PROSPERO (york.ac.uk), identifier CRD42023410382.

KEYWORDS

skin aging, fruit, fruit extract, oral, randomized controlled trials, meta-analysis

Introduction

Keeping skin soft and beautiful is a common desire, especially for women. Dry skin can accentuate wrinkles and make a person look older. As the largest organ of the body, the skin is the barrier between the cells and the external environment, covering most of the body (3). Regarding the mechanism of skin aging, it has been shown to be related to damage to collagen and elastic fibers by matrix metalloproteinases, extracellular matrix degradation, abnormal fibroblast function, oxidative stress and cell proliferation, apoptosis and inflammatory response, and light-induced DNA damage (4–6). Pigmentation, dryness, loss of elasticity and deepening of wrinkles are all significant changes in skin aging. Topical cosmetics are the most basic form of skin care (7, 8). Their advantages include high safety of topical application, but they act only on the skin surface, have limited effects, and uncomfortable skin reactions caused by topical agents, such as sensory reactions or subjective sensations (no signs of inflammatory events), may still be encountered after application (9–11). For example, facial skin is usually more sensitive than other parts of the body and can feel tingling and burning in those who use dermatological agents. At the same time, the skin, as living tissue, requires nutrients to remain healthy (12). The required nutrients come mainly from food and not only from topical preparations. Over the past 20 years, the trend toward achieving “beauty from within” has grown, leading individuals to adopt a healthier lifestyle by consuming nutrient-rich foods (13). Consequently, there has been a growing interest in methods that use dietary supplements or functional foods to delay or improve skin aging. Fruits, as an important component of a daily diet, contain a rich array of antioxidants, anti-inflammatory substances, vitamins, minerals, and polyphenolic compounds. They play a positive role in skin antioxidation, anti-inflammation, wrinkle reduction, and skin brightening. Consequently, there has been significant interest in the potential of consuming fruits or fruit extracts to slow down skin aging (14, 15). The aim is to restore the deepest layers of the skin, effectively improving surface appearance. This leads to the question: can fruits or fruit extracts improve skin aging, and if so, how specifically do they contribute? It has been reported that a large number of fruits or fruit extracts have beneficial effects on skin moisturization, anti-wrinkle properties, and removal of pigmentation, helping to make the skin appear younger (12, 13, 16). However, despite the importance of individual fruits or fruit extracts for skin care, there is a lack of research on their anti-aging effects. Many such randomized controlled trials (RCTs) often yield confusing and inconsistent results, presenting a challenge for consumers. Scientific evidence for the anti-aging effects of fruits or fruit extracts is generally scarce, usually stemming from *in vitro* or uncontrolled *in vivo* studies. Furthermore, few studies have provided sufficient and convincing scientific validation of their efficacy and safety.

Therefore, the purpose of this study is to conduct a detailed and Systematic review of the research on the intake of fruits and fruit extracts and their anti-skin aging effects, through extensive literature searches. By conducting Meta-analysis on randomized controlled trials, we aim to understand their efficacy and safety. This will lead to a better scientific understanding of the relationship between the intake of fruits or fruit extracts and skin aging, and its application in daily skincare.

Methods

This article follows the PRISMA (Reporting Guidelines for Systematic Evaluation and Meta-analysis) guidelines regarding accreditation, selection, eligibility, and inclusion. The study protocol was registered in advance in the PROSPERO database (No. CRD42023410382). (17).

Search strategy

Four databases, Pubmed, Embase, Web of Science, and Cochrane Library (Central Database), were searched for relevant literature from 2000–01 to 2023–03. Because according to the “results by year” function of Pubmed, the skin health of orally consumed fruits or fruit extracts has been extensively studied only in the last 23 years. A computerized search of the relevant literature was performed for the following terms: (1) skin aging or skin condition or skin hydration; (2) fruit or fruit extracts. These search terms were adapted accordingly for each database. Supplementary Table S1 provides a comprehensive description of the search method. In this paper, only English RCTs were selected.

Eligibility criteria

Studies were selected when they met all of the following inclusion criteria:

1) Study type. Only parallel group randomized controlled trials (RCTs) assessing skin hydration, minimal erythema dose (MED), skin elasticity, wrinkle depth, or transepidermal water loss (TEWL) in human subjects were included. Such trials can control for participant differences by randomization and parallel group design, reduce bias, and introduce a control group taking placebo or low-dose supplements to eliminate placebo effects. The published paper must be a peer-reviewed full-text article.
2) Type of participant. Healthy individuals of any gender or race between the ages of 18 and 70 years were included. The main criteria for exclusion were as follows: (A) pregnancy, breastfeeding, and various metabolic, cardiovascular, or liver diseases; (B) serious skin-related diseases such as atopic dermatitis, psoriasis, and vitiligo; and (C) regular consumption of any food, drug, or other supplement that affects skin conditions, including health foods, antioxidant supplements, retinoids, steroids, and any other hormonal products.
3) Type of intervention. Randomized controlled trials comparing fruit or fruit extracts with placebo or lower doses of the same fruit or fruit extract were eligible. Studies were eligible when the same protocol was used in the test and control groups, regardless of any additional lifestyle interventions used. Studies using different protocols between the test and placebo groups were excluded.
4) The seven included studies involved multiple ethnic groups, with a large proportion of Asians, so in the Meta-analysis study, ethnic subgroup analyses were conducted, grouping Asians (including Koreans, Japanese, and Chinese) and non-Asians

(including Americans and Italians). Both Asian and non-Asian subgroups were included in the outcome indicators of interest in this study.

Study selection

Three reviewers (H.L., L.W.and J.W.) independently screened titles and abstracts. Full texts were imported into Endnote (Version X9, Clarivate Analytics) for further review when they were deemed potentially eligible by the initial screening process. The full texts were then independently evaluated against the aforementioned inclusion criteria by reviewers (H.L. and L.W. or J.W.) to determine their eligibility for inclusion in the study. Throughout the process, whenever any question arose, we consulted with other researchers (J.F. and L.J.) or referenced relevant literature and books for clarification.

Data extraction

The characteristics of each included study are documented in Supplementary Table S2, including details of the study such as author(s), country and year of publication, characteristics of the study population (age, sex and health status), study duration, intervention content of each group, daily dose, the form of applications, testing environment [°C room temperature (R.T.) and % relative humidity (R.H.)], the start and end time of the study, the measurement instruments used, and the skin sites assessed. The safety of the intervention was assessed by comparing adverse events (AEs), treatment-related adverse effects (TEAs), and treatment-related discontinuations (TAW) in the intervention and placebo groups.

The other collected data, such as the baseline and endpoint measure (average) and its variability SD (standard deviation) or SE (standard error), were input into an Excel spreadsheet. Five outcomes were evaluated: skin hydration, minimum erythema dose (MED), overall skin elasticity (R2), transcutaneous water loss (TEWL), and wrinkle depth. When multiple measurement sites were provided in the study, facial and arm test areas commonly used by females were preferred as the measurement sites for assessing moisturizing effects. When data from different racial populations were provided, subgroup analysis would be performed, and studies from different races would be considered as independent studies. The study data with the longest observation period, particularly those with multiple time points of measurement results, were selected for analysis.

During the data collection process, whenever any issues arose, we consulted with other researchers to discuss and resolve them.

Quality assessment

Three reviewers (H.L. and L.W.or J.F.) evaluated the risk of bias (ROB) in the randomized controlled trials (RCTs) using the Cochrane Collaboration's assessment tool (18), which includes seven criteria: selection biases (SB), performance biases (PB), detection biases (DB), attrition biases (AB), reporting biases (RB), and other biases in the trial design or methodology. Examples of other biases include changes in life habits during the study, unstable conditions in test rooms that may affect the measurement results, and potential conflicts of interest. Each study was assessed for each of these criteria and rated as "low risk," "high risk," or "unclear." ROB was considered low when the study adequately reported methods without potential biases according to the Cochrane Collaboration's guidelines (18). Conversely, high ROB was assigned when the method used in the study could not remove underlying biases. If there was insufficient information to make a determination, or if the information provided was inadequate, the trial was labeled with an"unclear risk of bias."

Statistical analysis

Evaluation of overall effect size

The Meta-analysis of seven randomized controlled trials (RCTs) included in the analysis was performed using Review Manager 5.4 (19). The standardized mean differences (SMDs) of continuous variables were calculated using a random effects model in statistical analysis. The mean difference was obtained by subtracting the baseline value from the endpoint value. If changes in standard deviation (SDs) from baseline were not reported, the following formula based on the baseline and endpoint SDs of the treatment and placebo groups can be used to calculate the SD value:

$$SD = \sqrt{\begin{aligned}&(\mathrm{SD\_baseline} \wedge 2 + \mathrm{SD\_final} \wedge 2)\\ &-2 \times \mathrm{Corr} \times \mathrm{SD\_baseline} \times \mathrm{SD\_final}.\end{aligned}}$$

As baseline-endpoint correlation coefficients (Corr) were not reported in these studies, a Corr value of 0.5 was used (20), which is a value calculated in most articles with sufficient data and ranges from 0.4 to 0.6.

In addition, if an RCT does not compare with a placebo group and only compares the effects of low-dose and high-dose interventions, the high-dose group is considered as the intervention group, and the low-dose group is considered as the placebo group. The effect size of skin hydration, minimal erythema dose (MED), skin elasticity, transepidermal water loss (TEWL), and wrinkle depth were expressed in standard mean difference (SMD) with a 95% confidence interval (CI), as not all included studies reported these five outcomes using the same device and unit of measurement. $p < 0.05$, indicating statistical significance (21).

Evaluation of heterogeneity

Higgins I (I^2) was used to estimate heterogeneous statistics in the legend of each forest plot. The I^2 index represents the proportion of observed differences in effect sizes attributable to heterogeneity rather than sampling errors in the total variation between studies (22). For instance, an I^2 of 0% indicates that the entire difference in effect sizes is due to sampling error, while an I^2 of 100% implies that the variation in effect sizes is due entirely to true effects. I^2 values of 0–25%, 25–50%, 50–75%, and 75–100% are generally considered low, moderate, high, and very high heterogeneity, respectively (23).

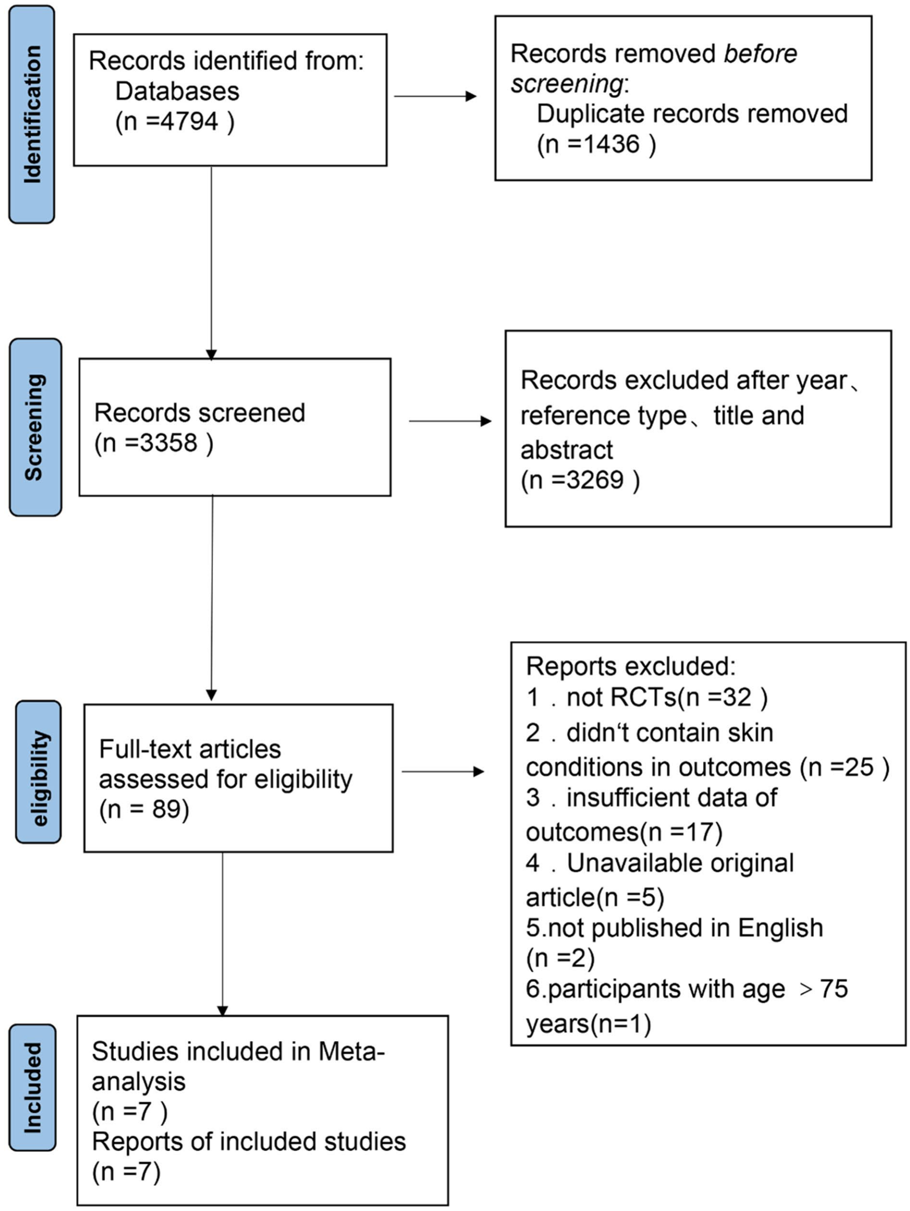

FIGURE 1
Flow diagram for study selection process.

Results

Identification of researches

A total of 4,794 references (Figure 1) were retrieved, including 795 from PubMed, 962 from Embase, 2,251 from Web of Science, and 786 from Cochrane Library. After screening, 7 randomized controlled trials were included in the Systematic review and Meta-analysis.

Risk of bias within researches

Considering that Nobile 2022 involved participants from both China and Italy, studies conducted with participation from different ethnic groups are considered as separate studies.

The review of every ROB item presented as percentages across the entire selected research as per the judgment of the researcher was displayed in Figure 2, and the judgment for every trial was described in Supplementary Figure S1. All studies have fully reported randomization, including the use of computer-generated randomization schedules and random number tables. Therefore, the selection bias of all studies is considered to be at low risk. Five studies (63%) reported a clear process of blinding for both participants and outcome assessors, while 36% of the studies had an unclear risk of bias. Two studies (25%) were identified as having low risk of detection bias, while the remaining studies had an unclear risk of bias. Attrition bias was low risk in 7 research works (88%), and 1 research (12%) was unclear. The risk of reporting bias was high in one study (12%), while concrete data were provided and trials were pre-registered in a publicly available trial registry

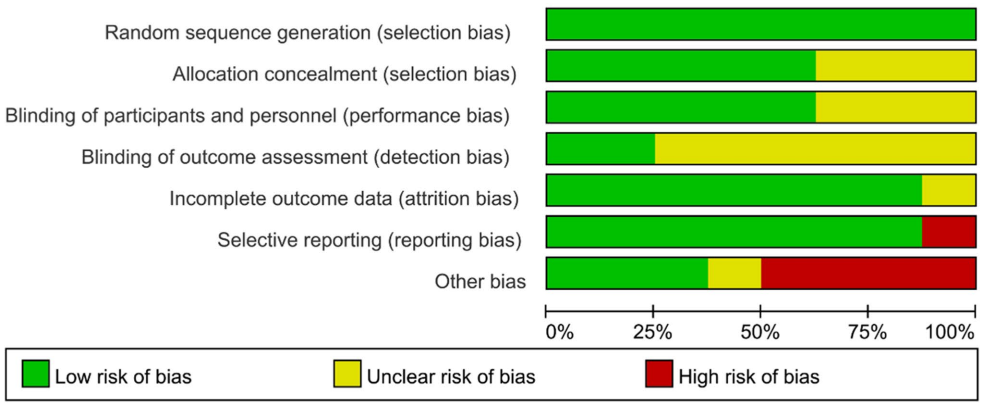

FIGURE 2
Summary of review authors' judgments for each risk of bias domain.

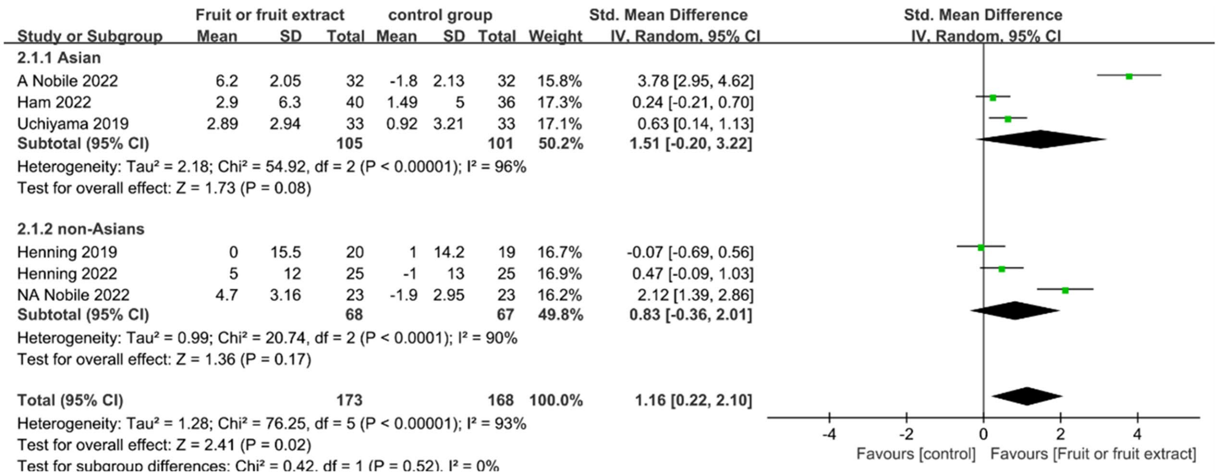

FIGURE 3
Forest plot of comparison: Fruit or fruit extract vs. placebo on skin hydration (SMD). The details of each study are reported in Supplementary Table S2. CI, confidence interval.

in seven studies (88%). Four studies (50%) were identified as having a high risk of "other biases," with commonly observed factors including potential conflicts of interest, maintenance of participants' living habits, and insufficient details regarding physical environmental conditions.

Research features

Supplementary Table S2 summarizes the main characteristics of each study. Seven studies were included in a Meta-analysis to evaluate the effect of oral intake of fruits or fruit extracts on improving skin aging. Among the seven trials included in the Meta-analysis, two involved Asian participants (Japanese and Korean). One study consisted of participants from China and Italy, and three trials were conducted among U.S. participants. The primary participants were women between the ages of 30 and 70 with visible signs of skin aging, such as wrinkles and dryness, at baseline levels.

Skin hydration

Skin hydration levels were assessed in five studies ($n=341$), including a study conducted in both China and Italy, which were considered as two independent studies. Three studies focused on Asian populations (24–26), and three studies included non-Asian populations (27, 28). Four studies examined the effects of different fruit extract formulations, including capsules and tablets (24–27), while one study involved direct ingestion of avocado (28). These studies lasted 8–12 weeks and evaluated skin hydration levels.

Figure 3 displays the forest plot of a Meta-analysis of six studies, which estimates the difference in skin hydration between intervention and placebo groups.

The results of the Meta-analysis of six studies showed no significant improvement in skin hydration in the Asian group after ingestion of fruit or fruit extract (SMD = 1.51; 95% CI: -0.2-3.22, $p=0.08$); and no significant difference in the non-Asian group compared to the placebo group after ingestion of fruit or fruit extract (SMD = 0.83; 95% CI: −0.36- 2.01, $p=0.41$). However, the ability of ingesting fruits or fruit extracts to promote skin hydration was supported by statistical evidence based on the overall effect size summary analysis (SMD = 1.16; 95% CI: 0.22–2.10, $p = 0.02$).Overall, both the Asian group ($I^2 = 96\%$) and the non-Asian group () exhibited a high degree of heterogeneity. Sensitivity analysis using the one-by-one exclusion method found that the heterogeneity significantly decreased($I^2 = 23\%$， $I^2 = 36\%$) when the study by Nobile 2022 was excluded, with no change in the pooled effect result for skin hydration (SMD = 0.35; 95%CI: 0.07–0.62,$p = 0.01$) (Supplemental Figure S2); the heterogeneity mainly originated from Nobile 2022, which may be due to the skin measurements differed in location, the study measured skin moisture on the back, which is less exposed to the external environment, while other studies measured skin hydration on the face and forearm, which are more exposed.

Skin elasticity

Skin elasticity was monitored in three studies ($n = 225$) lasting from 8 to 12 weeks. One study was carried out in both China and Italy and was considered as two independent studies. Two studies were included in the Asian population group (24, 25), whereas the other two studies were included in the non-Asian population group (24, 27). The four studies focused on different fruit extract formulations (including capsules and tablets).

Figure 4 displays the forest plot of a Meta-analysis of four studies, which estimates the difference in skin elasticity between intervention and placebo groups.

The Meta-analysis of four studies ($n = 225$) revealed no significant effect of consuming fruits and fruit extracts on increasing skin elasticity compared to placebo (SMD = 1.43; 95% CI: −0.07-2.93, $p = 0.06$) (Figure 4). No significant correlation was observed in both the Asian and non-Asian groups.In the Meta-analysis of these two subgroups, the overall study exhibited high heterogeneity (I^2 of 92 and 98%, respectively), which may be attributed to different sources of fruit extracts, including red-orange complex extract, *Citrus sinensis* extracts (PTE), and pomegranate extracts, different extraction methods such as standardized extracts and 50% ethanol extracts, as well as the differences in body parts of the tested subjects (back, eye contour, and arm).

Wrinkle depth

Four studies ($n = 280$) evaluated wrinkle depth over a period of 8 to 12 weeks. One of the trials included both Chinese and Italian participants, and studies with different ethnic groups were considered as independent studies. Therefore, three studies were included in the Asian group (24–26), while two studies were included in the non-Asian group (24, 29). Among them, the focus of four studies (24–26) was on different formulations of fruit extracts, including capsules and tablets, while one study (29) directly consumed mangoes.

Figure 5 displays the forest plot of a Meta-analysis of four studies, which estimates the difference in wrinkle depth between intervention and placebo groups.

Among the five studies, a total of 280 participants were included, and no significant improvement in wrinkle depth was observed after consuming fruit or fruit extracts (SMD = -0.73; 95% CI: −1.65-0.20, $p = 0.12$) (Figure 5). No significant correlation was also observed for subgroup analysis: Asian group (SMD = −1.04; 95% CI: −2.13-0.05, $p = 0.06$) or non-Asian group (SMD = −0.21; 95% CI: −2.61-2.20, $p = 0.87$). However, both analyses showed significant heterogeneity between studies ($I^2 = 92$ and 95%). Sensitivity analysis using the one-by-one exclusion method found that the results of the study changed when Fam 2020 was removed, and a significant improvement in skin wrinkle depth was observed after ingesting fruit extracts (SMD = -1.13; 95% CI: −1.97--0.28, $p = 0.87$) ($I^2 = 89\%$) (Supplemental Figure S3). This may be because the Fam 2020 study used a low-dose placebo group and a high-dose experimental group, while the rest of the studies used a placebo control group. Nevertheless, high heterogeneity still existed, possibly due to the complex composition of the supplement products, different extraction methods for the extracts, and differences in dosages between studies.

Study or Subgroup	Fruit or fruit extract Mean	SD	Total	control group Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
2.2.1 Asian								
A Nobile 2022	0.078	0.095	32	-0.004	0.012	32	25.8%	1.20 [0.66, 1.73]
Ham 2022	0.4	5.27	40	0.6	4.45	36	26.1%	-0.04 [-0.49, 0.41]
Subtotal (95% CI)			**72**			**68**	**51.9%**	**0.57 [-0.64, 1.78]**
Heterogeneity: Tau² = 0.70; Chi² = 12.02, df = 1 (P = 0.0005); I² = 92%								
Test for overall effect: Z = 0.92 (P = 0.36)								
2.2.2 non-Asians								
Henning 2019	-0.02	0.18	20	0	0.15	19	25.5%	-0.12 [-0.75, 0.51]
NA Nobile 2022	0.078	0.017	23	-0.006	0.015	23	22.6%	5.15 [3.91, 6.39]
Subtotal (95% CI)			**43**			**42**	**48.1%**	**2.49 [-2.67, 7.65]**
Heterogeneity: Tau² = 13.62; Chi² = 54.94, df = 1 (P < 0.00001); I² = 98%								
Test for overall effect: Z = 0.94 (P = 0.34)								
Total (95% CI)			**115**			**110**	**100.0%**	**1.43 [-0.07, 2.93]**
Heterogeneity: Tau² = 2.18; Chi² = 69.03, df = 3 (P < 0.00001); I² = 96%								
Test for overall effect: Z = 1.87 (P = 0.06)								
Test for subgroup differences: Chi² = 0.50, df = 1 (P = 0.48), I² = 0%								

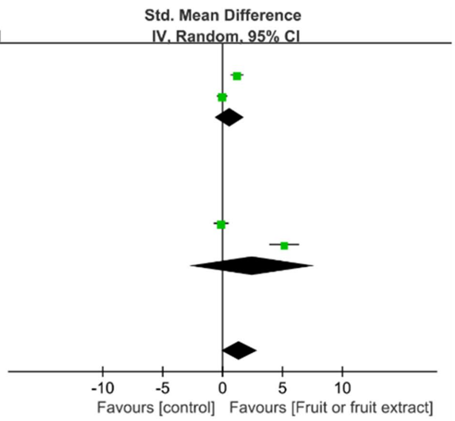

FIGURE 4
Forest plot of comparison: Fruit or fruit extract vs. placebo on skin elasticity (SMD). The details of each study are reported in Supplementary Table S2. CI, confidence interval.

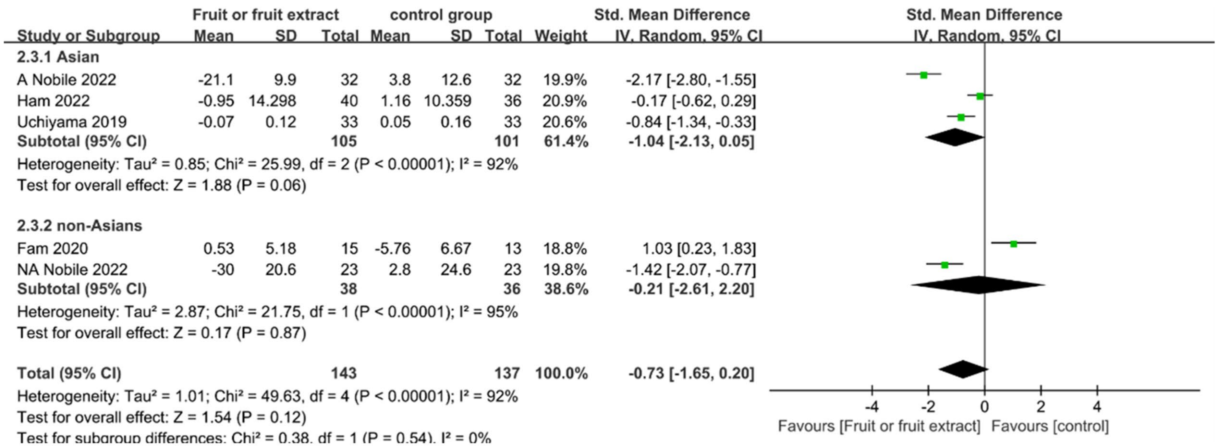

FIGURE 5
Forest plot of comparison: Fruit or fruit extract vs. placebo on wrinkle depth (SMD). The details of each study are reported in Supplementary Table S2. CI, confidence interval.

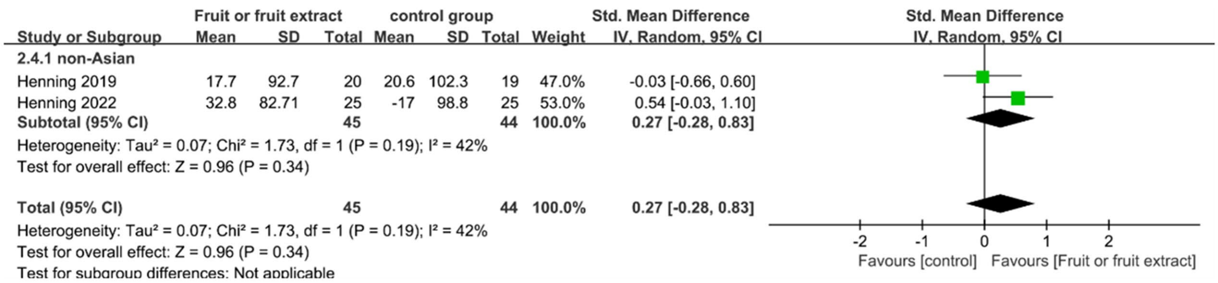

FIGURE 6
Forest plot of comparison: Fruit or fruit extract vs. placebo on MED (SMD). The details of each study are reported in Supplementary Table S2. CI, confidence interval.

Minimum erythema dose

MED was measured in two studies ($n = 89$), one with fruit extract intake (27) and the other with fruit intake (28). Both studies included participants from non-Asian groups and reported changes in MED after 8–12 weeks of fruit (27) or fruit extract (28) consumption.

Figure 6 displays the forest plot of a Meta-analysis of four studies, which estimates the difference in MED between intervention and placebo groups.

Among 89 participants in two studies, oral consumption of fruit or fruit extracts showed no statistical difference in MED compared to placebo (SMD = 0.27; 95% CI: −0.28-0.83; $p = 0.34$) (Figure 6). Meta-analysis showed moderate heterogeneity (I2 = 42%), largely due to a limited number of randomized controlled trials and the fact that the supplements consumed included both fruit extracts and whole fruits.

Transepidermal water loss

TEWL was measured in two studies ($n = 186$), one of which was conducted simultaneously in China and Italy and was considered as two independent studies. A total of 2 studies were included in the Asian group (24, 25) and 1 study was included in the non-Asian group (24). All studies investigated the effects of fruit extracts for a duration of 8 to 12 weeks.

Figure 7 displays the forest plot of a Meta-analysis of three studies, which estimates the difference in Transepidermal water loss (TEWL) between intervention and placebo groups.

A Meta-analysis of these three studies showed a significant difference between the fruit extract group and the placebo group (SMD = −6.54; 95% CI: −12.15- -0.92; $p = 0.02$) (Figure 7), indicating that the intake of fruit extracts has the ability to reduce TEWL. There was no significant difference in the change in TEWL for the Asian group (SMD = −8.11; 95% CI: −24.37-8.15; $p = 0.33$); the non-Asian group showed a significant improvement in TEWL (SMD = −4.09; 95% CI: −5.14- -3.04; $p < 0.00001$).However, there was high heterogeneity between the two studies, possibly due to differences in the tested areas (back, eye area), different types of fruit extracts (qingken orange extract, red orange (*Citrus sinensis* (L.) Osbeck) extract), and variations in extract preparation methods (standardized extract, 50% ethanol extract).

Discussion

This study selected seven randomized controlled trials investigating the effects of oral consumption of fruits or fruit extracts

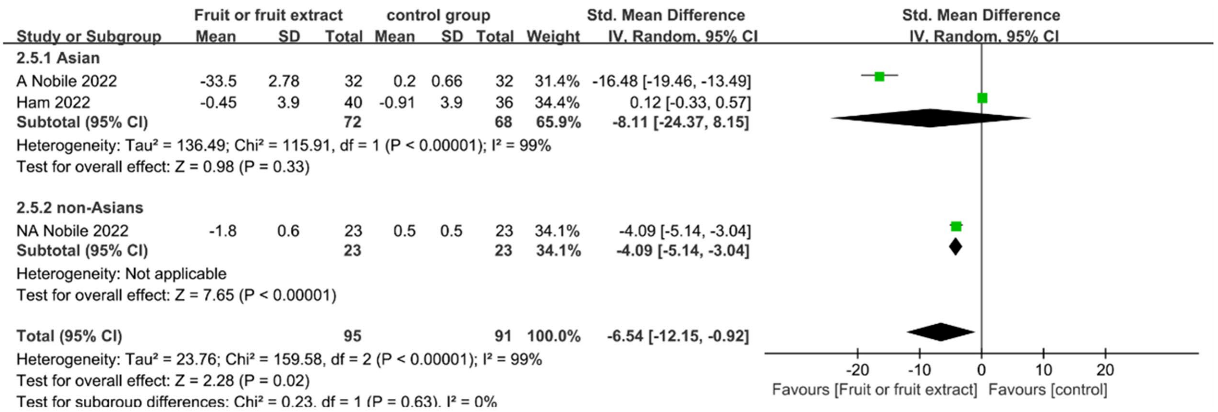

FIGURE 7
Forest plot of comparison: Fruit or fruit extract vs. placebo on TEWL (SMD). The details of each study are reported in Supplementary Table S2. CI, confidence interval.

on skin aging, involving a total of 387 healthy subjects. The main outcome measures were skin hydration, skin elasticity, wrinkle depth, minimum erythema dose (MED), and transcutaneous water loss (TEWL), to assess the overall impact of fruit or fruit extract intake on skin aging. Prior to this study, no Systematic review or Meta-analysis had been published specifically targeting fruits or fruit extracts. This study fills this gap by providing a comprehensive review and Meta-analysis, updating the evidence for the safety and efficacy of oral fruits and fruit extracts in improving skin aging.

Through Meta-analysis and systematic evaluation, we found that intake of fruits or fruit extracts can effectively improve skin aging in a number of ways, including skin hydration and transcutaneous water dissipation (TEWL). Skin hydration refers to the ability of the outermost layer of the skin – the stratum corneum or its degradation products – to bind to water (30). Many factors can influence the rate and extent of skin penetration, and skin hydration is one of the main factors that affect the rate and extent of skin absorption (31). Transcutaneous water loss (TEWL) is the loss of water from deep skin tissue through evaporation and diffusion across the epidermis. It is a form of skin water loss invisible to the naked eye, and its value reflects the amount of moisture evaporated from the skin surface and is an important parameter for assessing skin barrier function (32). The smaller the value of this parameter the better it is for skin barrier function and repair.Other outcome measures in the study included skin elasticity, wrinkle depth, and minimum erythema dose (MED). Ingestion of fruits or fruit extracts did not improve these skin conditions. The data on skin elasticity in this study mainly used the overall skin elasticity (R2) (33, 34), which provides a comprehensive picture of changes in skin elasticity. The observed indicator for wrinkles is wrinkle depth (35), as this indicator was clearly documented in the included literature and is more readily available.MED is the minimum UV radiation dose (Joules/cm2) or minimum exposure time required to produce the lowest erythema dose, and this indicator varies depending on individual skin photosensitivity and is referred to as phototype. This metric mainly assesses the effects of skin photoaging (36).

The mechanism of action of fruits and fruit extracts on the skin is complex and involves the presence of various nutrients and phytochemicals such as flavonoids, polyphenols, vitamins, alkaloids, anthocyanins, hydroxycinnamic acid, flavanones and ascorbic acid, which form antioxidants and other beneficial substances in the body's cellular system (37) and exert UV protection (38) and anti-inflammatory (39) roles. Studies in humans have shown that they are effective in reducing oxidative stress in people exposed to air pollution (40) and provide photoprotection against UV-induced erythema (41) and photoaging (42–44). Several studies have shown that the nutrients in fruits and fruit extracts promote the synthesis of collagen and elastin fibers and improve skin elasticity and firmness (45, 46), which includes various vitamins, with vitamin C showing a predominance, as vitamin C has strong redox properties and is essential for maintaining the active forms of prolyl and lysyl hydroxylases. Hydroxylation of proline and lysine is carried out by prolyl hydroxylase using ascorbic acid as a cofactor. Ascorbic acid deficiency leads to reduced hydroxylation of proline and lysine, which affects collagen synthesis (47). At the same time, additional studies have demonstrated that specific phytochemicals found in fruits and fruit extracts can inhibit the activity of tyrosinase, leading to a reduction in melanin formation and consequent skin whitening effects (48). As for the bioavailability of nutrients in fruits, it may be influenced by various factors, such as the type of nutrient, fruit variety, ripeness, preparation method and interaction with other food components (49). In general, fruits are a good source of various vitamins, minerals, fiber and phytochemicals. These nutrients are usually well absorbed by the body when consumed in whole form because the fiber in fruit slows digestion and promotes absorption of the best nutrients. For example, the bioavailability of vitamin C may be better when fruit is eaten raw rather than cooked, because heating degrades this nutrient (50). On the other hand, the bioavailability of certain phytochemicals, such as lycopene in tomatoes, can be enhanced when fruits are cooked or processed because heat helps break down plant cell walls and release nutrients (51). This article deals only with the consumption of fruits and fruit extracts without cooking or special processing. Overall, as natural plant-derived substances ingested as food, they have been shown to be safe for long-term intake, and the mechanisms of action of fruits and fruit extracts on the skin need further study.

This Systematic review and Meta-analysis has several limitations. Although the search strategy was comprehensive, some clinical trials may not have been identified. Our systematic and detailed search strategy should help identify all trials and reduce bias. Another limitation is that our standardized quality assessment and data analysis was based on the information and data reported in the study rather than the study itself. Although this study confirms the role of fruit consumption and fruit extracts in improving skin aging, the number of randomized controlled trials included in this article is small and skin aging is multifactorial, and the credibility of the study may be confounded by factors such as geographic climate. Therefore, further validation and refinement of this finding will require longer randomized controlled trials, larger sample sizes, and the use of objective dermatological methods to obtain more reliable results related to the effects of fruits and fruit extracts on skin health.

Most of the included trials did not mention adverse events (AEs), thus avoiding safety concerns. As we have shown, some trials did not include the necessary information on fruit extraction and origin that would be needed to properly compare trials in terms of effectiveness. Researchers studying such products should place greater value the importance of AE reporting

Conclusion

Overall, fruits or fruit extracts were significantly associated with skin aging in some aspects compared to placebo, but still no significant improvements were observed in many aspects. Further exploration of the magnitude of the effects of fruits and fruit extracts, especially in skin elasticity, wrinkle depth, and minimum erythema dose (MED), needs to be investigated through larger-scale and more rigorous studies. Many of the included studies had small samples and lacked satisfactory quality in terms of design and methodology. Future studies must ensure that trials are conducted and reported using methods that minimize bias as much as possible, and comply with the CONSORT statement on clinical trial reporting (52). We believe that as people's attention to skin aging deepens and such trials continue to be conducted, more and more evidence will emerge to support the positive effects of consuming fruits and fruit extracts on skin aging-related indicators.

Currently, only two observations are valid, including skin hydration and transcutaneous water dissipation (TEWL), while the evidence for recommendations against other aspects remains insufficient.

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.

Author contributions

HL, LW, JF, and LJ were suitable for the study design, literature searches, statistical analysis, and manuscript preparation. The study was supervised by JW. 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.2023.1232229/full#supplementary-material

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Oxidative balance score inversely associated with the prevalence and incidence of metabolic syndrome: analysis of two studies of the Korean population

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Background: Pro-oxidant/antioxidant imbalances leading to chronic inflammation and insulin resistance can contribute to the development of metabolic syndrome (MetS). Oxidative Balance Score (OBS), a comprehensive measure of exposure to pro- and anti-oxidants, represents an individual's total oxidative balance. This study aimed to evaluate the association between OBS and MetS using two large datasets.

Methods: We analyzed data from 2,735 adults older than 19 years from the 2021 Korean National Health and Nutritional Examination Survey (KNHANES) and 5,807 adults aged 40–69 years from the Korean Genome and Epidemiology Study (KoGES). In each dataset, OBS was categorized into sex-specific tertiles (T).

Results: In KNHANES, the odds ratios and 95% confidence intervals for prevalent MetS in T3, compared to T1, were 0.44 (0.29–0.65) in men and 0.34 (0.23–0.50) in women after adjusting for confounders. In KoGES, the hazard ratios and 95% confidence intervals for incident MetS in T3, compared to T1, were 0.56 (0.48–0.65) in men and 0.63 (0.55–0.73) in women after adjusting for confounders.

Conclusion: OBS appears to be inversely related to MetS, which suggests that adopting lifestyle behaviors that decrease oxidative stress could be an important preventive strategy for MetS.

KEYWORDS

oxidative balance score, pro-oxidant, antioxidant, metabolic syndrome, Korean Genome and Epidemiology Study

1. Introduction

Metabolic syndrome (MetS) is a cluster of metabolic dysregulations that place individuals at higher risk of type 2 diabetes and cardiovascular disease (1). The global prevalence of MetS has been estimated to be around 25% (2) and is continuously increasing in parallel with the prevalences of obesity and type 2 diabetes (3). According to a meta-analysis of global data, the prevalence of MetS is higher in the Eastern Mediterranean Region (32.9, 95% CI: 28.7–37.2) and the Americas (26.0, 95% CI: 22.7–29.4) (4). One report indicates that the prevalences of MetS in men and women in Korea in 2018 were 27.9 and 17.9%, respectively (5). Additional research suggests that people with MetS spend 1.6 times more on health care than those without MetS

($5,732 vs. $3,581) (6). Accordingly, public health experts should devote more attention to reducing the disease burden of MetS because it carries higher risks of cardiovascular mortality, all-cause mortality, and comorbidities, such as cerebrovascular disease, peripheral vascular disease, and cardiovascular disease, in addition to increased costs (7).

Both insulin resistance and chronic systemic inflammation are key factors in MetS (8). Emerging evidence demonstrates that systemic oxidant stress brought on by insulin resistance and excessive fatty acids activates a reciprocal interaction of downstream inflammatory pathways (9). As such, interest in means with which to control oxidative stress and chronic inflammation in order to mitigate the severity of comorbid chronic diseases and to prevent MetS is growing (10), and several studies have suggested that increasing consumption of dietary antioxidants and decreasing pro-inflammatory dietary behaviors can reduce oxidative stress levels and MetS incidence (11, 12).

In the literature, researchers have highlighted differences in onset and progression of MetS between men and women. These differences can be attributed to various factors, including hormones, adipose tissue distribution, genetics, and lifestyle factors (13). Oxidative balance score (OBS) is a useful tool for assessing an individual's oxidation–reduction balance, including dietary and lifestyle factors (14). Previous studies have suggested that combined measure of various pro-oxidants and anti-oxidants, including dietary and non-dietary factors, are closely associated with metabolic diseases (14–16). OBS could provide more comprehensive evaluation of oxidative stress and antioxidant capacity than individual markers alone. However, lack of standardized methodology for calculating OBS makes it difficult to compare scores across different populations or studies. Also, there is limited clinical utility for predicting disease risk (14, 17).

Although several studies have reported a relationship between OBS and MetS, results are inconsistent. A cross-sectional study conducted in Korea reported that OBS is inversely related to the risk of MetS in adults aged ≥40 years (18), whereas an Iranian cross-sectional study did not show a significant association between OBS and MetS components (19). Moreover, there is limited evidence of the association between OBS and the incidence of MetS from prospective cohort studies. Our study aimed to address a gap in current knowledge by investigating the association between OBS and MetS prevalence and incidence in two large, independent population-based datasets in a sex-specific manner.

2. Materials and methods

2.1. Study population

This study utilized two population-based cohorts: the 2021 Korea National Health and Nutrition Examination Survey (KNHANES) and the Korean Genome and Epidemiology Study (KoGES). The KNHANES is a nationwide representative population-based survey. The cohort profile was described in detail in a previous study (20). The 2021 KNHANES dataset included 7,090 participants aged 19 years and older. The KoGES Ansan and Ansung study is a community-based prospective cohort study. The study design and procedures have been described in detail in a previous study (21). The KoGES Ansan and Ansung cohort included 10,030 adults aged between 40 and 69 years. This survey was first conducted in 2001 and 2002, and participants were followed up every 2 years. In the present study, we included participants with up to eight follow-up evaluations conducted between 2017 and 2018.

A flowchart of the study population is provided in Figure 1 (KNHANES and KoGES). Among the 7,090 participants in the 2021 KNHANES dataset, 2,735 participants (1,185 men and 1,550 women) were finally included for analysis after excluding participants who had missing data with which to evaluate MetS ($n = 1,197$) and to calculate OBS ($n = 3,158$). Among the 10,030 participants in the KoGES dataset, 5,807 participants (2,921 men and 2,886 women) were finally included for analysis after excluding participants with missing data needed for MetS evaluation ($n = 3$), those with MetS at baseline ($n = 3,197$), those who lacked data needed to calculate OBS ($n = 457$), and those who did not participate in a follow-up after the baseline survey ($n = 566$).

All participants in KNHANES and KOGES provided informed consent for data collection. The study protocol conformed to the ethical guidelines of the 1964 Declaration of Helsinki and its later amendments. This study was approved by the Institutional Review Board (IRB) of Nowon Eulji Medical Center (IRB number: 2021-09-025).

2.2. Oxidative balance score assessment

OBS was calculated from data on six pro-oxidant factors and five antioxidant factors selected based on previous studies (14, 22–24), and data for all 11 factors were available in the both KHNANES and KoGES datasets. Table 1 presents the OBS assignment scheme. Pro-oxidant factors included saturated fatty acid (SFA), total iron intake, smoking status, drinking status, obesity status, and abdominal obesity status (14, 25, 26). Each query was scored 0, 1, or 2, except for abdominal obesity status, which was scored 0 or 1. The scores for SFA and total iron intake were assigned 0–2 points according to sex-specific tertile values (low, 2; intermediate, 1; high, 0) of each variable. The scores for never smokers, former smokers, and current smokers were 2, 1, and 0, respectively. The scores for non-drinkers, mild-to-moderate drinkers (1–29 g/day in men, 1–19 g/day in women), and heavy drinkers (≥30 g/day in men, ≥20 g/day in women) were 2, 1, and 0, respectively. Obesity received 0 points, overweight received 1 point, and normal weight received 2 points. Zero points were given for abdominal obesity and 1 point for no abdominal obesity. Antioxidant factors included physical activity (high intensity, 2; moderate intensity, 1; low intensity, 0) and intake of omega-3 poly-unsaturated fatty acid (PUFA) to omega-6 PUFA ratio, vitamin C, vitamin E, and beta-carotene, which were each assigned 0–2 points according to sex-specific tertile values (low, 0; intermediate, 1; high, 2) for each variable. Using OBS, which had a possible maximum of 21 points, we divided the individuals into tertile groups by sex. Higher OBS indicate higher antioxidant properties. In the KoGES and KNHANES datasets, OBS cut-off points were stratified into three categories: for men, T1 (≦11 for KoGES and ≦10 for KNHANES), T2 (12–13 for KoGES and 11–12 for KNHANES), and T3 (≧14 for KoGES and ≧13 for KNHANES); for women, T1 (≦12), T2 (13–14), and T3 (≧15) in both datasets.

2.3. Definition of MetS

Based on the National Cholesterol Education Program Adult Treatment Panel III definition as amended by the National Heart Lung and Blood Institute and the American Heart Association (27),

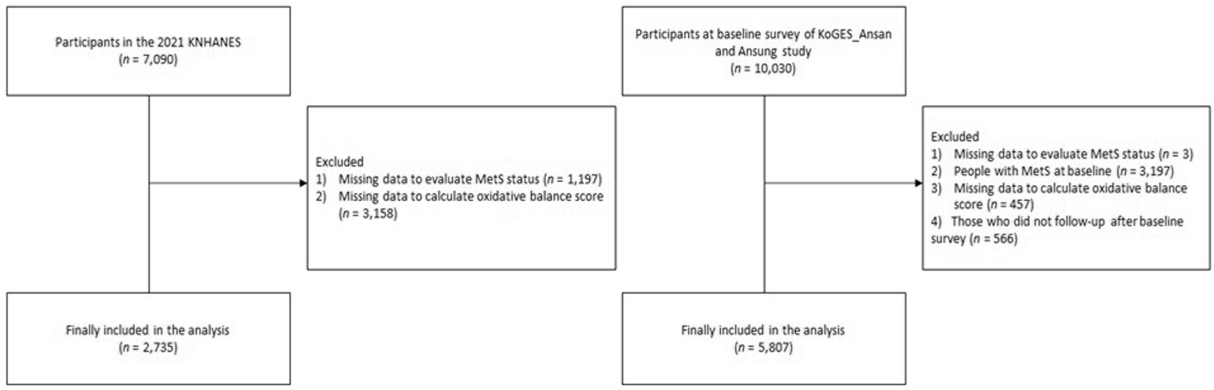

FIGURE 1
Flow chart of the study population.

TABLE 1 Oxidative balance score assignment scheme.

OBS components	Assignment scheme*
1. Saturated fatty acid [P]	0 = high (3rd tertile), 1 = intermediate (2nd tertile), 2 = low (1st tertile)
2. Total iron intake [P]	0 = high (3rd tertile), 1 = intermediate (2nd tertile), 2 = low (1st tertile)
3. Smoking status [P]	0 = current smoker, 1 = former smoker, 2 = never smoker
4. Drinking status [P]	0 = heavy drinker (≥30 g/day in men, ≥20 g/day in women), 1 = mild-to-moderate drinker (<30 g/day in men, <20 g/day in women), 2 = non-drinker
5. Overweight/obese [P]	0 = obese, 1 = overweight, 2 = normal
6. Abdominal obesity [P]	0 = abdominal obesity, 1 = normal
7. Omega-3/omega-6 PUFA ratio [A]	0 = low (1st tertile), 1 = intermediate (2nd tertile), 2 = high (3rd tertile)
8. Vitamin C intake [A]	0 = low (1st tertile), 1 = intermediate (2nd tertile), 2 = high (3rd tertile)
9. Vitamin E intake [A]	0 = low (1st tertile), 1 = intermediate (2nd tertile), 2 = high (3rd tertile)
10. Total beta-carotene intake [A]	0 = low (1st tertile), 1 = intermediate (2nd tertile), 2 = high (3rd tertile)
11. Physical activity [A]	0 = low (<7.5 METs-hr/day), 1 = moderate (7.5–30 METs-hr/day), 2 = high (>30 METs-hr/day)

*Low, intermediate, and high categories correspond to sex-specific tertile values among participants in the KoGES at the baseline survey or participants in the 2021 KNHANES. P, pro-oxidant; A, anti-oxidant; PUFA, poly-unsaturated fatty acid; MET, metabolic equivalent of task; KoGES, Korean Genome and Epidemiology Study; KNHANES, Korean National Health and Nutrition Examination Survey.

we defined MetS as the presence of three or more of the following: (1) abdominal obesity as a waist circumference ≥ 90 cm in men and ≥ 85 cm in women based on the 2018 Korean Society for the Study of Obesity guidelines (28); (2) fasting plasma glucose ≥ 5.6 mmol/L or use of oral hypoglycemic medication or insulin; (3) serum triglyceride concentration ≥ 1.7 mmol/L or use of lipid-lowering medication; (4) serum high-density lipoprotein (HDL) cholesterol concentration < 1.0 mmol/L in men or < 1.3 mmol/L in women; and (5) systolic blood pressure (SBP) ≥ 130 mmHg, diastolic blood pressure (DBP) ≥ 85 mmHg, or use of anti-hypertensive medication.

2.4. Covariates

Well-trained medical staff members performed the health examination sand health interviews following the KHANES protocol (20) and KoGES protocol, respectively (29). Body mass index (BMI) was calculated as a person's weight in kilograms divided by the square of their height in meters. Individuals with a BMI of at least 23 kg/m^2 but less than 25 kg/m^2 were considered overweight, and those with a BMI of ≥25 kg/m^2 were considered obese based on the 2018 Korean Society for the Study of Obesity guidelines (28). Abdominal obesity was defined as a waist circumference ≥ 90 cm in men and ≥ 85 cm in women based on the same guidelines (28). Mean blood pressure (MBP, mmHg) was calculated as DBP + 1/3 × (SBP – DBP) (30). Whole blood white blood count (WBC), plasma glucose, serum insulin, total cholesterol, triglycerides, HDL, cholesterol and C-reactive protein (CRP) were assessed using a Hitachi 700–110 Chemistry Analyzer (Hitachi, Ltd., Tokyo, Japan) after at least 8 h of fasting.

Information on smoking, alcohol consumption, physical activity, education level, and household income were obtained from self-reported questionnaires. A never smoker was defined as someone who had never smoked or had smoked fewer than 100 cigarettes in their lifetime. Former smokers were those who had quit smoking and had smoked more than 100 cigarettes during their lifetime. Current smokers were those who were active smokers at the time of the questionnaire and had smoked more than 100 cigarettes during their lifetime.

We further evaluated the daily alcohol intake (g/day) of each participant. Heavy drinkers were men who drank more than 30 g/day and women who drank more than 20 g/day. Mild-to-moderate drinkers were men who drank less than 30 g/day and women who drank less than 20 g/day. Non-drinkers were those who did not drink alcohol.

Physical activity was assessed using the metabolic equivalent of task–hours per week (MET-hrs/day) using the International Physical Activity Questionnaire (31). Participants were classified into low- (<7.5 METs-hr/day), moderate- (7.5–30 METs-hr/day), or high- (>30 METs-hr/day) intensity physical activity groups. An in-person interview concerning nutrition was conducted in the respondent's home.

Total energy intake and nutritional status were calculated using a validated 112-item food frequency questionnaire (FFQ) developed for KNHANES (32) and 103-item FFQ developed for KoGES (33). Information for each item was collected based on recalls of the average frequency and amount consumed per serving over the past year. From the FFQ, this study considered the total daily intake values of energy (kcal/day), SFA (g/day), total iron (mg/day), omega-3 PUFA to omega-6 PUFA ratio, vitamin C (mg/day), vitamin E (mg/day), and beta-carotene (μg/day).

Participants were classified into elementary/middle school, high school, and college/university education levels. Monthly household income was divided into three groups: <100 million Korean Won, 100–200 million Korean Won, and > 200 million Korean Won.

2.5. Statistical analysis

We conducted a normality test, and variables with normal distribution are presented as means ± standard deviations. Serum triglyceride, insulin, and CRP with non-normal distribution are presented as medians (25th percentile, 75th percentile). Continuous variables were compared using a one-way analysis of variance or the Kruskal–Wallis test according to the sex-specific OBS tertiles. All statistical analyses were conducted separately for men and women. Categorical variables are described as numbers (%) and were compared using the chi-square test. The dose–response relationship between OBS and the risk of incident MetS was determined using a Cox proportional hazard spline curve. Kaplan–Meier curves with the log-rank test were used to verify the cumulative incidence of MetS according to the sex-specific OBS tertiles. For KNHANES, we calculated the odds ratio (OR) and 95% confidence intervals (CI) for the prevalence of MetS. For KoGES, we completed univariable and multivariable Cox proportional hazard regression analyses to calculate the HRs and 95% CIs for incident MetS of the second tertile (T2) and highest tertile (T3) groups, compared with the referent lowest tertile (T1) group, in a sex-specific manner. In the fully adjusted model, we adjusted for age, education level, monthly household income, total energy intake, MBP, WBC, fasting plasma glucose, and serum total cholesterol levels based on each population characteristics. Confounders were determined as variables influencing exposure and outcome based on a literature review and univariate analysis (34, 35). We calculated variance inflation factor (VIF) values to assess the degree of multicollinearity among the variables, indicating that a VIF value of five or higher represents a high correlation of the variables (36). In this study, the maximum VIF value observed was 2.1, which corresponded to the variable 'age' in women (Supplementary Tables 1, 2). All statistical analyses were performed with SAS statistical software (version 9.4; SAS Institute Inc., Cary, NC, USA) and R software (version 4.1.1; R Foundation for Statistical Computing, Vienna, Austria). *P*-values less than 0.05 were regarded as statistically significant.

3. Results

3.1. Baseline characteristics of the study population

Table 2 shows the baseline characteristics of the men according to OBS tertile in the KoGES and KNHANES, respectively. In the KoGES, the T3 group had lower fasting glucose, insulin, total cholesterol, triglyceride, and WBC. T3 men had higher intake of SFA, total iron, omega-3 to omega-6 PUFA ratio, vitamin C, vitamin E, and beta-carotene. Since we excluded participants with existing MetS at the baseline survey of KoGES, the proportion of participants with two MetS components was lowest in the T3 group.

In the KNHANES, the T3 men had older age and lower MBP, insulin, triglyceride, and WBC. The proportion of MetS was significantly higher in T1 men. The T3 men had lower intake of SFA, and higher intake of omega-3 to omega-6 PUFA ratio, vitamin C, vitamin E, and beta-carotene. The T3 men exhibited lower proportions of current smokers, heavy drinkers, obese, and abdominal obesity and greater high intensity physical activity in both the KoGES and KNHANES.

Table 3 shows the baseline characteristics of the women according to OBS tertile in the KoGES and KNHANES, respectively. In the KoGES, the T3 women had lower MBP, insulin, total cholesterol, triglyceride, and WBC. The proportion of participants with two MetS components was lowest in the T3 group. T3 women had higher intake of SFA, total iron, omega-3 to omega-6 PUFA ratio, vitamin C, vitamin E, and beta-carotene.

In the KNHANES, the T3 women had older age and higher HDL-cholesterol, but lower insulin, triglyceride, and WBC. The proportion of MetS was significantly higher in T1 women. The T3 women had lower intake of SFA and higher intake of omega-3 to omega-6 PUFA ratio, vitamin C, vitamin E, and beta-carotene. The T3 women had lower proportions of current smokers, heavy drinkers, obese, and abdominal obesity and greater high intensity physical activity in both the KoGES and KNHANES.

3.2. Longitudinal association of OBS with incident MetS

During the mean 13.6-year follow-up period, 1,296 (44.4%) men and 880 (30.5%) women developed new-onset MetS in the KoGES. Baseline characteristics of men and women from KoGES are shown in Supplementary Table 3. Inverse relationships were noted between OBS and the risk of incident MetS in both men (Figure 2A) and women (Figure 2B). Figure 3 presents the cumulative incidence rates of MetS as Kaplan–Meier curves according to sex-specific OBS tertiles in the KoGES. The T3 group showed the lowest cumulative incidence of MetS, followed by the T2 and T1 groups, among both men (Figure 3A) and women (Figure 3B) (both log-rank test *p*-values <0.001).

Table 4 shows the independent association between OBS tertiles and MetS in the KoGES and the 2021 KNHANES. In the KoGES, the

TABLE 2 Baseline characteristics of men in the KoGES and 2021 KNHANES.

	Oxidative balance score tertiles							
	Men in KoGES				Men in KNHANES			
Variables	T1 (*n* = 1,128)	T2 (*n* = 977)	T3 (*n* = 816)	*p**	T1 (*n* = 420)	T2 (*n* = 310)	T3 (*n* = 455)	*p**
Age, years	51.3 ± 8.7	51.5 ± 9.0	51.5 ± 8.9	0.773	45.9 ± 17.1	50.0 ± 16.8	54.8 ± 17.4	<0.001
MBP, mmHg	95.3 ± 11.6	95.6 ± 12.1	95.1 ± 12.5	0.657	93.2 ± 10.7	91.0 ± 9.9	89.8 ± 10.4	<0.001
Glucose, mg/dL	87.2 ± 17.7	86.1 ± 15.5	85.3 ± 13.6	0.030	104.5 ± 25.3	103.8 ± 19.2	103.1 ± 23.6	0.659
Insulin, IU/	6.9 ± 3.8	6.5 ± 3.3	6.2 ± 3.0	<0.001	10.3 ± 6.7	9.7 ± 7.5	7.8 ± 5.6	<0.001
Total cholesterol, mg/dL	192.0 ± 34.8	192.0 ± 35.9	186.2 ± 34.4	<0.001	191.1 ± 39.1	186.1 ± 39.4	187.8 ± 39.0	0.201
Triglyceride, mg/dL	160.2 ± 99.7	147.4 ± 97.9	134.7 ± 82.9	<0.001	163.6 ± 164.1	142.1 ± 118.5	121.2 ± 75.7	<0.001
HDL cholesterol, mg/dL	45.7 ± 9.7	45.6 ± 9.4	46.2 ± 10.8	0.335	48.2 ± 12.0	47.9 ± 10.0	49.5 ± 11.8	0.087
WBC, 10^9/μL	6.9 ± 1.8	6.7 ± 1.8	6.4 ± 1.8	<0.001	6.5 ± 1.7	6.3 ± 1.6	5.9 ± 1.5	<0.001
Education level, n (%)				0.858				0.414
Elementary/middle school	475 (42.2%)	397 (40.8%)	329 (40.5%)		61 (14.5%)	44 (14.2%)	85 (18.7%)	
High school	412 (36.6%)	354 (36.4%)	296 (36.4%)		119 (28.3%)	87 (28.1%)	119 (26.2%)	
College/university	239 (21.2%)	221 (22.7%)	188 (23.1%)		240 (57.1%)	179 (57.7%)	251 (55.2%)	
Household income, n (%)				0.267				0.394
<100 million Korean Won	288 (25.7%)	266 (27.5%)	234 (28.7%)		125 (30.0%)	103 (33.3%)	165 (36.4%)	
100–200 million Korean Won	345 (30.8%)	278 (28.7%)	257 (31.6%)		145 (34.8%)	103 (33.3%)	145 (32.0%)	
>200 million Korean Won	487 (43.5%)	425 (43.9%)	323 (39.7%)		147 (35.3%)	103 (33.3%)	143 (31.6%)	
Energy intake, kcal/day	1895.2 ± 593.2	2034.1 ± 680.7	2178.1 ± 749.7	<0.001	2231.6 ± 975.2	2243.5 ± 917.7	2113.0 ± 758.9	0.063
Number of MetS components, n (%)				<0.001				<0.001
0	211 (18.7%)	273 (27.9%)	234 (28.7%)		103 (24.5%)	76 (24.5%)	146 (32.1%)	
1	405 (35.9%)	365 (37.4%)	353 (43.3%)		77 (18.3%)	59 (19.0%)	103 (22.6%)	
2	512 (45.4%)	339 (34.7%)	229 (28.1%)		85 (20.2%)	78 (25.2%)	97 (21.3%)	
≥3	–	–	–		155 (37.0%)	97 (31.3%)	109 (24.0%)	
Saturated fatty acid, g/day	9.8 ± 5.4	11.4 ± 6.9	12.1 ± 7.6	<0.001	21.2 ± 16.4	17.4 ± 12.9	14.2 ± 11.7	<0.001
Total iron intake, mg/day	17.6 ± 8.0	20.2 ± 9.6	22.8 ± 10.7	<0.001	11.5 ± 9.5	12.2 ± 7.7	11.5 ± 7.1	0.406
Smoking status, n (%)				<0.001				<0.001
Current smoker	727 (64.5%)	466 (47.7%)	215 (26.3%)		168 (40.0%)	90 (29.0%)	50 (11.0%)	
Former smoker	294 (26.1%)	309 (31.6%)	261 (32.0%)		171 (40.7%)	119 (38.4%)	208 (45.7%)	
Never smoker	107 (9.5%)	202 (20.7%)	340 (41.7%)		81 (19.3%)	101 (32.6%)	197 (43.3%)	
Drinking status, n (%)				<0.001				<0.001
Heavy drinker	320 (28.4%)	188 (19.2%)	62 (7.6%)		101 (24.0%)	35 (11.3%)	32 (7.0%)	
Mild to moderate drinker	637 (56.5%)	524 (53.6%)	375 (46.0%)		247 (58.8%)	169 (54.5%)	200 (44.0%)	
Non-drinker	171 (15.2%)	265 (27.1%)	379 (46.4%)		72 (17.1%)	106 (34.2%)	223 (49.0%)	
Obesity status, n (%)				<0.001				<0.001
Obese	491 (43.5%)	240 (24.6%)	102 (12.5%)		273 (65.0%)	150 (48.4%)	108 (23.7%)	
Overweight	313 (27.7%)	323 (33.1%)	226 (27.7%)		87 (20.7%)	82 (26.5%)	132 (29.0%)	
Normal weight	324 (28.7%)	414 (42.4%)	488 (59.8%)		60 (14.3%)	78 (25.2%)	215 (47.3%)	
Abdominal obesity, n (%)	157 (13.9%)	55 (5.6%)	18 (2.2%)	<0.001	148 (35.2%)	73 (23.5%)	59 (13.0%)	<0.001
Omega-3/omega-6 PUFA ratio	0.139 ± 0.040	0.155 ± 0.048	0.171 ± 0.056	<0.001	0.158 ± 0.175	0.204 ± 0.165	0.240 ± 0.176	<0.001
Vitamin C intake, mg/day	80.3 ± 58.6	122.3 ± 87.5	161.2 ± 122.8	<0.001	51.1 ± 165.5	77.6 ± 132.5	95.5 ± 86.1	<0.001
Vitamin E intake, mg/day	11.7 ± 5.7	14.6 ± 7.3	17.4 ± 8.0	<0.001	7.1 ± 4.6	8.4 ± 4.4	8.7 ± 3.8	<0.001

(Continued)

TABLE 2 (Continued)

	Oxidative balance score tertiles							
	Men in KoGES				Men in KNHANES			
Beta-carotene intake, μg/day	2564.1 ± 2134.9	3766.0 ± 3078.5	4905.9 ± 3687.3	<0.001	2183.1 ± 2501.2	3286.5 ± 3360.4	4350.8 ± 4891.9	<0.001
Physical activity, n (%)				<0.001				<0.001
Low (<7.5 METs-hr/day)	113 (10.0%)	39 (4.0%)	18 (2.2%)		242 (57.6%)	140 (45.2%)	153 (33.6%)	
Moderate (7.5–30 METs-hr/day)	720 (63.8%)	563 (57.6%)	428 (52.5%)		128 (30.5%)	122 (39.4%)	209 (45.9%)	
High (>30 METs-hr/day)	295 (26.2%)	375 (38.4%)	370 (45.3%)		50 (11.9%)	48 (15.5%)	93 (20.4%)	

**p* value for the comparison of the baseline characteristics among sex-specific tertile groups of oxidative balance score at the baseline survey.
Significance was set at $p < 0.05$.
KoGES, Korean Genome and Epidemiology Study; KNHANES, Korean National Health and Nutrition Examination Survey; MBP, mean blood pressure; HDL, high-density lipoprotein; CRP, C-reactive protein; MetS, metabolic syndrome; PUFA, poly-unsaturated fatty acid; MET, metabolic equivalent of task.

HRs (95% CIs) for new-onset MetS per increment in OBS were 0.90 (0.88–0.92) in men and 0.88 (0.86–0.91) in women. Compared to referent T1, the adjusted HRs (95% CIs) for new-onset MetS were 0.82 (0.72–0.93) in T2 and 0.56 (0.48–0.62) in T3 among men. Compared to referent T1, the adjusted HRs (95% CIs) for new-onset MetS were 0.71 (0.62–0.81) in T2 and 0.63 (0.55–0.73) in T3 among women.

In the 2021 KNHANES, the ORs (95% CIs) for MetS per increment in OBS were 0.89 (0.85–0.93) in men and 0.88 (0.84–0.93) in women. Compared to referent T1, the adjusted ORs (95% CIs) for MetS in T3 were 0.44 (0.29–0.65) in men and 0.34 (0.23–0.50) in women. When we conducted the subgroup analysis in adults aged 40–69 years from KNHANES, the significant association between OBS and incident MetS remained (T3 vs. T1, adjusted ORs and 95% CIs = 0.39 [0.24–0.65] in men and 0.26 [0.16–0.43] in women) (Supplementary Table 4).

4. Discussion

We found that OBS was independently and inversely related to the prevalence of MetS and incident MetS in men and women separately after adjusting for potential confounders in both cross-sectional and longitudinal datasets.

Higher OBS values were closely associated with a lower risk of incident MetS. Our findings are line with previous studies that have demonstrated the association with chronic diseases such hypertension (37), non-alcoholic fatty liver diseases (NAFLD) (38), cardiovascular diseases (39) and MetS (18). While limited studies have reported on the link between OBS and MetS in Caucasian populations, Annor et al. found an inverse association between OBS and hypertension, a component of MetS, in a racially diverse population (37). A cross-sectional study conducted in Korea found that higher OBS was associated with a lower risk of MetS (18). The study further revealed that individuals in the highest OBS quartile exhibited lower levels of inflammatory markers, including white blood cell count and C-reactive protein. A recent study has revealed that OBS exhibits an inverse association with NAFLD, a condition sharing a similar spectrum with metabolic syndrome (38).

In contrast to our current study, Noruzi et al. (19) reported an inverse association between higher OBS values and a reduced likelihood of MetS components, such as abdominal obesity and elevated DBP, among an Iranian population. However, their study did not find a significant association between OBS and the overall prevalence of MetS or other individual MetS components. Several factors could explain the disparities observed in the previous study. One contributing factor is that the previous Iranian study did not include comprehensive data on alcohol consumption, which is an important component of OBS. Additionally, differences in demographic factors, including age, ethnicity, and the composition of participant samples, could contribute to the observed differences. Furthermore, variations in the composition of OBS components and discrepancies in sample sizes may also contribute to these disparities.

Many studies have suggested various OBS values with different scoring schemes and with different types of anti-and pro-oxidant components, including dietary factors and lifestyle factors (14). Similar to previous studies, we aimed to construct an OBS using OBS components available in the KoGES and KNHANES as much as possible. Also, we found that higher scores for the OBS used in the current study were correlated with lower blood glucose, insulin, total cholesterol, triglyceride, and CRP.

There are several plausible explanations for the findings of this study. First, a higher OBS indicates a favorable antioxidant defense system, which can counteract excessive oxidative stress, a key contributor to MetS development (10). Previous studies have indicated that healthy lifestyles that include aerobic exercise, high consumption of vegetables, alcohol restriction, and body weight control are associated with decreased blood pressure, insulin resistance, and other related diseases by controlling oxidative stress (40, 41). Although the highest tertile group in KoGES showed the highest SFA and total iron intake, compared with the other tertile groups, this could be due to the tertile group having the highest total energy intake. However, considering that the relationship between OBS and incident MetS remained significant even after adjusting for total energy intake, the effect of antioxidants and lifestyle factors could outweigh that of the pro-oxidant components. Second, chronic systemic inflammation can contribute to the development of MetS. Chronic inflammation may worsen insulin action, increase blood pressure, and deteriorate lipid metabolism (42, 43). A randomized controlled trial found that participants in the highest quartile of white blood cell counts had a higher probability of having MetS than those in the lowest baseline sex-adjusted quartile (OR: 2.47, 95% CI: 2.03–2.99, p for trend <0.001) (44). Adopting a healthy dietary pattern characterized by a high intake

TABLE 3 Baseline characteristics of women in the KoGES and 2021 KNHANES.

	Oxidative balance score tertiles							
	Women in KoGES				Women in 2021KNHANES			
Variables	T1 (*n* = 828)	T2 (*n* = 1,027)	T3 (*n* = 1,031)	*p**	T1 (*n* = 611)	T2 (*n* = 481)	T3 (*n* = 458)	*p**
Age, years	51.6 ± 8.7	50.6 ± 8.6	49.1 ± 8.1	<0.001	47.7 ± 16.4	51.2 ± 16.3	54.8 ± 14.9	<0.001
MBP, mmHg	91.9 ± 12.5	91.3 ± 12.1	89.8 ± 12.1	<0.001	86.8 ± 11.0	86.0 ± 10.3	86.6 ± 11.4	0.478
Glucose, mg/dL	82.4 ± 12.0	81.3 ± 11.5	81.2 ± 13.0	0.061	98.9 ± 18.2	98.9 ± 18.5	98.5 ± 15.4	0.928
Insulin, IU/	7.9 ± 5.7	7.5 ± 4.0	7.2 ± 4.2	0.010	9.5 ± 6.2	8.8 ± 8.5	7.2 ± 5.0	<0.001
Total cholesterol, mg/dL	191.4 ± 35.0	187.2 ± 34.8	182.6 ± 32.1	<0.001	190.9 ± 37.4	189.0 ± 37.3	193.7 ± 38.3	0.165
Triglyceride, mg/dL	119.4 ± 49.0	117.1 ± 45.9	113.1 ± 51.2	0.017	112.2 ± 73.0	103.7 ± 67.6	97.0 ± 67.3	0.002
HDL cholesterol, mg/dL	48.2 ± 10.2	48.5 ± 10.3	48.6 ± 9.9	0.711	55.3 ± 12.9	56.2 ± 13.3	58.0 ± 13.5	0.005
WBC, 10^9/μL	6.3 ± 1.8	6.2 ± 1.7	6.0 ± 1.7	0.001	5.9 ± 1.6	5.6 ± 1.5	5.4 ± 1.5	<0.001
Education level, n (%)				<0.001				0.462
Elementary/middle school	558 (67.7%)	640 (62.7%)	540 (52.6%)		147 (24.1%)	115 (23.9%)	111 (24.2%)	
High school	210 (25.5%)	316 (31.0%)	383 (37.3%)		152 (24.9%)	131 (27.2%)	136 (29.7%)	
College/university	56 (6.8%)	65 (6.4%)	104 (10.1%)		312 (51.1%)	235 (48.9%)	211 (46.1%)	
Household income, n (%)				0.001				0.358
<100 million Korean Won	297 (36.4%)	346 (34.2%)	307 (30.3%)		193 (31.6%)	151 (31.5%)	152 (33.3%)	
100–200 million Korean Won	256 (31.4%)	320 (31.7%)	292 (28.8%)		223 (36.6%)	152 (31.7%)	152 (33.3%)	
>200 million Korean Won	263 (32.2%)	345 (34.1%)	414 (40.9%)		194 (31.8%)	177 (36.9%)	153 (33.5%)	
Energy intake, kcal/day	1727.4 ± 544.0	1805.4 ± 629.3	2120.2 ± 848.0	<0.001	1563.7 ± 611.1	1578.7 ± 601.8	1589.8 ± 535.0	0.766
Number of MetS components, n (%)				<0.001				0.001
0	108 (13.0%)	214 (20.8%)	272 (26.4%)		209 (34.2%)	178 (37.0%)	152 (33.2%)	
1	296 (35.7%)	423 (41.2%)	452 (43.8%)		137 (22.4%)	100 (20.8%)	133 (29.0%)	
2	424 (51.2%)	390 (38.0%)	307 (29.8%)		100 (16.4%)	95 (19.8%)	88 (19.2%)	
≥3					165 (49.4%)	108 (22.4%)	85 (18.6%)	
Saturated fatty acid, g/day	9.3 ± 5.0	10.6 ± 6.3	12.9 ± 9.3	<0.001	15.2 ± 11.3	12.9 ± 9.8	10.3 ± 7.7	<0.001
Total iron intake, mg/day	15.6 ± 6.5	18.3 ± 8.9	23.1 ± 12.4	<0.001	8.7 ± 7.0	8.5 ± 5.0	9.4 ± 5.3	0.035
Smoking status, n (%)				<0.001				<0.001
Current smoker	49 (5.9%)	26 (2.5%)	6 (0.6%)		54 (8.8%)	7 (1.5%)	2 (0.4%)	
Former smoker	17 (2.1%)	9 (0.9%)	3 (0.3%)		49 (8.0%)	16 (3.3%)	9 (2.0%)	
Never smoker	762 (92.0%)	992 (96.6%)	1,022 (99.1%)		508 (83.1%)	458 (95.2%)	447 (97.6%)	
Drinking status, n (%)				<0.001				<0.001
Heavy drinker	28 (3.4%)	10 (1.0%)	5 (0.5%)		53 (8.7%)	11 (2.3%)	1 (0.2%)	
Mild to moderate drinker	327 (39.5%)	282 (27.5%)	187 (18.1%)		273 (44.7%)	167 (34.7%)	110 (24.0%)	
Non-drinker	473 (57.1%)	735 (71.6%)	839 (81.4%)		285 (46.6%)	303 (63.0%)	347 (75.8%)	
Obesity status, n (%)				<0.001				<0.001
Obese	452 (54.6%)	354 (34.5%)	138 (13.4%)		272 (44.5%)	119 (24.7%)	52 (11.4%)	
Overweight	234 (28.3%)	306 (29.8%)	295 (28.6%)		122 (20.0%)	121 (25.2%)	99 (21.6%)	
Normal weight	142 (17.1%)	367 (35.7%)	598 (58.0%)		217 (35.5%)	241 (50.1%)	307 (67.0%)	
Abdominal obesity, n (%)	259 (31.3%)	151 (14.7%)	57 (5.5%)	<0.001	99 (16.2%)	44 (9.1%)	8 (1.7%)	<0.001
Omega-3/omega-6 PUFA ratio	0.134 ± 0.037	0.154 ± 0.053	0.175 ± 0.067	<0.001	0.177 ± 0.190	0.239 ± 0.249	0.295 ± 0.268	<0.001
Vitamin C intake, mg/day	82.8 ± 65.4	123.6 ± 110.1	194.1 ± 147.0	<0.001	38.2 ± 46.4	66.1 ± 78.7	106.3 ± 114.9	<0.001
Vitamin E intake, mg/day	10.3 ± 5.7	13.1 ± 7.1	18.3 ± 10.9	<0.001	5.6 ± 3.2	6.4 ± 3.6	7.4 ± 3.4	<0.001
Beta-carotene intake, μg/day	2095.2 ± 1456.3	3144.4 ± 2730.2	5089.7 ± 4572.1	<0.001	1757.6 ± 1782.7	2690.9 ± 2388.5	4602.5 ± 4064.0	<0.001
Physical activity, n (%)				<0.001				<0.001
Low (<7.5 METs-hr/day)	127 (15.3%)	75 (7.3%)	53 (5.1%)		357 (58.4%)	221 (45.9%)	170 (37.1%)	

(Continued)

TABLE 3 (Continued)

	Oxidative balance score tertiles							
	Women in KoGES				Women in 2021KNHANES			
Moderate (7.5–30 METs-hr/day)	560 (67.6%)	702 (68.4%)	648 (62.9%)		213 (34.9%)	223 (46.4%)	218 (47.6%)	
High (>30 METs-hr/day)	141 (17.0%)	250 (24.3%)	330 (32.0%)		41 (6.7%)	37 (7.7%)	70 (15.3%)	

*p value for the comparison of the baseline characteristics among sex-specific tertile groups of oxidative balance score at the baseline survey.
Significance was set at $p < 0.05$.
KoGES, Korean Genome and Epidemiology Study; KNHANES, Korean National Health and Nutrition Examination Survey; MBP, mean blood pressure; HDL, high-density lipoprotein; CRP, C-reactive protein; MetS, metabolic syndrome; PUFA, poly-unsaturated fatty acid; MET, metabolic equivalent of task.

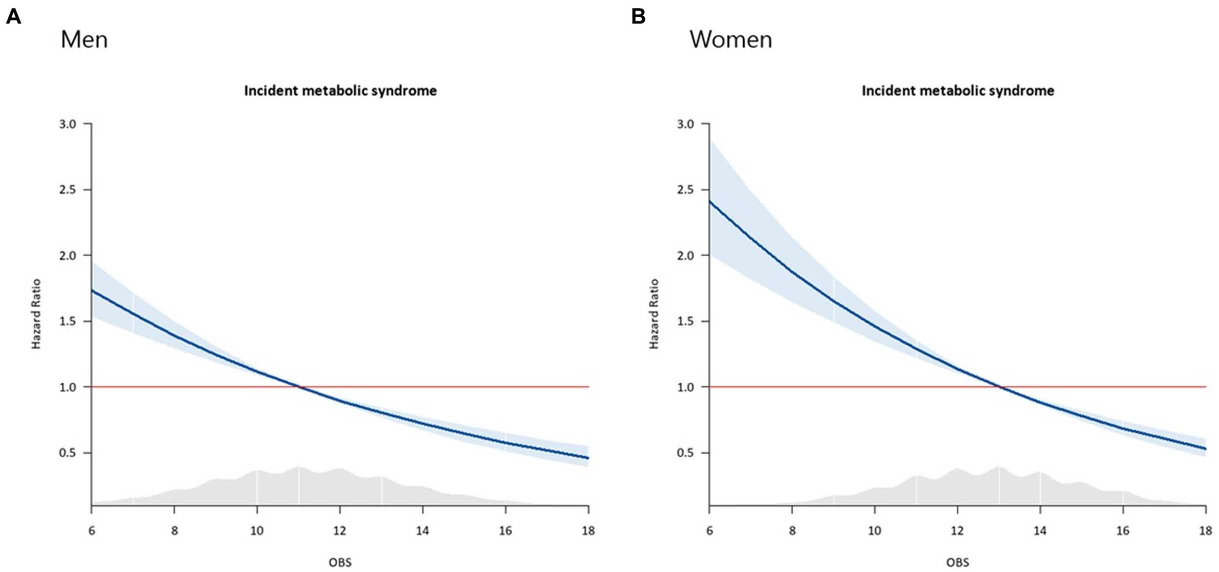

FIGURE 2
Cox proportional hazard spline curves showing an inverse relationship between oxidative balance scores and the risk of incident metabolic syndrome. The blue line illustrates changes in hazard ratios for the incidence of metabolic syndrome across a range of OBS values. The sky-blue area represents 95% confidence intervals. The red line indicates a hazard ratio of one. The gray shaded area denotes a density plot showing the distribution of the study population according to OBS values. **(A)** Men, **(B)** Women.

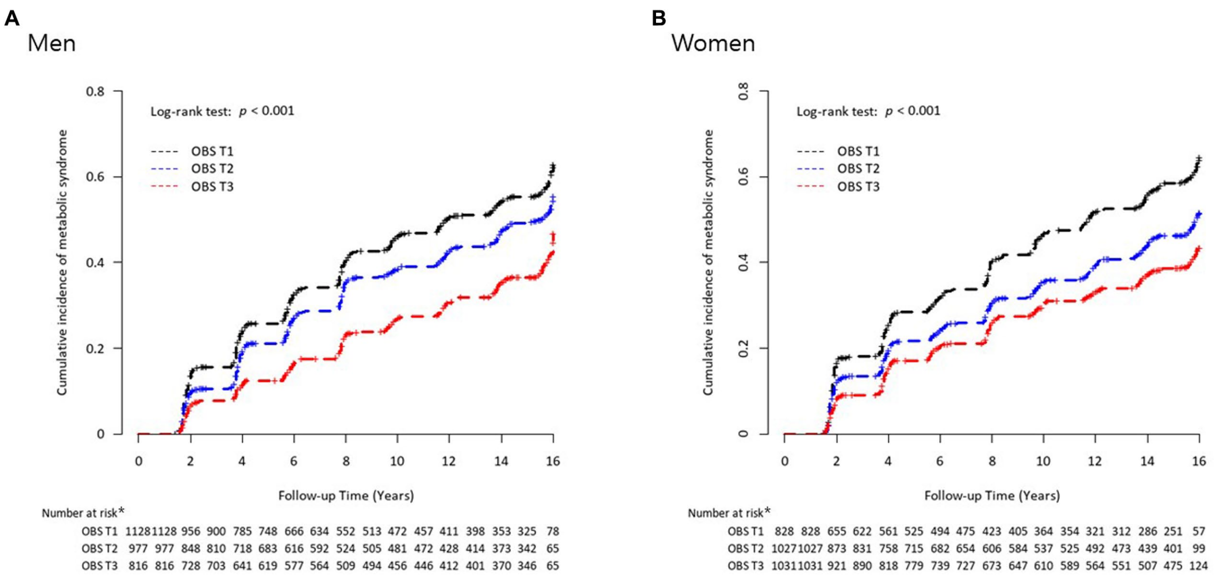

FIGURE 3
Kaplan–Meier curves of cumulative incidence rates of metabolic syndrome according to sex-specific oxidative balance score tertiles for **(A)** men and **(B)** women. *Number at risk refers to those individuals at each time point who are still under observation and have not yet experienced metabolic syndrome.

TABLE 4 Associations between oxidative balance score and incident/prevalent metabolic syndrome in KoGES and KNHANES.

KoGES								
Oxidative balance score tertiles	Numbers, n	New-onset MetS cases, n	Follow-up period, person-year	Incidence rate per 1,000 person-years	Unadjusted		Adjusted	
					HR (95% CI)	*p*	HR (95% CI)	*p*
Men								
Continuous (per increment)					0.90 (0.88–0.92)	<0.001	0.90 (0.88–0.92)	<0.001
T1	1,128	582	10028.0	58.0	1		1	
T2	977	440	9414.4	46.7	0.81 (0.72–0.92)	<0.001	0.82 (0.72–0.93)	0.002
T3	816	274	8610.4	31.8	0.56 (0.48–0.64)	<0.001	0.56 (0.48–0.65)	<0.001
Women								
Continuous (per increment)					0.88 (0.86–0.91)		0.90 (0.88–0.93)	<0.001
T1	828	452	7420.6	60.9	1		1	
T2	1,027	443	10264.1	43.2	0.71 (0.62–0.81)	<0.001	0.71 (0.62–0.81)	<0.001
T3	1,031	388	11217.2	34.6	0.57 (0.50–0.65)	<0.001	0.63 (0.55–0.73)	<0.001
KNHANES								
Oxidative balance score tertiles	Numbers, n	MetS cases, n		Prevalence rate of MetS, %	Unadjusted		Adjusted	
					OR (95% CI)	*p*	OR (95% CI)	*p*
Men								
Continuous (per increment)					0.89 (0.85–0.93)	<0.001	0.87 (0.81–0.92)	<0.001
T1	420	155		36.90	1		1	
T2	310	97		31.29	0.78 (0.57–1.06)	0.115	0.67 (0.45–1.00)	0.051
T3	455	109		23.96	0.54 (0.40–0.72)	<0.001	0.44 (0.29–0.65)	<0.001
Women								
Continuous (per increment)					0.88 (0.84–0.93)		0.80 (0.75–0.86)	<0.001
T1	611	165		27.00	1		1	
T2	481	108		22.45	0.59 (0.59–1.03)	0.085	0.58 (0.40–0.86)	0.006
T3	458	85		18.56	0.62 (0.46–0.83)	0.001	0.34 (0.23–0.50)	<0.001

Adjusted for age, education level, monthly household income, total energy intake, mean blood pressure, whole blood white blood cell count, fasting plasma glucose, and serum total cholesterol levels based on characteristics for each population. Significance was set at $p < 0.05$.

KoGES, Korean Genome and Epidemiology Study; KNHANES, Korean National Health and Nutrition Examination Survey; MetS, metabolic syndrome; HR, hazard ratio; CI, confidence interval.

of vegetables, nuts and fish, quitting smoking, and maintaining a healthy body weight are recognized as effective measures for mitigating chronic inflammation (45). We found that OBS was negatively correlated with CRP in the current study. Third, elevated OBS values have the potential to enhance insulin sensitivity, facilitate efficient glucose utilization, and reduce the risk of insulin resistance, which is a crucial factor in the development of MetS. Physical activity improves peripheral insulin sensitivity (46), while smoking can have detrimental effects on pancreatic β-cell function and insulin sensitivity (47).

Finally, the metabolic overburden of mitochondria, which causes incomplete β-oxidation and an accumulation of lipotoxicity, is a major contributor to both β-cell dysfunction and muscle insulin resistance (48). Moreover, a genome-wide association study revealed that the VEGF signaling pathway, glutathione metabolism, and the Rac-1 pathway were highly enhanced biological pathways associated with OBS and MetS (18). Follow-up studies are needed to determine differences in genetic variations, such as single nucleotide polymorphisms, according to OBS values.

This study has some limitations. First, there were differences in sociodemographic factors and some items for the OBS between individuals included in the analysis and those excluded from the analysis (Supplementary Tables 5, 6). Thus, there is a possibility of selection bias. However, we analyzed the KOGES and KNHANES datasets separately and observed significant results in each analysis. We also found significant associations between OBS and MetS in adults who aged 40–69 years in the KNHANES. Second, in the analysis of the KoGES dataset, only OBS values from the baseline survey were considered due to the unavailability of follow-up information specifically related to diet. All variables included in the OBS can change over time. Therefore, in future studies, the effect of changes in OBS over time on the incidence of MetS should be analyzed. Third, information about dietary components was obtained from FFQs. Although FFQs are useful for investigating nutritional status in large-scale epidemiology studies, they lack accuracy on absolute nutrient values, especially micronutrients, and may over- or under-report consumption of certain foods (49). Due to a lack of information, we could not consider blood micronutrient levels. In addition, there is a possibility of recall bias in the information on average intake over the past year. Fourth, each component included in the OBS may have distinct effects on MetS. It is important to consider an analysis method that takes into account the weights associated with each pro-oxidant and anti-oxidant component when assessing their impact on incident MetS. Further controlled clinical trials considering more detailed anti-and pro-oxidants components should be performed to confirm a causal relationship between OBS and incident MetS. Also, efforts are needed to construct a verified OBS. Finally, we could not consider the impact of all possible pro- and anti-inflammatory cytokines, including interleukin (IL)-1β, IL-4, IL-6, IL-10, and tumor necrosis factor-α.

Despite these limitations, we are the first to report a significant association between OBS and MetS in a large, population-based, prospective study with a long follow-up period. The present study found higher OBS values were significantly associated with lower incidences of MetS in two independent large population-based datasets. OBS-enhancing strategies, including maintaining a healthy weight, engaging in regular exercise, quitting smoking, and consuming antioxidant substances, may successfully reduce one's risk of developing MetS. It is imperative to conduct more randomized controlled studies to confirm the validity of authorized OBS diet recommendations for preventing MetS.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: the Korean Genome and Epidemiology Study data are available through a procedure described at: https://nih.go.kr/ko/main/main.do. The Korean National Health and Nutritional Examination Survey data are available through a procedure described at: https://knhanes.kdca.go.kr/knhanes/sub03/sub03_02_05.do.

Ethics statement

The studies involving humans were approved by IRB of Yongin Severance Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

H-MP, T-HH, J-HL, and Y-JK: study concept and design, acquisition, analysis, interpretation of data, drafting the manuscript, and approval of the final manuscript and had the final responsibility to submit the study for publication. J-HL and Y-JK: study concept and design, interpretation of data, supervision, and revising the manuscript. 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.2023.1226107/full#supplementary-material

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Impact of 4-week of a restricted Mediterranean diet on taste perception, anthropometric, and blood parameters in subjects with severe obesity

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Introduction: The study of taste functionality and its relation to human health is receiving growing attention. Obesity has been reported to cause alterations in sensory perception regarding system functionality and preferences. However, a small body of research addresses tastes perception and its modification with the achievement of body mass reduction through surgical intervention. Much fewer efforts have been made to evaluate the impact of mild restrictive nutritional intervention on gustatory functions. Thus, the objectives of this study were to determine if a dietary intervention of 4 weeks following a restricted balanced Mediterranean diet would affect the sweet and salty taste thresholds of subjects with severe obesity and could influence their anthropometric and blood parameters.

Methods: Fifty-one patients with severe obesity (*F*: 31; age: 43.7 $\pm$ 12.5; BMI = 47.6 $\pm$ 1.0) were enrolled in the study. The recognition threshold for sweet and salty taste and anthropometric and blood parameters were assessed before and after the 4-week weight loss program.

Results and Discussion: The Mediterranean diet has proven to be an effective treatment, significantly improving all anthropometric and blood parameters ($p < 0.05$) after 4 weeks of intervention. Moreover, the hypo-sodium treatment associated with the diet significantly improved the salty threshold ($p < 0.001$). No changes were detected for the sweet threshold. Collectively, these data highlight that dietary treatment might impact taste perception differently. Therefore, a taste-oriented nutritional intervention could represent a novel approach to developing more individualized, taste-oriented follow-up interventions to maintain sustainable and long-term weight loss.

KEYWORDS

taste acuity, hypocaloric balanced Mediterranean diet, taste-oriented nutritional reeducation, salt recognition threshold, obesity, weight loss program

1. Introduction

During the past three years, our world has been facing the coronavirus pandemic, and the loss of or alterations to taste and smell are one of the main symptoms of viral infection. Nowadays, more than ever before, clinicians and researchers in the field have recognized the importance of understanding, researching, and assessing sensory science for the promotion of health and the prevention of diseases (1). Thus, the need to improve chemo-sensation knowledge and perception in specific targets of consumers or patients fostered the application of psychophysiological studies to evaluate the senses of smell and taste quantitatively in various clinical and community settings through advances in sensory science.

Among various chronic diseases, obesity has been reported to cause alterations in sensory perception regarding system functionality and preferences (2). Indeed, individuals with obesity are reported to perceive tastes as less intense (3–6) and may need greater stimulation of taste and oral somatosensory systems to satisfy their reward, increasing the willingness to ingest energy-dense foods, particularly rich in sugars, fats, and salt. Indeed, several studies have emphasized the inverse relationship between the perception of sweet taste, salt taste and fat stimulus and nutritional status [e.g., (7–11)]. However, a small body of research addresses tastes perception and its modification with the achievement of body mass reduction through dietary or surgical intervention. While literature findings underscored a more robust and sustained impact of bariatric surgery in affecting taste perception (12, 13), much fewer efforts have been made to evaluate the impact of mild restrictive nutritional intervention on gustatory functions. It is still unclear if the changes in taste perception are a surgery-specific phenomenon or if it is a general phenomenon that accompanies weight loss. Indeed, the data concerning this topic still need to be more consistent (14). A link between diet and fat taste has been shown in two intervention studies, whereby the modification of fat content of the diet (i.e., low-fat dietary intervention) induced a significant weight reduction and positively influenced fat stimulus threshold, resulting in a decreased taste threshold (increased sensitivity) in both lean participants and those with obesity (15) and overweight and obese subjects (16). However, in this previous study (16), the low-fat diet consumption over the 6 weeks had no significant positive effect on sweet and salty detection thresholds.

On the contrary, the sweet taste threshold was decreased in women with obesity after diet-induced weight loss programs of 3 months (17, 18). Because data are limited and conflicting, it is therefore important to understand whether taste perception may change during a weight-loss program and how this may contribute to the success or failure of achieving a long-term dietary modification. Hypothetically, an individual following a taste-oriented diet could experience an improvement in taste system functionality, which may help to modify taste preferences and accomplish healthier dietary habits.

In 2010, the Mediterranean diet was awarded the recognition of UNESCO as an Intangible Heritage of Humanity (19). Several studies and guidelines indicate the Mediterranean diet as the non-pharmacological dietary approach of choice in the management of patients with severe obesity (20, 21). Literature data show that the Mediterranean diet has a protective role against non-communicable diseases and several benefits by shutting down low-grade inflammation. It is characterized by high consumption of whole cereals, fruit, legumes, vegetables, and nuts, a moderate use of dairy products, a low consumption of meat and poultry and a moderate consumption of alcohol. Its high content of plant and whole foods has been shown to increase satiety, and this may also improve adherence in individuals who need to lose weight on a hypocaloric diet (21, 22). As also shown in some studies, diets unbalanced in favor of some nutrients rather than others (e.g., low fat, high protein, or low carbohydrates) do not seem to be more effective than a balanced, moderately low-calorie diet as Mediterranean pattern (23, 24). This dietary pattern is characterized by a low content of processed foods, favoring the consumption of less processed foods with a lower content of sodium, simple sugars, and saturated fats instead. Moreover, several epidemiological and clinical studies have highlighted the positive effects of the Mediterranean diet on cardiovascular risk factors, obesity, metabolic syndrome, non-alcoholic fatty liver disease, and diabetes (21, 25–28).

Based on these premises, scientists and clinicians can utilize this information to develop more individualized, taste-oriented follow-up interventions to maintain sustainable and long-term weight loss, with the perspective of more personalized and precise nutritional therapy. Thus, the primary goal of this study was to determine if a weight loss dietary intervention of 4 weeks following a restricted balanced Mediterranean diet would affect the sweet and salty taste thresholds of subjects with severe obesity. Moreover, the second aim of the present study was to ascertain whether and to what extent dietary intervention could influence anthropometric and blood parameters.

2. Materials and methods

2.1. Patients recruitment

This study was carried out as part of a larger study (11), which aims to identify predictors that may play a role in successful weight loss and maintenance in obese subjects with eating disorders.

The study protocol was performed according to the principles established by the Declaration of Helsinki and approved by the Istituto Auxologico Italiano ethics committee (Approval registration number: 43C101). Written informed consent was obtained from all participants before entering the study.

The general study population consisted of adults of both sexes with severe obesity who self-referred to Istituto Auxologico Italiano and later enrolled as part of a 4-weeks weight loss program. Participants had to meet the following criteria: body mass index (BMI) > 30 kg/m^2 and 18–60 years of age. Participants were excluded if they were uncooperative, pregnant or breastfeeding, heavy smokers, had undergone bariatric surgery, or had a medical condition that affected their taste or weight-loss ability (i.e., thyroid disorders, presence of endocrine abnormalities associated with obesity, current or recent oral, nasal or sinus infections, major psychiatric disorders) (11). At the end of the recruitment phase, a total of fifty-one subjects (F: 60.7%) with severe obesity (BMI = 47.6 ± 7.2 kg/m^2) and a mean age of 43.7 ± 12.5 years were enrolled in the study.

2.2. Study outline

This study was a dietary intervention where participants, admitted for residential rehabilitation hospitalization, followed a hypocaloric balanced Mediterranean diet. All participants were required to attend one laboratory session at baseline and week 4, during which general condition, blood, and anthropometric measures were examined, and detection threshold tests for sucrose and sodium chloride using ascending forced choice triangle tests (29) were evaluated. Moreover, participants were required to complete the Binge Eating Scale (BES) (30) to assess the presence and severity of binge eating disorders.

Figure 1 illustrates the flow of the study.

2.2.1. Weight loss program

The 4 weeks weight loss program took place at Istituto Auxologico Italiano where the patients were admitted for rehabilitation hospitalization. Before receiving the dietary prescription, the patients underwent a complete nutritional and dietary assessment to evaluate their dietary habits. The trained dieticians generally reported a dietary pattern rich in ultra-processed foods higher in salt, sugars, and fats. Following the dietary assessment, a dietary plan is set that considers nutritional needs in relation to the subjects' basal metabolism calculated with Mifflin (31).

The patients were asked to follow the 4-week weight loss program (i.e., a personalized, restricted, balanced Mediterranean diet) (32). The hospital provided the meals, and generally, the dietary plan consisted of a calorie reduction of 15–30% of the usual intake, which was considered effective in ensuring adequate weight loss over time, with a macronutrient distribution based on the Mediterranean pattern (55% energy from carbohydrates, 25% energy from total fat, and 20% energy from protein). Table 1 shows the bromatological composition of the dietary intervention (3 dietary plans are reported as examples to show that the diet was personalized for each patient). The average dietary sodium content was 2000 mg sodium, corresponding to less than 5 grams of sodium chloride. In contrast, the diet's average content of simple sugars is about 10–12% of the total kcal. An example of a weekly dietary plan is reported in Supplementary Table S1.

A dietary approach of this type, combined with nutritional counseling, is associated with objectives such as adequate weight loss, consumption of complete and proper meals, acquiring and reinforcing correct eating habits, and improving recognition of the biological stimuli of hunger and satiety in the timing of meal consumption (33).

2.2.2. Anthropometric measures, biochemical analysis, and clinical evaluations

Using standard techniques, anthropometric measurements were performed after an overnight fast. At baseline, height (m) was measured to the nearest 0.1 cm using a SECA 217 vertical stadiometer (SECA, Hamburg, Germany). Body weight was measured to the nearest 100 g using a SECA 700 scale at baseline and week 4. BMI was calculated as weight (kg)/height (m^2) and classified according to the WHO cut-offs (34). Body composition [fat mass (FM) and fat free mass (FFM)] was assessed by Bioimpedance (BIA). The blood sample, analyzed during routine laboratory, was collected in a fasting state with a venous sampling and stored at −80°C until the analysis took place with the oxidase enzymatic method measured using a Cobas Integra 800 Autoanalyzer (Roche Diagnostics, Monza, Italy) for glucose. Colorimetric enzymatic assays measured using a Cobas Integra 800 Autoanalyzer were used to determine serum total cholesterol (Roche Diagnostics, Monza, Italy reference code 03039773190), LDL-cholesterol (Roche Diagnostics, Monza, Italy reference code 07005717), HDL-cholesterol (Roche Diagnostics, Monza, Italy reference code 07528566), and triglyceride levels (Roche Diagnostics, Monza, Italy reference code 20767107). Blood pressure was measured at baseline and week 4 using a manual sphygmomanometer. The clinical pharmacological examinations were performed on the same day. Based on blood and anthropometric parameters, for each subject, obesity-related comorbidities were defined as such: Hypertension [SBP ≥140 mm Hg; DBP ≥90 mm Hg; or use of antihypertensive drugs (35)]; Diabetes (fasting plasma glucose ≥126 mg/dL; or use of oral anti-glycemic medication or insulin (36); Dyslipidemia [HDL-cholesterol <35 mg/dL; triglycerides >200 mg/dL, (36)]; Metabolic syndrome (HDL-cholesterol <40 mg/dL in men and < 50 mg/dL in women, triglycerides ≥150 mg/dL, SBP ≥130 mm Hg or DBP ≥85 mm Hg, fasting plasma glucose ≥100 mg/dL. MS was defined as 3 or more of the above components (37). Participants with a BES score ≥ 18 were identified as binge eaters (30).

2.2.3. Salt and sweet thresholds

The detailed protocol was fully described in (11). In brief, the test was carried out according to International Organization for Standardization (29) using triangle tests with ascending forced choice methodology. Sweet and salty taste acuity was evaluated using filter

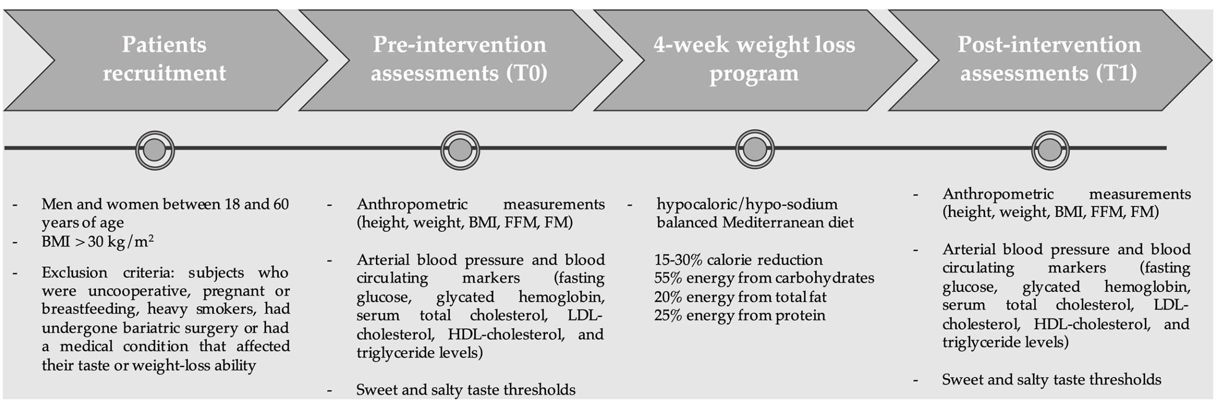

FIGURE 1
Study flow diagram.

TABLE 1 Example of bromatology composition of the restricted Mediterranean diet for three subjects.

	Energy (kcal)	Protein (g)	Protein (%)	Lipid (g)	Lipid (%)	Glucides (g)	Glucides (%)
Subject 1	1,500	71	19	43	26	210	55
Subject 2	1,650	75	18	45	24	240	58
Subject 3	1800	94	20	51	25	255	55
Average of all dietary plans	1,655 ± 205 [a]	79 ± 13	19	46 ± 4	26	228 ± 34	55

The average of all dietary plans of all patients involved has been reported as a mean ± standard deviation.

TABLE 2 General characteristics of the evaluated subjects (n = 51).

Variables	Values
Gender (n; %)	
F	31; 60.7%
M	20; 39.3%
Age (mean ± SD)	43.7 ± 12.5
Smoking status (n; %)	
Yes	14; 27.4%
No	37; 72.6%
Comorbidities	
Hypertension, (*n*; %)	41; 80.4%
F:M (*n*)	24:17
Diabetes, (*n*; %)	12; 23.5%
F:M (*n*)	6:6
Dyslipidemia, (*n*; %)	6; 11.8%
F:M (*n*)	1:5
Metabolic syndrome, (*n*; %)	21; 41.2%
F:M (*n*)	13:8
Binge eating disorder, (*n*; %)	15; 29.4%
F:M (*n*)	11:4

paper strips (Indigo Instruments – Cat#33814-Ctl; 47 × 6 × 0.3 mm) immersed in ten aqueous solutions with increasing concentrations of sucrose and sodium chloride, respectively. The best-estimated threshold was used for each participant to determine salty and sweet recognition thresholds (29).

2.3. Statistical analysis

2.3.1. Sample size calculation

A power calculation was conducted to determine the appropriate sample size for the study. Using data from a pilot study and previous research (16, 18), to detect a threshold difference of 0.2 mM sodium chloride between baseline and week 4, a sample size of $n = 41$ obese subjects will allow testing for medium effect sizes of $d = 0.4$ (repeated-measures t-test, $\alpha = 0.05$, $\beta = 0.8$) as calculated with G-power (version 3.13).

2.3.2. Data analysis

Data in the tables and figures are presented as mean ± SD unless otherwise indicated or medians with interquartile range (IQR = 75th – 25th percentile) for skewed data sets. Sucrose and NaCl detection thresholds were positively skewed and required logarithmic transformation to approximate a normal distribution. Bivariate correlations such as Pearson/Spearman correlation coefficients were used to determine whether there was a relation between taste thresholds and blood and anthropometric parameters. The bivariate correlations were performed at baseline (t0), and to know if the correlation changes after 4 weeks, a delta Δ (value at T1 – value at T0) was calculated for each variable and then correlated. McNemar's test was used to compare the number of subjects presenting comorbidities related to obesity pre- and post-intervention. Paired *t*-tests were used to analyze changes in sucrose and NaCl oral detection thresholds from baseline to week 4. The Wilcoxon signed-rank tests were used to analyze changes in blood and anthropometric measurements between baseline and week 4. All analyses were performed with SPSS software (version 27.1, IBM, Armonk, New York), and the criterion for statistical significance was $p \leq 0.05$.

3. Results

3.1. Characteristics of subjects

The general characteristics of the subjects are reported in Table 2. A total of 51 subjects with severe obesity (BMI = 47.6 ± 7.2 kg/m^2) and a mean age of 43.7 ± 12.5 years were enrolled in the study. 60.7% of the subjects were female, and 27.4% were smokers.

3.2. Significant correlation between blood and anthropometric parameters and salt and sweet taste thresholds

As far as the salty threshold is concerned, statistically significant correlations with anthropometric parameters are observed at the baseline. Indeed, weight ($r = 0.47$, $p < 0.001$) and BMI ($r = 0.33$, $p < 0.05$), correlated positively and significantly with the recognition threshold (Figures 2A,B, respectively), indicating that higher weight and BMI correspond to an increased perception threshold of the salty taste (i.e., a reduced sensitivity). A negative and significant correlation between the salt threshold and the levels of circulating high-density plasma lipoprotein (HDL-cholesterol, $\rho = -0.40$, $p < 0.01$; Figure 2C).

No significant correlations were observed for the sweet threshold besides the positive and significant correlation with fasting plasma glucose ($\rho = 0.27$, $p = 0.05$; Figure 2D), indicating that a reduced sweet sensitivity corresponds to higher concentrations of plasma glucose in the fasting state.

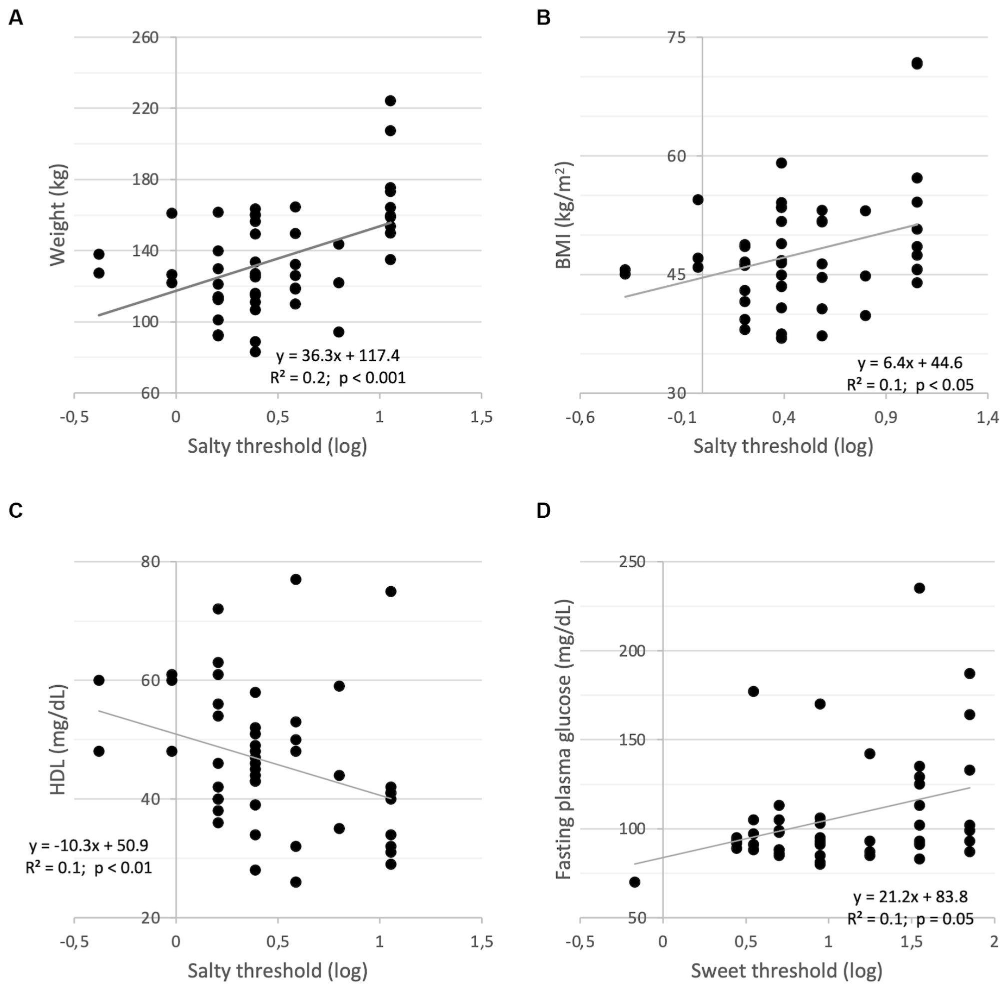

FIGURE 2
Correlations between salty threshold (log) and weight **(A)**; BMI **(B)**; HDL-cholesterol **(C)**; Correlation between sweet threshold (log) and fasting plasma glucose **(D)**.

To investigate whether the correlation changes after 4 weeks, bivariate correlation analyses were performed showing that changes in salty threshold positively correlates with changes in Δweight ($r = 0.29$, $p < 0.05$), ΔBMI ($r = 0.34$, $p < 0.05$), Δ%FM ($\rho = 0.33$, $p < 0.05$) and negatively with Δ%FFM ($\rho = 0.33$, $p < 0.05$). In addition, changes in sweet threshold positively correlated with fasting plasma glucose ($\rho = 0.25$), albeit not significantly ($p = 0.08$).

3.3. Significant amelioration of blood and anthropometric measures after the dietary intervention

During the dietary intervention, body weight ($p < 0.001$) and BMI ($p < 0.001$) decreased significantly in a manner similar to fat mass ($p < 0.001$). On the contrary, fat-free mass significantly increased ($p < 0.001$). Both systolic and diastolic blood pressure also improved significantly ($p \leq 0.001$). Moreover, the program was sufficient to determine a statistically significant improvement in all the blood parameters considered.

As regards the comorbidities related to obesity, the dietary intervention significantly reduced the number of subjects who presented values above the cut-off after the restricted Mediterranean diet (post-intervention: Hypertension $n = 9$, $\chi^2 = 26.4$, $p < 0.001$; Diabetes $n = 4$ $\chi^2 = 12.7$, $p < 0.01$; Dyslipidemia $n = 1$, $\chi^2 = 8.6$, $p = 0.12$; Metabolic syndrome $n = 5$, $\chi^2 = 9.0$, $p < 0.001$).

The anthropometric and blood characteristics of all the subjects pre- (baseline) and post- (4-weeks later) dietary intervention are reported in Table 3.

3.4. Impact of the dietary intervention on taste thresholds

The taste thresholds of saltiness and sweetness before and after dietary intervention are reported in Figure 3. There was a significant decrease in salt taste thresholds following the consumption of the hypocaloric balanced Mediterranean diet ($p < 0.001$) (Figure 3A). In contrast, the weight loss program had no significant effect on sweet thresholds ($p = 0.39$) (Figure 3B).

TABLE 3 Subjects' anthropometric and blood characteristics pre– (baseline) and post– (4-weeks later) dietary intervention.

Parameters	Pre-intervention	Post-intervention	*p*-values
Anthropometric data			
Weight (kg)	134.7 (4.0)[a]	127.4 (3.7)	< 0.001
Body mass index (kg/m^2)	47.6 (1.0)	45.1 (0.9)	< 0.001
Fat mass (%)	53.8 (0.07)	52.5 (0.07)	< 0.001
Fat free mass (%)	46.1 (0.06)	47.5 (0.06)	< 0.001
Arterial blood pressure			
Systolic blood pressure (mmHg)	137.5 (88.0–106.0)[a]	130.0 (120.0–130.0)	< 0.001
Diastolic blood pressure (mmHg)	80.0 (80.0–90.0)	80.0 (70.0–80.0)	0.001
Blood chemistry			
Fasting plasma glucose (mg/dL)	94.0 (88.0–106.0)	94.0 (84.0–101.0)	< 0.001
Glycate hemoglobin (mmol/mol)	39.0 (34.75–45.25)	37.0 (33.0–42.0)	< 0.001
Total cholesterol, mg/dL	183.6 (149.0–205.2)	154.2 (128.2–172.4)	< 0.001
HDL-cholesterol, mg/dL	44.0 (39.0–53.0)	41.0 (34.0–47.0)	< 0.001
LDL-cholesterol, mg/dL	109.0 (88.0–131.0)	82.0 (70.0–101.0)	< 0.001
Triglycerides, mg/dL	127.0 (95.0–191.0)	118.0 (86.0–165.0)	0.04

[a]Data are presented as mean ± SEM or medians with IQR.

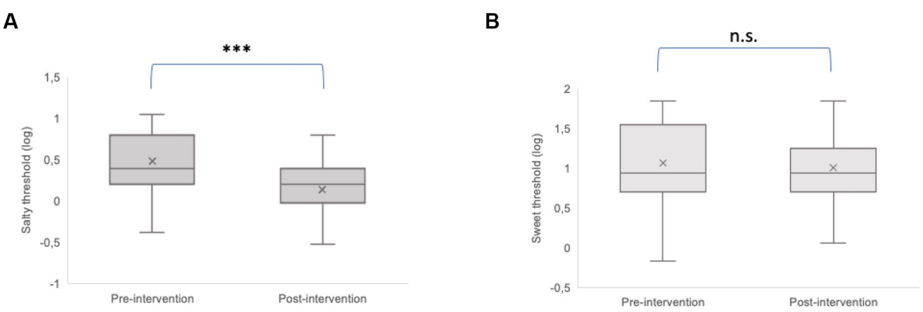

FIGURE 3
Comparison of the taste threshold of saltiness **(A)** and sweetness **(B)** before (pre-intervention) and after (post-intervention) weight loss intervention. The plots provide a representation of data distribution (the 'box'), the minimum and the maximum (the 'whiskers'), and the median (horizontal line) ± IQR (vertical line) within each group. Statistics according to *t*-test: ****p* < 0.001; n.s. not significant.

4. Discussion

This study aimed to assess whether a weight loss dietary intervention (i.e., restricted balanced Mediterranean diet) in people with severe obesity can modify various factors (i.e., anthropometric, blood, and sensory parameters), which are generally negatively affected by the pathological condition. It is well known that anthropometric parameters and lipid profile changes were more evident in subjects with severe obesity. Moreover, it has been suggested that obesity seems to affect taste functionality (2), probably due to an inflammatory response in the fungiform taste buds, which has been correlated with impairments in taste perception (38).

In the present study, this relationship was studied by analyzing the influence of anthropometric and blood parameters of patients with severe obesity on the thresholds of sweet and salt taste stimuli. The results at baseline showed a general increase in salty taste threshold (i.e., decreased acuity to saltiness) corresponding to an increase in weight and BMI, supporting the hypothesis that obese adults consume more salty foods and have reduced salt sensitivity and higher salt preference (39, 40). On the contrary, a negative correlation was found between HDL levels and salty taste recognition thresholds, in accordance with previous results (37, 41). Moreover, we found a direct correlation between fasting plasma glucose levels and sweet taste recognition threshold, suggesting a blunted sweet taste response in this group of subjects with severe obesity. Accordingly, an increase in taste thresholds has been previously associated with hyperglycemia and insulin resistance (42, 43). Noteworthy, these correlations also remain after 4 weeks when changes (Δ) were analyzed. In particular, the decrease in salty threshold is significantly correlated with a decrease in anthropometric parameters (i.e., weight, BMI, fat mass and fat-free mass), such that the greater the decrease in 4-weeks intervention, the greater the decrease in parameters related to obesity.

As regards the weight loss dietary intervention in patients with severe obesity, the restricted Mediterranean diet has proven to be an effective treatment, showing an improvement in all anthropometric and blood parameters after 4 weeks of intervention. Our results confirmed the efficacy of the dietetic treatment in ameliorating many risk markers associated with obesity, cardiovascular diseases, and metabolic syndrome (28, 44–46). The adequate consumption of plant and whole foods and unsaturated fatty acids and the synergic effect and mechanisms of specific nutrients have had a direct impact on all risk markers evaluated, namely, BMI, blood pressure, fasting blood glucose, high-density lipoprotein (HDL) cholesterol, and triglycerides, as well as systemic inflammation that characterized subjects with severe obesity. The adherence to the Mediterranean diet could have facilitated these interesting results. Indeed, recent research has shown that greater adherence to the Mediterranean diet is effective for prevention and management of different diseases and is associated with a significant improvement in health status (25, 47–49). In the present study, the residential and controlled inpatient setting have guaranteed a greater adherence of the patients to the dietary treatment. Nevertheless, the hypo-sodium treatment associated with the diet (average sodium content between 1,500 and 2000 mg per day) significantly improved salt thresholds. Indeed, the interesting finding of this study is that salty taste thresholds significantly decreased (i.e., increased sensitivity to sodium chloride) after the 4-week diet. Our results align with previous findings (50), whereby one week of sodium restriction improves the taste threshold for the salty taste in patients with chronic kidney disease and healthy subjects. On the contrary, the study of Newman and colleagues (16) did not report such improvement. This discrepancy could be due to the type of dietary treatment chosen (i.e., fat-taste-oriented) since the low-fat intake provided in (16) specifically affected and improved the fat-taste thresholds.

Although previous studies highlighted a positive effect of the dietary intervention on the thresholds (18, 51) and preferences (52) for sweet taste, no modifications in sweet taste thresholds - corresponding to changes in consumption of sugars and carbohydrates from baseline to the end of dietary intervention - were highlighted in the present study. A possible explanation for the conflicting results may lie in the different duration of dietary treatments since all the cited studies planned dietary interventions longer than 3 months (i.e., from 12 to 30 weeks). Seeing as how differently the hypocaloric balanced Mediterranean diet affected the sweet and salty thresholds, we can speculate that this discrepancy could be due to the different trans-duction mechanisms related to the taste receptors for salt and sweet (sodium channel vs. G-protein coupled receptors, respectively). The decreases in salty taste threshold are specific to the sodium restriction throughout the four weeks. They could be due to changes in the background concentration of salivary Na^+ to which the taste receptors are adapted, which is much easier to achieve than a modification in sweet-sensitive type II receptor cells (53). Thus, it is possible that the dietary approach proposed in the present study would need to be followed over a more extended period of time before definitive changes in sweet taste thresholds would be seen.

Nevertheless, our preliminary results seemed promising and could greatly impact an individual's dietary behaviors. Indeed, it is noted that a reduction in the usual amount of oral sodium intake is one of the candidate factors influencing the recognition threshold for salty taste (54). Also, the preference for salty foods may change with an acute transient increase in salt preference. Then, after weeks or months on a low-sodium diet, a shift in the opposite direction was reported (55).

To the best of our knowledge, this is the first study that assessed the effects of the restricted balanced Mediterranean diet on salt and sweet taste thresholds in patients with severe obesity. Investigating whether taste functionality in this kind of patient might be improved by dietary intervention would increase understanding of how to develop more precise taste-oriented nutritional therapy and improve the compliance of these subjects to the treatment.

A limitation of this work lies in the duration of the treatment. This study examined the effects of short-term weight loss on eating behavior and taste perception. However, future work should consider investigating the efficacy of dietary treatments of longer duration, which may lead to better results on both salty and sweet taste thresholds. In addition, even if the sample size was appropriate to observe a moderate effect size, the number of participants is still limited, and no separation by sex or age could be performed. Moreover, for simplicity and design clarity, only two taste stimuli (i.e., NaCl and sucrose) were used in the current study and presented using filter paper strips. Still, future studies should evaluate other taste stimuli (e.g., sour, bitter, and fat) and examine the effects of dietary intervention on taste perception and preferences using more ecologically relevant food stimuli (i.e., solid stimuli containing texture and smell in addition to taste). Finally, our study cannot determine whether taste perception and eating behavior in our obese subjects were different from lean subjects because a control group in the study design was not included.

5. Conclusion

In conclusion, our results showed an improvement in taste thresholds of saltiness, thanks to a re-education of the taste buds through a controlled sodium diet. This approach led to a better taste perception, improving its sensitivity by approaching normal values. It can be hypothesized that the weight loss will continue following the dietary plan after hospitalization, and taste thresholds may also improve further. This would lead to good dietary compliance over time, as the hedonistic satisfaction first sought through highly palatable foods can also be satisfied with consuming healthier foods with lower caloric density. Therefore, targeting the taste could represent a new approach to weight control to prevent the risk of therapeutic failure and identify new personalized intervention strategies.

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 humans were approved by Istituto Auxologico Italiano Ethics Committee (Approval registration number: 43C101). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

CC, SM, EP, and SB: conceptualization, methodology, and resources. CC and EP: formal analysis. CC and SM: investigation and writing—original draft preparation. CC, SM, LG, MS, EP, and SB: writing—review and editing. CC: visualization. EP and SB: supervision and project administration. SB: funding acquisition. 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.2023.1196157/full#supplementary-material

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The effects of pro-, pre-, and synbiotics supplementation on polycystic ovary syndrome: an umbrella review of meta-analyses of randomized controlled trials

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Background: Synbiotics, refer to a combination of probiotics and prebiotics in a form of synergism that beneficially affect the host's health by alternating the composition and/or function of the gut microbiota. Numerous meta-analyses of randomized clinical trials have proven that pro, pre-, and synbiotics supplementation has health outcomes in women with polycystic ovary syndrome (PCOS). However, the strength and quality of this evidence in aggregate have not yet been synthesized in great detail.

Methods: PubMed, Scopus, Web of Sciences, and Google Scholar were searched up to March 2023. We pooled the mean difference and its 95% confidence interval (CI) by applying a random-effects model.

Results: Overall, nine meta-analyses including a total of 12 trials were identified. The results of the present study indicated that probiotic supplementation significantly reduced the homeostatic model assessment for insulin resistance (HOMA-IR; WMD: −0.29, 95% CI: −0.57 to −0.02, $p = 0.03$, $n = 4$; moderate certainty) and fasting glucose concentration (FGC; WMD: −7.5 mg/dL, 95% CI: −13.60 to −0.51, $p = 0.03$; $n = 4$; low certainty). Moreover, synbiotic supplementation had beneficial effects on glycemic control, lipid profile, and hormonal parameters, but the certainty of the evidence was rated as low to very low. However, supplementation with pro−/synbiotics did not affect inflammation and oxidative stress in women with PCOS. Furthermore, waist/hip circumference, fasting glucose concentration, lipid profile, dehydroepiandrosterone sulfate, high-sensitivity C-reactive protein, and hirsutism score were significantly reduced after prebiotics supplementation with low certainty of evidence.

Conclusion: Although pro-, pre-, and synbiotics supplementation had beneficial effects on some PCOS-related outcomes, the certainty of the evidence was rated as low to very low. Therefore, further well-designed RCTs might help to confirm our findings in women with PCOS.

KEYWORDS
synbiotics, meta-analysis, probiotics, prebiotics, polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy that affects women of reproductive age, particularly in the early to late reproductive stages (15–35 years) (1, 2). As defined in 2003 by the Rotterdam Consensus Declaration, the onset of two out of these following features is a sign of PCOS: oligo or anovulation, hyperandrogenism, and polycystic ovaries (3, 4). Depending on diagnostic criteria it is estimated that between 5 and 21% of women worldwide are affected by PCOS (5). Major complications of PCOS include insulin resistance (IR), glucose intolerance, type 2 diabetes mellitus, dyslipidemia, cardiovascular disease (6), hirsutism (7), acne, alopecia (8), and high C-reactive protein (9). The financial burden of PCOS, including the costs of initial diagnosis and reproductive endocrine complications, was estimated at $ 3.7 million per year in the United States and taking into account the cost of pregnancy-related and long-term complications, it has risen to $8 million per year (10).

Multiple pathophysiological mechanisms are assumed due to the heterogeneity of the PCOS characteristics. Hyperinsulinemia and insulin resistance, exaggerated LH pulse frequency and amplitude, and enhanced ovarian or adrenal androgen production, are the main presumed causes of PCOS (11, 12).

Recent studies regarding probiotics, "live microorganisms which when administered in adequate amounts confer a health benefit on the host," demonstrated that the administration of probiotics can decrease intestinal permeability, modify the immune system of the gastrointestinal tract and prevent the growth of pathogenic bacteria (13–15). The term prebiotic is used as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (16). Short-chain fatty acids (SCFAs) from the metabolism of prebiotics, decrease inflammatory markers and subsequently reduce insulin resistance (17). The presence of a combination of living microorganisms and substrate(s) that host microorganisms use to their advantage and which benefits the host's health is called synbiotics (18). Synbiotics administration was associated with significant improvement in fasting plasma glucose (FPG), homeostatic model assessment for insulin resistance (HOMA-IR) and body mass index (BMI) (19).

A substantial number of systematic reviews and meta-analyses (SRMAs) of randomized controlled trials on the effects of pro-, pre-, and synbiotics supplementation on PCOS-related outcomes (6, 20–22) have been conducted in recent years. Regardless of the high number of SRMAs, there is still some uncertainty about the efficacy of each prebiotic, probiotics, and synbiotics supplement separately. There is also currently no available data to support the certainty of the evidence for each estimate and the amount of impact detected based on the minimal clinically important differences (MCID). Also, the strength and quality of this evidence in aggregate have not yet been synthesized in great detail. Therefore, this umbrella review aims to examine systematic reviews to determine the effectiveness of pro-, pre-, and synbiotics on hormonal parameters, glycemic control markers, blood lipids, anthropometric indices, and inflammatory and oxidative stress biomarkers in women with PCOS and update the evidence.

Methods

The current umbrella review was designed based on the protocols of the Cochrane Handbook for Systematic Reviews of Interventions on overviews of systematic reviews (23). The protocol of this umbrella review was registered in the International Prospective Register of Systematic Reviews (PROSPERO) database (https://www.crd.york.ac.uk/PROSPERO, CRD42021281029).

Search strategy

The systematic search was conducted in major databases including PubMed, Web of Science, Scopus, and Google Scholar until 22 March 2023, with no restrictions on publication time or language. Detailed information relating to the search strategy of databases as well as the medical subject headings (MeSH) and text words in our search strategy to identify relevant studies are provided in Supplementary Table 1. We also added other literature that was found by manually reviewing related published SRMAs of RCTs evaluating the effects of pro-, pre-, and synbiotics supplementation in women with PCOS. Moreover, the references list of any related meta-analyses was manually reviewed to collect further eligible studies.

Eligibility and study selection

Relevant studies were selected based on the PICOS (population/intervention/comparison/outcome) framework: P (women with polycystic ovary syndrome), I (pro-, pre- and synbiotics supplementation), C (placebo), O (PCOS-related outcomes), and study design (SRMAs of RCTs). Two authors (ST and NP) independently selected meta-analyses in this umbrella review if they met the following criteria: (1) SRMAs of RCTs that were conducted in the people of any age with a diagnosis of polycystic ovary syndrome; (2) received at least one oral probiotic, prebiotic, or synbiotics supplementation compared to a control group; (3) reported weighted

Abbreviations: PCOS, Polycystic ovary syndrome; GRADE, Grading of recommendations assessment development and evaluations; CI, Confidence interval; WMD, Weighted mean differences; RCTs, Randomized clinical trials; IR, Insulin resistance; SCFAs, Short-chain fatty acids; FPG, Fasting plasma glucose; HOMA-IR, Homeostatic model assessment for insulin resistance; BMI, Body mass index; SRMAs, Systematic reviews and meta-analyses; MCID, Minimal clinically important differences; TC, Total cholesterol; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; VLDL-C, Very low-density lipoprotein cholesterol; TG, Triglyceride; WC, Waist circumference; TAC, Total antioxidant capacity; GSH, Glutathione; MDA, Malondialdehyde; NO, Nitric oxide; hs-CRP, High-sensitivity c-reactive protein.

or standardized mean differences (MDs) along with 95% confidence intervals (CIs); (4) reported at least one potential outcomes in published SRMAs of RCTs including hormonal parameters [dehydroepiandrosterone (DHEA), total testosterone (TT), and sex hormone-binding globulin (SHBG)], hirsutism score, fasting glucose concentration (FGC levels), markers for insulin (fasting insulin levels, HOMA-IR, and QUICKI), blood lipids [total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and triglyceride (TG) levels], anthropometric indices (body weight, BMI, and waist circumference), inflammatory- and oxidative stress biomarkers [total antioxidant capacity (TAC), glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO), and high-sensitivity c-reactive protein (hs-CRP)]. We excluded studies with insufficient data and other study designs. We also excluded primary trials in the meta-analysis if they: (1) were trials without a control group; (2) used pro-, pre-, and synbiotics supplementation in combination with other nutrients. If more than one published meta-analysis for a given outcome was available, we selected only the publication with the higher number of primary trials (24). Also, we have manually reviewed the reference lists of other meta-analyses to identify additional relevant trials.

Data extraction

NP extracted the following data from eligible meta-analyses using a pre-designed abstraction form: first author's name, country, publication year, number of primary studies, and participant number. Furthermore, for each primary RCTs from included meta-analyses, we also extracted the following required data: First author, country, publication year, effect size, participant number, duration of intervention, and the dose of supplementation.

Assessment of methodological quality

A Measurement Tool to Assess Systematic Reviews (AMSTAR-2) scale (25) was used to evaluate the methodological quality of included meta-analysis by two independent researchers (ST and SZM). Disagreements were resolved by consensus with the third researcher (SSH). We also carried out the quality of primary trials including each eligible meta-analysis using the Cochrane risk-of-bias tool for randomized trials (RoB) (26). According to this systematic bias assessment, the overall quality of primary studies was scored as good, fair, or weak (Supplementary Table 2).

The AMSTAR 2 tool (25) was applied to assess the quality of conduct of the included meta-analyses of randomized controlled trials. Instrument (AMSTAR 2) retains 10 of the original domains, and has 16 items in total.

Data synthesis and statistical analysis

For each health outcome, the largest meta-analysis with a maximum number of RCTs was selected, as well as primary trials that were ignored in the biggest meta-analyses were also added (Table 1). Then, we recalculated the MD and its 95% CI by applying a random-effects model in each meta-analysis that was included in our umbrella review (27). To evaluate the possibility of publication bias, we used Egger's test method (28). Heterogeneity across studies was estimated by Cochran Q and I^2 statistics, in which I^2 values greater than 50% or $p < 0.05$ were considered as significant (29). Statistical analyses were conducted using STATA version 14 software (Stata Corp, College Station, Texas, United States).

Grading of the evidence

The certainty of the evidence was rated according to the Grading of Recommendations Assessment, Development and Evaluations (GRADE) (30). The GRADE consists of five domains: risk of bias in the individual studies, inconsistency, indirectness, imprecision, and publication bias. As a result, high, medium, low, or very low-GRADE ratings were considered for the certainty of evidence. The MCID for the estimations was determined using previous data in the literature, and in the absence of sufficient evidence, we used half of the baseline SDs for that outcome (31). Supplementary Table 3 demonstrates the MCID values utilized in the current umbrella review.

Results

Literature search

We identified a total of 91 meta-analyses studies through initial electronic searches. After removing 17 duplicated studies, 62 publications were assessed based on reviewing titles and abstracts. Of those, 12 records remained for full-text revision. Among them, three articles were excluded due to the full text being unavailable (32) and performed on other patients (33, 34). Overall, nine meta-analyses were finally included in this umbrella review. The flow diagram of the study selection process is illustrated in Figure 1. Through the screening primary studies of included meta-analyses, five RCTs were excluded for either of the following reasons: full text being unavailable ($n = 2$) (35, 36) and using probiotics in combination with other interventions ($n = 3$) (37–39). Detailed reasons for the exclusion of primary trials by full-text assessing are provided in Supplementary Table 4. Overall, nine meta-analyses (6, 20–22, 40–44) reporting 12 RCTs (45–55) met the eligibility criteria for the final analysis in this umbrella review.

Study characteristics (Description of original RCTs)

Of the 12 primary trials included in this review, four studies with six arms used synbiotics (21, 46, 52), two trials used prebiotics (53, 54), and the remaining used probiotics (45, 47–49, 51, 55). Seven trials were double-blind (45, 47–50, 52, 55) and four trials were triple-blind placebo-controlled trials (46, 53, 54), while one trial was a single-blinded clinical trial (51). Included trials were published between 2017 and 2021. All primary studies were conducted in Iran (45–50, 52–55) and Egypt (51). The follow-up duration among primary studies varied between 8 and 12 weeks and the dosage of probiotic or synbiotic supplementation ranged from 2×10^8 to 3×10^{10} CFU/day. Characteristics of eligible primary studies are illustrated in Table 2.

TABLE 1 General characteristics of the published meta-analyses investigating the effects of pro-pre/synbiotic supplementation in patients with polycystic ovary syndrome.

Author, year	No. of primary trials	Number of primary trials included from other meta-analyses	Types of supplementation	Outcome	Sample	Dose (range, mg)	Follow-up (range, weeks)	ES	Effect size (95%CI)	*p* value	I^2 (%)	*p* heterogeneity
					Size							
(6)	12	0	Probiotics	Body weight	731	$\geq 2\times 10^8$ CFU	8–24 weeks	SMD	−0.02 (−0.36,0.31)	0.892	66.20%	0.007
			Synbiotic			$<2\times 10^8$ CFU			−0.12 (−0.49,0.25)	0.534	53.50%	0.009
			Prebiotics						−0.61 (−1.12,−0.10)	0.019	NA	NA
(6)	13	1	Probiotics	BMI	791	$\geq 2\times 10^8$ CFU	8–24 weeks	SMD	−0.03 (−0.24,0.19)	0.823	31.80%	0.174
			Prebiotics			$<2\times 10^8$ CFU			−0.13 (−0.53,0.26)	0.508	58.90%	0.063
			Synbiotic						−0.66 (−1.17,-0.15)	0.012	NA	NA
(6)	5	0	Probiotics	WC	316	$\geq 2\times 10^8$ CFU	8–24 weeks	SMD	Overall	Overall	Overall	Overall
			Prebiotics			$<2\times 10^8$ CFU			0.37 (−0.78,1.53)	0.052	95.50%	0
			Synbiotic									
(6)	4	-	Probiotics	HC	256	$\geq 2\times 10^8$ CFU	8–24 weeks	SMD	Overall	Overall	Overall	Overall
			Prebiotics			$<2\times 10^8$ CFU			−0.25 (−0.78,0.27)	0.34	76.9	0.005
			Synbiotic									
(19)	3	0	Probiotics	Ferriman–Gallway score	855	$\geq 2\times 10^9$ CFU	8–12 weeks	SMD	0.15 (−0.21,−0.51)	0.07	0	0.41
			Prebiotics			$<2\times 10^9$ CFU			−0.56 (−1.07,−0.06)		-	-
			Synbiotic						−0.23 (−0.74,0.28)		-	-
(6)	8	0	Probiotics	FGC	496	$\geq 2\times 10^8$ CFU	8–24 weeks	SMD	−0.96 (−1.86,−0.07)	0	90.50%	0.03
			Prebiotics			$<2\times 10^8$C			−6.98 (−8.32,−5.63)	-	NA	0
			Synbiotic						−0.36 (−0.87,0.15)	0.04	67.70%	0.16
(6)	7	0	Probiotics	HOMA-IR	434	$\geq 2\times 10^8$ CFU	8–24 weeks	SMD	−0.74 (−1.25,−0.23)	0.005	73.10%	0.011
			Synbiotic			$<2\times 10^8$ CFU			−0.74 (−1.59,0.11)	0.08	87.20%	0
(6)	6	0	Probiotics	Insulin-sensitivity check index	379	$\geq 2\times 10^8$ CFU	8–24 weeks	SMD	3.65 (0.71,6.58)	0.015	98.10%	0
			Synbiotic			$<2\times 10^8$ CFU			0.92 (−0.12,1.96)	0.084	91.00%	0
(6)	7	0	Probiotics	FINS	434	$\geq 2\times 10^8$ CFU	8–24 weeks	SMD	−0.70 (−1.13,-0.26)	0.002	63.60%	0.041
			Synbiotic			$<2\times 10^8$ CFU			−0.67 (−1.54,0.20)	0.13	87.90%	0
(6)	7	1	Probiotics	TG	428 + 118	$\geq 2\times 10^8$ CFU	8–12 weeks	SMD	−0.50 (−0.80,−0.20)	0.001	0.00%	0.92
			Prebiotics			$<2\times 10^8$ CFU			−4.41 (−5.35,−3.48)	0	NA	NA
			Synbiotic						−0.14 (−0.47,0.20)	0.42	25.20%	0.26

(Continued)

TABLE 1 (Continued)

Author, year	No. of primary trials	Number of primary trials included from other meta-analyses	Types of supplementation	Outcome	Sample Size	Dose (range, mg)	Follow-up (range, weeks)	ES	Effect size (95%CI)	*p* value	I^2 (%)	*p* heterogeneity
(6)	7	1	Probiotics	TC	428 + 118	≥2 × 10^8 CFU	8–12 weeks	SMD	−0.26 (−0.85,0.32)	0.4	0.00%	0.43
			Prebiotics			<2 × 10^8 CFU			−7.52 (−8.95,−6.08)	0	NA	NA
			Synbiotic						−0.28 (−0.56,0.01)	0.12	0.00%	0.5
(6)	7	1	Probiotics	HDL-c	428 + 118	≥2 × 10^8 CFU	8–12 weeks	SMD	−0.17 (−0.98,0.63)	0.67	85.90%	0.001
			Prebiotics			<2 × 10^8 CFU			4.28 (3.37,5.20)	0	NA	NA
			Synbiotic						0.09 (−0.48,0.65)	0.76	72.70%	0.026
(6)	7	1	Probiotics	LDL-c	428 + 118	≥2 × 10^8 CFU	8–12 weeks	SMD	−0.13 (−0.42,0.17)	0.4	0.00%	0.43
			Prebiotics			<2 × 10^8 CFU			−5.57 (−6.69,-4.46)	0	NA	NA
			Synbiotic						−0.22 (−0.51,0.06)	0.12	0.00%	0.5
(6)	4	1	Probiotics	VLDL-c	235 + 118	≥2 × 10^8 CFU	8–12 weeks	SMD	−0.48 (−0.78,−0.18)	0.002	0.00%	0.95
			Synbiotic			<2 × 10^8 CFU			−0.32 (−0.83,0.19)	0.21	NA	NA
(6)	9	1	Probiotics	CRP	558 + 118	≥2 × 10^8 CFU	8–12 weeks	SMD	Overall	Overall	Overall	Overall
			Synbiotic			<2 × 10^8 CFU			−0.63 (−1.37,0.10)	0.089	93.90%	0
			Prebiotics									
(22)	4	0	Probiotics	NO	240	≥2 × 10^8 CFU	8–12 weeks	SMD	Overall	Overall	Overall	Overall
			Synbiotic			<2 × 10^8 CFU			0.33 (0.08, 0.59)	0.01	0.00%	0.39
(22)	4	1	Probiotics	TAC	240 + 86	≥2 × 10^8 CFU	8–12 weeks	SMD	Overall	Overall	Overall	Overall
			Synbiotic			<2 × 10^8 CFU			0.64 (0.38,0.90)	<0.001	0.00%	0.58
(22)	4	0	Probiotics	GSH	240	≥2 × 10^8 CFU	8–12 weeks	SMD	Overall	Overall	Overall	Overall
			Synbiotic			<2 × 10^8 CFU			0.26 (0.01,0.52)	0.04	0.00%	0.57
(22)	4	1	Probiotics	MDA	240 + 86	≥2 × 10^8 CFU	8–12 weeks	SMD	Overall	Overall	Overall	Overall
			Synbiotic			<2 × 10^8 CFU			−0.90 (−1.16,−0.63)	<0.001	0.00%	0.63
(22)	6	1	Probiotics	TT	326	≥2 × 10^8 CFU	8–12 weeks	SMD	Overall	Overall	Overall	Overall
			Synbiotic			<2 × 10^8 CFU			−0.58 (−0.82,−0.34)	<0.001	10.40%	0.34
(58)	3	1	Probiotics	DHEAS	182 + 62	≥2 × 10^8 CFU	8–12 weeks	SMD	0.00 (−0.51,0.51)	1	Overall	Overall
			Synbiotic			<2 × 10^8 CFU			−0.31 (−0.82,0.20)	0.24	0.00%	0.57
			Prebiotics						−0.36 (−0.86,0.14)	0.16		

(Continued)

TABLE 1 (Continued)

Author, year	No. of primary trials	Number of primary trials included from other meta-analyses	Types of supplementation	Outcome	Sample Size	Dose (range, mg)	Follow-up (range, weeks)	ES	Effect size (95%CI)	p value	I^2 (%)	p heterogeneity
(22)	4	0	Probiotics	SHBG	240	≥2 × 10^8 CFU	8–12 weeks	SMD	Overall	Overall	Overall	Overall
			Synbiotic			<2 × 10^8 CFU			0.46 (0.08,0.85)	0.01	55.70%	0.08

Overall: synbiotic, prebiotic, probiotic supplementation. BMI, Body mass index; CI, Confidence interval; DHEAS, Dehydroepiandrosterone sulfate; FGC, Fasting glucose concentration; FI, Fasting insulin; GSH; Glutathione; HOMA-IR, Homeostasis model assessment-estimated insulin resistance; HDL, High density lipoprotein; hs-CRP, High sensitive c-reactive protein; LDL, Low density lipoprotein; MDA, Malondialdehyde; NO, Nitric oxide; QUICKI, Quantitative insulin sensitivity check index; SHBG, Sex hormone binding globulin; TC, Total cholesterol; TG, Triglycerides; TAC, Total antioxidant capacity; TT, Total testosterone; VLDL, Very low density lipoprotein; WC, Waist circumference; and wk, Week.

Methodological quality

According to AMSTAR 2 scores, two meta-analyses were classified as high-quality studies (6, 42), four meta-analyses were performed with a low-quality method (21, 22, 40, 44), and the other three meta-analyses were performed with a critically low-quality method (20, 41, 43). Detailed AMSTAR scores for each meta-analysis are presented in Supplementary Table 5.

Findings from the meta-analysis

Probiotic supplementation in patients with PCOS

Six primary trials from nine systematic reviews and meta-analyses evaluated the impact of probiotic supplementation in patients with PCOS. We found moderate-certainty evidence that probiotic supplementation significantly reduced HOMA-IR compared to the control group (WMD: −0.29, 95% CI: −0.57 to −0.02, $p = 0.03$) with no significant between-study heterogeneity ($I^2 = 33.8\%$, $p = 0.20$). There was also low certainty of evidence that probiotic supplementation had a significant effect on FGC (WMD: −7.5 mg/dL, 95% CI: −13.60 to −0.51, $p = 0.03$), VLDL-C (WMD: −50.40 mg/dL, 95% CI: −9.91 to −0.89, $p = 0.01$), WC (WMD: 0.86 cm, 95% CI: 0.38–1.33, $p < 0.001$), TT (WMD: −0.40 ng/mL, 95% CI: −0.73 to −0.07, $p = 0.017$), SHBG level (WMD: 25.40 nmol/L, 95% CI: 12.50–38.30, $p < 0.001$), TAC (WMD: 107.10 mmol/L, 95% CI: 8.95–1.61, $p < 0.001$), MDA (WMD: 1.10 µmol/L, 95% CI: 0.59–1.61, $p < 0.001$), and hirsutism score (WMD: -1.50, 95% CI: −2.50 to −0.85, $p < 0.001$). However, supplementation with probiotics had no significant effects on other outcomes (Table 3). The results of GRADE are described in Supplementary Table 6. We could not perform subgroup analyses due to the small number of primary studies.

Synbiotics supplementation in patients with PCOS

Overall, four primary clinical trials with six arms from nine meta-analyses were included in the analyses to evaluate the effects of synbiotics supplementation in women with PCOS. There was low certainty of evidence that synbiotic supplementation had a significant reduction in WC (WMD: −2.70 cm, 95% CI: −4.28 to −1.12, $p = 0.001$), fasting insulin (SMD: −0.90, 95% CI: −1.24 to −0.57, $p < 0.001$), HOMA-IR (WMD: −0.82, 95% CI: −1.09 to −0.56, $p < 0.001$), VLDL-C (WMD: −4.40 mg/dL, 95% CI: −7.19 to −1.61, $p = 0.002$), TC (WMD: −10.57 mg/dL, 95% CI: −20.83 to −0.31, $p = 0.04$), LDL-C (WMD: −21.58 mg/dL, 95% CI: −41.62 to −1.53, $p = 0.03$), TT (WMD: −0.13 ng/mL, 95% CI: −0.18 to −0.09, $p < 0.001$), and hirsutism score (WMD: −1.20, 95% CI: −2.11 to −0.29, $p = 0.01$). We also observed that synbiotics supplementation significantly increased SHBG (WMD: 19.30 nmol/L, 95% CI: 2.26–36.34, $p = 0.02$) compared to the placebo with low certainty of evidence. Moreover, pooled analysis suggested the significant effect of synbiotics consumption on QUICKI (WMD: 0.01, 95% CI: 0.00–0.01, $p = 0.03$), and TG (WMD: −15.37 mg/dL, 95% CI: −22.53 to −8.21, $p = 0.001$), but the certainty of the evidence was rated as very low. Intake of synbiotics supplementation had no significant effect on other outcomes in women with PCOS (Table 4). Detailed GRADE scores for each outcome are shown in Supplementary Table 7.

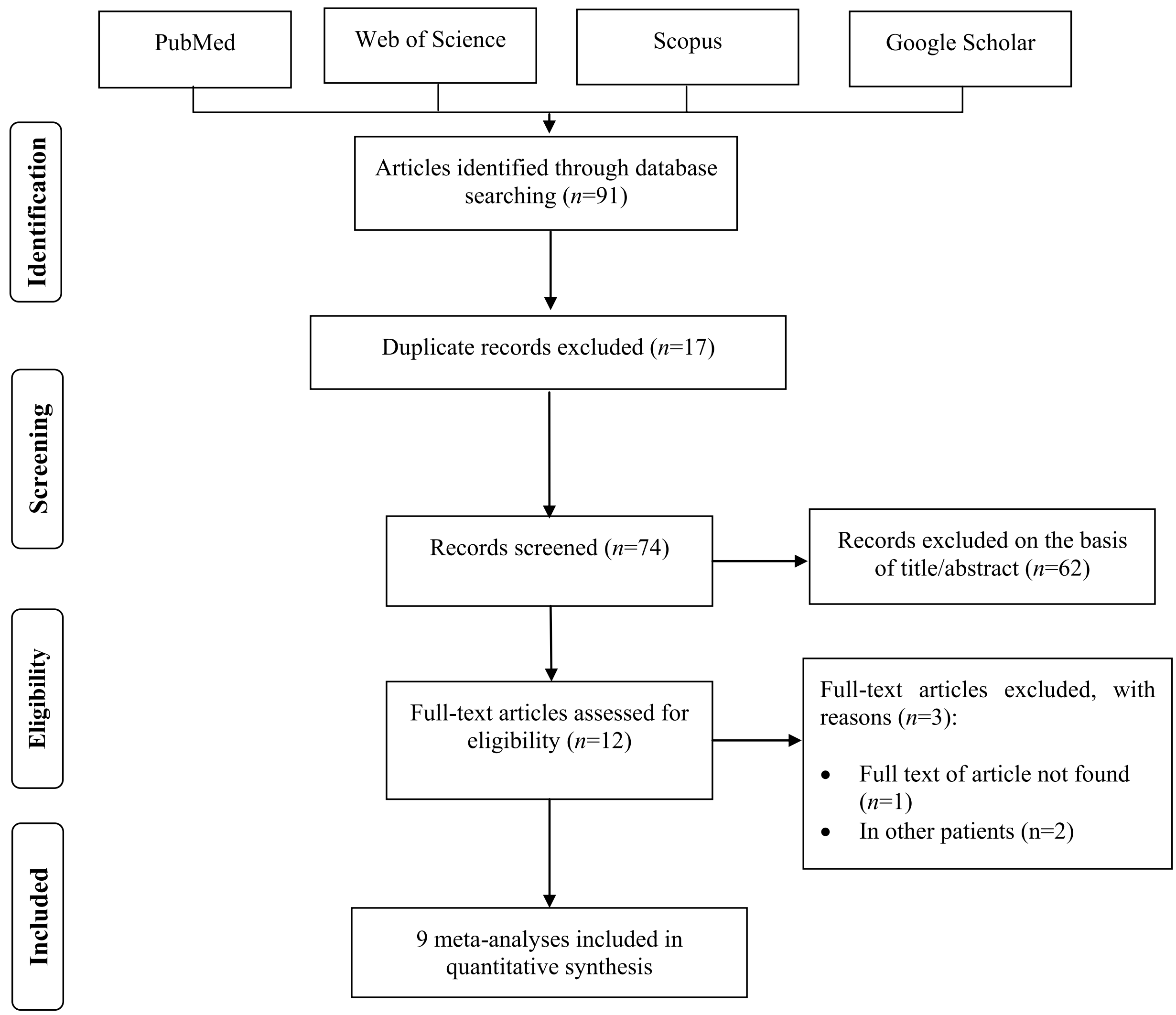

FIGURE 1
Literature search and review flow diagram for selection of umbrella review meta-analyses.

Prebiotic supplementation in patients with PCOS

The effect of prebiotic supplementation in women with PCOS was examined in two primary studies from two meta-analyses. There was low certainty of evidence that supplementation with prebiotics significantly reduced WC (WMD: −5.10 cm, 95% CI: −8.60 to −1.60, $p = 0.004$), hip circumference (HC; WMD: −4.60 cm, 95% CI: −7.47 to −1.73, $p = 0.002$), FGC (WMD: −15.14 mg/dL, 95% CI: −20.38 to −9.90, $p = 0.003$), TG (WMD: −31.12 mg/dL, 95% CI: −49.63 to −12.61, $p = 0.06$), TC (WMD: −34.83 mg/dL, 95% CI: −52.47 to −17.19, $p < 0.001$) LDL-C (WMD: −37.65 mg/dL, 95% CI: −52.09 to −22.69, $p < 0.001$), DHEA-S (WMD: −0.84 μg/mL, 95% CI: −1.52 to −0.16, $p = 0.01$), hs-CRP (WMD: −1.94 mg/L, 95% CI: −3.27 to −0.61, $p = 0.00$), and hirsutism score (WMD: -1.68, 95% CI: −3.19 to −0.17, $p = 0.02$). However, prebiotic supplementation did not have a significant effect on other outcomes in women with PCOS (Table 5). Detailed GRADE evidence for prebiotic supplementation in patients with PCOS was presented in Supplementary Table 8.

Publication bias

We found statistically significant publication bias regarding the levels of HDL-C (Egger's = 0.01) following intake of probiotic supplementation, and the levels of FGC after supplementation with synbiotics (Egger's = 0.04). Therefore, we did the trim-and-fill method to detect sources of bias and found results similar to the original. No evidence of publication bias based on Egger's tests was observed in other outcomes (Tables 3–5).

Discussion

The present work was performed on meta-analyses of RCTs to comprehensively assess the effects of pro-, pre-, and synbiotics supplementation on PCOS-related outcomes. We evaluated the evidence using the well-known GRADE tool and to provide better comparisons between outcomes, the available data were reanalyzed using random effects analysis. Our findings are important because there is limited evidence-based support for use of pro-, pre-, and synbiotics supplements in the management of PCOS-related outcomes.

The results of the present study showed probiotic supplementation significantly reduced HOMA-IR, FGC, and VLDL. In addition, synbiotics supplementation was found to have beneficial effects in the reduction of WC, fasting insulin, HOMA-IR, TG, VLDL, TC, LDL-c,

TABLE 2 Characteristics of eligible primary studies on the effects of pro-pre/synbiotic supplementation in patients with polycystic ovary syndrome.

First author (Country; year)	RCT design (Blinding)	Supplementation	Strains	Mean age (year)	Mean BMI (kg/m²)	Sample size (Supplementation/Placebo)	Duration (weeks)	Intervention		Outcomes
								Treatment group	Control group	
(59)	Parallel (Double)	Probiotic	*Lactobacillus acidophilus, Lactobacillus casei*, and *Bifidobacterium bifidum*	25	25	60 (30/30)	12	2×10^9 CFU	Placebo (ND)	FGC, TC, LDL, HDL, TG, Insulin, HOMA-IR, QUICKI, Weight, and BMI
(60)	Parallel (Double)	Synbiotic	*Lactobacillus casei, Lactobacillus ramnosousa, Lactobacillus plantroum*, and *Bacillus koagolans*, indicousa	30	26	56 (23/23)	8	2×10^8 CFU	Placebo (water + pomegranate flavoring)	FGC, Insulin, HOMA-IR, QUICKI, Weight, BMI, Testosterone, LH, and FSH
(61)	Parallel (Double)	Probiotic	*Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus fermentum,* and *Lactobacillus gasseri*	30	26	60 (30/30)	12	2×10^9 CFU	Placebo (ND)	Weight, BMI, HsCRP, and WC
(62)	Parallel (Double)	Probiotic	*Lactobacillus acidophilus, Lactobacillus casei, and Bifidobacterium bifidum*	27	23	60 (30/30)	12	2×10^9 CFU	Placebo (starch)	Testosterone, SHBG, DHEA, HsCRP, NO, TAC, GSH, MDA, NO, and mF-G
(63)	Parallel (Double)	Synbiotic	*Lactobacillus acidophilus* 3×10^{10} CFU/g, *Lactobacillus casei* 3×10^9 CFU/g, *Lactobacillus bulgaricus* 5×10^8 CFU/g, *Lactobacillus rhamnosus* 7×10^9 CFU/g, *Bifidobacterium longum* 1×10^9 CFU/g, *Bifidobacterium breve* 2×10^{10} CFU/g, *Streptococcus thermophilus* 3×10^8 CFU/g, and prebiotic Inulin (fructooligosaccharide)	28	32	99 (50/49)	12	500 mg	Placebo (starch)	FGC, Cho, LDL, HDL, TG, BP, Weight, BMI, and WHR

(Continued)

10.3389/fnut.2023.1178842

TABLE 2 (Continued)

First author (Country; year)	RCT design (Blinding)	Supplementation	Strains	Mean age (year)	Mean BMI (kg/m^2)	Sample size (Supplementation/Placebo)	Duration (weeks)	Intervention		Outcomes
								Treatment group	Control group	
(64)	Parallel (Double)	Synbiotic	*Lactobacillus acidophilus* 2×10^9 CFU/g, *Lactobacillus casei* 2×10^9 CFU/g, and *Bifidobacterium bifidum* 2×10^9 CFU/g plus 0.8 g inulin	25	27	60 (30/30)	12	2×10^9 CFU	Placebo (ND)	Testosterone, SHBG, DHEA, HsCRP, NO, TAC, GSH, MDA, NO, mF-G, Weight, and BMI
(65)	Parallel (Double)	Probiotic	Lactobacillus delbruekii, *Lactobacillus fermentum*	30	34	60 (30/30)	12	1×10^9 CFU	ND	FGC, Cho, LDL, HDL, TG, Insulin, HOMA-IR, HsCRP, Weight, and BMI
(66)	Parallel (Double)	Synbiotic	*Lactobacillus acidophilus* 2×10^9 CFU/g, *Lactobacillus casei* 2×10^9 CFU/g, *Bifidobacterium bifidum* 2×10^9 CFU/g plus 0.8 g inulin	27	27	60 (30/30)	12	2×10^9 CFU	Placebo (starch)	FGC, TC, LDL, HDL, TG, Insulin, HOMA-IR, QUICKI, Weight, and BMI
(67)	Parallel (Double)	Prebiotic	20 g of resistant Dextrin	31	25	62 (31/31)	12	20 g	Placebo (Maltodextrin)	Weight, BMI, and WC
(56)	Parallel (Double)	Probiotic	*Lactobacillus casei* 7×10^9 CFU/g, *Lactobacillus acidophilus* 2×10^9 CFU/g, *Lactobacillus rhamnosus* 1.5×10^9 CFU/g, *Lactobacillus bulgaricus* 2×10^8 CFU/g, *Bifidobacterium breve* 2×10^{10} CFU/g, *Bifidobacterium longum* 7×10^9 CFU/g, and *Streptococcus thermophiles* 1.5×10^9 CFU/g	25	25	72 (36/35)	8	500 mg	Placebo (Maltodextrin)	FGC, Insulin, HOMA-IR, and CRP

(Continued)

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TABLE 2 (Continued)

First author (Country; year)	RCT design (Blinding)	Supplementation	Strains	Mean age (year)	Mean BMI (kg/m^2)	Sample size (Supplementation/ Placebo)	Duration (weeks)	Intervention		Outcomes
								Treatment group	Control group	
(68)	Parallel (Double)	Prebiotic	20g of resistant Dextrin	31	25	62 (31/31)	12	20 g	Placebo (Maltodextrin)	TC, LDL, HDL, TG, HsCRP, Testosterone, and DHEA
(47)	Parallel (Double)	Synbiotic	*Lactobacillus casei, Lactobacillus ramnosousa,* and *Lactobacillus plantroum,* and *Bacillus koagolans,* indicousa	30	26	56 (23/23)	8	2×10^8 CFU	Placebo (water + pomegranate flavoring)	TC, LDL, HDL, TG, HsCRP, TAC, MDA, and BP

BMI, Body mass index; FGC, Fasting glucose concentration; HOMA-IR, Homeostatic model assessment of insulin resistance; QUICKI, Quantitative insulin sensitivity check index; LH, Luteinizing hormone; FSH, Follicle stimulating hormone; BP, Blood pressure; MDA, Malondialdehyde; TAC, Total antioxidant capacity; Hs-CRP, High sensitive C-reactive protein; TG, Triglyceride; TC, Total cholesterol; LDL, Low-density lipoprotein; HDL, High-density lipoprotein; GSH, Glutathione; NO, nitric oxide; DHEA, Dehydroepiandrosterone; and SHBG, Sex hormone binding globulin.

TT, and hirsutism score. Moreover, we found prebiotic supplementation significantly reduced WC, HC, FGC, TG, TC, LDL-c, dehydroepiandrosterone sulfate, hs-CRP, and hirsutism score. In contrast, our study showed that probiotic supplementation significantly increased WC, SHBG, TAC, and MDA parameters. It was also found synbiotics supplementation significantly increased SHBG and QUICKI. These findings should, however, be interpreted with some caution due to the following reasons: Firstly, almost all of the significant findings in the analyses received low and very low-quality evidence based on the GRADE tool. Only moderate quality of evidence was found for the effects of probiotics on the HOMA-IR index. None of the included meta-analyses considered this critical point and their findings were judged based on statistical differences. The included meta-analyses in this umbrella review were also evaluated for methodological accuracy using the AMSTAR tool. According to this method, three meta-analyses showed critically low quality, three showed low quality, and two showed high quality. The meta-analyses were rated as low and critically low-quality methods because did not register the protocol of the meta-analysis, had no comprehensive search strategies, did not report the reasons for excluded studies, and did not discuss the possible risk of bias in primary studies. Secondly, most of the analyses were performed on limited number of studies (≤5) with less than 12 months of follow up duration. It is interesting that for some outcomes only one RCT was available, so the results seem unreliable. Thirdly, our results showed high evidence of statistical heterogeneity between the studies in some analyses which weakens the clinical certainty of the results (56, 57). Unfortunately, a low number of primary RCTs made it impossible to conduct subgroup analyses, so we were unable to find sources of heterogeneity between studies ($n < 10$). Fourthly, the effects of an intervention on selected outcomes are not solely based on statistical significance but should also be judged on clinical relevance. For example, the results of the current umbrella review showed inconsistent findings regarding the potential effects of pro-, pre-, and synbiotics supplementation on WC in patients with PCOS. Accordingly, probiotic supplementation slightly, but not clinically important, increased WC (0.86 cm) compared to the control group. In contrast, synbiotics and prebiotic supplementation decreased WC by nearly −2.7 and −5.10 cm, respectively. Of course, these findings with low-quality evidence were obtained from data from only two trials for probiotics and one trial for synbiotics and prebiotics. Also, possible explanations for this inconsistency might be the short duration of the interventions. It is recommended that extend the treatment period for central obesity beyond 12 weeks (70, 71). Fifthly, it is imperative to consider strain-specific efficacy when using probiotics or symbiotics in the treatment or prevention of disease. The efficacy of potential probiotic strains varies according to experimental studies (72). As a result, it is important to determine whether the microbes can survive from ingestion to delivery to the target organ, whether the microbes are capable of interfering with pathogenesis (usually using animal models of disease), and whether they can be sustained from ingestion to administration (73). Interestingly, among 127 studied Lactobacillus strains, only 3% were found to be capable of being used as probiotics due to their ability to survive in the target organ and to withstand bile and stomach acidity (74). In addition, over 170 Lactobacillus species were examined in depth, revealing significant differences in resistance to antibiotics and probiotic potential (75). A probiotic strain's presence or absence of the

TABLE 3 The effects of probiotic supplementation in women with PCOS.

Outcomes (unit)	Number of trials (arms)	Number of participants	Follow-up (range), wk	Dose (range), CFU	Effect size (95% CI)	*p* value	I^2 (%)	$p_{heterogeneity}$	Egger's test	Certainty of evidence (GRADE)[1]
Body weight (kg)	4	309	12	$1–3\times10^9$	0.25 (−1.37, 1.88)	0.759	97.1	<0.001	0.500	Low
BMI (kg/m²)	5	409	12	$1\times10^9–2\times10^{12}$	0.44 (−0.23, 1.12)	0.199	94.3	<0.001	0.264	Low
Waist circumference (cm)	2	189	12	$1\times10^9–3\times10^{10}$	0.86 (0.38, 1.33)	<0.001	0.0	0.496	-	Low
Hip circumference (cm)	1	99	12	3×10^{10}	−0.60 (−1.09, 2.29)	0.487	-	-	-	Low
Fasting glucose concentration (mg/dL)	4	331	8–12	$2\times10^9–3\times10^{10}$	−7.05 (−13.60, −0.51)	0.035	93.7	<0.001	0.781	Low
Fasting insulin	4	331	8–12	$2\times10^9–3\times10^{10}$	−0.40 (−0.94, 0.15)	0.152	82.6	0.01	0.098	Low
HOMA-IR	4	331	8–12	$2\times10^9–3\times10^{10}$	−0.29 (−0.57, −0.02)	0.037	33.8	0.209	0.536	Moderate
QUICKI	3	231	8–12	$2–7\times10^9$	0.01 (−0.0, 0.01)	0.240	62.0	0.072	0.627	Low
Triglycerides (mg/dL)	3	259	12	$2\times10^9–2\times10^{12}$	−39.51 (−95.42, 16.40)	0.166	97.5	<0.001	0.224	Low
Very low-density lipoprotein (mg/dL)	1	60	12	2×10^9	−50.40 (−9.91, −0.89)	0.019	-	-	-	Low
Total cholesterol (mg/dL)	3	259	12	$2\times10^9–2\times10^{12}$	−4.29 (−19.62, 11.04)	0.584	89.4	<0.001	0.428	Low
HDL cholesterol (mg/dL)	3	259	12	$2\times10^9–2\times10^{12}$	6.20 (−5.38, 17.77)	0.294	53.5	0.117	0.013	Low
LDL cholesterol (mg/dL)	3	259	12	$2\times10^9–2\times10^{12}$	−3.80 (−8.93, 1.32)	0.146	99.0	<0.001	0.874	Low
Total testosterone (ng/mL)	1	60	12	2×10^9	−0.40 (−0.73, −0.07)	0.017	-	-	-	Low
Dehydroepiandrosterone sulfate (μg/mL)	1	60	12	2×10^9	0.17 (−0.01, 0.35)	0.063	-	-	-	Low
Sex hormone-binding globulin (nmol/L)	1	60	12	2×10^9	25.40 (12.50, 38.30)	<0.001	-	-	-	Low
C-reactive protein (mg/L)	2	171	8–12	$7\times10^9–3\times10^{10}$	0.92 (−0.57, 2.40)	0.226	73.3	0.053	-	Very low
high-sensitivity C-reactive protein (mg/L)	3	250	12	$1\times10^9–2\times10^{12}$	0.50 (−1.92, 2.93)	0.684	99.1	<0.001	0.307	Low
Nitric oxide (μmol/L)	1	60	12	2×10^9	1.80 (−1.49, 5.09)	0.284	-	-	-	Low
Total antioxidant capacity (mmol/L)	1	60	12	2×10^9	107.10 (8.95, 205.25)	0.032	-	-	-	Low
Glutathione (GSH; μmol/L)	1	60	12	2×10^9	70.80 (−5.39, 146.99)	0.069	-	-	-	Low
Malondialdehyde (μmol/L)	1	60	12	2×10^9	1.10 (0.59, 1.61)	<0.001	-	-	-	Low
Hirsutism score	1	60	12	2×10^9	−1.50 (−2.15, −0.85)	<0.001	-	-	-	Low

[1]GRADE, Grading of recommendations assessment, development, and evaluation.
BMI, Body mass index; CFU, Colony-forming unit; CI, confidence interval; and wk, week.

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TABLE 4 The effects of synbiotic supplementation in women with PCOS.

Outcomes (unit)	Number of trials (arms)	Number of participants	Follow-up (range), wk	Dose (range), mg/d	Effect size (95% CI)	*p* value	I^2 (%)	$p_{heterogeneity}$	Egger's test	Certainty of evidence (GRADE)[1]
Body weight (kg)	3 (4)	304	8–12	2×10^9–2×10^8	−0.19 (−0.79, 0.42)	0.546	40.2	0.170	0.131	Moderate
BMI (kg/m²)	3 (4)	304	8–12	2×10^9–2×10^8	−0.06 (−0.33, 0.21)	0.668	56.8	0.074	0.070	Low
Waist circumference (cm)	1 (2)	184	8	2×10^8	−2.70 (−4.28, −1.12)	0.001	0.0	0.747	-	Low
Hip circumference (cm)	1 (2)	184	8	2×10^8	−0.03 (−1.75, 1.69)	0.970	0.0	0.964	-	Low
Fasting glucose concentration (mg/dL)	2 (3)	244	8–12	2×10^8–2×10^9	−1.94 (−3.95, 0.08)	0.060	0.0	0.722	0.040	Low
Fasting insulin	2 (3)	244	8–12	2×10^8–2×10^9	−0.90 (−1.24, −0.57)	<0.001	0.0	0.479	0.644	Low
HOMA-IR	2 (3)	244	8–12	2×10^8–2×10^9	−0.82 (−1.09, −0.56)	<0.001	0.0	0.438	0.717	Low
QUICKI	2 (3)	244	8–12	2×10^8–2×10^9	0.01 (0.0, 0.01)	0.037	85.6	0.001	0.647	Very low
Triglycerides (mg/dL)	2 (3)	232	8–12	2×10^8–2×10^9	−15.37 (−22.53, −8.21)	0.001	0.0	0.554	0.742	Very low
Very low density lipoprotein (mg/dL)	1	60	12	2×10^9	−4.40 (−7.19, −1.61)	0.002	-	-	-	Low
Total cholesterol (mg/dL)	2 (3)	232	8–12	2×10^8–2×10^9	−10.57 (−20.83, −0.31)	0.043	35.3	0.217	0.245	Low
HDL cholesterol (mg/dL)	2 (3)	232	8–12	2×10^8–2×10^9	3.02 (−2.57, 8.62)	0.289	80.4	0.006	0.937	Very low
LDL cholesterol (mg/dL)	2 (3)	232	8–12	2×10^8–2×10^9	−21.58 (−41.62, −1.53)	0.035	47.0	0.151	0.424	Low
Total testosterone (ng/mL)	2 (3)	244	8–12	2×10^8–2×10^9	−0.13 (−0.18, −0.09)	<0.001	22.7	0.274	0.245	Low
Dehydroepiandrosterone sulfate (µg/mL)	1	60	12	2×10^9	−0.30 (−0.72, 0.12)	0.160	-	-	-	Low
Sex hormone-binding globulin (nmol/L)	1	60	12	2×10^9	19.30 (2.26, 36.34)	0.026	-	-	-	Low
high-sensitivity C-reactive protein (mg/L)	2 (3)	232	8–12	2×10^8–2×10^9	−0.15 (−0.39, 0.09)	0.216	90.0	<0.001	0.626	Very low
Nitric oxide (µmol/L)	1	60	12	2×10^9	5.20 (1.52, 8.88)	0.006	-	-	-	Low
Total antioxidant capacity (mmol/L)	2 (3)	232	8–12	2×10^9–3×10^{10}	−0.10 (−0.42, 0.23)	0.566	62.5	0.070	0.306	Very low
Glutathione (GSH; µmol/L)	1	60	12	2×10^9	−2.60 (−49.70, 44.50)	0.914	-	-	-	Low
Malondialdehyde (µmol/L)	2 (3)	232	8–12	2×10^9–3×10^{10}	−0.27 (−0.45, 0.09)	0.003	40.4	0.187	0.258	Low
Hirsutism score	1	60	12	2×10^9	−1.20 (−2.11, −0.29)	0.010	-	-	-	Low

[1]GRADE, Grading of recommendations assessment, development, and evaluation.
BMI, Body mass index; CFU, Colony-forming unit; CI, confidence interval; and wk, Week.

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TABLE 5 The effects of prebiotic supplementation in women with PCOS.

Outcomes (unit)	Number of trials (arms)	Number of participants	Follow-up (range), wk	Dose (range), mg/d	Effect size (95% CI)	*p* value	I^2 (%)	$p_{heterogeneity}$	Egger's test	Certainty of evidence (GRADE)[1]
Body weight (kg)	1	62	24	20,000	−2.80 (−6.83, 1.23)	0.173	-	-	-	Low
BMI (kg/m^2)	1	62	24	20,000	−1.40 (−2.95, 0.15)	0.077	-	-	-	Low
Waist circumference (cm)	1	62	24	20,000	−5.10 (−8.60, −1.60)	0.004	-	-	-	Low
Hip circumference (cm)	1	62	24	20,000	−4.60 (−7.47, −1.73)	0.002	-	-	-	Low
Fasting glucose concentration (mg/dL)	1	62	12	20,000	−15.14 (−20.38, −9.90)	0.003	-	-	-	Low
Triglycerides (mg/dL)	1	62	12	20,000	−31.12 (−49.63, −12.61)	0.064	-	-	-	Low
Total cholesterol (mg/dL)	1	62	12	20,000	−34.83 (−52.47, −17.19)	<0.001	-	-	-	Low
HDL cholesterol (mg/dL)	1	62	12	20,000	7.20 (−1.63, 11.40)	0.001	-	-	-	Low
LDL cholesterol (mg/dL)	1	62	12	20,000	−37.65 (−52.09, −22.69)	<0.001	-	-	-	Low
Dehydroepiandrosterone sulfate (μg/mL)	1	62	12	20,000	−0.84 (−1.52, −0.16)	0.016	-	-	-	Low
High-sensitivity C-reactive protein (mg/L)	1	62	12	20,000	−1.94 (−3.27, −0.61)	0.004	-	-	-	Low
Hirsutism score	1	62	12	20,000	−1.68 (−3.19, −0.17)	0.029	-	-	-	Low

[1]GRADE, Grading of recommendations assessment, development, and evaluation.
BMI, Body mass index; CI, Confidence interval; and wk, week.

different factors could explain why some strains are effective in some types of diseases but are not effective in others. However, a direct comparison of different strains is relatively uncommon, and multiple trials for the same strain or mixture are not common for the same disease. Strain-specificity can be accounted for by including only probiotics belonging to the same strain in meta-analyses. Another strategy is conducting subgroup analyses with the same probiotic strains within each sub-group. The results of previous research showed that not all probiotic strains are as effective as originally believed based on subgroup analyses and re-analysis of the data (76–78). This critical point was not taken into account by any of the meta-analyses that included in this umbrella review. Our review on the primary included RCTs also showed that all of those studies intervened by mixture of probiotic strains. Among them, two trials intervened by symbiotic formulas with the same probiotic and prebiotic mixture (50, 52) and two by capsules with the same probiotic mixture (45, 48) while others contained different strains of probiotics. Accordingly, due to the lack of included primary studies, we were unable to perform subgroup analyses to cover this important note in detail.

The main mechanisms behind these beneficial effects of pro-, pre-, and synbiotics on PCOS-related outcomes are still unclear. However, one possible explanation may be due to the effects of these compounds on short-chain fatty acids (SCFAs), the main by-products of fermentation in the intestinal lumen. The production of SCFAs has been shown to influence intestinal mucosal integrity, resulting in reduced inflammation, microbial endotoxins, and insulin resistance. In addition, the SCFAs play a role in the regulation of food intake and blood glucose homeostasis through the regulation of the secretion of gut peptides such as peptide YY and glucagon-like peptide-1 (79). Moreover, it has been suggested that the SCFAs inhibit the activation of the rate-limiting enzyme in the cholesterol production pathway, hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase), which leads to lower cholesterol metabolism and better lipid metabolism (80). Regarding sex hormones and hirsutism score, it has been found that probiotics or synbiotic supplements increase mucin formation, enhance bowel function, and reduce the quantity of gram-negative (inappropriate) bacteria in the colon. These modifications lessen the transmission of lipopolysaccharides (LPS) along the mucous wall and metabolic endotoxemia, which can ultimately result in improvements in insulin receptor function, lower levels of insulin, and increased levels of normal ovarian function, which in turn reduce the production of androgens such as DHEA, FAI, and testosterone (81, 82). As well, a limited number of RCTs with a short duration (less than 12 weeks) make it impossible to draw any conclusions regarding the impact of pro-pre- and synbiotic supplementation on PCOS-related outcomes, which adds to the importance of further studies in this area.

Our study had some strengths. This is the first study evaluating the effects of pro-, pre-, and synbiotic supplementation on several outcomes in patients with PCOS. To conduct this review, we selected the largest meta-analyses for each outcome, excluded RCTs without inclusion criteria, and recalculated effect sizes for each outcome, whenever possible. In addition, the certainty of the evidence was assessed using the GRADE tool. As a valid and acceptable tool, it helps the findings of systematic reviews to be more elucidative and informative. Accordingly, our review showed that, in most cases, the results of the meta-analyses were accompanied by small effect sizes and low or very low certainty of the evidence.

Our study has some limitations that should be considered. First, since the primary studies were limited to Iran and Egypt, these findings seem to have limited generalizability. Second, the number of studies for each outcome was limited and only one study has been conducted on the effects of prebiotics on PCOS-related outcomes. Third, the validity of our findings is impacted by considerable heterogeneity in some pooled results. Of course, we were unable to perform subgroup analyses to detect potential sources of heterogeneity because there were less than 10 trials available for each analysis. Forth, different probiotic and synbiotic supplementation across trials and the pooling of their effects added uncertainty to the interpretation of specific findings to each outcome. For example, although in the pooled data analysis probiotic supplementation improved FGC levels, synbiotic supplementation did not show any significant result. Fifth, the included meta-analyses did not obtain data from unpublished information, which may lead to publication bias. Sixth, it is impossible to fully control the confounding effects of other components of the diet via statistical methods, therefore, the effects of a pro-prebiotic and synbiotic supplementation may be partially mediated by other diet components. Seventh, the results of this study may be also cofounded by other PCOS-related lifestyle factors, such as body weight, age, and levels of physical activity. There were few primary studies, so we were unable to conduct subgroup analyses to take these factors into account.

Conclusion

In conclusion, the results of the present umbrella review suggests the beneficial effects of probiotics and synbiotics supplementation on the HOMA-IR index. However, the results originated from pooled data of the low number of RCTs with a maximum duration of 12 weeks. Also, we could not find a conclusive finding for other outcomes because of some important limitations such as small sample sizes in primary trials, small pooled effect sizes, and low or very low certainty in the evidence. Therefore, further well-designed RCTs with the following criteria might help to confirm or reject our findings in patients with PCOS: studies with different races and larger sample sizes; comparing the effects of different types of pro-, pre-, and synbiotic supplements on specific outcomes, RCTs with longer periods and larger sample sizes to assess and compare the effects of different dose of supplements, reporting all potential side effects following probiotics supplementation, and comparing the effects of different probiotics, prebiotics, and synbiotics to the promotion of evidence about the effects of these different interventions.

Our review generated several key messages for clinicians and patients, notably those who are eager for an adjuvant approach to the treatment of PCOS. Even though there are a variety of pathways that support the advantages of pro/pre and synbiotic supplementation in women with PCOS, it is critical to highlight that the magnitude of the effect was not clinically important, and the certainty of the evidence was low and very low. It is critical to highlight that there is insufficient data to support their obvious and long-term clinical effects.

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.

Author contributions

ST, SS-B, and KD performed data interpretation, design, search, and statistical analysis. NP collated the data. SZ-M, MR, SS, and MT arbitrated the study quality. ST, YJ, and SA contributed to writing the manuscript. HM and SS-B revised the draft manuscript. 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.2023.1178842/full#supplementary-material

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Dietary and lifestyle oxidative balance scores are independently and jointly associated with nonalcoholic fatty liver disease: a 20 years nationally representative cross-sectional study

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Background: Oxidative stress is an important contributor to the progression of nonalcoholic fatty liver disease (NAFLD), but whether dietary and lifestyle pro- and antioxidants may have combined or independent effects on NAFLD, and advanced liver fibrosis (AHF) remains unclear. We aimed to elucidate the relationship between a well-established oxidative balance score (OBS) and NAFLD/AHF.

Methods: This was a cross-sectional study. We included adult participants with complete data from the National Health and Nutrition Examination Survey 1999–2018. Survey-weighted adjusted multivariate regression analyses were used to examine the association of all OBS with NAFLD/AHF. A combination of restricted cubic splines, mediation analysis, stratified analysis, and sensitivity analysis were used to further elucidate these associations.

Results: We included 6,341 eligible adult participants with prevalence of NAFLD and AHF of 30.2 and 13.9%, respectively. In the fully adjusted model, the highest quartile of OBS, dietary OBS, and lifestyle OBS were associated with 65, 55, and 77% reduced risk of NAFLD, respectively, compared with the reference population, respectively. However, all OBS were not associated with the risk of AHF. All OBS were nonlinearly associated with risk of NAFLD and had a more pronounced reduced risk for OBS, dietary OBS, and lifestyle OBS after exceeding 26, 21, and 5 points, respectively. OBS may exert a protective effect indirectly through inflammation, oxidative stress, and glycolipid metabolism markers. Stratification and sensitivity analyses demonstrate the robustness of our findings.

Conclusion: All OBS were nonlinearly and negatively associated with NAFLD risk. These effects may exert indirectly through inflammation, oxidative stress, and glycolipid metabolism markers.

KEYWORDS

nonalcoholic fatty liver disease, NHANES, advanced liver fibrosis, oxidative stress, antioxidant

Introduction

Nonalcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease globally, with a course ranging from simple hepatic steatosis to the more severe form, nonalcoholic steatohepatitis (NASH), and a small percentage can progress to cirrhosis, liver cancer, and lead to death (1). The diagnosis of NAFLD is determined by the evidence of the presence of hepatic steatosis (accumulation of more than 5% fat in hepatocytes) confirmed by noninvasive markers/imaging/biopsy and the exclusion of secondary chronic liver injury (e.g., excessive alcohol consumption, viral hepatitis, etc.) (2). Generally, one in three adults worldwide may suffer from NAFLD, and its incidence continues to increase at an alarming rate (3). Better understanding of NAFLD/NASH and risk stratification by recognizing modifiable risk factors will help alleviate this public health burden.

Although the pathogenesis of NAFLD is still not fully understood, the accepted perspective is the 'multiple hit' hypothesis. Multiple factors including environmental exposures, unhealthy diet/lifestyle, and genetics/epigenetics have been shown to contribute to the following metabolic dysregulation, immune dysregulation, inflammation, and fibrosis through a variety of pathophysiological mechanisms (4). Oxidative stress caused by events such as mitochondrial dysfunction and endoplasmic reticulum stress has been well defined as an important hallmark for the progression from simple steatosis to NASH with more severe inflammation and fibrosis (5). Therefore, modulation of reactive oxygen species (ROS) production is currently an important research direction for therapies targeting NAFLD, especially NASH. Currently, several agents targeting anti-oxidative stress including vitamin E have shown improvement in NASH in preclinical experiments and clinical trials (6).

However, the impact of comprehensive dietary/lifestyle modifiable interventions related to oxidative stress on NAFLD and more severe cohorts such as those with advanced fibrosis is still lacking. Awareness of the impact of these modifiable risk factors and timely intervention may be of great value in the prevention of NAFLD progression and remission of NASH. To fill this knowledge gap, we employ a previously well-established oxidative balance score (OBS) in a nationally representative survey, the National Health and Nutrition Examination Survey (NHANES), to examine its effects on NAFLD and advanced liver fibrosis (AHF) for the first time.

Method

Study design and population

The NHANES is a series of nationally representative, multi-stage, cross-sectional surveys with complex probability sampling. It was reviewed and approved by the NCHS Ethics Review Board, and informed consent was obtained from all participants. Further information can be found on the official website.

We conducted the analysis using ten consecutive cycles of NHANES 1999–2018. From the initial 101,316 individuals, we first excluded all those missing data for the US Fatty Liver Index (USFLI) (n = 71,814) and OBS components (n = 15,672). Second, we excluded those younger than 18 years or pregnant (n = 385), those with other liver diseases such as chronic hepatitis, excessive alcohol intake (≥3 drinks/day for men and ≥2 drinks/day for women), and cancer (n = 4,829), and those with extreme total energy intake (n = 1,065). Finally, we excluded populations with missing data on included covariates (n = 1,195) and those with missing mediating variables (n = 15). The final number of eligible adult participants was 6,341 (Figure 1).

Composition of OBS

We calculated the OBS based on previous well-established studies (7–9). The OBS used in this study consisted of 16 dietary factors and 4 lifestyle measures that have previously been shown to be associated with oxidative stress (Supplementary Table S1). These components were divided into pro-oxidants and antioxidants based on their effect on oxidative stress. Specifically, pro-oxidants consisted of dietary total fat, dietary iron intake, alcohol consumption, body mass index (BMI), and serum cotinine, while antioxidants included dietary fiber, carotenoids, riboflavin (vitamin B2), niacin, vitamin B6, total folate, vitamin B12, vitamin C, vitamin E, calcium, magnesium, zinc, copper, selenium intake, and physical activity. Dietary intake information was obtained from two 24 h dietary review questionnaires consisting of a 24 h dietary recall interview conducted at a Mobile Examination Center and a telephone follow-up 3–10 days later through the USDA's Automated Multiple-Pass Method. The average of the two 24 h recall dietary interviews was used to proxy its intake. The OBS components and intakes for food or beverage consumed in the 24 h prior to the interview were collected and recorded by the NHANES computer-assisted dietary interview system. Instead, dietary nutrient intake was assessed according to the University of Texas Food Intake Analysis System and the USDA's Food and Nutrient Database for Dietary Studies. We did not include intake of nutrients from dietary supplements and medications based on the previous study as these data were lacking or poorly provided in full in many cycles (9). Notably, we used serum nicotine as a proxy for smoking based on previous studies, as it incorporated both active and passive smoking levels. More information on the data collection can be found in the previous study (9).

Our allocation scheme is described in Table 1. Most of the components were assigned according to their sex-specific tertiles, with antioxidants assigned 0, 1, and 2 points from tertile 1 to 3, respectively, and pro-oxidants assigned the reverse. Instead, alcohol consumption was assigned a score based on recognized intake criteria (0 point for men ≥2 drinks/d and ≥1 drinks/d for women, 1 point for men <2 drinks/d and <1 drinks/d for women; and 2 points for <12 drinks/year). In addition, we divided OBS into dietary and lifestyle OBS according to the source of the components to explore their combined and independent effects on NAFLD/AHF, respectively.

Definition of NAFLD/AHF

Suspected NAFLD was defined according to the USFLI, which was developed using the NHANES database, which has

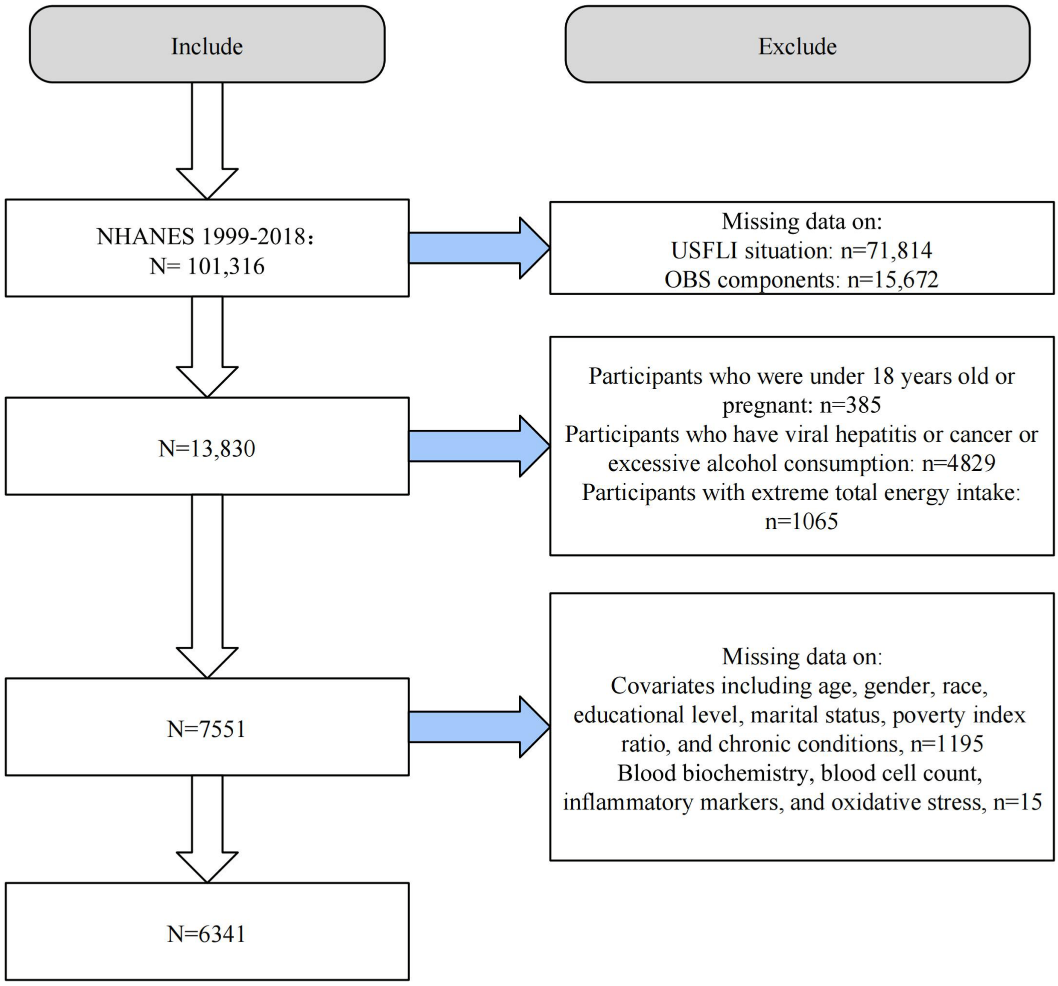

FIGURE 1
Flow chart of study population selection. USFLI, US fatty liver index; OBS, oxidative balance score; NHANES, the National Health and Nutrition Examination Survey.

moderately improved accuracy compared to the FLI in the multi-ethnic US population (10). USFLI was developed based on ethnicity, age, gamma glutamyltransferase (GGT), waist circumference (WC), fasting insulin, and fasting glucose with the following formula: USFLI = $(e^{-0.8073 \times \text{non-Hispanic black} + 0.3458 \times \text{Mexican American} + 0.0093 \times \text{age} + 0.6151 \times \ln(\text{GGT}) + 0.0249 \times \text{WC} + 1.1792 \times \ln(\text{insulin}) + 0.8242 \times \ln(\text{glucose}) - 14.7812})/(1 + e^{-0.8073 \times \text{non-Hispanic black} + 0.3458 \times \text{Mexican American} + 0.0093 \times \text{age} + 0.6151 \times \ln(\text{GGT}) + 0.0249 \times \text{WC} + 1.1792 \times \ln(\text{insulin}) + 0.8242 \times \ln(\text{glucose}) - 14.7812}) \times 100$. A USFLI $\geq$30 was considered to have putative NAFLD with an area under the receiver operating characteristic curve of 0.80 (10). AHF was defined as NAFLD fibrosis score (NFS) > 0.676 or fibrosis 4 index (FIB-4) > 2.67 or aspartate aminotransferase (AST)/platelet ratio index (APRI) > 1 in the presence of NAFLD (11). NFS has been validated to diagnose AHF non-invasively and accurately using the formula: NFS = −1.675 + 0.037 × age + 0.094 × BMI + 1.13 × impaired fasting glycemia or diabetes (yes = 1, no = 0) + 0.99 × AST/alanine aminotransferase (ALT) − 0.013 × platelet −0.66 × albumin (12). The formula for FIB-4 is: FIB-4 = (age × AST)/(Platelet counts × (SQRT(ALT))) (13). The formula for APRI is: APRI = ([AST/upper limit of normal (ULN)]/Platelet counts) × 100 (14). In the 1999–2000 cycle of NHANES, AST = 40 U/L was used as ULN, and 33 U/L was used as ULN in subsequent years (15).

Covariates

Based on previous relevant studies, we selected several possible covariates, including age, gender (male or female), ethnicity (Mexican American, non-Hispanic black, non-Hispanic white, other Hispanic, or other races), education (<high school, high school, or >high school), family income to poverty ratio (PIR), marital status, total energy intake, and chronic comorbid conditions including a range of other cardiometabolic diseases, i.e., diabetes, hypertension, and self-report cardiovascular disease (CVD) (including coronary heart disease, stroke, heart attack, congestive heart failure, and angina). The criteria for diagnosing hypertension was that a doctor says someone has high blood pressure, is taking antihypertensive medication, or that their blood pressure values are in the hypertensive range (16). Diabetes was diagnosed based on if a doctor said that a person has diabetes, abnormal blood glucose/glucose tolerance test, or was taking related medication (17).

TABLE 1 OBS allocation scheme.

	Male			Female		
	0	1	2	0	1	2
Dietary OBS components						
Dietary fiber (g/d)	<12.6	<19.7	≥19.7	<10.1	<16.35	≥16.35
Carotene (RE/d)	<99.04	<306.64	≥306.64	<98.25	<383.96	≥383.96
Vitamin B2(mg/d)	<1.79	<2.69	≥2.69	<1.34	<2.02	≥2.02
Niacin (mg/d)	<20.68	<29.75	≥29.75	<14.528	<21.86	≥21.86
Vitamin B6 (mg/d)	<1.59	<2.40	≥2.40	<1.13	<1.77	≥1.77
Total folate (mcg/d)	<316	<492	≥492	<251	<389	≥389
Vitamin B12 (mcg/d)	<3.36	<6.2	≥19.13	<2.22	<4.22	≥4.22
Vitamin C (mg/d)	<42.5	<113.21	≥113.21	<38.01	<98.5	≥98.5
Vitamin E (ATE) (mg/d)	<5.82	<9.43	≥9.43	<4.54	<7.52	≥7.52
Calcium (mg/d)	<6.46	<1072.5	≥1072.5	<499.5	<849	≥849
Magnesium (mg/d)	<257	<361.28	≥361.28	<187	<283.43	≥283.43
Zinc (mg/d)	<9.75	<15.1	≥15.1	<6.73	<10.73	≥10.73
Copper (mg/d)	<1.12	<1.57	≥1.57	<0.85	<1.28	≥1.28
Selenium (mcg/d)	<94.94	<141.85	≥141.85	<67.83	<99.5	≥99.5
Total fat (g/d)	≥107.42	<107.42	<69.83	≥75.775	<75.775	<50.965
Iron (mg/d)	≥19.165	<19.165	<12.88	≥14.315	<14.315	<9.65
Lifestyle OBS components						
Physical activity (MET-minute/week)	<417.9	<1136.8	≥1136.8	<274	<846	≥846
Alcohol (drinks/d)	≥2 drinks/d	<2 drinks/d	<12 drinks/year	≥1 drinks/d	<1 drinks/d	<12 drinks/year
Body mass index (kg/m2)	≥29.16	<29.16	<25.55	≥28.63	<28.63	<23.74
Cotinine (ng/mL)	≥1.08	<1.08	<0.038	≥0.171	<0.171	<0.035

OBS, oxidative balance score.

Mediation variables

We speculated that OBS may have indirect effects on NAFLD through several other pathways. First, oxidative stress-related markers may be important mediators, and second, given the close association of oxidative stress with inflammation and metabolism, we considered the potential mediating effects of these markers (Figure 2). To investigate whether OBS acts indirectly on NAFLD through mediators related to inflammation, oxidative stress, and glycolipid metabolism, we performed a mediation analysis. We selected several important biomarkers of these relevant aspects in NAFLD based on the literature, including blood neutrophils (18), ALT/AST (19), serum albumin (20), uric acid (UA) (21), bilirubin (22), GGT (23), triglycerides (TG) (24), total cholesterol (TC) (24), high-density lipoprotein (HDL)-cholesterol (24), and fasting blood glucose (25). Detailed laboratory tests and data collection instruments are publicly available.[1]

Statistical analysis

All analyses were conducted using EmpowerStats software and R version 4.1.3. Due to the complex design of NHANES, we appropriately weighted our data analysis according to the NHANES survey reporting guidelines for the survey. For continuous variables, we used the survey-weighted mean (95% confidence interval [CI]), and the value of p was calculated by survey-weighted linear regression. For categorical variables, we used survey-weighted percentages (95% CI), and value of ps were calculated by survey-weighted Chi-square tests. We used multivariate logistic regression analysis to examine the association between OBS and NAFLD/AHF. We constructed three multivariate regression models: first, we constructed model 1 (unadjusted model), which did not adjust for any covariates; model 2 adjusted for age, sex, race, marital status, education level, and energy intake (kcal/day); model 3 was based on model 2 and added adjustments for diabetes, hypertension, and CVD to model 2. Restricted cubic splines (RCS) were used to describe the nonlinear relationship between the OBS and NAFLD/AHF. The curve fitting term is defined by the RCS function from the rms package, and the degrees of freedom (or knots) are determined according to the magnitude of the P for nonlinear value.

To investigate whether OBS may influence the risk of NAFLD through other pathways, we performed a mediation analysis by selecting possible markers as mediating variables. The total effect of OBS on the risk of NAFLD (c) includes the direct effect of OBS (c') and the indirect effect through mediating variables ($a*b$), and $c = c' + a*b$. For the markers of inflammation, oxidative stress, and glycolipid metabolism included in this study, we performed individual mediator

1 https://wwwn.cdc.gov/nchs/nhanes/Default.aspx

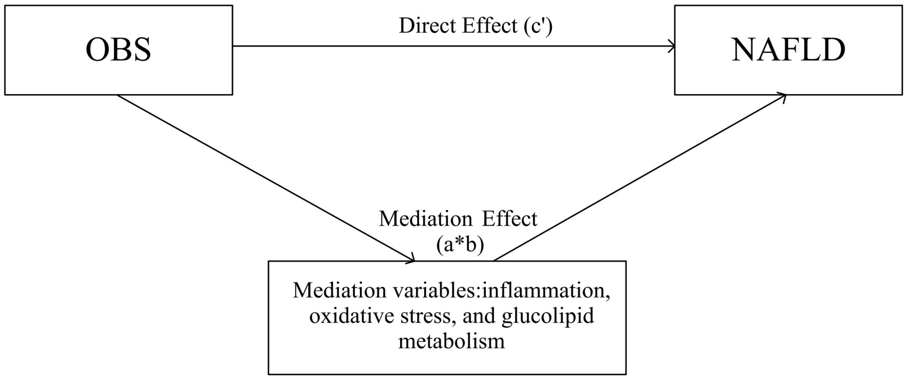

FIGURE 2
Schematic diagram of the mediation effect. OBS, oxidative balance score; NAFLD, nonalcoholic fatty liver disease.

analyses based on previous studies and calculated direct effects in the association of OBS with risk of NAFLD and indirect effects through these markers. We calculated the proportion of the total effect mediated by the included mediating variables. All mediation analyses were adjusted for all covariates. In addition, stratified and sensitivity analyses were performed to test the consistency of the findings across subgroups and the stability of the results.

Results

Baseline characteristics

6,341 adult participants (representing a weighted US population of 129,919,267) were included in our study. 1,917 and 266 individuals were diagnosed with NAFLD and AHF (prevalence of 30.2 and 13.9%, respectively). We divided the quartiles according to OBS, and all indicators including PIR, gender, race, education level, marital status, daily energy intake, presence of diabetes, and hypertension were significantly different (all $p < 0.05$), except for age and presence of CVD, which did not differ between quartiles. As the quartiles of OBS gradually increased, participants were wealthier, had a higher proportion of women, a higher proportion of non-Hispanic whites, a higher level of education, a higher proportion of singles, a higher daily energy intake, and a lower prevalence of diabetes and hypertension (Table 2). We also divided the population according to the presence of NAFLD and AHF. Those with NAFLD differed significantly on all covariates except for PIR and daily energy intake, which did not differ from those without NAFLD. OBS (both dietary and lifestyle OBS) were significantly lower in the NAFLD population (all $p < 0.0001$). However, while OBS and dietary OBS differed in patients with AHF compared to non-AHF (1,917 patients with NAFLD had accessible diagnostic data), baseline lifestyle OBS showed no difference in the population ($p = 0.786$) (Supplementary Tables S2, S3).

Multivariate regression analysis

Three models were constructed to explore the relationship between OBS and NAFLD/AHF when used as continuous or categorical variables. Model 1 was an unadjusted model, model 2 was adjusted for age, sex, race, marital status, education level, and daily energy intake, and model 3 was adjusted for all covariates. In all three models, OBS (continuous) (including dietary OBS and lifestyle OBS) was negatively associated with the risk of NAFLD (all $p < 0.0001$). When OBS was used as a categorical variable, the risk of NAFLD gradually decreased with increasing quartiles in all models (P for trend <0.0001). In the fully adjusted model, OBS was associated with a 65% lower risk of NAFLD in Q4 compared to Q1 (reference) (odds ratio [OR] (95% CI) = 0.352 (0.260, 0477), $p < 0.0001$). Similarly, in model 3, dietary OBS and lifestyle OBS in Q4 compared with Q1 reduced the risk of NAFLD by 55 and 77%, respectively, (all $p < 0.0001$) (Table 3). However, OBS (continuous and categorical) was only associated with the risk of AHF in the unadjusted model. There was no association with the risk of AHF in the adjusted models (all $p > 0.05$) (Supplementary Table S4).

Nonlinear relationship exploration

We examined the nonlinear relationship between OBS and risk of NAFLD using RCS models. OBS, dietary OBS, and lifestyle OBS were all nonlinearly associated with risk of NAFLD (all P nonlinearity <0.0001). In addition, the inflection points of the nonlinear curves for the relationship between OBS, dietary OBS, and lifestyle OBS and NAFLD were 26, 21, and 5, respectively (Figure 3). We therefore performed a threshold effect analysis based on the inflection point. Interestingly, OBS (both dietary and lifestyle OBS) was significantly negatively associated with NAFLD risk both before and after the inflection point. After the inflection point, OBS was more significantly associated with a reduced risk of NAFLD, suggesting that OBS, dietary OBS, and lifestyle OBS were associated with a more significant reduction in NAFLD risk after exceeding 26, 21, and 5 points, respectively (Supplementary Table S5).

Mediation analysis

We then explored whether OBS indirectly affects NAFLD risk through markers related to inflammation, oxidative stress, and glycolipid metabolism. There was a significant direct effect of OBS with NAFLD risk in the presence of each individual mediating variable (all $p < 0.0001$). Indirect mediation effects were present for all included mediators (all $p < 0.0001$) except ALT and AST (all $p > 0.05$). The

TABLE 2 Baseline data according to OBS quartiles.

	Q1	Q2	Q3	Q4	Value of *p*
Mean ± SD	15.408 ± 2.908	22.671 ± 1.677	27.987 ± 1.395	34.674 ± 2.896	
Sample *N*	*n* = 1,475	*n* = 1,602	*n* = 1,438	*n* = 1826	
Weighted *N*	*N* = 32,585,939	*N* = 31,950,344	*N* = 28,849,243	*N* = 36,533,742	
OBS Dietary	11.662 (11.425,11.899)	18.445 (18.281,18.609)	23.298 (23.139,23.457)	28.980 (28.785,29.176)	<0.0001
OBS Lifestyle	3.541 (3.435,3.647)	4.166 (4.049,4.282)	4.649 (4.529,4.769)	5.467 (5.384,5.551)	<0.0001
Age	46.876 (45.533,48.220)	48.310 (47.136,49.484)	48.218 (46.291,50.146)	46.838 (45.649,48.027)	0.1248
PIR	2.929 (2.805,3.053)	3.307 (3.176,3.438)	3.458 (3.302,3.614)	3.506 (3.364,3.648)	<0.0001
Gender					0.0050
Male	55.535 (51.278,59.713)	55.786 (52.545,58.979)	51.776 (47.847,55.683)	47.550 (44.240,50.883)	
Female	44.465 (40.287,48.722)	44.214 (41.021,47.455)	48.224 (44.317,52.153)	52.450 (49.117,55.760)	
Race					<0.0001
Mexican American	5.490 (4.437,6.774)	5.413 (4.268,6.843)	5.316 (4.176,6.746)	5.336 (4.207,6.746)	
Non-Hispanic Black	14.535 (11.710,17.904)	10.090 (8.308,12.203)	7.057 (5.555,8.927)	4.991 (3.979,6.244)	
Non-Hispanic White	67.538 (63.187,71.606)	74.469 (70.455,78.107)	77.732 (74.237,80.874)	79.492 (76.795,81.949)	
Other Hispanic	5.484 (3.556,8.369)	4.842 (2.684,8.583)	3.158 (2.144,4.629)	3.476 (2.496,4.823)	
Other race	6.952 (4.537,10.512)	5.187 (3.919,6.836)	6.737 (5.010,9.001)	6.705 (5.212,8.587)	
Education level					<0.0001
Under high school	6.091 (4.799,7.703)	5.564 (3.682,8.323)	2.943 (2.036,4.237)	2.572 (1.954,3.379)	
High school	40.855 (36.428,45.436)	36.273 (32.559,40.158)	28.498 (24.296,33.108)	22.072 (19.058,25.412)	
More than high school	53.054 (48.394,57.662)	58.164 (54.258,61.969)	68.560 (64.019,72.771)	75.356 (71.808,78.591)	
Marital					0.0010
Single	67.915 (64.192,71.424)	76.400 (73.108,79.404)	73.157 (69.382,76.624)	75.228 (71.497,78.618)	
No single	32.085 (28.576,35.808)	23.600 (20.596,26.892)	26.843 (23.376,30.618)	24.772 (21.382,28.503)	
Daily energy intake(kcal/day)	1617.133 (1552.994,1681.271)	1927.349 (1877.948,1976.750)	2089.965 (2025.818,2154.112)	2307.397 (2249.890,2364.905)	<0.0001
Diabetes					0.0008
No	86.982 (84.206,89.333)	86.471 (83.601,88.905)	89.566 (87.007,91.669)	92.149 (90.125,93.786)	
Yes	13.018 (10.667,15.794)	13.529 (11.095,16.399)	10.434 (8.331,12.993)	7.851 (6.214,9.875)	
Hypertension					<0.0001
No	60.749 (56.496,64.845)	60.319 (56.379,64.129)	64.278 (60.048,68.297)	72.997 (68.969,76.679)	
Yes	39.251 (35.155,43.504)	39.681 (35.871,43.621)	35.722 (31.703,39.952)	27.003 (23.321,31.031)	
CVD					0.1491
No	91.714 (89.477,93.511)	91.323 (89.526,92.837)	93.198 (90.873,94.964)	93.962 (91.764,95.601)	
Yes	8.286 (6.489,10.523)	8.677 (7.163,10.474)	6.802 (5.036,9.127)	6.038 (4.399,8.236)	

OBS, oxidative balance score; SD, standard deviation; PIR, family income to poverty ratio; CVD, cardiovascular disease. For continuous variables: survey-weighted mean (95% CI), value of *p* was by survey-weighted linear regression. For categorical variables: survey-weighted percentage (95% CI), value of *p* was by survey-weighted Chi-square test.

average mediated proportions of blood neutrophils, serum albumin, GGT, UA, bilirubin, TC, TG, HDL-cholesterol, and fasting glucose were 9.3, 7.6, 13.3, 13.9, 5.03, 0.9, 13.8, 29.3, and 3.6%, respectively. (All $p < 0.0001$, except for TC where *P* was 0.0120) (Table 4).

Stratified analysis

To investigate whether the association of OBS with the risk of NAFLD was consistent across subgroups, we performed a stratified analysis. Notably, the *P* for interaction was >0.05 for all subgroups (age, sex, race, education level, marital status, diabetes, hypertension, and CVD), indicating that our findings were consistent across all subgroups (Figure 4).

Sensitivity analysis

To verify the stability of our results, we performed a sensitivity analysis. We obtained similar results after dividing the OBS (including dietary and lifestyle OBS) by tertile or quintile (Supplementary Table S6). Second, we did not adjust for daily

TABLE 3 Multivariate regression analysis of OBS and the risk of NAFLD.

	Model 1 OR (95% CI), *P*	Model 2 OR (95% CI), *P*	Model 3 OR (95% CI), *P*
OBS	0.960 (0.950, 0.971) <0.0001	0.945 (0.933, 0.957) <0.0001	0.947 (0.934, 0.960) <0.0001
OBS Quartile			
Q1	ref.	ref.	ref.
Q2	0.992 (0.807, 1.220) 0.940	0.841 (0.660, 1.072) 0.165	0.801 (0.616, 1.042) 0.101
Q3	0.839 (0.676, 1.042) 0.115	0.688 (0.545, 0.870) 0.002	0.696 (0.541, 0.895) < 0.0001
Q4	0.442 (0.348, 0.561) < 0.0001	0.342 (0.258, 0.454) < 0.0001	0.352 (0.260, 0477) < 0.0001
p for trend	<0.0001	<0.0001	<0.0001
OBS Dietary	0.970 (0.958, 0.982) < 0.0001	0.956 (0.942, 0.970) < 0.0001	0.957 (0.942, 0.973) <0.0001
OBS Dietary Quartile			
Q1	ref.	ref.	ref.
Q2	1.020 (0.840, 1.241) 0.836	0.890 (0.703, 1.127) 0.336	0.802 (0.614,1.050) 0.111
Q3	0.908 (0.744,1.107) 0.339	0.751 (0.604, 0.934) 0.011	0.763 (0.593, 0.981) 0.037
Q4	0.568 (0.449, 0.718) < 0.0001	0.453 (0.341, 0.602) < 0.0001	0.446 (0.330, 0.604) < 0.001
p for trend	<0.0001	<0.0001	<0.0001
OBS Lifestyle	0.691 (0.656, 0.727) < 0.0001	0.676 (0.644, 0.709) < 0.0001	0.691 (0.656, 0.728) < 0.0001
OBS Lifestyle Quartile			
Q1	ref.	ref.	ref.
Q2	0.766 (0.597, 0.98) 0.037	0.762 (0.584, 0.994) 0.0466	0.780 (0.596, 1.020) 0.071
Q3	0.458 (0.365, 0.574) < 0.0001	0.430 (0.343, 0.540) < 0.0001	0.460 (0.366, 0.579) < 0.0001
Q4	0.226 (0.175, 0.291) < 0.0001	0.212 (0.163, 0.275) < 0.0001	0.226 (0.171, 0.299) < 0.0001
p for trend	<0.0001	<0.0001	<0.0001

OBS, oxidative balance score; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; 95% CI, 95% confidence interval; CVD, cardiovascular disease. Model 1 was an unadjusted model, model 2 adjusted for age, sex, race, marital status, education level, and daily energy intake, and model 3 was adjusted for age, sex, race, marital status, education level, daily energy intake, diabetes, hypertension, and CVD.

energy intake in the multivariate regression analysis. Consistently, the associations of OBS, dietary OBS, and lifestyle OBS with NAFLD all remained similarly significant (Supplementary Table S7). These results indicate a robust stability of our findings.

Discussion

To the best of our knowledge, this study is the first to elucidate the effects of dietary and lifestyle modifiable pro- and antioxidants on NAFLD/AHF in a real-world setting using a comprehensive composite indicator of oxidative stress, the OBS. By analyzing data from a US nationally representative cross-sectional study over 20 years, we showed that higher OBS scores are nonlinearly associated with lower risk of NAFLD and that similar effects are present for independent dietary OBS and lifestyle OBS, supporting the critical impact of OBS on the onset and progression of NAFLD. Furthermore, through mediation analysis, we conclude that OBS may indirectly mediate NAFLD risk through effects on inflammation, oxidative stress, and glycolipid metabolism. Notably, OBS (both dietary OBS and lifestyle OBS) were not associated with the risk of AHF in patients with NAFLD.

Impaired oxidative stress signaling is closely related to the pathogenesis of NAFLD/NASH based on the critical mechanism of overproduction of ROS leading to cellular damage, senescence, and death, and can lead to disruption of lipid metabolism, impaired insulin signaling, and dysregulation of innate immune signaling pathways (26). Mechanistically, oxidative stress is essentially due to an imbalance between pro-oxidants (e.g., superoxide anions, hydroxyl radical, and hydrogen peroxide) and antioxidants (superoxide dismutase, glutathione peroxidase, and vitamins A, C, E, etc.) in the body (27). In the pathophysiology of NAFLD/NASH, lipotoxicity in the liver leads to impaired function of several ROS-generating organelles, such as mitochondria, endoplasmic reticulum, and peroxisomes, resulting in the release of abundant ROS and causing an imbalance in redox signaling. Accumulating experimental evidence suggests that modulation of redox signaling in NAFLD/NASH may attenuate disease progression and can serve as a potential target. Targeting nuclear factor E2-related factor 2, a redox-regulated transcription factor, has been demonstrated to improve NASH by ameliorating lipotoxicity, inflammation, and oxidative stress (28). In addition, scattered clinical data suggest that supplementation with a variety of dietary antioxidants such as vitamin E, vitamin C, and hesperidin, as well as lifestyle changes such as exercise, may improve clinical indicators in part by reducing oxidative stress in patients with NAFLD/NASH (29–31).

As there are still no approved drugs for the treatment of NAFLD/NASH, dietary and lifestyle changes remain the cornerstone of NAFLD management (32). Because of the current lack of real-world evidence on whether dietary and lifestyle pro- and antioxidants have integrated and independent effects on the onset and progression of NAFLD, we used a previously well-established composite score that assesses the pro- and antioxidants properties of components of dietary and lifestyle sources. The OBS is a tool used to assess overall redox homeostasis in individuals and has been shown to be associated with colorectal cancer, CVD, and chronic kidney disease in previous epidemiological studies

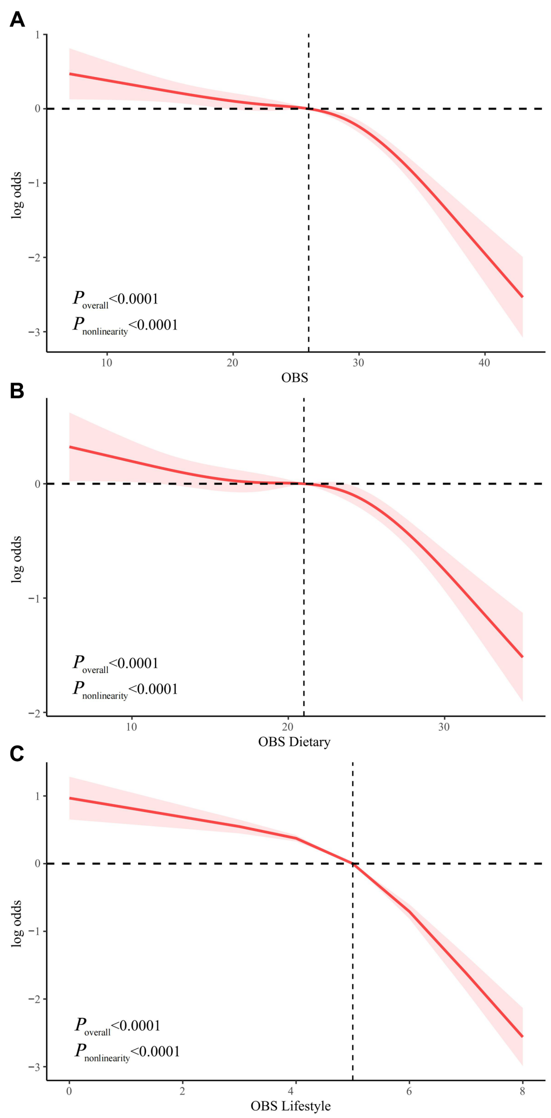

FIGURE 3
Non-linear relationship of all OBS with NAFLD. **(A)** Non-linear relationship between OBS and NAFLD. **(B)** Non-linear relationship between dietary OBS and NAFLD. **(C)** Non-linear relationship between lifestyle OBS and NAFLD. OBS, oxidative balance score; NAFLD, nonalcoholic fatty liver disease.

(33). More recent studies using NHANES have suggested that higher OBS is associated with improved cognitive function and depressive symptoms, and reduced risk of periodontitis and diabetes compared with those with the lowest OBS (7, 8, 34, 35). After adjustment for all included confounders, OBS treated as both a continuous and a categorical variable, was associated with the risk of NAFLD but not AHF. Compared to Q1, the risk of NAFLD was reduced by approximately 65% in the population with OBS located in Q4 (p trend <0.0001). Notably, both higher dietary OBS and lifestyle OBS were also independently associated with reduced risk of NAFLD, with approximately 55 and 77% reductions in the Q4 population compared to the reference population, respectively. Thus, lifestyle OBS appears to be associated with a greater reduction in NAFLD risk than dietary OBS. The underlying mechanisms remain unclear, but a recent study using NHANES III showed that physical activity is more important than diet for the prognosis of patients with metabolic-associated fatty liver disease (MAFLD) and sarcopenia (36). Future mechanistic studies are needed to further explore the relevant mechanisms.

We then further investigated the nonlinear relationship between OBS and risk of NAFLD. Both independent and combined dietary OBS and lifestyle OBS were nonlinearly associated with the risk of NAFLD (p nonlinearity <0.0001). The inflection points for OBS, dietary OBS, and lifestyle OBS were 26, 21, and 5 points, respectively. We used threshold effect analysis to show that OBS, dietary OBS, and lifestyle OBS were associated with a more pronounced reductions in NAFLD risk after 26, 21 and 5 points, respectively, which may have important implications for the dietary and lifestyle management of patients with NAFLD.

We then hypothesized that OBS may exert beneficial effects indirectly through relevant markers. Therefore, we next investigated whether OBS indirectly reduces the risk of NAFLD via these pathways of inflammation, oxidative stress, and glycolipid metabolism. Not surprisingly, most of these potential biomarkers may mediate the protective effect of OBS against NAFLD, although ALT and AST do not appear to mediate the effect. HDL-cholesterol, UA, and TG may have mediated the highest proportions, at 29.3, 13.9, and 13.8%, respectively. HDL-cholesterol mediates the regulation of lipid metabolism mainly by promoting reverse cholesterol transport, antioxidant, and anti-inflammatory mechanisms, and previous studies have demonstrated that both diet and lifestyle management can improve HDL-cholesterol levels (37). Conversely, TG has been shown to be a core biomarker of lipid dysregulation in patients with NAFLD (24). Moreover, the role of UA in anti-oxidative stress has been well established. Our study suggests that OBS may indirectly reduce the risk of NAFLD mainly through these markers, and future studies are needed to further validate our findings.

We performed a stratified analysis to elicit whether OBS still had relevant effects in different subgroups. In all subgroups, we did not observe significant differences between groups (all P for interaction >0.05), suggesting the consistency of our study across subgroups and that OBS may reduce NAFLD risk in people with different characteristics.

To test the stability of our results, we performed a sensitivity analysis. After dividing OBS (including dietary OBS and lifestyle OBS) into tertiles and quintiles, we obtained similar significant negative correlations with NAFLD risk. Consistently, a similar effect remained after not adjusting for daily energy intake in multivariate regression analysis. These findings justify the stability of our results, and the conclusion that OBS is associated with a reduced risk of NAFLD is robust.

Our results show that in the real world, healthy dietary and lifestyle habits with higher antioxidant properties are associated with a significant reduction in the risk of NAFLD. In fact, healthy eating habits have been widely shown to be associated with a reduced risk of NAFLD/MAFLD and liver fibrosis. A variety of dietary quality indices, including the Dietary Inflammatory Index (DII), the Mediterranean diet, the Dietary Approach to Stop Hypertension, the Alternate Healthy Eating Index diet, and the Healthy Eating Index-2015 are associated with the risk of NAFLD/MAFLD and liver fibrosis, with the higher DII being associated with lower diet quality, while the rest of the indices are considered healthy eating indices (38–40). Lifestyle changes are also recognized as having a fundamental role in the improvement of NAFLD. Obesity is a major contributor to the development and progression of NAFLD and there is strong evidence that a 10% weight loss is effective in improving the histological characteristics of NASH patients (41). Physically active individuals have an approximately 30% reduced risk of NAFLD compared to inactive individuals (40). In

TABLE 4 Mediation analysis of the relationship between OBS and NAFLD.

	Direct effect (average)	Value of *p*	Mediation effect (average)	Value of *p*	Proportion-mediated (average)	*p*-value
Separate mediators						
Neutrophils	−0.116 (−0.133, −0.099)	<0.0001	−0.012 (−0.015, −0.009)	<0.0001	0.093 (0.066, 0.126)	<0.0001
ALT	−0.1120 (−0.141, −0.094)	<0.0001	−0.001 (−0.005, 0.003)	0.5740	0.009 (−0.024, 0.049)	0.5740
AST	−0.129 (−0.150, −0.106)	0.0060	0.002 (−0.0001, 0.008)	0.0540	−0.017 (−0.064, 0.004)	NA
Albumin	−0.117 (−0.134, −0.100)	<0.0001	−0.010 (−0.012, −0.007)	<0.0001	0.076 (0.054, 0.102)	<0.0001
GGT	−0.109 (−0.125, −0.090)	<0.0001	−0.017 (−0.025, −0.010)	<0.0001	0.133 (0.080, 0.197)	<0.0001
UA	−0.111 (−0.127, −0.094)	<0.0001	−0.018 (−0.022, −0.014)	<0.0001	0.139 (0.109, 0.177)	<0.0001
Bilirubin	−0.121 (−0.139, −0.103)	<0.0001	−0.006 (−0.009, −0.004)	<0.0001	0.0503 (0.032, 0.073)	<0.0001
TG	−0.111 (−0.129, −0.091)	<0.0001	−0.018 (−0.024, −0.009)	<0.0001	0.138 (0.071, 0.184)	<0.0001
TC	−0.127 (−0.143, −0.109)	<0.0001	−0.001 (−0.002, −0.0002)	0.0120	0.009 (0.002, 0.017)	0.0120
HDL-cholesterol	−0.091 (−0.107, −0.075)	<0.0001	−0.038 (−0.044, −0.032)	<0.0001	0.293 (0.246, 0.351)	<0.0001
Fasting glucose	−0.120 (−0.137, −0.101)	<0.0001	−0.005 (−0.008, −0.001)	<0.0001	0.036 (0.015, 0.069)	<0.0001

OBS, oxidative balance score; NAFLD, nonalcoholic fatty liver disease; NA, not available; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase; UA, uric acid; TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein.

addition, smoking and low-moderate alcohol consumption have been suggested to be associated with the development and severity of NAFLD (42–44). Our results highlight that both independent and combined healthy diet and lifestyle in terms of oxidative stress can significantly reduce the incidence and development of NAFLD.

Interestingly, OBS did not appear to be associated with progression of fibrosis in patients with NAFLD. An important randomized controlled clinical trial showed that vitamin E was associated with improved histological features including hepatic steatosis and lobular inflammation and reduced biochemical markers such as ALT and AST, but not with improvement in fibrosis, in patients with NASH (45). However, a recent meta-analysis concluded that vitamin E was associated with at least ≥1 fibrosis stage improvement compared with placebo (46). Using NHANES, a study showed an association between serum vitamin C, choline, alpha-carotene, and vitamin B12 and liver fibrosis (47). Similarly, other antioxidants may be associated with fibrosis improvement in patients with NAFLD (48). Currently, the underlying mechanisms remain unclear, but an important reason may be that OBS is a composite index that includes both pro-oxidants and antioxidants, and therefore its combined effect cannot be explained by the alleviation of fibrosis by antioxidants alone. Therefore, future high-quality prospective studies are needed to further confirm our findings.

A point that warrants the need for cautious interpretation is the possibility of selection bias and compromised generalizability of the results as our study population went from an initial population of 101,316 to a final population of 6,341. However, several explanations can be offered as follows. First, the exclusion criteria implemented in our study were all rigorous. The fasting blood glucose and insulin tests required for the diagnosis of USFLI were only performed in a subset of participants, yet this also ensured the reliability of the NAFLD diagnosis (compared to FLI) because the fasting blood tests excluded dietary interferences and ensured that the samples were fresh. We excluded the population with missing covariates, which is more likely to ensure the reliability of the results in subsequent regression analyses adjusting for confounders. Finally, and most importantly, our analyses were all appropriately weighted and considered complex sampling design according to the NHANES study guidelines (49), i.e., probabilistic sampling to ensure that the overall sample was represented by a smaller sample. Therefore, we believe that weighting all analyses to represent the overall population and rigorous exclusion criteria ensure the reliability and generalizability of the results. More large-sample studies are needed in the future to validate our conclusions.

Our study has several important strengths. First, we used a nationally representative population-based study to conduct our investigation, and the results are generalizable and representative. Second, we included possible covariates based on previous studies, which greatly reduced the effect of confounding factors. We further explored the direct and indirect effects of OBS on NAFLD using mediation analysis, which showed potential beneficial effects of OBS through inflammation, oxidative stress, and glycolipid metabolism. Stratified and sensitivity analyses highlighted the robustness and stability of our findings. However, our study also has limitations. First, our study is a cross-sectional study, and thus residual confounders may still exist, and causal relationships cannot be drawn. Second, our study was conducted in the US population, and findings for other races and countries need to be further explored in future studies. Finally, we diagnosed NAFLD/AHF using non-invasive markers rather than liver biopsy, and thus may lack accuracy. However, because of the cost of liver biopsy, the potential risks of the operation, and the inability to generalize in large population-based studies, as well as the proven good accuracy of non-invasive scores, diagnosis using non-invasive markers is therefore also well characterized and these effects are unlikely to affect the reliability of the results.

Conclusion

Dietary and lifestyle OBS had joint and independent protective effects on the risk of NAFLD in a nationally representative cross-sectional study and showed a non-linear relationship. However, no association was found between OBS and AHF risk. The population with OBS in the highest quartile had a 65% reduction in NAFLD risk compared to the lowest quartile. OBS may indirectly affect the development of NAFLD through inflammation, oxidative stress, and

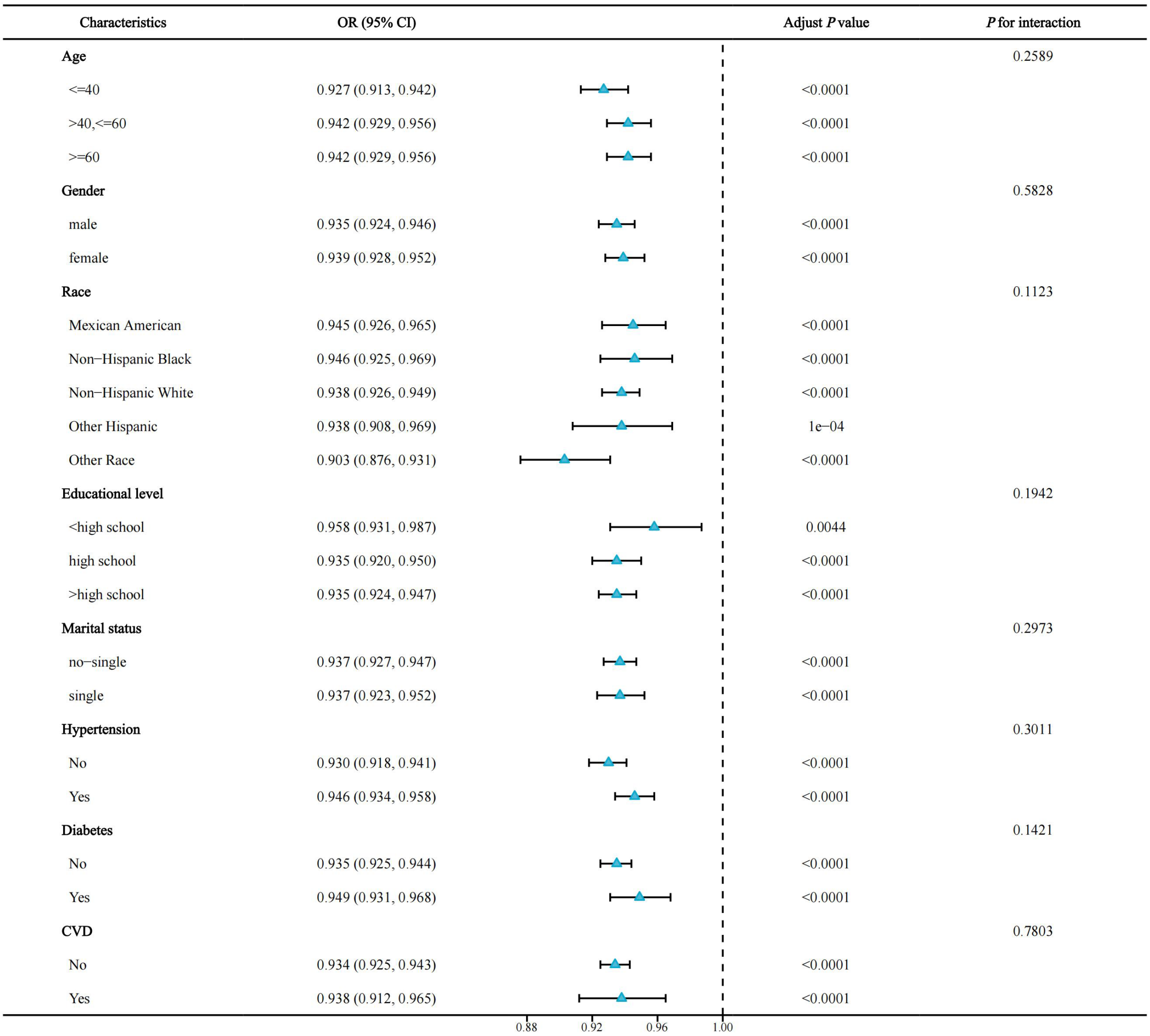

FIGURE 4
Forest plot for stratified analysis. OR, odds ratio; 95% CI, 95% confidence interval; CVD, cardiovascular disease.

glycolipid metabolism. These findings may be instrumental in stratifying the risk of NAFLD in the general population and allowing for interventions.

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 humans were approved by The National Center for Health Statistics (NCHS) Research Ethics Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

YL: Conceptualization, Formal analysis, Software, Writing – original draft. MC: Supervision, Writing – review & editing.

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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.2023.1276940/full#supplementary-material

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Glossary

NAFLD	Nonalcoholic fatty liver disease
AHF	Advanced liver fibrosis
OBS	Oxidative balance score
ROS	Reactive oxygen species
NHANES	National Health and Nutrition Examination Survey
NCHS	National Center for Health Statistics
USFLI	US fatty liver index
BMI	Body mass index
GGT	Gamma glutamyltransferase
WC	Waist circumference
NFS	NAFLD fibrosis score
FIB-4	Fibrosis 4 index
AST	Aspartate aminotransferase
APRI	AST/platelet ratio index
ALT	Alanine aminotransferase
ULN	Upper limit of normal
PIR	Family income to poverty ratio
CVD	Cardiovascular disease
UA	Uric acid
TG	Triglycerides
TC	Total cholesterol
HDL	High-density lipoprotein
95% CI	95% confidence interval
RCS	Restricted cubic splines
OR	Odds ratio
DII	Dietary Inflammatory Index

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Relationship between three dietary indices and health-related quality of life among rural elderly in China: a cross-sectional study

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Purpose: This study aimed to explore the association between health-related quality of life (HRQOL) and diet quality using three evidence-based dietary indices among older people in rural China.

Methods: This cross-sectional study included 1,258 rural older people (mean age 72.32 years; 55.6% female). HRQOL was assessed using the European Five Dimension Health Scale (EQ-5D), and dietary intake was assessed using a Food Frequency Questionnaire. Three dietary scoring indices, including the Alternate Healthy Eating Index, Dietary Approaches to Stop Hypertension, and Dietary Diversity Score (DDS), were calculated to assess and analyze the relationship between these dietary indices and quality of life.

Results: The EQ-5D score was 0.95 ± 0.10, and the EQ-Visual Analog Scale (VAS) score was 76.76 ± 14.44. All three groups with higher dietary indices had higher quality of life scores. After controlling for covariates in multivariate adjusted binary logistic regression analyzes, participants in the top tertile of DDS had higher quality of life scores than those in the bottom tertile. DDS was consistently associated with EQ-5D (Model 2: OR = 1.567, $p = 0.001$; Model3: OR = 1.351, $p = 0.044$) and EQ-VAS (Model 2: OR = 1.830, $p < 0.001$; Model 3: OR = 1.383, $p = 0.047$), significantly different from the other groups.

Conclusion: Older people in rural China who adhere to various foods experience a better quality of healthy life.

KEYWORDS

elderly, rural, dietary, quality of life, HRQOL

1. Introduction

As the world's population grows older, aging is becoming a serious social problem. By 2050, the number of people over 60 years worldwide will double from 1.4 billion in 2015 to 2.1 billion (1). According to the latest census results in China, 18.70 and 13.50% of the total population are aged ≥60 years old and ≥65 years old, respectively. The township population accounts for 36.11% of the total population (2). Due to urbanization, most young people start to work and live in the cities, while the older people remain in the rural areas (3). As a result, the quality of life of the elderly living in rural areas has attracted attention (4, 5). Older people in rural China generally have inadequate nutritional intake (6), which is extremely detrimental to their nutritional health (7). The risk of malnutrition among Chinese elders is 12.6% (8). Several cross-sectional and cohort studies have shown negative associations between healthy and adequate nutritional status and chronic disease risk (9–11). Additionally, nutrition is closely related to the health-related quality of life (HRQOL) of rural older people.

HRQOL is a multidimensional indicator of overall health. It can be defined as a composite confounding criterion of social relationships, personal emotions, independence, etc., significantly related to the quality of life consequences of an individual's health status. Many scales can be used to evaluate the quality of a healthy life, such as the Brief Health Questionnaire, the World Health Organization (WHO) Quality of Life Scale (WHOQOL), and the Quality of Well-Being Scale (QWB) (12). Quality of life assessment tools for older adults include the 36-item Brief Health Status Inventory (SF-36), the European Five Dimension Health Scale (EQ-5D), and the WHOQOL. The EQ-5D has feasibility properties in the elderly population (13). The Chinese version of the EQ-5D scale has been widely used in China and has good reliability and validity (14). Therefore, we chose the EQ-5D to assess the quality of life of older people in China.

Adequate nutrition is an essential component of healthy aging (15). It is well known that the nutritional status of older people is critical to health. However, with increasing age and declining physical functions, older people may develop a loss of appetite and decreased ability to chew (16). This can lead to weight loss and malnutrition. Moreover, the income level of older people affects their nutritional status: research has shown that financial hardship is associated with nutritional problems (17). Most older people in rural China have low economic incomes, limiting their ability to afford a high-quality diet or maintain good nutrition. It is, thus, of significant interest to evaluate the dietary quality of older Chinese people in rural areas.

Diet Quality Index (DQI) is a common index to evaluate diet quality, such as the Mediterranean (MED) (18), the Alternate Healthy Eating Index (AHEI) (19), the Dietary Approaches to Stop Hypertension (DASH) (20), and the Dietary Diversity Score (DDS) (21). Existing studies have mostly analyzed the correlation between one DQI or different DQIs and different diseases (22–25). A large study investigated the association of diet quality, assessed by the AHEI, MEDAS, and DDS score, with health status (all-cause mortality, cardiovascular disease mortality or morbidity, cancer mortality or morbidity, type 2 diabetes, and neurodegenerative disease risk) (26). However, there are few studies on the relationship between quality of life and DQI in older people, especially in rural Chinese older people (27). In this study, three dietary index scores were selected to assess the quality of life of older people in rural China.

2. Materials and methods

2.1. Study design and study population

This cross-sectional study included 1,258 people (≥65 years) living in rural China. All participants in the study were volunteers who signed informed consent forms.

As shown in Figure 1, 1,280 older people were invited to this study (94.4% participation rate), and 22 participants were excluded because they could not complete all questionnaires. Therefore, data from 1,258 older people (mean age 72.32 ± 6.00 years; 55.6% female) were analyzed.

2.2. Dietary survey

The Food Frequency Questionnaire (FFQ, containing 97 food items), which has been used in studies of Chinese residents with good results and high reliability, was used to measure the dietary intake of respondents in the past 3 months (28, 29). Based on the latest version of the Chinese food composition table, we constructed different nutritional variables such as whole grains, vegetables, fruits, red meat, sugary drinks, alcohol, trans fatty acids, omega fatty acids (EPA + DHA), and sodium.

2.3. Dietary quality indices

Using the dietary intake data derived from the FFQ, we selected the following DQIs: AHEI, DASH, and DDS. The three dietary index scores were derived based on the protocols of Stephanie (9), Teresa (30), and Jin (31), respectively.

The AHEI score consisted of 11 items. We assigned a maximum score of 10 to each item if the recommended intake was achieved and a minimum score of 0 for minimum intake. The total score ranged from 0 to 110, depending on the level of intake.

The DASH score was based on the intake level of all people, regardless of the total intake in the entire population. Each food category was divided into quintiles and there were eight food groups in total. For foods beneficial to health, such as fruits, vegetables, nuts, and legumes, we assigned a categorical score: quintile 1 and quintile 5 were scored 1 and 5, respectively. However, for sugary drinks and red meat, the scale was reversed, and quintile 1 was assigned a score of 5. The total DASH score ranged from 8 to 40 points.

The DDS score was developed by Kant et al. (32). We combined the Chinese Dietary Guidelines and the dietary recommendations of the Chinese Nutrition Association to change the DDS score to a more suitable scale for China (31). Foods were divided into nine categories. The food groups consumed by the study subjects in a week were investigated. One point was assigned to each food group consumed, regardless of the number of times and the amount of intake, and the same food group was not repeatedly scored. The total possible score was nine.

2.4. HRQOL

The EuroQol Five-Dimensional Scale (EQ-5D) consists of two parts: the Five-Dimensional Health Description System and the Visual Analog Scale (EQ-VAS) (33, 34). The five-dimensional health description system includes five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension corresponds to five levels, describing 3,125 health states (35). The EQ-VAS is a visual scale from 0 (worst health self-rated status) to 100 (best health self-rated status) and is an individual's assessment of their health status on the day of measurement. This study used the utility value score system developed by Luo et al. (36) to evaluate the

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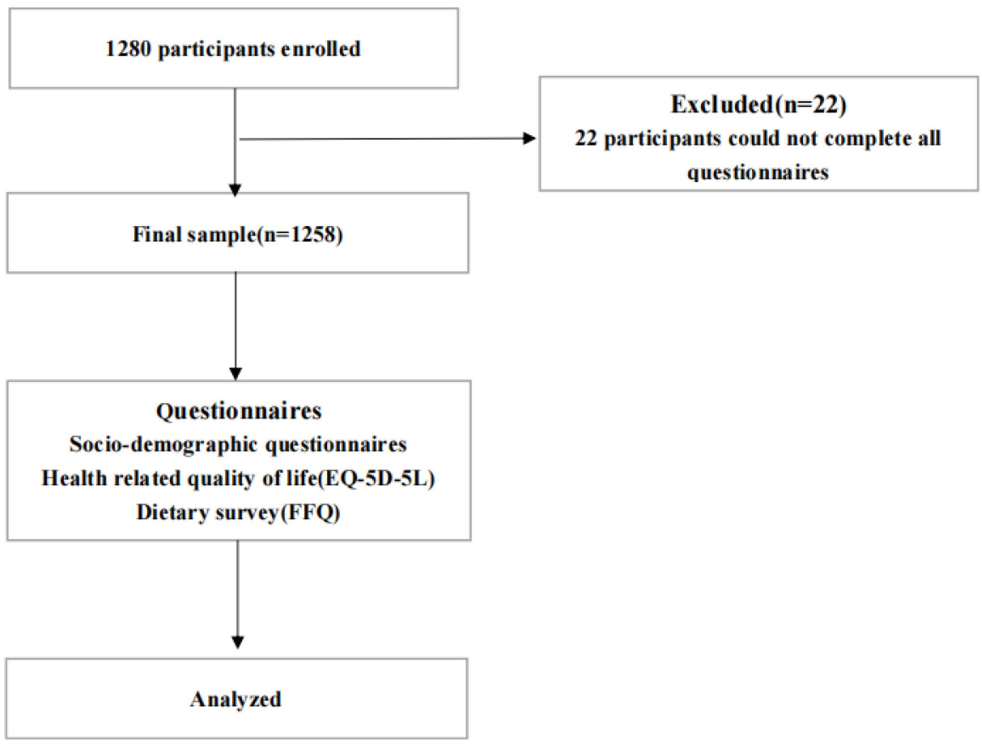

FIGURE 1
Flow chart for the course of study.

health utility index of the Chinese population. The dimensions and degrees were multiplied together, and the results were summed to give a health utility value for each individual. The EQ-5D health utility score ranges from −0.391 to 1: the higher the score, the better the health status.

2.5. Statistical analysis

Based on the distribution of each dietary index score and number of participants, we divided participants into three groups: T1, T2, and T3. However, due to the concentration of DASH and DDS scores, we grouped the concentrated values as one category to complete the grouping—many participants in the DASH and DDS groups had the same score value and could not be separated into the two groups, resulting in differences in the number of participants in each group. Chi-square tests were used to compare categorical variables between quartiles of dietary pattern scores. For continuous variables, we first tested the normality assumption using the Shapiro–Wilk test. Non-normally distributed variables were normalized using a logarithmic transformation. One-way analysis of variance was then used to compare continuous variables between dietary pattern scores. HRQOL was categorized into a high and a low group according to its distribution and analyzed using binary logistic regression. The multivariate adjustment model employed binary logistic regression: Model 1 did not adjust for any factors; Model 2 adjusted for gender, age, and body mass index; Model 3 additionally adjusted for smoking, alcohol consumption, chronic illness, cognitive status, residence, income, and education (each factor that had an impact on the quality of life of older people was selected based on a one-way analysis of the Supplementary Table).

All data analyzes were performed using the Statistical Package for Social Sciences software (version 25), and all significance levels were two-sided p-values <0.050. Categorical data were expressed as percentages. Continuous variables were expressed as arithmetic means and log-transformed variables at 95%.

3. Results

3.1. Three different HRQOLs with basic participant characteristics

A total of 1,258 participants were included in this study; 55.6% ($n = 699$) of the participants were female, with a mean age of 72.32 ± 6.00 years. T3 in the DASH group had the lowest percentage of smokers (23.7%) and the lowest percentage of hypertensive patients (33.7%) and T3 group of DDS had the lowest proportion of malnutrition (16.4%) and the lowest proportion of MCI (14.8%).Three-quarters (75.4%) of the participants were married, and 1,056 (83.9%) lived with their families. Participants in the highest group (T3) of each diet index score were more likely to be educated, married, live with their families, be highly active, have a higher income, and have good nutritional and cognitive status (Table 1).

TABLE 1 Characteristics of study participants.

	Total	AHEI			DASH			DDS		
	(*n* = 1,258)	T1 (*n* = 443)	T2 (*n* = 399)	T3 (*n* = 416)	T1 (*n* = 259)	T2 (*n* = 510)	T3 (*n* = 489)	T1 (*n* = 471)	T2 (*n* = 299)	T3 (*n* = 488)
Age	72.32 ± 6.00	72.99 ± 6.23	72.53 ± 6.00	71.41 ± 5.67	72.80 ± 6.14	72.51 ± 6.235	71.88 ± 5.67	73.06 ± 6.44	72.15 ± 5.80	71.72 ± 5.63
BMI (%)										
Underweight	39 (3.1)	17 (3.8)	14 (3.5)	8 (1.9)	9 (3.5)	19 (3.7)	11 (2.2)	20 (4.2)	9 (3.0)	10 (2.0)
Normal	406 (32.3)	143 (32.3)	130 (32.6)	133 (32.0)	96 (37.1)	168 (32.9)	142 (29.0)	163 (34.6)	95 (31.8)	148 (30.3)
Overweight	625 (49.7)	217 (49.0)	192 (48.1)	216 (51.9)	116 (44.8)	249 (48.8)	260 (53.2)	222 (47.1)	148 (49.5)	255 (52.3)
Obesity	188 (14.9)	66 (14.9)	63 (15.8)	59 (14.2)	38 (14.7)	74 (14.5)	76 (15.5)	66 (14.0)	47 (15.7)	75 (15.4)
Education (%)										
Illiteracy	575 (45.7)	230 (51.9)	183 (45.9)	162 (38.9)	123 (47.5)	266 (52.2)	186 (38.0)	257 (54.6)	149 (49.8)	169 (34.6)
Primary school	450 (35.8)	149 (33.6)	142 (35.6)	159 (38.2)	91 (35.1)	161 (31.6)	198 (40.5)	152 (32.3)	101 (33.8)	197 (40.4)
Secondary school	233 (18.5)	64 (14.4)	74 (18.5)	95 (22.8)	45 (17.4)	83 (16.3)	105 (21.5)	62 (13.2)	49 (16.4)	122 (25.0)
Married (%)										
Single	17 (1.4)	6 (1.4)	3 (0.8)	8 (1.9)	5 (1.9)	9 (1.8)	3 (0.6)	9 (1.9)	2 (0.7)	6 (1.2)
married	949 (75.4)	320 (72.2)	295 (73.9)	334 (80.3)	185 (71.4)	369 (72.4)	395 (80.8)	343 (72.8)	220 (73.6)	386 (79.1)
Widowed and divorced	292 (23.2)	117 (26.4)	101 (25.3)	74 (17.8)	69 (26.6)	132 (25.9)	91 (18.6)	119 (25.3)	386 (79.1)	96 (19.7)
Residence (%)										
alone	202 (16.1)	76 (17.2)	65 (16.3)	61 (14.7)	42 (16.2)	89 (17.5)	71 (14.5)	79 (16.8)	59 (19.7)	64 (13.1)
With family	1,056 (83.9)	367 (82.8)	334 (83.7)	355 (85.3)	217 (83.8)	421 (82.5)	418 (85.5)	392 (83.2)	240 (80.3)	424 (86.9)
Tobacco smoking (%)										
Yes	313 (24.9)	115 (26.0)	90 (22.6)	108 (26.0)	80 (30.9)	117 (22.9)	116 (23.7)	110 (23.4)	70 (23.4)	133 (27.3)
No	945 (75.1)	328 (74.0)	309 (77.4)	308 (74.0)	179 (69.1)	393 (77.1)	373 (76.3)	361 (76.6)	229 (76.6)	355 (72.7)
Alcohol consumption (%)										
Yes	334 (26.6)	102 (23.0)	94 (23.6)	138 (33.2)	75 (29.0)	126 (24.7)	133 (27.2)	99 (21.0)	77 (25.8)	158 (32.4)
No	924 (73.4)	341 (77.0)	305 (76.4)	278 (66.8)	184 (71.0)	384 (75.3)	356 (72.8)	372 (79.0)	222 (74.2)	330 (67.6)
Annual income (%)										
≤3,000	236 (18.8)	119 (26.9)	75 (18.8)	42 (10.1)	30 (11.6)	126 (24.7)	80 (16.4)	100 (21.2)	63 (21.1)	73 (15.0)
>3,000	1,022 (81.2)	324 (73.1)	324 (81.2)	374 (89.9)	229 (88.4)	384 (75.3)	409 (83.6)	371 (78.8)	236 (78.9)	415 (85.0)
Hypertension (%)										
Yes	444 (35.3)	156 (35.2)	133 (33.3)	155 (37.3)	87 (33.6)	192 (37.6)	165 (33.7)	164 (34.8)	94 (31.4)	186 (38.1)
No	814 (64.7)	287 (64.8)	266 (66.7)	261 (63.7)	172 (66.4)	318 (62.4)	324 (66.3)	307 (65.2)	205 (68.6)	302 (61.9)

(Continued)

10.3389/fnut.2023.1259227

TABLE 1 (Continued)

	Total	AHEI			DASH			DDS		
	(*n* = 1,258)	T1 (*n* = 443)	T2 (*n* = 399)	T3 (*n* = 416)	T1 (*n* = 259)	T2 (*n* = 510)	T3 (*n* = 489)	T1 (*n* = 471)	T2 (*n* = 299)	T3 (*n* = 488)
Diabetes (%)										
Yes	235 (18.7)	75 (16.9)	84 (21.1)	76 (18.3)	29 (11.2)	52 (10.2)	63 (12.9)	48 (10.2)	31 (10.4)	65 (13.3)
No	1,023 (81.3)	368 (83.1)	315 (78.9)	340 (81.7)	230 (88.8)	458 (89.8)	426 (87.1)	423 (89.8)	268 (89.6)	423 (86.7)
Activity time (%)										
<2h	373 (29.7)	145 (32.7)	124 (31.1)	104 (25.0)	86 (33.2)	156 (30.6)	131 (26.8)	170 (36.1)	96 (32.1)	107 (21.9)
≥2h	885 (70.3)	298 (67.3)	275 (68.9)	312 (75.0)	173 (66.8)	354 (69.4)	358 (73.2)	301 (63.9)	203 (67.9)	381 (78.1)
Nutrition (%)										
Health	1,010 (80.3)	364 (82.2)	313 (78.4)	333 (80.0)	220 (84.9)	387 (75.9)	403 (82.4)	371 (78.8)	231 (77.3)	408 (83.6)
Malnutrition	248 (19.7)	79 (17.8)	86 (21.6)	83 (20.0)	39 (15.1)	123 (24.1)	86 (17.6)	100 (21.2)	68 (22.7)	80 (16.4)
Cognition (%)										
Health	944 (75.0)	308 (69.5)	304 (76.2)	332 (79.8)	185 (71.4)	364 (71.4)	395 (80.8)	313 (66.5)	215 (71.9)	416 (85.2)
MCI	314 (25.0)	135 (30.5)	95 (23.8)	84 (20.2)	74 (28.6)	146 (38.6)	94 (19.2)	158 (33.5)	84 (28.1)	72 (14.8)

BMI, body mass index; AHEI, Alternate Healthy Eating Index; DASH, Dietary Approaches to Stop Hypertension; DDS, Dietary Diversity Score.

3.2. Univariate analysis of different dietary quality indices on HRQOL

The EQ-5D and EQ-VAS scores were 0.95 ± 0.10 and 76.76 ± 14.44, respectively (Table 2). Across the three different dietary index scores, the T3 group had the highest EQ-5D and EQ-VAS scores (Table 2). The EQ-5D score in T3 of AHEI was 0.96 ± 0.08, DASH was 0.95 ± 0.09, and DDS was 0.96 ± 0.08; the EQ-VAS score for AHEI was 78.88 ± 15.11, DASH was 78.59 ± 14.55, and DDS was 80.06 ± 13.10. The number of people with health problems was lowest in the highest scoring group (T3) on the dimensions assessing quality of life. There were differences in self-care ($p = 0.005$) and anxious/depression ($p = 0.012$) scores by levels of AHEI. By contrast, only self-care scores ($p = 0.009$) varied by levels of DASH. Meanwhile, scores in all dimensions (mobility: $p = 0.001$, self-care: $p < 0.001$, pain/discomfort: $p = 0.002$, anxious/depression: $p = 0.035$) differed by levels of DDS.

3.3. Multifactorial effects of different dietary quality indices on HRQOL

Controlling for covariates in multivariate adjusted binary logistic regression analyzes, participants in the top tertile of DDS had higher quality of life scores than those in the bottom tertile (Tables 3, 4). The higher the DDS score, the greater the chance that the EQ-5D (Model 2: OR = 1.567, $p = 0.001$; Model 3: OR = 1.351, $p = 0.044$) will be in a high classification, which is similar to the trend for the EQ-VAS (Model 2: OR = 1.830, $p < 0.001$; Model3: OR = 1.383, $p = 0.047$). The EQ-VAS (Model 2: OR = 0.694, $p = 0.041$; Model 3: OR = 0.636, $p = 0.016$) was negatively associated with quality of life in the T2 score group of DASH. No significant association was found between higher AHEI scores and HRQOL after adjusting for covariates ($p > 0.05$).

4. Discussion

This study applied the European Five-Dimensional Health Scale (EQ-5D) to investigate the quality of life in a sample of older people in rural Chinese. We found that most of the older people in rural areas were farmers, and due to the low economic level and degenerative changes in old age, they were prone to various health problems. Finding ways to improve their quality of life is a difficult but urgent issue.

The EQ-5D health utility score was 0.95, similar to the Chinese population standard of EQ-5D-5L (0.946) (37). By contrast, the EQ-5D health utility score was higher than the score (0.94) found by Yang et al. (33). Additionally, the score in the study was higher than that of students in the United Kingdom (UK) (0.90 ± 0.167) (38) and Canada (0.89 ± 0.14) (39), and the score in older people in Vietnam (0.80 ± 0.20) (40). These findings suggest that older people in rural China have a higher quality of life. In a survey of older people in five cities in China, the EQ-5D health utility score was close to 1, and the local older people had better quality of life. The EQ-VAS score was based on the self-perception and health evaluation of the study participants on the day of assessment. The EQ-VAS score in this study was 76.97, which was lower than that of students in the United Kingdom (38) and Canada (39) and higher than that of older people in Vietnam (40). One possible reason is that, due to cultural

TABLE 2 Univariate analysis of three dietary index scores and HRQOL.

	Mobility *n* (%)	Self-care *n* (%)	Usual Activities *n* (%)	Pain/ discomfort *n* (%)	Anxious/ Depression *n* (%)	EQ-5D utility value Mean ± SD	EQ-VAS score Mean ± SD
Total	138 (11.0)	116 (9.2)	158 (12.6)	370 (29.4)	133 (10.6)	0.95 ± 0.10	76.76 ± 14.44
AHEI							
T1	60 (13.5)	55 (12.4)	68 (15.3)	131 (29.6)	57 (12.9)	0.94 ± 0.10	76.24 ± 13.08
T2	43 (10.8)	36 (9.0)	49 (12.3)	126 (31.6)	47 (11.8)	0.94 ± 0.12	75.78 ± 14.98
T3	35 (8.4)	25 (6.0)	41 (9.9)	113 (27.2)	29 (7.0)	0.96 ± 0.08	78.88 ± 15.11
χ^2 /Z	5.805	10.544	5.938	1.921	8.788	231.189	54.514
P	0.055	0.005	0.051	0.383	0.012	0.004	0.001
DASH							
T1	34 (13.1)	27 (10.4)	38 (14.7)	66 (25.5)	31 (12.0)	0.95 ± 0.10	76.81 ± 15.58
T2	59 (11.6)	59 (11.6)	69 (13.5)	158 (31.0)	59 (11.6)	0.94 ± 0.11	75.49 ± 13.56
T3	45 (9.2)	30 (6.1)	51 (10.4)	146 (19.9)	43 (8.8)	0.95 ± 0.09	78.59 ± 14.55
χ^2 /Z	2.986	9.370	3.509	2.577	2.707	214.069	59.791
P	0.225	0.009	0.173	0.276	0.258	0.034	<0.001
DDS							
T1	66 (14.0)	64 (13.6)	75 (15.9)	155 (32.9)	66 (14.0)	0.93 ± 0.12	74.88 ± 14.86
T2	39 (13.0)	20 (6.7)	41 (13.7)	91 (30.4)	30 (10.0)	0.94 ± 0.10	75.20 ± 15.00
T3	33 (6.8)	32 (6.6)	42 (8.6)	124 (25.4)	37 (7.6)	0.96 ± 0.08	80.06 ± 13.10
χ^2 /Z	14.628	17.157	12.159	6.689	10.604	247.932	74.400
P	0.001	<0.001	0.002	0.035	0.005	<0.001	<0.001

AHEI, Alternate Healthy Eating Index; DASH, Dietary Approaches to Stop Hypertension; DDS, Dietary Diversity Score.

traditions, Chinese people were more reluctant to report health problems than Western populations and lacked confidence in their health status (41, 42).

As we predicted, older adults who adhered to all three dietary index scores at univariate analysis had higher HRQOL scores. In particular, the group with the highest dietary pattern score had the highest HRQOL. Older adults typically experience a decline in physical and mental abilities, including mobility and digestion, putting them at higher risk for malnutrition (43, 44). This indicates that compliance with dietary patterns leads to higher quality of life.

The AHEI did not show any correlation with HRQOL after multifactor adjustment, so we speculated that the AHEI may not be suitable for the dietary patterns and habits of older people in rural China.

The DASH dietary index score was associated with the EQ-VAS after multifactorial analysis. The T2 group showed a negative correlation with HRQOL (OR < 1): according to our analysis of the scores, the groups with greater differences were in the fruit category and red meat. Participants in the T2 group consumed more red meat, which may have been associated with lower EQ-VAS scores. More importantly, because of the concentration of scores, only one population was scored in the T2 group, which was representative. This needs to be explored in further studies. This might be related to the specific dietary structure of older people in rural China, which only suggests that the DASH and AHEI dietary patterns were not suitable for the dietary patterns of older people living in rural China and does not indicate a lack of association between such diets and quality of life.

DDS is a simple and efficient way to assess diet quality as an indicator of nutritional and health status (35). DDS was used to assess nutritional adequacy and overall diet quality and was considered a key indicator of high diet quality in different populations (25, 45). The higher the DDS dietary pattern score, the higher the HRQOL score of older adults, which is consistent with the results of previous studies (46, 47). Previous studies have suggested that higher DDS scores in Chinese adults may be associated with higher protein intake (48), which may improve the risk of malnutrition in older adults. After multifactorial analysis, we found that adherence to DDS dietary index scores after adjusting for other confounders had a better effect on HRQOL older adults in rural China. The DDS dietary index score performed well in EQ-5D and EQ-VAS scores in the high-scoring subgroup. This indicates that adherence to the DDS dietary pattern improves the quality of life and life experience of older people in rural China. This was similar to the findings of Poorrezaeian (49), which illustrated that DDS is a protective factor against depression in older adults, with each unit increase in DDS associated with a 39% reduction in the risk of major depression. Mina Poorrezaeian (50) also found that anxiety scores were significantly lower in people with high dietary diversity than in those with low dietary diversity, and the two were negatively correlated. This further suggests that DDS may be a protective factor for older people's mental quality of life and that adherence to DDS can enhance older people's self-rated quality of life (EQ-VAS).

Meanwhile, vegetables and fruits are the main sources of antioxidants, and the intake of diverse foods may increase antioxidant capacity (48). Moreover, diverse food intake may promote healthier

TABLE 3 Estimated parameters of the EQ-5D multifactor analysis and mixed model.

		Model1			Model2			Model3		
		OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*
AHEI	T1	1.0			1.0			1.0		
	T2	1.092	(0.825–1.445)	0.537	1.058	(0.795–1.406)	0.700	0.989	(0.737–1.327)	0.940
	T3	1.298	(0.980–1.719)	0.069	1.177	(0.883–1.570)	0.266	1.069	(0.790–1.447)	0.664
DASH	T1	1.0			1.0			1.0		
	T2	0.944	(0.690–1.290)	0.715	0.999	(0.726–1.375)	0.995	1.053	(0.756–1.467)	0.759
	T3	1.016	(0.741–1.394)	0.919	1.010	(0.731–1.393)	0.954	0.951	(0.681–1.329)	0.770
DDS	T1	1.0			1.0			1.0		
	T2	1.123	(0.834–1.512)	0.445	1.108	(0.819–1.501)	0.505	1.001	(0.730–1.372)	0.996
	T3	1.646	(1.259–2.152)	<0.001	1.567	(1.191–2.060)	0.001	1.351	(1.008–1.811)	0.044

AHEI, Alternate Healthy Eating Index; DASH, Dietary Approaches to Stop Hypertension; DDS, Dietary Diversity Score; OR, odds ratio; 95% CI, 95% confidence interval.
Model 1: No adjustment for any factor; Model 2: Adjusts for gender, age, BMI; Model 3: Adds adjustments for smoking, alcohol consumption, chronic illness, cognitive status, residence, income, education.

TABLE 4 Estimated parameters of the EQ-VAS multifactor analysis and mixed model.

		Model1			Model2			Model3		
		OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*
AHEI	T1	1.0			1.0			1.0		
	T2	0.931	(0.678–1.279)	0.660	0.910	(0.661–1.254)	0.566	0.885	(0.633–1.237)	0.474
	T3	1.408	(1.044–1.900)	0.025	1.310	(0.966–1.776)	0.083	1.272	(0.919–1.761)	0.147
DASH	T1	1.0			1.0			1.0		
	T2	0.669	(0.473–0.944)	0.022	0.694	(0.489–0.985)	0.041	0.636	(0.441–0.918)	0.016
	T3	1.171	(0.841–1.631)	0.349	1.158	(0.828–1.620)	0.392	0.990	(0.695–1.409)	0.953
DDS	T1	1.0			1.0			1.0		
	T2	1.038	(0.731–1.474)	0.836	1.033	(0.725–1.472)	0.857	0.880	(0.604–1.282)	0.507
	T3	1.898	(1.415–2.521)	<0.001	1.830	(1.364–2.454)	<0.001	1.383	(1.005–1.903)	0.047

AHEI, Alternate Healthy Eating Index; DASH, Dietary Approaches to Stop Hypertension; DDS, Dietary Diversity Score; OR, odds ratio; 95% CI, 95% confidence interval.
Model 1: No adjustment for any factor; Model 2: Adjusts for gender, age, BMI; Model 3: Adds adjustments for smoking, alcohol consumption, chronic illness, cognitive status, residence, income, education.

gut flora (51). Studies have demonstrated a positive correlation between dietary diversity and healthy gut microbial stability, which may all contribute to the fact that a diverse diet may improve the quality of life in older adults. Dietary fiber, phenolic compounds, and carotenoids, which are abundant in vegetables and fruits, also reduce the level of inflammation in the body (52). Increased inflammation is thought to be a potential mechanism for the development of mental illness (53). This suggests that adherence to DDS not only increases HRQOL scores in older adults but may also increase satisfaction with life through the modulation of mental health in older adults.

The energy and nutrient intake among older Chinese adults is inadequate, and most older people are at high risk of nutritional deficiencies, especially those living in rural areas with lower levels of education and low household income (54). During our survey, we learned that most older people in rural areas still work on the farm and eat the food they grow daily. The diets of rural Chinese older adults have a single food intake and a carbohydrate-based diet with an insufficient intake of fruits and vegetables. Therefore, a DDS dietary index score that increases food diversity will increase the intake of different nutrients for rural older people. Additionally, consuming different types of food is associated with greater psychological comfort to older people due to problems such as swallowing and will give them a sense of good appetite and a strong body.

We hope future researchers will focus more on older people in rural areas, where their intake of a single food type and poor education predispose them to neglect. Owing to urbanization and the declining physical condition of older adults, it is crucial that they receive more attention and support from society for a healthier and thriving old age.

5. Limitations

This study was cross-sectional. Consequently, more specific reasons and results cannot be derived. Due to the older age and the lower educational levels, older adults in rural areas may express their physical condition poorly using questionnaires. More objective indicators should, therefore, be used for analysis. To better represent the national level of older people, samples should be taken from multiple regions and analyzed. Additionally, the diet of older people is best investigated in four different seasons to reflect their dietary status better. Although we have adjusted for multiple confounding factors, we could not adjust for all possible confounding factors.

6. Conclusion

This study adds to the evidence that adherence to the DDS dietary pattern is associated with a higher quality of life. The diets of older people in rural China are mainly carbohydrate-based, with a single type of food intake and a low intake of fruit, vegetables, and milk. We found a positive association between DDS and quality of life. Therefore, we recommend that older people should consume a variety of foods daily. With a balanced intake of various nutrients, increasing the intake of more food groups, especially fruits and vegetables, is likely to improve the quality of life of older people in rural areas.

Finally, society and the government should pay more attention to the health status and quality of life of older people. Not only dietary issues but also psychological and social issues can affect the quality of life of older people in their later years, which all require the attention of society.

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.

Author contributions

CY: Data curation, Formal analysis, Investigation, Software, Writing – original draft, Writing – review & editing. PL: Investigation, Writing – original draft. WH: Writing – review & editing. YZ: Data curation, Investigation, Writing – original draft. CL: Writing – review & editing. TG: Data curation, Formal analysis, Writing – review & editing. FZ: Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

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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.2023.1259227/full#supplementary-material

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The effect of flaxseed supplementation on sex hormone profile in adults: a systematic review and meta-analysis

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Inconsistent data suggest that flaxseed supplementation may have a role in sex hormones. We aimed to carry out a systematic review and meta-analysis of randomized controlled trials (RCTs) investigating effects of flaxseed supplementation on sex hormone profile. PubMed, Scopus, Embase, Cochrane Library, Web of Science databases, and Google Scholar were searched up to March 2023. Standardized mean difference (SMD) was pooled using a random-effects model. Sensitivity analysis, heterogeneity, and publication bias were reported using standard methods. The quality of each study was evaluated with the revised Cochrane risk-of-bias tool for randomized trials, known as RoB 2. Finding from ten RCTs revealed that flaxseed supplementation had no significant alteration in follicle-stimulating hormone (FSH) (SMD: −0.11; 95% CI: −0.87, 0.66: $p = 0.783$), sex hormone-binding globulin (SHBG) (SMD: 0.35; 95% CI: −0.02, 0.72; $p = 0.063$), total testosterone (TT) levels (SMD: 0.17; 95% CI: −0.07, 0.41; $p = 0.165$), free androgen index (FAI) (SMD = 0.11, 95% CI: −0.61, 0.83; $p = 0.759$), and dehydroepiandrosterone sulfate (DHEAS) (SMD: 0.08, 95%CI: −0.55, 0.72, $p = 0.794$). Flaxseed supplementation had no significant effect on sex hormones in adults. Nevertheless, due to the limited included trials, this topic is still open and needs further studies in future RCTs.

KEYWORDS

flaxseed, sex hormones, gender, systematic review, meta-analysis

1. Introduction

Sex hormones and their harmony have been considered as an important issue, due to their interactions with body tissues. Estrogen and progesterone are the main female sex hormones that their production and secretion are triggered by pituitary hormones including follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (1). Additionally, LH is the main regulatory hormone for testosterone in men (2). Besides the regulation of the reproductive system and maturation, these sex hormones have other critical roles in health status. Such that, estrogen protectively influences bone mass (3), immune system (4), and cardiovascular system (5). Muscle mass is also strengthened by androgens (6). Nevertheless, disharmony in sex hormones is involved in the incidence of several diseases including osteoarthritis (7), obesity, metabolic syndrome, polycystic ovary syndrome (PCOS), cardiovascular disease (CVD) (8), and different types of cancers (9, 10). Therefore, the prevention and management of disharmony in sex hormones are very worthful.

The synthesis and metabolism of sex hormones are influenced by various factors including genetics, lifestyle, dietary intake, physical activity and environmental factors (11, 12). Based on evidence, flaxseed might influence the sex hormone levels due to its phytoestrogens content such as lignans. The main lignan content of flaxseed is secoisolariciresinol diglycoside which is converted to the mammalian lignans enterolactone and enterodiol by intestinal bacteria (13). Due to similar structure of lignans to sex hormones, they could inhibit the aromatase activity and elevate the sex hormone-binding globulin (SHBG) synthesis in adipose tissues and the liver. Lignans could also bind to testosterone and increase its excretion in bile. Moreover, it has anti-cancer properties due to its inhabitation effect on cell proliferation and mammary tumor incidence (14, 15).

Although the lignan contents of flaxseed could regulate the sex hormones and consequently influence the incidence of related disease, the exact effect of flaxseed supplementation on sex hormones was not clear. Previous studies suggested that the consumption of flaxseed could alter the metabolism of estrogen in postmenopausal women (16). They illustrated that flaxseed supplementation could change serum levels of only some sex hormones (17) or even influence the metabolism and urinary levels of some estrogen metabolites (18). Hutchins et al. (19) reported that consumption of 5 or 10 g/day ground flaxseed for 7 weeks resulted in reduced *estradiol* levels in postmenopausal women. Moreover, a clinical trial illustrated the favorable effect of flaxseed on sex hormones, only in overweight and obese women (20). Haidari et al. (21) also found that supplementation with 3 g/day of flaxseed for a period of 12 weeks did not result in any significant reduction in serum testosterone or SHBG levels in patients with PCOS. Regarding the controversial findings of previous studies, we aimed to examine the probable effect of flaxseed intake on sex hormones such as FSH, SHBG, free androgen index (FAI), total testosterone (TT), and dehydroepiandrosterone sulfate (DHEAS) through a comprehensive systematic review and meta-analysis of randomized clinical trials (RCTs).

2. Method

The current meta-analysis was provided according to the PRISMA guideline (22). The protocol of the present study has been approved by the ethics committee of Isfahan University of Medical Sciences (identifier: IR.MUI.RESEARCH. REC.1402.031 and grant number: 140215).

2.1. Search strategy

A comprehensive systematic search was applied on Web of Science, Google Scholar, EMBASE, PubMed, and Scopus up to March 2023 without any restriction in language or publication year. Additionally, we checked the reference list of related articles to avoid missing the eligible studies. The details of keywords and search strategy in each database were provided in Supplementary Table S1.

2.2. Inclusion and exclusion criteria

Studies were eligible to be included if met the following requirements: (1) had randomized clinical controlled trial design; (2) investigated the influence of flaxseed on sex hormones or their binding proteins including [total testosterone, SHBG, follicle-stimulating hormone (FSH), free androgen index (FAI) and dehydroepiandrosterone Sulfate (DHEAS)]; (3) performed on men and women 18 years or older; (4) reported the change values of the mentioned variables or their values before and after intervention in both control and in treatment groups. Nevertheless, we excluded studies if: (1) used the combination of flaxseed with other substances or exercise; (2) had not control group; (3) did not apply random allocation; (4) did not report changes of the interested variables or their values before and after the intervention; (5) investigated the pregnant women, and children; and (6) gray literatures, patents, dissertations.

2.3. Data extraction

Two investigators independently performed data extraction and the principle researcher supervised this process (G.A). The necessary information was extracted including the duration, location, design and publication year of included studies, first author's last name, the mean age, health status, and the number of included subjects, flaxseed dosage, and the mean ± standard deviation changes of sex hormones or their values before and after intervention in both groups. In studies with insufficient data, authors were requested to send more information by email.

2.4. Quality assessment

Two authors (VM and MV) independently evaluated the risk of bias for each study with RoB 2. The assessment focused on 5 different domains of each study: allocation concealment, random sequence generation, selective reporting, blinding of outcome assessment, and incomplete outcome data. For each section, algorithms assessed the potential bias (low risk, unclear risk, or high risk) (23).

2.5. Statistical analysis

Using the mean ± standard deviation (SD) changes of sex hormones in intervention and control groups, standardized mean difference (SMD) and 95% confidence intervals were estimated as the overall estimates (24). The Cochran's Q test and inconsistency index (I-squared) were used to determine the between-study heterogeneity. Such that, $I^2 \geq 75\%$ and p-value of Q statistic <0.1 were defined as high between-study heterogeneity (25). In cases with significant between-study heterogeneity, subgroup analysis was applied to find the source of heterogeneity. Additionally, meta-regression was performed for continuous variables. Using the sensitivity analysis, the individual effect of each study on the overall effect size was examined. Through the use of funnel plots, Begg's and Egger's tests, we assessed the publication bias (26). All analyses were conducted through the use of Stata Statistical Software version 14 (Stata Corp, College Station, TX, United States).

3. Results

3.1. Selection and characteristics of studies

A total of 1,691 articles were found through the initial literature search of PubMed, Embase, Cochrane, and Web of Science databases, of which 522 were duplicates, and 874 articles were excluded through screening titles and abstracts. Finally, 10 out 16 RCTs were included in the meta-analysis. The systematic review literature screening flow chart is shown in Figure 1. The characteristics of the included RCTs are outlined in Table 1. All included studies were conducted between 1998 and 2020. Of the 10 included studies, five of them were conducted in USA and two in Iran, two in Brazil, and one in Canada. The intervention duration of the studies ranged from 4.5 to 24 weeks; the mean age of the 49 years; and patient and control sample sizes ranged from 34 to 81. The risk of bias was assessed as shown in Figure 2. According to the RoB2, six of ten studies had high quality.

3.2. Effects of flaxseed on FSH

The pooled results showed that flaxseed supplementation no significantly decreased FSH (SMD: −0.11; 95% CI: −0.87, 0.66: $p = 0.783$, $I^2 = 87.3\%$, $p < 0.001$) (Figure 3). Flaxseed supplementation in studies with an intervention duration of ≥12 weeks contributes to a more significant reduction in FSH level (Table 2). Removing an individual study at a time by sensitivity analysis did not affect the results. Begg's test revealed no significant in identifying small-study effects ($p = 0.452$).

3.3. Effect of flaxseed on SHBG

Meta-analysis of data from six RCTs with seven arms revealed no significant alteration in SHBG following flaxseed supplementation (SMD: 0.35; 95% CI: −0.02, 0.72; $p = 0.063$, $I^2 = 71.3\%$, $p = 0.002$) (Figure 4). Subgroup analysis indicated that flaxseed supplementation in patients with PCOS, an intervention duration of ≥12 weeks and mean age of ≤50 years had a significant effect in increasing SHBG (Table 2). By removing Chang et al. study, the non-significant effect of flaxseed on SHBG levels became significant (SMD: 0.47; 95% CI: 0.16, 0.79; $p < 0.05$). The result of Begg's tests was not significant in identifying small-study effects ($p = 0.207$).

3.4. Effect of flaxseed on TT

The results of the meta-analysis indicated that flaxseed did not significantly increase TT levels (SMD: 0.17; 95% CI: −0.07, 0.41; $p = 0.165$, $I^2 = 27.7\%$, $p = 0.227$) (Figure 5). Performing subgroup analysis revealed that the effects of flaxseed on TT levels in men with prostate cancer were more robust than the entire sample (Table 2). By removing Chang et al. study, the non-significant effect of flaxseed on TT levels became significant (SMD: 0.26; 95% CI: 0.31, 0.49; $p < 0.05$). There were no significant small-study effects with using Begg's test ($p = 0.296$).

3.5. Effect of flaxseed on FAI

Results did not show any meaningful effect of flaxseed supplementation on FAI (SMD = 0.11, 95% CI: −0.61, 0.83; $p = 0.759$, $I^2 = 87.6\%$, $p < 0.001$) (Figure 6). No significant difference in overall effect size was shown after removing each study using sensitivity analysis.

3.6. Effect of flaxseed on DHEAS

Flaxseed supplementation led to no significant increase in DHEAS (SMD: 0.08, 95%CI: −0.55, 0.72, $p = 0.794$, $I^2 = 77.5\%$, $p = 0.012$) (Figure 7).

4. Discussion

In the present systematic review and meta-analysis, we summarized the available data from 10 trials which investigated the effect of flaxseed supplementation on sex hormones. This study to the best of our knowledge is the first in this field of research. The meta-analysis did not show a significant effect of flaxseed on FSH, FAI, DHEAS, TT, and SHBG levels in comparison with control group in adults. However, subgroup analyses showed that flaxseed supplementation in subjects with ≤50 years old, an intervention duration of ≥12 weeks and with PCOS significantly increased SHBG. In addition, we observed an increase in TT following flaxseed supplementation in subgroup of men, and subjects with prostate cancer. Results from most trials in this area are in line with our study. In terms of estrogenic effects of flaxseed, it did not change FSH, FAI levels and contradicted previous results that flaxseed has estrogenic properties (16, 18). It should be taken into account that few RCTs have examined the effect of flaxseed supplementation on sex hormones. Consequently, further RCTs are required to reach a firm conclusion about the effect of flaxseed in different durations and doses on sex hormones.

The effects of dietary components on circulating sex hormone levels are potentially of great importance for the prevention of hormone-associated complaints. The endocrine system is a complex network of hormones and glands that decline with age includes a reduction in testosterone levels of 0.5–1% per year in men, and of estrogen in women, that initiates around 30 years of age (27, 28). The decline in testosterone levels in men is related to loss of muscle mass and strength, and moreover testosterone/dihydrotestosterone supplementation can increase muscle strength (29). Flaxseed is a rich source of several biologically active compounds such as plant lignin secoisolariciresinol diglucoside, which is metabolized to the mammalian lignans enterolactone and enterodiol by intestinal bacteria (13). These products are basically similar to endogenous sex

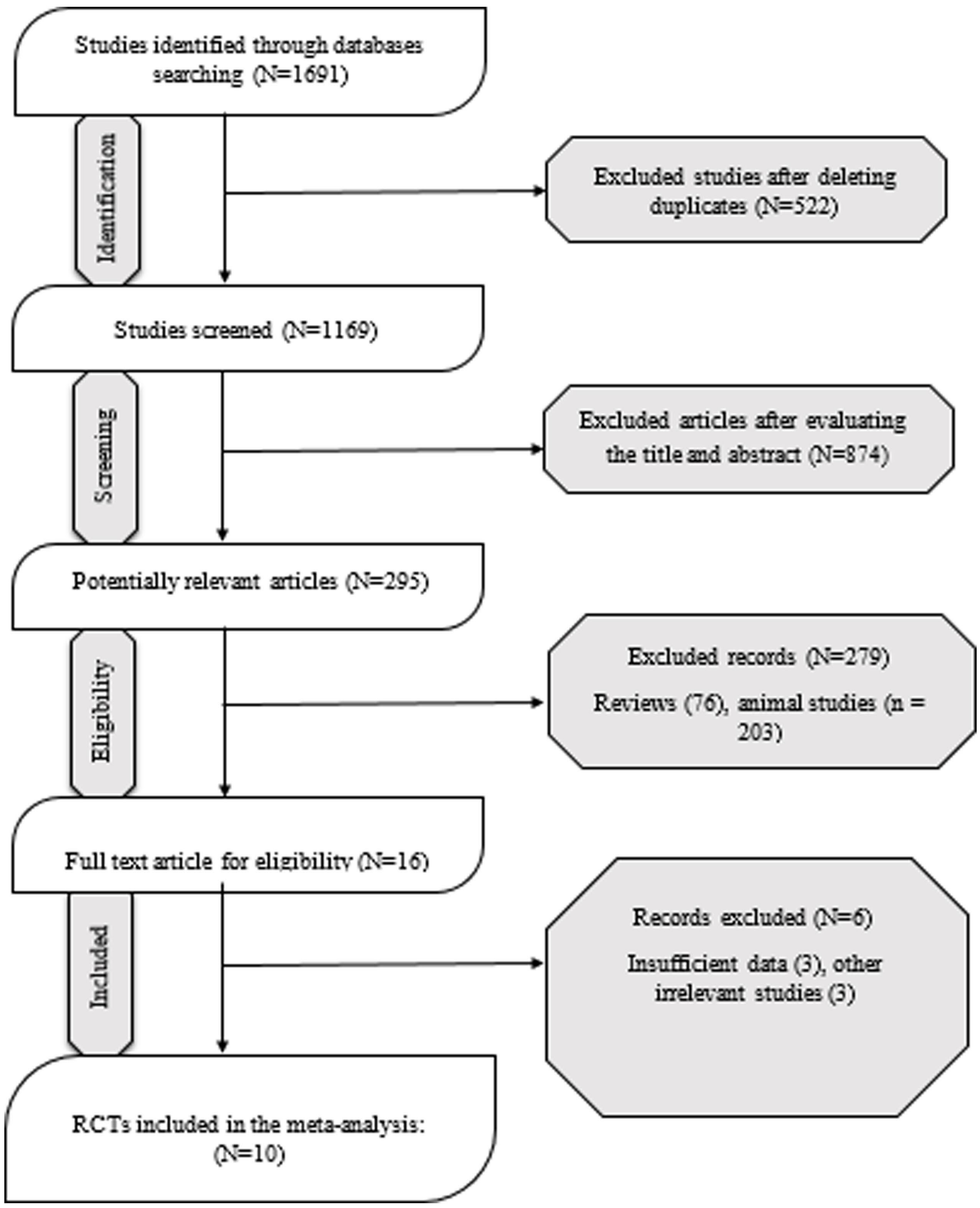

FIGURE 1
Flow diagram of study selection.

hormones and have been revealed to exert weak hormonal properties and prevent carcinogenesis in animal studies (30, 31). For at least two decades, flaxseed, as well as lignans has been examined for their capability has been investigated for their capability to prevention of hormone-related cancers such as breast cancer (30, 32–34). Moreover, lignan has been revealed to decrease testosterone by binding it to enterohepatic circulation and 5α-reductase, the enzyme that converts testosterone to dihydrotestosterone (35, 36).

Morton et al. (37) reported that high lignan diets may be protective against prostate cancer, which is related to high androgen levels. Additionally, lignans have been reported to stimulate SHBG synthesis in the liver and interact with SHBG to change biological activity of circulating androgens and estrogens (38, 39). However, we cannot conclude that flaxseed consumption imposed a significant effect on FSH, FAI, and DHEAS in adult subjects. This might be due to the inconsistencies between the included trials, such as (a) different duration of the intervention period; (b) form and dose of flaxseed supplements used in the trials. In included trials, heterogeneity exists with regard to the role of flaxseed supplementation in lowering sex hormones levels. One potential source of heterogeneity was the intervention duration; where a short period may not be adequate to elicit significant alterations in sex hormones levels. It is also possible that an effect was actually existent but was unobserved in the current study due to the small sample sizes of the included trials, which resulted in a low statistical power for finding of significant results, and obviously represents a viable avenue for future studies.

The mechanism by which flaxseed supplementation could affect sex hormones is, currently, not well understood. Evidence from RCT by Chang et al. (17), provides some support for flaxseed's role in changing estrogen metabolism rather than preventing estrogen

TABLE 1 Study characteristics of included studies.

Author, year	Design	Participants, *n*	Health condition	Age, year	Intervention		Duration (week)
					Treatment group	Control group	
Arjmandi, US, 1998	RA/DB/ crossover	F: 34	Postmenopausal women	Int: 55.44, Con: 57.54	38,000 mg/day whole flaxseed	Sunflower seed	6
Lucas, USA, 2002	RA/DB/parallel	F: 36 Int: 20, Con: 16	Postmenopausal women	Int: 54, Con: 55	40,000 mg/day ground whole flaxseed	Wheat	12
Wahnefried, USA, 2008	RA/SB/parallel	M: 81 Int: 40, Con: 41 M: 80 Int: 40, Con: 40	Prostate Cancer	Int: 60.2, Con: 58.2 Int: 59.3, Con: 59.2	30,000 mg/day flaxseed-supplemented diet, 30,000 mg/day flaxseed-supplemented diet+ low-fat diet	Usual diet, low-fat diet	4.5
Patade, USA, 2008	RA/SB/parallel	F: 26 Int: 17, Con: 9	Postmenopausal women	47-63	30,000 mg/day flaxseed	Control	12
Simbalista, Brazil, 2009	RA/DB/parallel	F: 38 Int: 20, Con: 18	Postmenopausal women	Int: 52, Con: 52.7	25,000 mg/day ground flaxseed	Wheat bran	12
Vargas, USA, 2011	RA/DB/parallel	F: 34 Int: 17, Con: 17	PCOS	Int: 29.4, Con: 28.9	3,500 mg/day flaxseed oil (capsule)	Soybean oil	6
Colli, Brazil, 2012	RA/parallel	F: 53 Int: 28, Con: 25 F: 47 Int: 22, Con: 25	Menopausal	Int: 53.57, Con: 56.57 Int: 54.16, Con: 56.57	1,000 mg/d flaxseed Extract (lignan) 90,000 mg/d flaxseed meal	Collagen	24
Mirmasoumi, Iran, 2017	RA/DB/parallel	F: 60 Int: 30, Con: 30	PCOS	Int: 28.4, Con: 27	1000 mg/day flaxseed oil (capsule)	Liquid paraffin	12
Chang, Canada, 2018	RA/SB/parallel	F: 99 Int: 48, Con: 51	Postmenopausal women	60	15,000 mg/day ground flaxseed	Usual diet	7
Haidari, Iran, 2020	RA/parallel	F: 41 Int: 21, Con: 20	PCOS	Int: 27.21, Con: 26.13	30,000 mg/day brown milled flaxseed powder + lifestyle modification	Lifestyle modification	12

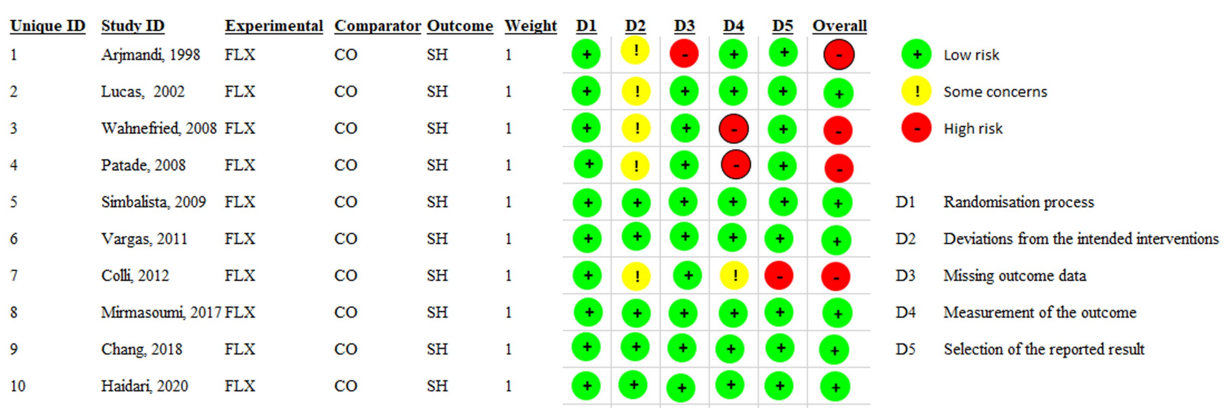

FIGURE 2
Risks of bias assessed by RoB2 for each included study (*n* = 10). FLX, Flaxseed; CO, Control; SH, Sex hormone.

TABLE 2 Subgroup analyses for the effects of Flaxseed supplementation on sexual hormone.

	No	No of participants	SMD (95% CI)[a]	P-within[b]	I^2 (%)[c]	P-heterogeneity[d]
Flaxseed on FSH						
Overall	6	209	−0.11 (−0.87, 0.66)	0.783	87.3	<0.001
Duration (week)						
<12	4	134	0.40 (−0.25, 1.05)	0.227	70.0	0.019
≥12	2	75	−1.05 (−1.47, −063)	<0.001	0.0	0.552
Flaxseed on SHBG						
Overall	7	431	0.35 (−0.02, 0.72)	0.063	71.3	0.002
Age (year)						
≤50	3	135	0.38 (0.04, 0.72)	0.027	0.0	0.878
>50	4	296	0.36 (−0.26, 0.97)	0.261	85.0	<0.001
Gender						
Men	2	161	0.42 (−0.41, 1.24)	0.321	85.3	0.009
Women	5	270	0.32 (−0.14, 0.78)	0.169	69.4	0.011
Duration (week)						
<12	4	294	0.20 (−0.33, 0.72)	0.464	79.5	0.002
≥12	3	137	0.57 (0.18, 0.95)	0.004	18.0	0.295
Study population						
PCOS	3	135	0.38 (0.04, 0.72)	0.027	0.0	0.878
Prostate cancer	2	161	0.42 (−0.41, 1.24)	0.321	85.3	0.009
Post-menopausal	2	135	0.32 (−0.98, 1.63)	0.627	90.5	<0.001
Flaxseed on total testosterone						
Overall	6	395	0.17 (−0.07, 0.41)	0.165	27.7	0.227
Age (year)						
≤50	3	135	0.02 (−0.32, 0.36)	0.906	0.0	0.980
>50	3	260	0.27 (−0.15, 0.69)	0.205	65.1	0.057
Gender						
Men	2	161	0.48 (0.17, 0.79)	0.003	0.0	0.600
Women	4	234	−0.04 (−0.30, 0.22)	0.906	0.0	0.956
Duration (week)						
<12	4	294	0.24 (−0.10, 0.57)	0.464	49.5	0.115
≥12	2	101	0.00 (−0.39, 0.39)	0.004	0.0	0.998
Study population						
PCOS	3	135	0.02 (−0.32, 0.36)	0.906	0.0	0.980
Prostate cancer	2	161	0.48 (0.17, 0.79)	0.003	0.0	0.600
Post-menopausal	1	99	−0.12 (−0.51, 0.27)	0.551	–	–
Flaxseed on FAI						
Overall	4	262	0.11 (−0.61, 0.83)	0.759	87.6	<0.001
Gender						
Men	2	161	0.59 (−0.10, 1.29)	0.094	79.0	0.029
Women	2	101	−0.41 (−1.65, 0.82)	0.514	88.7	0.003
Study population						
PCOS	2	101	−0.41 (−1.65, 0.82)	0.514	88.7	0.003
Prostate cancer	2	161	0.59 (−0.10, 1.29)	0.094	79.0	0.029

SMD, standardized mean difference; CI, confidence interval.[a]Obtained from the Random-effects model.
[b]Refers to the mean (95% CI).
[c]Inconsistency, percentage of variation across studies due to heterogeneity.
[d]Obtained from the Q-test.

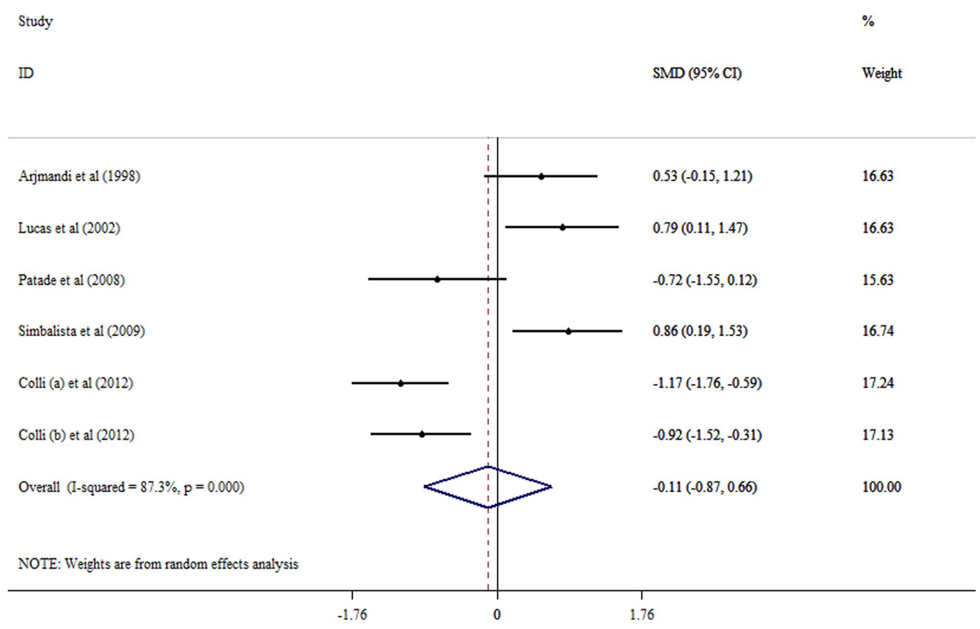

FIGURE 3
Forest plot detailing mean difference and 95% confidence intervals (CIs) the effects of flaxseed supplementation on FSH levels.

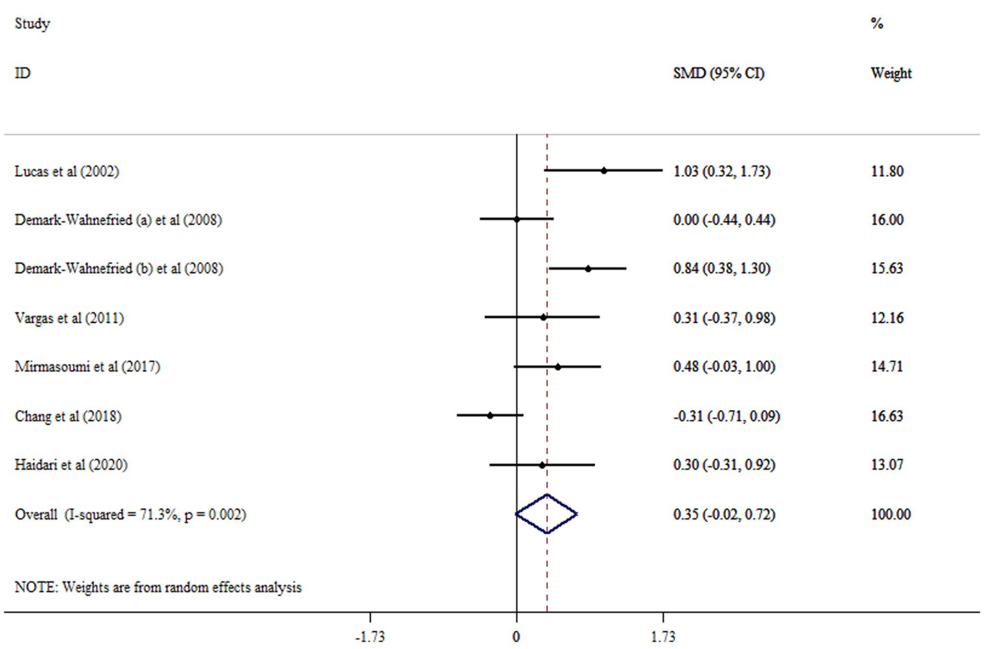

FIGURE 4
Forest plot detailing mean difference and 95% confidence intervals (CIs) the effects of flaxseed supplementation on SHBG levels.

synthesis. It has been suggested that flaxseed may change the profile of estrogen metabolites by changing the activity of cytochrome P450 enzymes responsible for estrogen hydroxylation (40, 41). Moreover, lignans have been proposed to exert anticancer effects by competing with estrogens for binding to estrogen receptors, resulting in changed estrogen-sensitive gene expression, and consequently, reduced cell proliferation and improved apoptosis (42). Another mechanism by which lignans appears to have a role in controlling sex hormones may

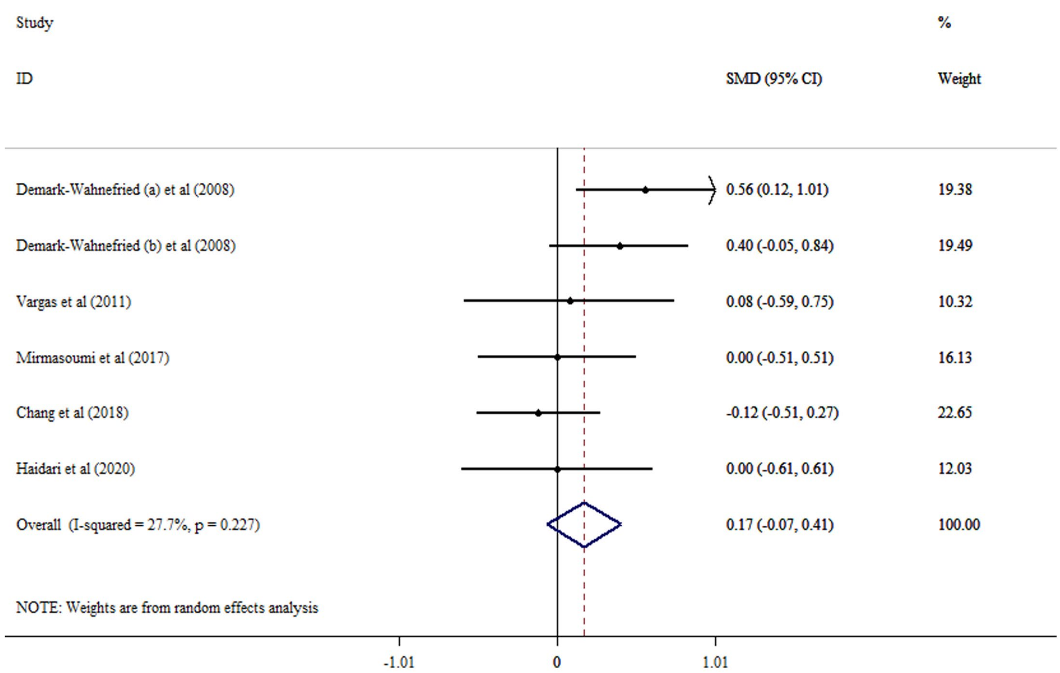

FIGURE 5
Forest plot detailing mean difference and 95% confidence intervals (CIs) the effects of flaxseed supplementation on TT levels.

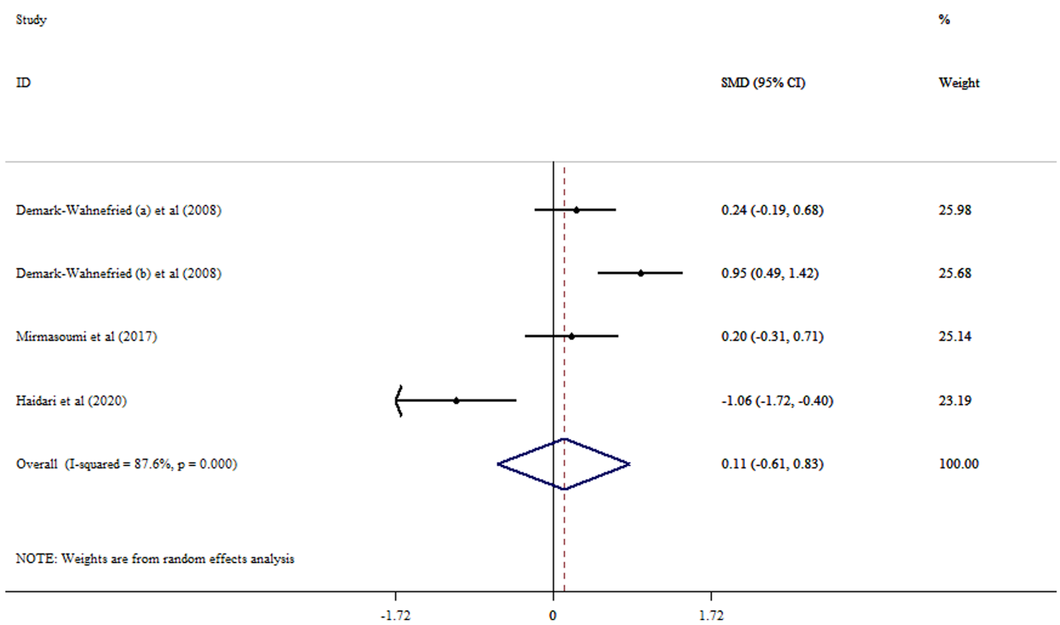

FIGURE 6
Forest plot detailing mean difference and 95% confidence intervals (CIs) the effects of flaxseed supplementation on FAI levels.

be due to its interact with enzymes involved in hormone metabolism and synthesis to control relative levels of circulating sex hormones (43). *In vitro* studies have proposed that lignans may decline estrogen synthesis by impeding the aromatase enzyme responsible for altering androstenedione and testosterone to estrone and estradiol, respectively.

4.1. Strengths and limitations

This is the first systematic review and meta-analysis of RCTs examining the effect of flaxseed supplementation on sex hormones. The lack of publication bias as evidenced by Egger's

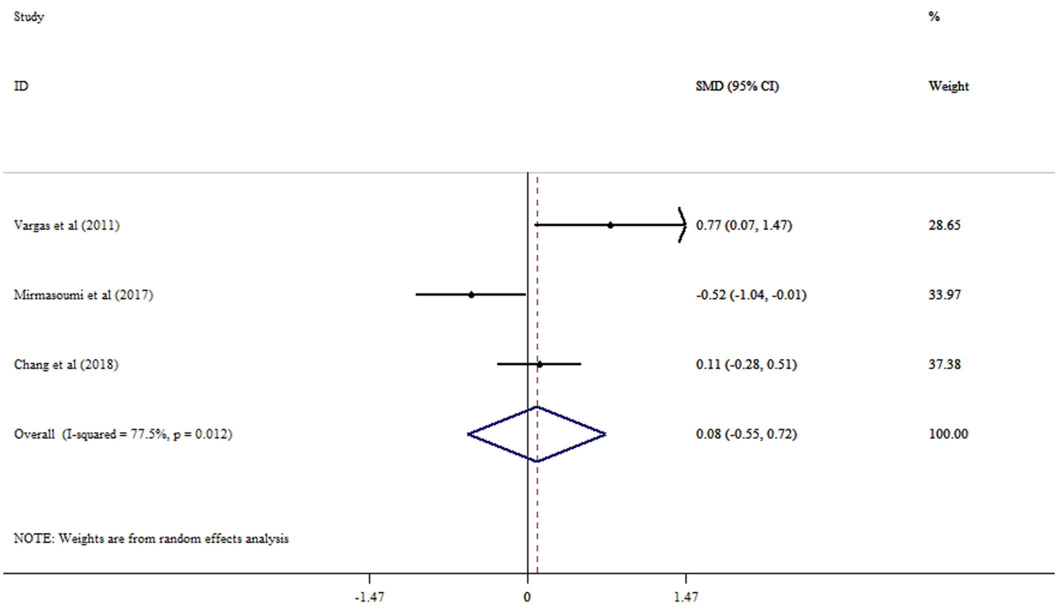

FIGURE 7
Forest plot detailing mean difference and 95% confidence intervals (CIs) the effects of flaxseed supplementation on DHEAS levels.

and Begg's test propose that our result was reliable, since trials which could potentially change the overall results were not evident. Moreover, most of the included trials were designated as low risk of bias according to Cochrane risk of bias tool. However, there are some limitations that can be addressed in future studies. The number of RCTs that met the eligibility criteria for inclusion in the meta-analysis, as well as the number of men included in these trials, were limited. This made it challenging to accurately assess the clinical effectiveness of flaxseed on sex hormone. Included studies were conducted on participants with varying health conditions (PCOS, postmenopausal women, and prostate cancer), and only a small proportion of the participants were young and healthy. It was not possible to examine the effects of flaxseed on other sex hormones due to inadequate dataset. Studies about the effect of flaxseed supplementation on FSH, FAI, and DHEAS were very low. So, more studies are needed to confirm our findings about these hormones. The included studies involved participants with diverse health status. Another limitation is the difference in the form of flaxseed, the preparation method, and period of intervention. Moreover, the number of trials and included subjects were small.

5. Conclusion

In conclusion, we found no significant effect of flaxseed on sex hormones in adults. However, according to subgroup analyses flaxseed supplementation increased SHBG in subjects with ≤50 years old and with PCOS, and TT in men. Nevertheless, due to the discussed limitations of the included trials, this topic is still open and needs further studies in future RCTs.

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 authors.

Ethics statement

The study protocol was approved and registered by the ethics committee of Isfahan University of Medical Sciences (identifier: IR.MUI.RESEARCH. REC.1402.031).

Author contributions

VM and MV was responsible for designing and coordinating the study. KK, FT, VM, AM, and MV were responsible for data collection, data analysis, and data interpretation in the manuscript. MV and MN were responsible for the statistical work and for writing the manuscript. GA was responsible for reviewing 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.2023.1222584/full#supplementary-material

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Effects of omega-3 fatty acid supplementation on nutritional status and inflammatory response in patients with stage II-III NSCLC undergoing postoperative chemotherapy: a double-blind randomized controlled trial

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Background: The primary objective of this study was to investigate the effects of oral omega-3 fatty acids in lowering the risk of malnutrition and improving the inflammatory response in patients with stage II-III lung cancer receiving postoperative chemotherapy.

Methods: One hundred and three lung cancer patients identified as being at risk for malnutrition according to the 2002 nutritional risk screening criteria were randomized into either the omega-3 fatty acid supplementation group or the placebo group during postoperative chemotherapy. Data on anthropometric parameters, laboratory nutritional indicators, and inflammatory markers were collected, and changes and differences between the two groups were compared and analyzed.

Results: Sixty three patients were included in the final analysis. The baseline information of the two groups of patients was comparable ($p > 0.05$). After 12 weeks, patients in the treatment group exhibited significantly higher levels of hemoglobin (11.26 ± 1.25 vs.10.60 ± 0.94, $p = 0.021$) and serum albumin (45.38 ± 5.06 vs.42.66 ± 5.06, $p = 0.036$) compared with those in the placebo group. Meanwhile, the levels of inflammatory factors C-reactive protein (2.16 ± 1.06 vs. 4.11 ± 1.72, $p < 0.001$), interleukin-1 (6.61 ± 2.19 vs.10.85 ± 3.61, $p < 0.001$), interleukin-6 (2.48 ± 1.20 vs. 4.53 ± 0.98, $p < 0.001$), interleukin-8 (9.26 ± 2.69 vs. 39.01 ± 6.53, $p < 0.001$), and tumor necrosis factor-α (1.88 ± 0.60 vs. 4.07 ± 0.97, $p < 0.001$) were significantly decreased in the treatment group. In contrast, differences in weight, BMI, upper arm circumference, triceps skinfold thickness, triglycerides, cholesterol, and IFN-γ between the two groups were not statistically significant ($p > 0.05$). Finally, in the treatment group, the levels of hemoglobin (10.89 ± 1.15 vs. 11.82 ± 1.21, $p = 0.042$), triglyceride (0.92 ± 0.29 vs. 1.03 ± 0.22, $p = 0.043$), and cholesterol (3.56 ± 0.82 vs. 4.23 ± 0.88, $p = 0.045$) were higher in stage II patients after the intervention compared with stage III patients.

Conclusion: Supplementation with omega-3 fatty acids improved nutritional status and reduced chronic inflammatory responses in patients with stage II-III non-small cell lung cancer undergoing postoperative chemotherapy.

Clinical Trial Registration: AEA RCT Registry, identifier AEARCTR-0007165.

KEYWORDS

lung cancer, chemotherapy, inflammatory response, nutritional status, omega-3 fatty acids

Introduction

As is well documented, lung cancer remains the leading cause of cancer-related deaths worldwide. According to GLOBOCAN statistics on cancer in 185 countries in 2020 (1), approximately 1.8 million individuals are estimated to die annually from lung cancer, accounting for 18% of the total cancer mortality. Its occurrence is closely related to smoking, air pollution, occupational exposure, and environmental factors (2–4). Surgical-based multimodal treatment is recommended for all patients eligible for surgery after a comprehensive evaluation. Meanwhile, postoperative adjuvant chemotherapy assists in eliminating postoperative residual cancer cells, thereby minimizing the risk of postoperative recurrence and improving patients' postoperative survival time (5, 6).

However, postoperative chemotherapy is prone to cause adverse reactions such as nausea, vomiting, diarrhea, anorexia, neutropenia, and thrombocytopenia. Additionally, a proportion of patients manifest a deterioration in nutritional status, leading to decreased immune function, a higher risk of infection-related complications, and eventually cancer-related fatigue (7). These effects can have detrimental impacts on the physical, psychological, familial, and social well-being of patients. Consequently, enhancing the physical condition of postoperative patients has been the spotlight of the field of oncology.

Omega-3 polyunsaturated fatty acids (ω-3 PUFA), also referred to as n-3 PUFA, are fundamental components of a healthy human diet and encompass a-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA) (8). ALA is an essential fatty acid found mainly in plant oils. DPA is a vital intermediate product aiding in the conversion of ALA into EPA and DHA, yet the human body is unable to efficiently synthesize it owing to the low activity of enzymes involved in its conversion, resulting in a drastic limitation of its conversion capacity (9). Earlier studies have reported that ω-3 PUFA supplementation can increase skeletal muscle mass, modulate inflammatory responses, reduce the risk of gastrointestinal reactions, attenuate anorexia, improve the prognosis of cancer patients, and confer tolerance to chemotherapy, radiotherapy, and surgery in patients with non-small cell lung cancer (NSCLC), thus prolonging their survival time (10–14).

Numerous studies have extensively studied and established the anti-inflammatory and immunomodulatory benefits of ω-3 PUFA in cancer patients (15–18). Nevertheless, studies on its effect on lung cancer patients at risk of malnutrition receiving postoperative chemotherapy are scarce. Furthermore, clinical studies have reported that the influence of ω-3 PUFA on cancer progression and nutritional status is controversial (19). Therefore, this study aimed to investigate the effect of ω-3 PUFA on the nutritional status and inflammatory response of postoperative NSCLC patients receiving chemotherapy.

Materials and methods

Study design

This was a double-blind, randomized, placebo-controlled trial. One hundred and three patients who attended the Department of Thoracic Surgery of Lu'an Hospital, Anhui Medical University, from May 2021 to December 2022 and were diagnosed with NSCLC by postoperative pathological biopsies were recruited. Nutritional risk Screening 2002 (NRS-2002) was used to assess the clinical nutritional risk of each patient before surgery, and this scoring scale was used to identify the malnutrition of patients. These patients were randomized to either the ω-3 PUFA supplementation group or the placebo group. This study was approved by the Ethics Committee of the Affiliated Lu'an Hospital of Anhui Medical University (approval number: 2021LL009) and conformed to the Declaration of Helsinki, and all patients were fully informed and signed the informed consent form.

Inclusion criteria

Patients preoperatively assessed for surgical intervention, with an NRS-2002 score equal to or greater than 3 points (risk of malnutrition), histopathology-confirmed postoperative stage II-III NSCLC, postoperative assessment of life expectancy exceeding 3 months, and consent to continue with postoperative adjuvant chemotherapy.

Exclusion criteria

Refusal to continue chemotherapy after surgery or intolerance to chemotherapy; intolerance to fish or fish oil preparations; poorly controlled cardiovascular and renal diseases, diabetes, gastrointestinal disease, and severe infections; refusal to participate in the randomized trial; incomplete information during the study.

Intervention

Eligible patients were randomized by the clinical secretary according to a randomized list into two groups: The ω-3 PUFA supplementation group (treatment group) and placebo group. The treatment group received ω-3 polyunsaturated fatty acid gel capsules (1.6 g EPA/day and 0.8 g DHA/ day, no other additives or antioxidants), while the placebo capsules consisted of sunflower oil (2.4 g/day). The shape, size, and mass of the gel capsules were identical, and both investigators and patients were blinded to group assignment. The start of the intervention was 1 week postoperatively when there were no obvious complications, and they were instructed to take it before meals at a fixed time every day. A return form was distributed to record daily medication and adverse events and retrieved at each visit to the hospital for chemotherapy. Patients were encouraged to report perceived adverse events to the investigator by telephone or other means. The choice of chemotherapy regimen was based on the postoperative pathological type, chemotherapeutic drug sensitivity report and body surface area. All patients strictly adhered to the standard cancer treatment protocols.

Assessment method

Anthropometric measurements reflect nutritional status, comprising weight, height, and body mass index (BMI). Patient body weight was measured under fasting conditions in the morning, without shoes, while wearing the same patient uniform. The right mid-arm circumference was also recorded. Triceps skinfold thickness was measured using a Harpenden caliper. Nutrition-related laboratory indicators were routinely tested by the clinical laboratory of the hospital. Among them, hemoglobin was determined by optical absorption, albumin was determined by turbidimetry, and triglycerides and cholesterol were determined by enzymatic methods. The levels of inflammatory markers [C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-1(IL-1), interleukin-6(IL-6), interleukin-8 (IL-8), and interferon-γ (IFN-γ)] were measured by mature ELISA kits. Specifically, CRP levels were determined using a Human High Sensitivity ELISA Kit (Anisan™, Tehran, Iran), whilst serum TNF-α, IL-1, IL-6, IL-8, and IFN-γ levels were measured using a human cytokine-specific ELISA kit (Biosource Europe, Belgium). These kits have been widely used in multiple studies and experiments, and their performance and stability have been thoroughly validated.

Statistical analysis

Statistical analyses were conducted using SPSS version 22.0 for Windows (SPSS Inc., Chicago, IL, United States). The Shapiro–Wilk method was used to assess data normality. Measurement data were expressed as mean ± standard deviation (Mean ± SD), and independent samples t-test was used to compare the means of two consecutive normally distributed variables. The Mann–Whitney U test was employed to compare the means of two groups of non-normally distributed variables. The Pearson χ^2-test was used to compare the proportions of two variables. The hypothesis test level was set to $\alpha = 0.05$ and was considered statistically significant at $p < 0.05$.

Results

This trial initially enrolled 103 patients, of whom 12 did not meet the inclusion criteria for this trial, 7 declined to participate, and 2 requested direct inclusion in the treatment group and declined random assignment based on clinical data and eligibility criteria. Based on a computerized random assignment list, 82 patients were randomly assigned to the treatment and placebo groups. After 12 weeks of intervention, patient follow-up results were recorded. In the treatment group, 5 patients were excluded due to poor adherence, and 6 additional patients who refused to participate in follow-up visits were also excluded. In the placebo group, five patients refused to participate in follow-up visits, and three were excluded owing to poor adherence to medications. Finally, 63 subjects were enrolled (Figure 1). The treatment group ($n = 30$) consisted of 18 males (60%) and 12 females (40%) with a mean age of (62.50 ± 4.88) years. Similarly, there were 20 males (60.6%) and 13 females (39.4%) in the placebo group ($n = 33$) with a mean age of (61.81 ± 7.15) years. The baseline demographics and clinical characteristics of enrolled participants were comparable (Table 1). Adverse reactions during chemotherapy in the two groups were found to be gastrointestinal symptoms (Treatment group, $n = 18$ vs. Placebo group, $n = 21$, $p = 0.885$), Blood system symptoms (Treatment group, $n = 5$ vs. Placebo group, $n = 7$, $p = 0.646$), other symptoms such as rash, insomnia, etc. (Treatment group, $n = 2$ vs. Placebo group, $n = 3$, $p = 0.913$). After 12 weeks of intervention, there were no statistical differences in anthropometric parameters between the two groups, including weight, BMI, upper arm circumference, and triceps skinfold thickness (Table 2). Among the nutrition-based laboratory indicators, hemoglobin (11.26 ± 1.25 vs. 10.60 ± 0.94), serum albumin (45.38 ± 5.06 vs. 42.66 ± 5.06), and the change of serum albumin before and after intervention (4.50 ± 6.34 vs. 0.32 ± 5.58) were significantly different between the two groups ($p < 0.05$). There was no significant difference in triglycerides and cholesterol levels after the intervention. However, there were significant differences in the changes of triglyceride and cholesterol before and after intervention between the two groups ($p < 0.05$).

Analyzing the results of inflammatory factor assays exposed that after the intervention, the levels of CRP and TNF-α were significantly lower in the treatment group compared with those in the placebo group (2.16 ± 1.06 vs. 4.11 ± 1.72, $p < 0.001$ and 1.88 ± 0.60 vs. 4.07 ± 0.97, $p < 0.001$). Likewise, the levels of IL-1, IL-6, and IL-8 were also significantly lower in the treatment group than those in the placebo group (6.61 ± 2.19 vs.10.85 ± 3.61, 2.48 ± 1.20 vs. 4.53 ± 0.98, and 9.26 ± 2.69 vs. 39.01 ± 6.53, respectively, $p < 0.001$). We found statistically significant differences between the two groups when comparing the differences in changes in CRP, IL-1, IL-6, IL-8, and TNF-α before and after the intervention ($p < 0.05$). Moreover, the level of IFN-γ was numerically lower in the treatment group than that in the placebo group (9.22 ± 2.86 vs. 10.50 ± 3.34), but the difference was not statistically significant ($p = 0.099$) (Table 3).

Comparing post-treatment laboratory parameters in patients with stage II ($n = 18$) and stage III ($n = 12$) NSCLC in the treatment group uncovered that the levels of hemoglobin (11.82 ± 1.21 vs. 10.89 ± 1.15, $p = 0.042$), triglycerides (1.03 ± 0.22 vs. 0.92 ± 0.29, $p = 0.044$) and cholesterol (4.23 ± 0.88 vs. 3.56 ± 0.82, $p = 0.045$) were significantly elevated in patients with stage III NSCLC compared to those with stage II NSCLC. The anthropometric indices and inflammatory factor levels were similar between stage II and III patients, and only the change in IFN-γ before and after the intervention was statistically different between the two stages ($p < 0.05$) (Table 4).

Discussion

In the multimodal treatment of lung cancer, patients undergoing postoperative chemotherapy have garnered extensive attention. This randomized controlled trial investigated the influence of oral ω-3 PUFA in reducing the risk of malnutrition and the levels of inflammatory biomarkers in patients with stage II-III NSCLC

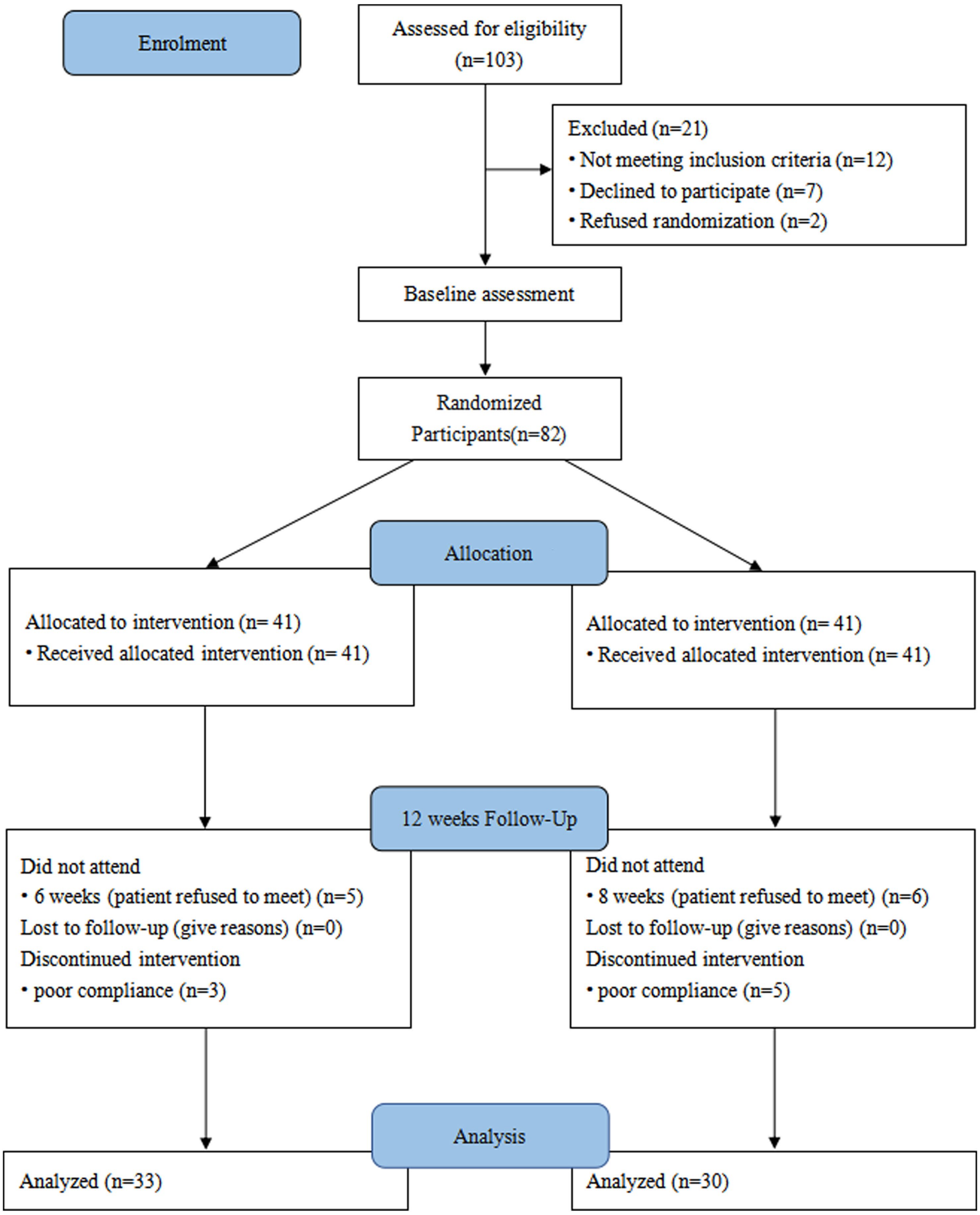

FIGURE 1
Consolidated standards of reporting trials (CONSORT) diagram. A patient flow diagram is shown.

undergoing postoperative adjuvant chemotherapy. Differences in metrics between the two randomly assigned groups suggested that supplementation of ω-3 PUFA during the early postoperative period lowered the risk of malnutrition in patients while alleviating the inflammatory response during chemotherapy.

Several studies have described that ω-3 PUFA results in beneficial health outcomes in various fields of medicine (20–23). Evidence from *in vitro* trials, studies utilizing animal experimental models, and epidemiological analyses advocate the use of ω-3 PUFA for multi-targeted tumor therapy, which has consistently yielded comparable results in clinical trials (24). Nonetheless, the mechanism by which ω-3 PUFA inhibits tumor progression has not been elucidated so far. ω-3 PUFA is hypothesized to regulate cell replication during proliferation and differentiation, interfere with cell cycle, and disrupt cell growth via the modulation of apoptosis and necrosis in tumor cells (25, 26). In addition, ω-3 PUFA can act through mechanisms such as inhibition of arachidonic acid derivatives, interference with oxygen radicals, and reactive oxygen species

TABLE 1 Baseline characteristics of participants.

Variable	Treatment group (*n* = 30)	Placebo group (*n* = 33)	*p*-value
Gender (N[%])			0.961
Male	18 (60.0)	20 (60.6)	
Female	12 (40.0)	13 (39.4)	
Age (year)	62.50 ± 4.88	61.81 ± 7.15	0.658
NRS-2002	3.23 ± 0.50	3.24 ± 0.50	0.943
Type of NSCLC (N[%])			
Adenocarcinomas	16 (53.3)	19 (57.6)	0.735
Squamous-cell carcinoma	11 (36.7)	10 (30.3)	0.593
Large cell carcinomas	1 (3.3)	1 (3.0)	0.945
Others	2 (6.7)	3 (9.1)	0.722
Stage of NSCLC (N[%])			
IIa	9 (30.0)	12 (36.4)	0.533
IIb	9 (30.0)	7 (21.2)	0.424
IIIa	8 (26.7)	10 (30.3)	0.750
IIIb	4 (13.3)	3 (9.1)	0.593
IIIc	0 (0.0)	1 (3.0)	0.336
Chemotherapy regimens (N[%])			
Cisplatin	11 (36.7)	10 (30.3)	0.593
Cisplatin and docetaxel	15 (50.0)	17 (51.5)	0.904
Cisplatin and pemetrexed disodium	4 (13.3)	6 (18.2)	0.599

NRS, nutritional risk screening. All variables are expressed as mean ± standard deviation (Mean ± SD) or frequency (percent). The Pearson χ^2-test was used for the comparison of gender, type of NSCLC, stage of NSCLC, and chemotherapy regimens, the Mann–Whitney U test was used for the comparison of NRS-2002 scores, and the independent samples *t*-test was used for the comparison of age.

production (27, 28), all of which are based on the action of EPA and DHA. In a double-blind trial, Van der Meij et al. administered n-3 PUFA to non-surgically treated NSCLC patients while the other group received an isocaloric control supplement and evinced that EPA 2.02 + DHA 0.92 g positively enhanced not only physical activity but also the overall prognosis of NSCLC patients (29). Additional trials are warranted on the dose–response relationship of ω-3 PUFA, considering that the influence of its individual components on quality of life is elusive. According to previous studies, (19, 30–32) the administration of oral ω-3 PUFA (EPA1.6 g/day+ DHA 0.8 g/day) is safe and effective, in line with the findings of our previous study (33).

Patients undergoing post-operative chemotherapy frequently suffer from nausea, anorexia, anxiety, and even transient weight loss during the early stage. Indeed, any form of cancer treatment demands increased protein and energy support. Albumin is a good indicator to directly reflect the nutritional status of patients. The normal synthesis and maintenance of hemoglobin, a protein found in red blood cells, requires a variety of nutrients, including iron, vitamin B12, folate, protein, and vitamin C. Poor nutrition and an unbalanced diet can both lead to insufficient hemoglobin synthesis, which can cause anemia. Maintaining adequate nutrient intake is essential for maintaining healthy hemoglobin levels. In a study carried out by Kaya et al., tumor patients had significantly lower postoperative serum albumin levels compared to their preoperative levels, whereas patients on an ω-3 PUFA-enriched diet experienced a dramatic reduction in albumin loss, indicating that ω-3 PUFA contributed to the nutritional recovery of postoperative patients (34). This conclusion is in agreement with our current research results that ω-3 PUFA supplementation improves the nutritional status of postoperative patients. Specifically, significant differences in hemoglobin and serum albumin levels were observed in patients after 12 weeks of oral ω-3 PUFA supplementation compared to those in the placebo group. In contrast, despite increasing, anthropometric parameters of patients taking oral sunflower oil were comparable to baseline levels. This may be ascribed to differences in the included population and the duration of the study. Murphy et al. uncovered an interaction between plasma n-3 PUFA levels and the rate of skeletal muscle change in NSCLC patients during chemotherapy (2.5 months) sessions and pointed out that the accelerated rate of muscle loss in patients with concurrent sarcopenia was closely related to plasma n-3 PUFA deficiency (35). However, the majority of studies have used the change in values from clinical trials as the study endpoint and have provided elaboration or hypotheses on the mechanism.

Inflammation is an important marker of cancer, and many studies have identified chronic inflammation as correlated with a poor prognosis (36, 37). Cytokines such as IL-1, IL-6, IL-8, TNF-α, and IFN-γ play a role in promoting inflammation in the inflammatory response. When the body is exposed to infection, injury, or other inflammatory stimuli, these cytokines are released by immune cells, initiating the inflammatory process. They cause vasodilation, increase blood flow, and attract immune cells such as white blood cells to damaged areas to fight potential pathogens or repair tissue damage. A prospective randomized controlled study conducted by Liang et al. included 42 patients who underwent radical colorectal cancer surgery

TABLE 2 Nutritional status and blood biochemical indices of patients in treatment group and placebo group.

Characteristics		Treatment group (n = 30)	Placebo group (n = 33)	p-value
Weight (Kg)	Before	60.72 ± 5.41	60.51 ± 6.59	0.889*
	After	61.70 ± 5.40	61.29 ± 6.78	0.792*
	Δ	0.97 ± 1.73	0.78 ± 1.59	0.638*
Height (cm)		166.23 ± 4.76	165.73 ± 5.86	0.710*
BMI (Kg/m^2)	Before	21.99 ± 1.97	22.02 ± 1.97	0.960*
	After	22.35 ± 2.04	22.29 ± 1.99	0.910*
	Δ	0.36 ± 0.63	0.27 ± 0.60	0.596*
Upper arm circumference (mm)	Before	24.24 ± 1.85	24.43 ± 1.62	0.670*
	After	24.41 ± 1.87	24.54 ± 1.58	0.767*
	Δ	0.17 ± 0.26	0.11 ± 0.40	0.688#
Triceps skinfold thickness (mm)	Before	12.31 ± 1.81	12.82 ± 2.02	0.296*
	After	12.51 ± 1.84	13.02 ± 1.98	0.295*
	Δ	0.20 ± 0.31	0.19 ± 0.26	0.591#
Hemoglobin (g/dL)	Before	10.64 ± 1.28	10.64 ± 1.42	0.994*
	After	11.26 ± 1.25	10.60 ± 0.94	0.021*
	Δ	0.62 ± 1.45	−0.04 ± 1.27	0.164#
Albumin (g/dL)	Before	40.89 ± 6.60	42.33 ± 6.00	0.336*
	After	45.38 ± 5.06	42.65 ± 5.06	0.036*
	Δ	4.50 ± 6.34	0.32 ± 5.58	0.006#
Triglyceride (mmol/L)	Before	0.95 ± 0.31	0.96 ± 0.24	0.308#
	After	0.96 ± 0.26	0.92 ± 0.25	0.685#
	Δ	0.01 ± 0.26	−0.04 ± 0.15	0.049#
Cholesterol (mmol/L)	Before	3.96 ± 0.92	3.84 ± 0.83	0.582#
	After	3.83 ± 0.89	3.95 ± 0.89	0.604*
	Δ	−0.13 ± 0.45	0.11 ± 0.42	0.030#

BMI, Body mass index; Δ, Difference between post-intervention and pre-intervention. All variables are expressed as mean ± standard deviation (Mean ± SD). *p, Independent samples t-test for normally distributed continuous variables. #p, Mann–Whitney U test for non-normally distributed continuous variables, $p < 0.05$ indicates a statistical difference.

TABLE 3 Inflammatory factors of patients in treatment group and placebo group.

Variable		Treatment group (n = 30)	Placebo group (n = 33)	p-value
CRP (mg/L)	Before	4.89 ± 2.00	4.24 ± 1.36	0.208#
	After	2.16 ± 1.06	4.11 ± 1.72	<0.001*
	Δ	−2.73 ± 1.87	−0.13 ± 1.88	<0.001*
IL-1 (pg/mL)	Before	11.46 ± 4.81	11.15 ± 3.59	0.741#
	After	6.61 ± 2.19	10.85 ± 3.61	<0.001#
	Δ	−4.85 ± 5.10	−0.29 ± 4.52	<0.001*
IL-6 (pg/mL)	Before	4.71 ± 0.86	4.60 ± 0.98	0.831#
	After	2.48 ± 1.20	4.53 ± 0.98	<0.001*
	Δ	−2.23 ± 1.53	−0.07 ± 1.40	<0.001*
IL-8 (pg/mL)	Before	37.96 ± 6.69	38.72 ± 5.88	0.640#
	After	9.26 ± 2.69	39.01 ± 6.53	<0.001#
	Δ	−28.70 ± 8.12	0.28 ± 8.35	<0.001#
TNF-α (pg/mL)	Before	4.10 ± 1.17	3.97 ± 1.13	0.670*
	After	1.88 ± 0.60	4.07 ± 0.97	<0.001*
	Δ	−2.22 ± 1.19	0.09 ± 1.41	<0.001*
IFN-γ (pg/mL)	Before	10.44 ± 3.85	11.21 ± 2.99	0.376*
	After	9.22 ± 2.86	10.50 ± 3.34	0.099#
	Δ	−1.22 ± 4.68	−0.71 ± 4.02	0.645*

Δ, Difference between post-intervention and pre-intervention. All variables are expressed as mean ± standard deviation (Mean ± SD). *p, By independent samples t-test. #p, By Mann–Whitney U test.

TABLE 4 Anthropometric parameters, nutritional indicators and inflammatory factors in patients with stage II and III NSCLC in the treatment group.

Variable		Stage II (*n* = 18)	Stage III (*n* = 12)	*p*-value
Weight (Kg)	Before	60.48 ± 6.31	61.09 ± 3.91	0.745*
	After	61.53 ± 6.36	61.95 ± 3.73	0.821*
	Δ	1.05 ± 1.71	0.86 ± 1.82	0.539#
BMI (Kg/m²)	Before	21.88 ± 2.34	21.16 ± 1.33	0.710*
	After	22.26 ± 2.34	22.49 ± 1.57	0.766*
	Δ	0.38 ± 0.62	0.33 ± 0.67	0.657#
Upper arm circumference (mm)	Before	24.30 ± 2.09	24.15 ± 1.51	0.832*
	After	24.46 ± 2.11	24.33 ± 1.54	0.849*
	Δ	0.16 ± 0.25	0.18 ± 0.29	0.797#
Skinfold thickness (mm)	Before	11.98 ± 1.55	12.82 ± 2.10	0.217*
	After	12.19 ± 1.64	12.99 ± 2.10	0.249*
	Δ	0.21 ± 0.33	0.17 ± 0.29	0.752*
Hemoglobin (g/dL)	Before	10.37 ± 1.32	11.04 ± 1.15	0.159*
	After	10.88 ± 1.15	11.82 ± 1.21	0.042*
	Δ	0.52 ± 1.52	0.78 ± 1.39	0.433#
Albumin (g/dL)	Before	40.41 ± 6.98	41.61 ± 6.21	0.525#
	After	45.28 ± 5.13	45.53 ± 5.17	0.897*
	Δ	4.88 ± 6.02	3.93 ± 7.04	0.694*
Triglyceride (mmol/L)	Before	0.95 ± 0.35	0.96 ± 0.25	0.341#
	After	0.92 ± 0.29	1.03 ± 0.22	0.044#
	Δ	−0.03 ± 0.31	0.07 ± 0.13	0.235#
Cholesterol (mmol/L)	Before	3.63 ± 0.89	4.44 ± 0.77	0.016*
	After	3.56 ± 0.82	4.23 ± 0.88	0.045*
	Δ	−0.07 ± 0.39	−0.21 ± 0.55	0.044#
CRP (mg/L)	Before	4.99 ± 2.11	4.74 ± 1.91	0.747*
	After	2.31 ± 1.03	1.95 ± 1.11	0.378*
	Δ	−2.68 ± 1.79	−2.79 ± 2.07	0.672#
IL-1 (pg/mL)	Before	12.51 ± 4.91	9.89 ± 4.39	0.176#
	After	6.89 ± 1.98	6.19 ± 2.49	0.374#
	Δ	−5.62 ± 5.70	−3.70 ± 4.00	0.322*
IL-6 (pg/mL)	Before	4.66 ± 0.87	4.78 ± 0.87	0.725*
	After	2.45 ± 1.38	2.52 ± 0.91	0.611#
	Δ	−2.22 ± 1.71	−2.26 ± 1.28	0.966#
IL-8 (pg/mL)	Before	38.11 ± 7.29	37.74 ± 5.97	0.966#
	After	9.24 ± 2.50	9.29 ± 3.07	0.799#
	Δ	−28.87 ± 8.91	−28.45 ± 7.14	0.866#
TNF-α (pg/mL)	Before	4.16 ± 1.11	4.01 ± 1.30	0.738*
	After	1.93 ± 0.60	1.80 ± 0.61	0.579*
	Δ	−2.23 ± 0.98	−2.20 ± 1.49	0.961*
IFN-γ (pg/mL)	Before	12.04 ± 3.69	8.05 ± 2.78	0.004*
	After	8.73 ± 2.74	9.96 ± 2.99	0.253*
	Δ	−3.31 ± 4.07	1.91 ± 3.77	0.001*

Δ, Difference between post-intervention and pre-intervention. All variables are expressed as mean ± standard deviation (Mean ± SD). *p, By independent samples *t*-test, #p, By Mann–Whitney U test.

and received either soybean oil supplementation (SO group) or a combination of ω-3 PUFA and soybean oil (FO group) as total parenteral nutrition post-surgery and found that IL-6 and TNF-α levels were lower in patients in the FO group than those in the SO group during the early postoperative period (38). Weiss et al. also observed a significant decrease in IL-6 levels in perioperative patients following fish oil intervention, as well as a corresponding decrease in TNF-α released by monocytes (39). Consistent with our results, there was a significant decrease in the level of both IL-6 and TNF-α in patients taking ω-3 PUFA after a 12-week dosing intervention. Compared to the study by Weiss et al., our study focused on the effect of ω-3 PUFA in chemotherapy-induced chronic inflammation, given that acute inflammatory responses in the perioperative period may be influenced by confounding factors such as the duration of surgery, antibiotic use, and the resolution of complications.

CRP is regarded as a classical inflammatory biomarker in oncology studies (40, 41), and its level is correlated with malignant progression and treatment-associated complications in lung cancer patients (42, 43). TNF-α also participates in the development of malignancies as an endogenous tumor-promoting factor. A meta-analysis established that expression levels of inflammatory factors such as CRP, TNF-α, and IL-6 in adults administered n-3 PUFA were significantly low; consequently, n-3 PUFA could be considered and recommended as an adjuvant anti-inflammatory agent (44). In a clinical trial including 64 oncology patients, significant differences in TNF-α, IL-1β, IL-6, and IFN-γ levels were detected in patients supplemented with EPA 2.0 g per day, but ω-3 PUFA did not significantly lower CRP levels compared to the control group (45). This result differs from our results, wherein significant changes in the levels of inflammatory factors were identified in patients in the treatment group, with the exception of IFN-γ ($p = 0.099$). We hypothesize that this discrepancy may be attributed to the limited sample size in the current study and the varying levels of serum IFN-γ in patients with tumors originating from different sites.

In addition to this, in this investigation, we compared the corresponding changes in study outcomes in the treatment group for NSCLC before and after the administration of the drug for different periods. The level of hemoglobin and triglycerides did not differ in the treatment group before the intervention, while statistical differences appeared after 12 weeks of intervention, indicating that ω-3 PUFA was more effective than stage II patients in promoting a positive shift in nutritional risk in stage III NSCLC patients, while in terms of anti-inflammatory effects, ω-3 PUFA did not show a particular advantage for stage III patients. Because of the difference between the baseline values of cholesterol before intervention in the two stages of patients, although the prognosis has changed significantly, the role of cholesterol still needs further clinical research. Van der Meij also found that n-3 PUFA exerted an anti-inflammatory effect in patients with stage III NSCLC receiving multimodal therapy (29), although no within-group comparisons were made between stage IIIa and IIIb patients. Some limitations of our study should also be acknowledged. For instance, the possibility of sampling error and bias due to the small sample size of stage II and III patients cannot be excluded.

An innovative randomized controlled trial was conducted to prospectively explore the relationship between ω-3 PUFA, malnutrition, and inflammatory factors in a specific population. We postulate that ω-3 PUFA may serve as an adjuvant therapy for patients undergoing chemotherapy after lung cancer surgery. However, this study exclusively included patients at nutritional risk, ultimately resulting in a small sample size. Furthermore, the results may not be representative of a broader population, and the study possesses inherent limitations typical of trials of this nature. In addition, this study did not include a dose-escalation test of ω-3 PUFA and did not examine the dose–response effect on patients. Nonetheless, the results of this trial provide compelling evidence for the beneficial effects of ω-3 PUFA in NSCLC patients undergoing postoperative chemotherapy.

Conclusion

ω-3 PUFA supplementation during postoperative adjuvant chemotherapy may enhance their nutritional status while mitigating inflammatory responses in patients at nutritional risk diagnosed with stage II-III NSCLC.

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 studies involving humans were approved by the Ethics Committee of the Affiliated Lu'an Hospital of Anhui Medical University (approval number: 2021LL009). The studies were conducted in accordance with the local legislation and institutional requirements. The 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

LG: Investigation, Software, Writing – original draft. MC: Conceptualization, Supervision, Validation, Writing – review & editing. MZ: Data curation, Formal analysis, Writing – review & editing. CN: Methodology, Supervision, Writing – review & editing. QH: Project administration, Supervision, Writing – review & editing.

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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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Effect of medium-chain triglycerides supplements and walking on health-related quality of life in sedentary, healthy middle-aged, and older adults with low BMIs: a randomized, double-blind, placebo-controlled, parallel-group trial

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Introduction: To extend individuals' healthy life expectancies, the improvement of subjective health and quality of life (QOL) has been increasingly prioritized, alongside the improvement of their physical functioning. Reports have indicated that intake of medium-chain triglycerides (MCTs) benefits the physical health of older individuals requiring nursing care, and athletes, and healthy individuals. But there are few studies investigating the effects of MCTs on subjective health and QOL. The present study sought to evaluate the combined effects of 12-week MCTs supplements and moderate-intensity walking exercise on the subjective health and QOL of middle-aged and older adults aged 60–74 with low BMIs (< 24 kg/m^2) and who had no exercise habits.

Methods: A placebo-controlled, double-blind, parallel-group trial was conducted. Three MCTs supplement groups with different doses and fatty acid compositions were compared with a control group. The study used the SF-36v2 questionnaire to assess subjective health and health-related QOL (HRQOL).

Results: The result showed significant improvements in the scores on subscales of the physical QOL, such as Physical functioning and General health, and summary scores on the mental QOL, compared to the control.

Conclusion: It is estimated that the combination of continuous intake of MCTs and walking exercise may affect HRQOL and improve subjective physical and mental health in sedentary, healthy, middle-aged and older adults.

Clinical trial registration: https://rctportal.niph.go.jp/s/detail/um?trial_id=UMIN000046861, UMIN000046861.

KEYWORDS
medium-chain fatty acids, medium-chain triglycerides, octanoic acid, decanoic acid, tiredness, vitality, mental health, subjective fatigue

1 Introduction

In Japan in 2021, the percentage of people aged 65 years and older comprised 28.9% of the total population. This is one of the highest such percentages in the world, and it is estimated to reach 31.2% by 2030 (1). Among this aging population, the number of people requiring long-term care and support continues to increase (2, 3). This trend not only leads to a decrease in their quality of life (QOL) (4), but also contributes to the costs of health care and long-term care (5, 6). Furthermore, the gap between average life expectancy and healthy life expectancy (i.e., the period during which some kind of support or long-term care is required) has remained at around 10 years for both men and women over the past dozen years (1). Increasing the number of people who can live independently, and extending healthy life expectancy, also reduces the burden on the people around them.

In the prevention of the necessity of long-term care, efforts have been made to improve not only the physical functions of older people, but also their QOL and subjective health status (7). It has been reported that improving physical function alone does not improve QOL, including subjective health status, in community-dwelling older adults in Japan (8). Additionally, subjective health has been reported to correlate with healthy life expectancy (9). Therefore, it is important to address not only the physical aspects of health, but also to improve QOL and subjective health in order to extend healthy life expectancy.

Medium-chain fatty acid (MCFA) is the broad term used to refer to straight-chain saturated fatty acids with 6–12 carbon chains. Medium-chain triglycerides (MCTs) composed of MCFA have the property of being preferentially converted into energy in the body after ingestion, unlike ordinary fats and oils, which consist of long-chain triglycerides (LCTs) (10). In clinical studies, a continuous daily consumption of 6 g of MCTs has been reported to improve physical function and body composition in older people. At a long-term care hospital, this MCTs intake led to an improvement in blood albumin levels, an indicator of malnutrition (11). In addition, significant improvements in grip strength, walking speed, and muscle mass were observed in nursing home residents (12, 13). Furthermore, animal studies have reported that continuous intake of MCTs increased mitochondrial biosynthesis (14) and activation of pathways involved in albumin synthesis in the liver (15). A case study of athletes reported a reduction in subjective fatigue with continuous intake of MCTs (16).

Regarding the effects of MCTs on mitochondrial function, which is associated with fatigue, animal studies have reported that both octanoic acid and decanoic acid increase mitochondrial biosynthesis and fatty acid oxidation-related gene expression, respectively (17, 18). Continuous intake of MCTs has been reported to increase fat oxidation during exercise in normal weight (19) and overweight (20) individuals having no exercise habits. Thus, continuous consumption of MCTs may benefit the physical health of people who need long-term care, and athletes, and normal-weight and overweight individuals. However, few studies have examined the effects of MCTs supplements on QOL and subjective health, and the effects of differing MCFA compositions and intake of MCTs are unknown.

In this study we investigated the effects of continuous intake of MCTs and moderate-intensity exercise on QOL and subjective health in healthy, middle-aged and older men and women with low BMIs, who had no exercise habits. In addition, three MCTs supplement groups were established to investigate the effect of MCTs doses and fatty acid composition.

2 Methods

Details of ethics, sample size, inclusion and exclusion criteria of subjects, study design and allocation of participants, management during the intervention period, dietary intervention, physical exercise intervention, lifestyle survey, dietary survey were described previously (21).

2.1 Ethics

The study was conducted in compliance with the Declaration of Helsinki (revised in 2013), the Japanese Ethical Guidelines for Medical and Health Research Involving Human Subjects, and the Japanese Act on the Protection of Personal Information (22, 23). We obtained approval from the ethics committees of the Yoga Allergy Clinic (approval number 21000023, approval date May 13, 2022). We registered this clinical trial in UMIN-CTR before the recruitment of participants (UMIN000046861).[1] The measurement of HRQOL was a secondary endpoint of this study.

2.2 Sample size

In a previous study of MCTs in a test diet, their effect on physical function was confirmed in 21 cases; older adults in nursing homes, with BMIs of 23 kg/m2 or less and aged 65 years or older, were fed 6 g of MCTs per day for three months (12). In the present study the amounts of MCTs in the test supplement were 6 g and 2 g, being equal to and one-third of the amount in the previous study. The number of cases in this study was set at 30 per group (120 in total), on the assumption that the number of cases required would increase and on the expectation that there would be drop-outs during the study and that some patients would be ineligible for analysis.

2.3 Subjects

Participants were Japanese males and females aged 60–74 years, with a BMI in the range from 19 kg/m^2 to less than 24 kg/m^2, who exercise or walk less than once a week, and for less than 30 min each time, and who did not routinely (for more than four hours a week) provide childcare or nursing care. Individuals who were instructed by a doctor to refrain from walking or exercising, or who were serious illness were excluded.

2.4 Study design and allocation of participants

The study was conducted as a randomized, double-blind, parallel-group trial with a placebo as the control diet. A party not involved in the trial (the test diet allocation manager) divided the subjects into four groups by stratified randomization: test diet groups (three types)

1 https://rctportal.niph.go.jp/s/detail/um?trial_id=UMIN000046861

and control diet group. The allocation factors were age, sex, and blood albumin level. After allocation, the test diet allocation manager confirmed that there were no significant differences between the four groups in age, sex, and blood albumin levels. The test diet allocation manager kept the test diet allocation list strictly confidential until the key was opened, and blinding was maintained for all parties except the test diet allocation manager.

2.5 Management during the intervention period

During the study period, the participants were instructed to avoid excessive physical activity, other than exercise interventions, to maintain normal lifestyle habits. If they performed non-routine household chores requiring physical effort (cleaning, redecoration and repair of the house, shoveling snow) or recreational trips for unavoidable reasons, they were instructed to record the details of these activities on the lifestyle questionnaire.

2.6 Dietary intervention

During the study period, one packet of the supplement containing 3 g of oil was taken as a supplement after any two of the three daily meals, breakfast, lunch, and dinner (two packets per day, 6 g of fats and oils). The oils in the supplements were LCTs (rapeseed oil from The Nisshin OilliO Group, Ltd., Tokyo, Japan) and MCTs (Nisshin MCT oil, Nisshin MCT-C10R, also from The Nisshin OilliO Group, Ltd.).

The four supplements were: the control supplement, Decanoic acid supplement, High-dose octanoic acid supplement, and Low-dose octanoic acid supplement. The daily amounts of LCTs, MCTs, and MCFAs contained in MCTs per 6 g of oil in the supplements are shown in Table 1. The subjects were instructed to maintain their pre-intervention dietary habits during the study period, except for the consumption of supplements.

The three MCT supplement groups were established for the following reasons. First, the MCTs (High-dose octanoic acid supplement in this study) which Abe et al. found to improve muscle strength and other parameters in persons requiring nursing care, were established to establish whether there would be a similar improvement in the subjects in this study (13). Second, a reduced dose group (Low-dose octanoic acid supplement) was established to test whether a similar effect would be observed at a lower dose. Among the MCFAs, octanoic acid and decanoic acid have been reported to have different effects on mitochondrial function (17, 24). High-dose octanoic acid supplement comprised MCTs rich in octanoic acid. Therefore, MCTs rich in decanoic acid (Decanoic acid supplement) were used to examine differences in the effects on subjective health between the consumption of MCTs rich in decanoic acid and MCTs rich in octanoic acid. LCTs in edible oils consumed daily were used as control.

2.7 Physical exercise intervention

During the study period, participants were instructed to walk for 40 ± 10 min on two days each week and to record their performance on the lifestyle questionnaire. They were instructed to maintain their habitual walking speed. Exercise was to be performed on non-consecutive days of the week whenever possible. For safety reasons, the following indoor exercises were used as an alternative on days when it was raining or extremely hot.

(1) Subjects having access to a walking machine, treadmill, etc., could use it to walk at a normal walking speed for the prescribed duration.
(2) For subjects having no such facilities, foot stamping on a flat surface for a specified duration at a pace equivalent to habitual walking on the spot, without moving, was an acceptable alternative to walking outdoors.
(3) If such alternative indoor exercise was carried out, it was to be recorded on the lifestyle questionnaire.

If any exercise could not be carried out for some unavoidable reason, the reason was to be recorded on the lifestyle questionnaire.

In a Japanese guideline (the Physical Activity Reference for Health Promotion 2013), normal walking was introduced as 3.0 metabolic equivalents (METs), strolling as 3.5 METs, and walking at a slightly faster pace as 4.3 METs (25). In this study, neither slow nor fast walking was instructed, and walking at a habitual pace was encouraged. Therefore, the subjects' exercise was estimated to be 3.0–3.5 METs, which was defined as moderate intensity walking.

2.8 Lifestyle survey

Participants were asked to report their living conditions during the study period, such as consumption of the test diet, physical condition, medication, exercise, and alcohol consumption, using the lifestyle questionnaire.

TABLE 1 Content of LCTs, MCTs, and MCFAs of MCTs in 6 g of oil in the supplement.

		Control supplement	Decanoic acid supplement	High-dose octanoic acid supplement	High-dose octanoic acid supplement
LCTs	g/day[1]	6	-	-	4
MCTs	g/day[1]	-	6	6	2
Octanoic acid (8:0)	g/day[2]	(−)	(1.40, 1.95)	(3.72, 4.14)	(1.24, 1.38)
Decanoic acid (10:0)	g/day[2]	(−)	(3.47, 4.00)	(1.06, 1.46)	(0.352, 0.487)

[1]Values show the amount of content. [2]Lower and upper limits are shown. -, (−) Content is zero.

2.9 Adverse events

During the study period, adverse events were investigated daily via the lifestyle questionnaire. If an adverse event occurred, the investigator immediately took the necessary and appropriate action, assessed the adverse event, and graded it for causal relation to the test diets as follows: none; probably none; may have; probably yes; yes; not assessable. Events other than those judged by the investigator to be "probably none" or "none" in causal relation to the test diets were considered to be adverse events.

2.10 Dietary survey

During the 12-week intervention period, dietary surveys of the normal diet without supplements were conducted before the intervention, and for four, eight, and 12 weeks after the intervention. Dietary surveys were conducted using a brief, self-administered diet history questionnaire (BDHQ).

2.11 Measurements

During the 12-week intervention period, subjective health and knee extension strength were measured before the intervention, and four weeks, eight weeks, and 12 weeks after the intervention.

The SF-36v2 (Japanese standard version), a HRQOL questionnaire, was used to assess subjective health. The SF-36 is the world's most widely used questionnaire to assess subjective health, and its reliability and validity have been scientifically verified in Japan. It measures subjective health in eight subscales: physical function (PF), role physical (RP), bodily pain (BP), general health perception (GH), vitality (VT), social functioning (SF), role emotional (RE), and mental health (MH). In addition, the eight subscales can be analyzed together into three component summary scores: the physical component summary (PCS) consisting of PF, BP, GH, RP, SF, and VT; mental component summary (MCS) consisting of BP, GH, SF, RE, VT, and MH; and role-social component summary (RCS) consisting of BP, GH, RP, SF, and RE (26, 27).

Knee extension strength of both right and left legs was measured, using a lower limb muscle strength measuring device (Locomo Scan-II; ALCARE Co., Ltd., Tokyo, Japan).

2.12 Statistical analysis

The analysis was performed in accordance with the statistical analysis plan.

Subject characteristics and nutrient intakes calculated from BDHQs are shown as mean ± standard deviation. For HRQOL survey score data, actual value and change values, which are post-intervention values (at four, eight, and 12 weeks of intake) relative to before the intervention (week 0 of intake), are shown as mean ± standard deviation.

Multiple comparisons were made of nutrient intakes during the intervention period, to compare the control supplement and MCTs supplements. First, the equal variance was tested for each data set, using Levene's test. If Levene's test result was significant, Steel's test was conducted; if not significant, Dunnett's test was conducted.

HRQOL survey score data were first compared among the four groups by multiple comparisons for the values at week 0.

If there were no significant differences, a linear mixed model was used, with diet and time as fixed effects and subject as a random effect, to test for interactions between diet and time effects for the longitudinal measurements (at four, eight, and 12 weeks of intake). If no interaction was found, a linear mixed model equation without an interaction term was used to test for the diet effect for the longitudinal measurements. If a diet effect was found, multiple comparisons were performed between the four groups at each intervention period (four, eight, and 12 weeks of intake). If an interaction was found, a one-way analysis of variance was used to confirm the dietary effect. If there were significant differences, multiple comparisons were performed between the four groups at each intervention period (four, eight, and 12 weeks of intake). Multiple comparisons between the four groups were first tested for equal variance by Levene's test. We then performed Steel's test if equivariance was found, or Dunnett's test if no equivariance was found.

For within-group comparisons of data at weeks 4, 8, and 12, based on week 0, equal variance was tested by Levene's test, and multiple comparisons by Steel's test if significant, or by Dunnett's test if not.

Descriptive statistics of the analyzed data were calculated using Microsoft Excel for Office 365 MSO (Microsoft Japan Corporation, Tokyo, Japan). R statistical software, v4.1.0 for Windows (R Core Team, Vienna, Austria) was used for statistical processing. In all significant difference analyses, a risk ratio of less than 5% was considered significant, and a risk ratio of 5–10% was considered a tendency.

2.13 Exploratory analysis

A Pearson's correlation test was conducted to determine the relationship between subjective health and muscle strength for exploratory analysis. Correlation coefficients were calculated using change values from 0 to 12 weeks in the SF-36v2 score and knee extension strength data as variables. In addition, we calculated partial correlation coefficients for SF-36v2 and knee extension strength, adjusting for gender, age, and fat free mass as covariates. Regarding gender, we assigned dummy variables as male = 1 and female = 2 for the analysis.

3 Results

Some of the data from this study (nutrients intake and knee extension strength) has been reported previously (21).

3.1 Analysis of subjects

A flowchart of the subjects of the analysis is shown in Figure 1. Those who gave written consent ($n = 258$) were assessed for eligibility

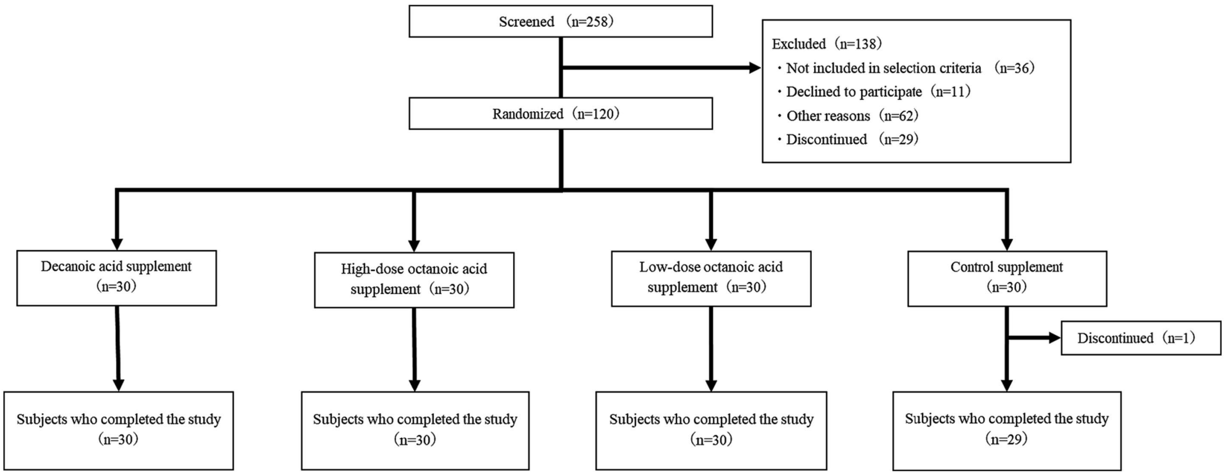

FIGURE 1
Flowchart of study subjects.

and 138 were excluded. The eligible subjects ($n = 120$) were enrolled and randomized into four groups of 30. Of those who completed the study ($n = 119$), those excluded by the statistical analysis plan were included in the analysis as per protocol set (PPS) ($n = 112$). A characteristic of the study participants has been described previously (21).

In this study, compliance was properly maintained. As a result, 89.9% of the subjects consumed 2 packets of test supplement daily during intervention. The remaining subjects also had an intake rate of over 98%. In addition, during the study period, walking was performed by all participants two days per week, although some participants performed the alternative indoor exercise due to the weather.

3.2 Adverse events

There were no adverse events attributed to the test supplement during this study.

3.3 Nutrient intake

There were no significant differences in nutrient intakes between the control supplement group and MCTs supplement groups, before or during the study period. Nutrient intakes were reported previously (21).

3.4 Test results of SF-36v2

The actual values and change values in each SF-36v2 score are shown in Table 2. There were no significant differences in any of the pre-intervention scores between the control group and MCTs supplement groups. In the control group there was no significant change in any of the SF-36 scores during the intervention period, compared to the pre-intervention period. As a result of the 12-week intervention, physical function (PF), general health (GH), vitality (VT) and mental component summary (MCS) showed significant increases in scores in all MCT supplement groups compared to the control group, either in actual or change scores. In addition, there was a significant increase in mental health (MH) scores in the Decanoic acid supplement and Low-dose octanoic acid supplement groups, compared to the control group. There were no significant changes in role-physical (RP), body pain (BP), social functioning (SF) and role-mental (RE), nor in physical component summary (PCS) or role-social component summary (RCS).

3.5 Correlation between SF-36v2 score and knee extension strength

The details of knee extension strength were reported previously (21). The results of the knee extension muscle strength measurements at 0 and 12 weeks are shown in Table 3. At week 12 of the intervention, all MCT supplement groups showed an increase from baseline in right knee extension strength, compared to the control group. For left knee extension strength, only the Decanoic acid supplement and Low-dose octanoic acid supplement groups showed higher values than the control group. The correlation coefficients between knee extension strength and SF-36 are shown in Table 4. A weak positive correlation was found between knee extension strength and MCS in Decanoic acid supplement group (0.40, $p < 0.05$). There was a positive correlation between knee extension strength and VT in Low-dose octanoic acid supplement group (0.42, $p < 0.05$). In contrast, in High-dose octanoic acid supplement group, a small positive correlation was found between right knee extension strength and PF (0.38, $p < 0.05$), but no correlation was found between VT, MH, MCS, and knee extension strength. This trend was similar when partial correlations were calculated using age, sex, and fat free mass as adjustment variables (Supplementary Tables S1–S4).

4 Discussion

In this study, the enrolled subjects were aged 60–74 years, had no exercise habits, and had an average BMI of 21.5 kg/m^2, which is

 10.3389/fnut.2023.1296896

TABLE 2 Variation in SF-36v2 score with consumption of MCT supplements or control supplement.[1]

	Week	Control supplement		Decanoic acid supplement		High-dose octanoic acid supplement		Low-dose octanoic acid supplement	
		Actual value	Change value	Actual value	Change value	Actual value	Change value	Actual value	Change value
Physical functioning (PF)	0	90.7 ± 1.7		90.7 ± 1.7		93.0 ± 1.6		95.0 ± 0.9	
	4	89.5 ± 1.8	−1.2 ± 0.8	94.1 ± 1.3 *	3.3 ± 1.6	95.4 ± 1.2 *	2.3 ± 1.0	96.8 ± 0.7 *	1.8 ± 0.6
	8	91.9 ± 1.9	1.2 ± 1.4	94.4 ± 1.3	3.7 ± 1.2	96.6 ± 0.9 *	3.6 ± 1.1	97.0 ± 0.6 *	2 ± 0.8
	12	92.2 ± 1.5	1.6 ± 1.1	96.1 ± 1.1 *	5.4 ± 1.6	96.8 ± 1.0 *	3.8 ± 1.0	97.9 ± 0.5 *	2.9 ± 0.7
Role-physical (RP)	0	91.8 ± 2.6		96.3 ± 2.1		92.6 ± 3.2		95.8 ± 2.0	
	4	95.3 ± 1.7	3.5 ± 1.6	95.8 ± 2.3	−0.5 ± 1.4	97.3 ± 1.6	4.7 ± 2.7	97.1 ± 1.5	1.3 ± 1.5
	8	95.1 ± 1.9	3.2 ± 2.1	96.3 ± 2.1	0.0 ± 1.2	97.5 ± 1.2	4.9 ± 2.6	98.4 ± 1.3	2.7 ± 1.6
	12	95.0 ± 1.9	3.2 ± 1.7	97.5 ± 1.3	1.2 ± 1.1	98.9 ± 0.7	6.2 ± 2.7	98.9 ± 0.7	3.1 ± 1.7
Bodily pain (BP)	0	82.6 ± 3.0		83.1 ± 3.3		83.4 ± 2.8		84.5 ± 3.5	
	4	85.2 ± 3.0	2.6 ± 2.8	86.6 ± 3.1	3.4 ± 3.3	87.7 ± 2.4	4.4 ± 2.1	92.0 ± 2.2	7.5 ± 2.7
	8	86.3 ± 2.9	3.7 ± 2.1	92.4 ± 2.0	9.3 ± 3.2	88.9 ± 2.5	5.5 ± 2.4	91.6 ± 2.9	7.2 ± 2.5
	12	88.7 ± 2.9	6.1 ± 2.5	93.6 ± 2.1	10.5 ± 3.5	90.6 ± 2.3	7.3 ± 1.8	93.9 ± 2.4	9.4 ± 2.6
General health (GH)	0	73.4 ± 2.0		78.9 ± 2.4		78.7 ± 2.5		80.0 ± 2.5	
	4	72.9 ± 2.4	−0.5 ± 1.3	80.8 ± 2.6	2.0 ± 1.4	81.4 ± 2.6 *	2.7 ± 1.3	81.8 ± 2.5 *	1.8 ± 0.8
	8	73.7 ± 2.4	0.3 ± 1.4	83.1 ± 2.5*	4.3 ± 1	82.0 ± 2.7	3.3 ± 1.7	83.5 ± 3.0 *	3.6 ± 1.5
	12	72.9 ± 2.4	−0.5 ± 1.4	84.0 ± 2.3 *	5.1 ± 1.2 *	83.6 ± 2.7 *	4.9 ± 1.9	85.6 ± 2.5 *	5.6 ± 1.3 *
Vitality (VT)	0	74.0 ± 2.8		74.1 ± 2.6		75.0 ± 2.2		78.2 ± 2.3	
	4	75.9 ± 3.1	1.9 ± 1.6	79.2 ± 3.1	5.1 ± 1.5	80.4 ± 2.0	5.4 ± 1.6	82.2 ± 2.2	4.0 ± 1.3
	8	74.2 ± 3.6	0.2 ± 2.1	79.2 ± 2.5	5.1 ± 0.9	81.1 ± 2.3	6.0 ± 1.8 *	84.2 ± 2.2 *	6.0 ± 1.4 *
	12	73.7 ± 3.3	−0.2 ± 2.2	82.9 ± 2.3 *	8.8 ± 1.3 *	81.9 ± 2.3	6.9 ± 1.9 *	86.4 ± 2.2 *	8.2 ± 1.7 *
Social functioning (SF)	0	92.2 ± 2.9		88.0 ± 3.9		91.1 ± 3.1		94.2 ± 2.4	
	4	93.5 ± 2.5	1.3 ± 2.1	94.4 ± 3.0	6.5 ± 2.8	98.7 ± 1.0	7.6 ± 2.6	97.8 ± 1.6	3.6 ± 2.1
	8	95.3 ± 2.0	3.0 ± 1.9	98.1 ± 1.1	10.2 ± 3.6	98.7 ± 1.0	7.6 ± 2.6	98.7 ± 1.0	4.5 ± 2.4
	12	95.3 ± 2.4	3.0 ± 1.8	97.7 ± 1.2	9.7 ± 3.9	98.7 ± 1.0	7.6 ± 2.6	100.0 ± 0.0	5.8 ± 2.4
Role-emotional (RE)	0	93.4 ± 2.0		93.2 ± 3.3		93.5 ± 2.2		95.8 ± 1.7	
	4	94.3 ± 1.9	0.9 ± 1.3	96.0 ± 2.3	2.8 ± 3.6	96.7 ± 1.5	3.3 ± 1.7	99.4 ± 0.6	3.6 ± 1.6
	8	93.7 ± 2.4	0.3 ± 2.2	98.1 ± 1.3	4.9 ± 3.0	96.1 ± 1.7	2.7 ± 1.6	99.4 ± 0.4	3.6 ± 1.7
	12	95.4 ± 1.8	2.0 ± 1.6	97.2 ± 1.5	4.0 ± 2.4	97.0 ± 1.4	3.6 ± 1.7	100.0 ± 0.0	4.2 ± 1.7
Mental health (MH)	0	82.1 ± 2.4		84.8 ± 2.0		83.4 ± 1.9		84.5 ± 2.4	
	4	83.4 ± 2.3	1.4 ± 1.4	86.3 ± 2.2	1.5 ± 1.4	86.8 ± 1.7	3.4 ± 1.6	88.9 ± 1.8	4.5 ± 1.5
	8	84.3 ± 2.2	2.2 ± 1.9	89.6 ± 1.2	4.8 ± 1.3	87.1 ± 1.8	3.8 ± 1.2	90.2 ± 1.6 *	5.7 ± 1.8
	12	83.3 ± 2.0	1.2 ± 1.8	91.5 ± 1.0*	6.7 ± 1.6	89.1 ± 1.9	5.7 ± 1.3	91.6 ± 1.8 *	7.1 ± 2.0
Physical component summery (PCS)	0	50.8 ± 1.1		52.6 ± 1.1		52.8 ± 1.0		53.4 ± 1.0	
	4	50.6 ± 1.1	−0.3 ± 0.6	53.2 ± 0.9	0.6 ± 1.2	53.4 ± 0.8	0.6 ± 0.6	54.2 ± 0.7 *	0.8 ± 0.8
	8	51.5 ± 0.9	0.6 ± 0.7	53.6 ± 0.8	1.0 ± 0.9	54.1 ± 0.8	1.3 ± 0.7	54.3 ± 0.9	0.8 ± 0.9
	12	52.0 ± 1.0	1.1 ± 0.8	54.4 ± 0.8	1.8 ± 0.9	54.5 ± 0.8	1.7 ± 0.7	54.9 ± 0.7 *	1.4 ± 0.8
Mental component summery (MCS)	0	59.1 ± 1.1		60.0 ± 1.2		60.2 ± 1.0		60.8 ± 1.2	
	4	59.6 ± 1.3	0.5 ± 0.7	61.8 ± 1.3	1.7 ± 0.7	62.1 ± 1.0	1.9 ± 0.6	63.0 ± 1.2	2.2 ± 0.5
	8	59.5 ± 1.3	0.4 ± 0.9	63.2 ± 0.9	3.2 ± 0.6 *	62.4 ± 1.1	2.2 ± 0.6	63.8 ± 1.3 *	3.1 ± 0.8 *
	12	59.0 ± 1.3	−0.1 ± 0.9	64.4 ± 0.9 *	4.4 ± 0.7 *	63.2 ± 1.2	3.0 ± 0.6 *	65.0 ± 1.3 *	4.3 ± 0.8 *
Role-social component summery (RCS)	0	51.1 ± 1.1		49.9 ± 1.5		49.7 ± 1.3		50.9 ± 1.0	
	4	52.2 ± 1.1	1.0 ± 0.6	50.7 ± 1.2	0.9 ± 1.1	51.8 ± 0.7	2.1 ± 1.1	51.6 ± 0.8	0.7 ± 0.8
	8	52.1 ± 1.0	0.9 ± 0.9	51.3 ± 0.9	1.4 ± 1.1	51.3 ± 0.7	1.6 ± 1.1	51.7 ± 0.9	0.8 ± 0.8
	12	52.4 ± 1.0	1.3 ± 0.7	50.6 ± 0.7	0.7 ± 1.1	51.3 ± 0.7	1.7 ± 1.1	51.4 ± 0.7	0.5 ± 0.9

[1]Values are shown as mean ± standard deviation. *Significant difference compared to control group ($p < 0.05$, Dunnett test).

TABLE 3 Muscle strength of each group during the intervention period and their changes from the baseline.[1]

		Control supplement		Decanoic acid supplement		High-dose octanoic acid supplement		Low-dose octanoic acid supplement	
	Week	Actual values	Change values	Actual values	Change values	Actual values	Change values	Actual values	Change values
Knee extension strength									
Right, *N*	0	309.3 ± 21.0		306.4 ± 19.6		326.3 ± 30.1		254.6 ± 19.9	
	12	356.7 ± 16.5	47.3 ± 14.7	426.0 ± 20.3†	119.6 ± 17.3	456.8 ± 26.3*,†	130.5 ± 18.3	391.3 ± 23.0†	136.7 ± 19.1
Left, *N*	0	291.8 ± 19.9		308.0 ± 21.1		323.0 ± 24.3		244.0 ± 18.1	
	12	356.6 ± 20.1	64.8 ± 15.9	433.0 ± 20.2*,†	125.1 ± 13.0	441.6 ± 23.1*,†	118.6 ± 17.9	379.6 ± 20.7†	135.6 ± 21.7

[1]Values are shown as mean ± standard deviation.
*Significantly different from control group ($p < 0.05$, Dunnett test).
†Significantly different from the baseline ($p < 0.05$, Dunnett test).

TABLE 4 Correlation coefficients between knee extensor strength and SF-36 score at 12 weeks of intervention.

	Control supplement		Decanoic acid supplement		High-dose octanoic acid supplement		Low-dose octanoic acid supplement	
	Right knee extension	Left knee extension	Right knee extension	Left knee extension	Right knee extension	Right knee extension	Right knee extension	Left knee extension
Physical functioning (PF)	0.03	−0.10	−0.05	−0.01	0.22	0.38 *	0.36	0.25
Role-physical (RP)	0.23	0.06	−0.03	−0.10	−0.13	−0.02	0.25	0.23
Bodily pain (BP)	0.33	−0.18	0.53 **	0.04	−0.05	−0.14	−0.03	0.21
General health (GH)	0.34	0.18	0.39 *	0.57 **	0.12	0.03	0.16	0.11
Vitality (VT)	0.25	0.05	0.14	−0.06	−0.01	−0.04	0.42 *	−0.03
Social functioning (SF)	−0.08	0.22	0.27	−0.09	0.16	0.20	0.30	0.17
Role-emotional (RE)	0.13	0.14	0.21	0.34	−0.01	0.08	0.10	−0.23
Mental health (MH)	0.03	0.10	0.06	−0.08	−0.08	0.04	0.15	−0.17
Physical component summery (PCS)	0.33	−0.21	0.17	0.16	0.05	0.04	−0.02	0.33
Mental component summery (MCS)	0.23	0.23	0.40*	0.40 *	0.06	0.06	0.24	0.24
Role-social component summary (RCS)	−0.17	0.25	0.07	−0.02	−0.04	0.11	0.23	−0.07

*$p < 0.05$ (Pearson's correlation coefficient). **$p < 0.01$ (Pearson's correlation coefficient).

generally below average for Japanese people of that age group (28). The intervention consisted of continuous supplements of MCTs combined with moderate-intensity equivalent walking for 12 weeks. The results showed significant improvements in scores on several subscales of the SF-36 in all MCTs supplement groups, compared to the control group. Therefore, regardless of the octanoic and decanoic acid composition, it is speculated that the combination of a continuous daily intake of 2 g or more of MCTs and walking may have an effect on subjective health and HRQOL in healthy middle-aged and older adults with lower BMIs and no exercise habits.

This study evaluated the effects of continuous MCTs intake and a moderate-intensity exercise intervention on mental and physical aspects of subjective health and social functioning, using the SF-36. The scoring of the subjects, based on the national standard score of the SF-36 at baseline (26), showed that all scores were above 50 points. There were significant increases in PF, GH, VT, and MCS in all MCTs supplement groups at week 12, compared to the control. MH also increased significantly in Decanoic acid supplement and Low-dose octanoic acid supplement groups. On the other hand, there were no significant changes in RP or BP, and there was no change in PCS, a summary score of physical health. The results indicated that the biggest effects of this intervention were in improving the mental aspect of QOL among the mental, physical, and social aspects of health. It also suggested improvements in PF, GH, and VT, which are subscales of PCS, and in GH, which is a subscale of RCS. Therefore, MCTs intake and walking have been shown to potentially improve mental aspects of QOL in middle-aged and older adults with no exercise habits and low BMIs.

Previous studies have shown a correlation between physical activity and HRQOL scores (29, 30). A study of healthy individuals aged 60–89 years in the U.S. reported higher HRQOL scores in the group that engaged in more than one hour of moderate-intensity physical activity per week, compared with the group that engaged in less than one hour per week (30). Referring to previous studies in the

U.S., the present study predicted an improvement in HRQOL scores with increased physical activity. However, our control group showed no significant changes in all SF-36 items, as measured before and after the intervention.

On the other hand, because middle-aged and older adults with no exercise habits were subjected to exercise, there was a possibility that mental and physical fatigue associated with the walking exercise might affect the SF-36 score. However, the control group in this study showed no significant changes in physical health, mental status, or items representing the frequency of feeling tired. It is possible that in addition to the increase in physical activity, the consumption of MCTs may have affected subjective health.

In Japan it is recommended to exercise at least two days a week, for at least 30 min per session, to reduce the risk of age-related decline in functional capability in daily living, and to extend the period of independent living by increasing the intensity and frequency of physical activity (31). This is comparable to the exercise load in the present study, but no participants dropped out of the study because of the walking exercise intervention. Therefore, the results may be valuable in that continued MCTs intake, combined with relatively easy exercise, may have improved HRQOL in the subjects of this study.

There were significant increases in the MCTs supplement groups, compared to the control, especially in the subscales of mental aspects of health, such as VT, MH and GH. Their summary score, MCS, was also significantly increased. In a study investigating the effects of MCTs on mental health, the effects of medium-and long-chain triglycerides (MLCTs) on depression-like symptoms were reported in animal studies. One study suggested that the intake of MLCTs alleviated depression-like behavior and had an antidepressant-like effect compared to the intake of LCTs in mice subjected to the stress of continuous forced swimming (32). This trend is similar to the result of the present study, which shows a significant improvement in MH and MCS scores, which indicate a depressed mental state. It is speculated that consumption of MCTs may affect subjective health, especially the mental aspect of health.

On the other hand, there was a significant change in PF, a subscale that significantly affects the physical aspect of health, although there was no change in PCS. The PF-related questions include an assessment of the walking and score has been reported to correlate with walking function (33). Therefore, in addition to the improvement in walking function resulting from the walking exercise as an intervention, lowering the psychological barrier to walking is thought to be one of the reasons for the increased PF score. Additionally, in the MCTs supplement groups, there was a significant increase in lower limb strength after the intervention, compared to the control group. It has been reported that continuous intake of MCTs increases metabolism-related enzymes in skeletal muscle (14, 34). Therefore, in our MCTs supplement groups, it is hypothesized that increased fatty acid oxidation capacity provided an ample energy supply for muscle activation in skeletal muscle. This increase in energy supply may have contributed to the improvement in muscle strength, consequently leading to the increase in PF scores.

The SF-36 is an assessment that measures subjective physical and mental health, but it is also used to assess subjective fatigue. Subjective fatigue is a subjective feeling of awareness of the presence of physical and mental fatigue. VT, which represents the frequency of experiencing fatigue, has been used as a measure of physical fatigue in several clinical studies and its validity has been reported (35, 36). In addition, MH is an indicator of levels of anxiety and depression, which have been reported to correlate with fatigue (37, 38). Moreover, both MH and MCS have been reported to have a strong negative correlation with mental fatigue, a subscale of the MFI-20 used as a fatigue assessment method (MCS $r = -0.563$, MH -0.550, VT -0.574, SF -0.500) (39). Intake of coenzyme Q10, which has been reported to have fatigue-reducing effects, resulted in increased SF-36 VT, MH, and MCS scores, alongside a decreased salivary secretory immunoglobulin A secretion rate — a biological indicator of stress closely related to fatigue (40). In addition, VT has been used as a measure of physical fatigue, while MH and MCS have been used as indicators of mental fatigue (41). Although biomarkers were not evaluated in the present study, and the direct relationship to fatigue is not clear, significant increases in VT, MH, and MCS were observed in the MCTs supplement groups. It can be speculated that the combination of MCTs intake and moderate-intensity exercise may reduce subjective physical and mental fatigue in middle-aged and older adults. It is also considered that this reduction in fatigue might contribute to the improvements in SF-36 scores.

Several mechanisms have been proposed for the perception of fatigue. It has been suggested that decreased adenosine triphosphate (ATP) production due to mitochondrial dysfunction, and cellular inflammation caused by oxidative stress, are related to fatigue (42, 43). Coenzyme Q10 and imidazole dipeptide are food ingredients that reduce mental and physical fatigue. These mechanisms of action have been reported to increase in mitochondrial ATP production and antioxidant effects (44), as well as anti-inflammatory effects via antioxidant effects (45).

Excessive production of reactive oxygen radicals in muscle and nerve cells due to over-exertion causes oxidation and damage to cells and intracellular proteins and lipids. Whether it is exercise-induced fatigue or psychological work-related fatigue, cytokines produced by the immune system cells that detect this damage are sent to the cranial nervous and endocrine systems, leading to the perception of fatigue. It has been suggested that fatigue can be delayed if sufficient energy is not available to repair the cells at that time (46). Indeed, increased levels of oxidative stress and decreased efficiency of mitochondrial ATP production have been reported in patients with chronic fatigue syndrome (CFS) (47–49). Providing sufficient ATP by maintaining mitochondrial function and suppressing oxidative stress *in vivo* may be important in reducing subjective fatigue, as it leads to a reduction in fatigue.

Studies have reported that MCTs or octanoic acid and decanoic acid each work to increase mitochondrial biosynthesis and activation of metabolism-related enzymes in tissues such as the cranial nervous system and skeletal muscle (14, 17, 24, 34, 50) and decrease oxidative stress (46). Moreover, β-hydroxybutyrate, a ketone converted from MCT, has been reported to increase protein expression of Mn-SOD and catalase (51). Therefore, we speculate that such an increase in ATP production and antioxidant capacity may have been the underlying mechanisms for the improvement in SF-36 scores in this study, indicating alleviation of subjective fatigue. However, further research is needed to elucidate these mechanisms.

In this study we examined the effects of MCT dose and fatty acid composition on HRQOL, in combination with walking exercise. Our Low-dose octanoic acid supplement group, which had the lowest daily MCTs intake, and Decanoic acid supplement group, which had an octanoic acid intake similar to the Low-dose octanoic acid supplement group, had significant changes in several SF-36 items, compared to the control group. The High-dose octanoic acid supplement group had fewer items that showed significant differences from the control. One reason for this may have been differences in muscle mass and strength at baseline. Although no statistically significant differences were found between the groups, knee extension strength at week 0 was approximately 20 N and 70 N greater in the High-dose octanoic acid supplement group than in the Decanoic acid supplement and High-dose octanoic acid supplement groups, respectively (Table 3). In the Decanoic acid supplement and Low-dose octanoic acid supplement groups there were significant increases in both left and right knee extension strengths at the change value, compared to the control diet group. No significant change in right knee extension strength was observed in the High-dose octanoic acid supplement group. The high muscle mass at baseline may have had at least a small effect on this result.

We analyzed the correlations between the changes in knee extension strength and SF-36 scores during the intervention. We found a weak to moderate positive correlation in scores related to subjective fatigue (such as MCS or VT) only in the Decanoic acid supplement and Low-dose octanoic acid supplement groups. This means there was an association between increased lower limb muscle strength and improvement in the mental subjective health subscale in the Decanoic acid supplement and Low-dose octanoic acid supplement groups. It is speculated that the increase in lower limb muscle strength associated with MCTs consumption may have had a small positive effect on subjective health and fatigue. It has been reported that SF-36 scores are more likely to decrease in individuals with reduced muscle strength (52), suggesting that physical function correlates with HRQOL. In conclusion, these results suggest that a certain daily intake (1.24 g) of octanoic acid as MCFA, which constitutes MCTs, in combination with walking exercise, might improve subjective health and reduce subjective fatigue by increasing muscle strength in middle-aged and older adults with low BMIs and no exercise habits.

Octanoic acid has been shown to increase the amount of acylated ghrelin in the stomach (53), and to activate Akt/mTOR, which is involved in protein metabolism (15). In fact, it has been reported that continuous intake of octanoic acid-rich MCTs increased plasma albumin concentrations (11), skeletal muscle mass and strength (12, 13). Therefore, it has been speculated that the antioxidant effect of albumin (54) and the increase in muscle strength may have influenced the present results. On the other hand, the mechanism by which decanoic acid intake increases muscle strength is not fully understood. This study was also unable to clarify the effects of different amounts of decanoic acid intake on HRQOL and subjective fatigue. Further studies are required, to investigate the mechanism by which decanoic acid affects muscle strength in animals and humans, to confirm the detailed dose–response. In this case, to establish the effects of decanoic acid dose, muscle strength and muscle mass at the baseline should be considered when assigning subjects.

Other factors that may affect this study include the fact that MCTs have been reported to be more likely to cause gastrointestinal discomfort than LCTs (55). However, the discomfort of ingesting MCTs was not considered to have a significant impact because the subjects' adherence to supplement consumption during the study period was high (over 90%) and they did not report any discomfort.

In this study, healthy men and women with no exercise habits undertook walking, a moderate-intensity exercise, twice a week. SF-36 was evaluated before and after the intervention. There are several limitations to the study. Firstly, whether walking or MCT had a greater effect on HRQOL is not clear. Secondly, the effect on middle-aged and older adults with a habit of daily exercise is unknown. Thirdly, the effect on subjective health at moderate or higher exercise intensities is unknown. Lastly, the effect on fatigue is unknown because biomarkers of fatigue were not measured.

5 Conclusion

Using SF-36v2, this study investigated the effects of 12 weeks of MCTs supplements plus walking equivalent to moderate-intensity exercise twice a week on subjective health and HRQOL in middle-aged and older adults with no exercise habits. The results showed that regardless of the MCFA composition of the MCTs and the amount of MCTs, the combination of continuous intake of 2 g or more of MCTs per day and walking exercise significantly improved scores on subscales of the physical aspect of QOL and summary scores on the mental aspect of QOL, compared with the LCTs intake used as a comparison. It is estimated that continuous intake of MCTs and walking might have affected HRQOL and improved physical and mental subjective health in middle-aged and older healthy adults with low BMIs.

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 studies involving humans were approved by Yoga Allergy Clinic (approval number 21000023, approval date May 13, 2022). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

HI: Conceptualization, Formal analysis, Writing – original draft. KK: Conceptualization, Investigation, Writing – review & editing. SW: Project administration, Supervision, Writing – review & editing. NN: Conceptualization, Methodology, Supervision, Writing – review & editing. TM: Supervision, Writing – review & editing.

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Conflict of interest

Authors HI, KK, SW, and NN were employed by the Nisshin OilliO Group, Ltd., Tokyo, Japan. The company is engaged in the manufacture and sale of products related to MCTs.

The remaining 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.

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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.2023.1296896/full#supplementary-material

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The association of the Affordable Care Act with nutrient consumption in adults in the United States

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The Patient Protection and Affordable Care Act, more commonly known as the ACA, was legislation passed in the United States in 2010 to expand access to health insurance coverage for millions of Americans with a key emphasis on preventive care. Nutrition plays a critical role in overall wellness, disease prevention and resilience to chronic illness but prior to the ACA many Americans did not have adequate health insurance coverage to ensure proper nutrition. With passage of the ACA, more individuals received access to nutritional counseling through their primary care physicians as well as prescription vitamins and supplements free of charge. The objective of this study was to evaluate the impact of a national health insurance reform on nutrient intake among general population, including more vulnerable low-income individuals and patients with chronic conditions. Using data from the National Health and Nutrition Examination Survey (NHANES), we identified 8,443 adults aged 21 years and older who participated in the survey before (2011–2012) and after the ACA (2015–2016) implementation and conducted a subgroup analysis of 952 respondents who identified as Medicaid beneficiaries and 719 patients with a history of cancer. Using pre-post study design and bivariate and multivariable logistic analyses, we compared nutrient intake from food and supplementation before and after the ACA and identified risk factors for inadequate intake. Our results suggest that intake of micronutrients found in nutrient-dense foods, mainly fruit and vegetables, has not changed significantly after the ACA. However, overall use of nutritional supplements increased after the ACA (p = 0.05), particularly magnesium (OR = 1.02), potassium (OR = 0.76), vitamin D (both D2, and D3, OR = 1.34), vitamin K (OR = 1.15) and zinc (OR = 0.83), for the general population as well as those in our subgroup analysis Cancer Survivors and Medicaid Recipients. Given the association of increased use of nutritional supplements and expansion of insurance access, particularly in our subgroup analysis, more research is necessary to understand the effect of increasing access to nutritional supplements on the overall intake of micro- and macronutrients to meet daily nutritional recommended allowances.

KEYWORDS

nutritional supplements, Affordable Care Act, cancer, nutritional status, National Health and Nutrition Examination Survey

1 Introduction

The World Health Organization, in collaboration with United Nation's Children's Fund (UNICEF), Food and Agricultural Organization of the United Nations (FAO) and World Food Program (WFP) has emphasized the role of public policy in modifying food systems to improve people's nutrition and health (1). Although global food production of calories has kept pace with population growth, the common prioritization of quantity and profitability over nutritional value has meant healthy diets remain unaffordable for over 40% of the world's population. At the same time, a surplus of availability of highly processed foods, which are often calorie-dense but nutrient-poor, contribute to the alarming rise in diet-related diseases, such as diabetes, heart disease and certain cancers. The Patient Protection and Affordable Care Act, a public policy more commonly known as the ACA, was passed in the United States in 2010 to expand access to health insurance coverage for millions of Americans. Historically the United States spends far more on health care than comparable countries, but with less optimal health outcomes. This is partly due to years of underinvestment in preventive health services (2). The ACA also expanded access through the authorization of Medicaid expansion in many states to address issues of health equity in vulnerable communities. Medicaid is a public health insurance program jointly funded by federal and state governments to provide coverage for low-income Americans (3). The ACA is widely considered one of most significant regulatory overhauls of the United States health care industry by requiring insurance companies to reprioritize preventive health services through free-of-charge coverage for what are deemed "Essential Health Benefits." These mandated services include the provision of wellness and preventive care, including blood pressure screenings, cholesterol checks, cancer screenings, vaccinations, and nutrition counseling for all individuals (3, 4).

Prior to the passage of the ACA, previous studies of nutrient intake using national survey data suggested that the majority of the US adult population did not meet the recommended daily intake of nutrient-dense foods which may contribute to high rates of chronic diseases, such as hypertension and type 2 diabetes (5). Starting in 2016 the ACA required most health plans, including Medicaid programs, to cover annual nutrition counseling for people at high risk of chronic diseases at no charge, which previouly were not universally covered (6). The ACA further expanded the Prevention and Public Health Fund which provided direct funding in communities to provide chronic disease self-management and diabetes prevention programming, as well as funding for preventing health services programming (7). Many of these initiatives included access to nutritional counseling provided by registered dietitians and other trained practitioners.

Abbreviations: ACA, Affordable Care Act; BMI, body mass index; CAPI, computer-assisted personal interviewing; CHF, congestive heart failure; CI, confidence interval; COPD, chronic obstructive pulmonary disease; DFE, dietary folate equivalent; DNI, Daily Nutrient Intake; FPL, Federal poverty limit; g, gram; HTN, hypertension; IPR, income to poverty ratio; mcg, microgram; mg, milligram; n, number; NCHS, National Center for Health Statistics; NH, non-Hispanic; NHANES, National Health and Nutrition Survey; OR, odds ratio; PS, propensity score; PUFAs, polyunsaturated fatty acids; RDA, Recommended Daily Allowances; SD, standard deviation; SE, standard error; US, United States; VLFS, very low food security.

In addition to being a vital component of general health and wellness and staying healthy, nutrition therapy can be an important component of adjunct care in treating most all disease states including obesity, heart failure, diabetes, hypertension, COPD and arthritis (8–10). Specifically, cancer patients are at significant risk for malnutrition and cachexia because cancer and cancer treatments have a serious negative impact on a patient's ability to consume and absorb food and nutrients (11, 12). Cancer patients are at risk throughout the, treatment continuum and into survivorship often falling prey to nutrient shortfalls (11). Therefore, dietary supplements have been used to treat cancer patients and patients with cardiovascular disease. Dietary supplements have been found to reduce the risk of cardiovascular disease, cancer, and all-cause mortality (13). A recent study by Shaver and colleagues found that patients with cancer that use nutrition supplements are less likely to be hospitalized and have a lower probability of dying post follow-up (14). More specifically, calcium supplementation has been found to reduce the risk of cancer in some patients, particularly lung cancer patients (15, 16). In 2017 the American Society for Nutrition encouraged more research on preventive health service policies particularly that examine the role nutrition and nutritional supplementation play in disease prevention and chronic care management (17).

To our knowledge, this is the first study that examined the association with the Affordable Care Act on nutrient intake among adult Americans. We hypothesize that after the ACA, more patients received nutritional counseling that in turn, led to better food choices and/or increased supplement intake to improve overall health and wellbeing. Using data from the National Health and Nutrition Examination Survey (NHANES), an ongoing national initiative, our study assessed whether the improved access to preventive care under a national health insurance reform (the ACA), improved the overall intake of the key nutrients among general population and low income individuals and patients with chronic nutrition-related diseases. In our analysis, we examined nutrients consumed with food separately from nutritional supplementation to account for differences in demand and consumption behavior regarding general goods and services, like food, and insurance-subsidized services like prescription nutrition supplementation.

2 Methods

2.1 Study design and population

The study was based on the data from the National Health and Nutrition Examination Survey (NHANES). The National Health and Nutrition Examination Survey (NHANES) is designed to assess the health and nutritional status of adults and children in the United States. The survey is unique in that it combines interviews and physical examinations. NHANES is a major program of the National Center for Health Statistics (NCHS) that receives millions of dollars from the federal budget. NCHS is part of the Centers for Disease Control and Prevention (CDC) and has the responsibility for producing vital and health statistics for the Nation. Several hundreds of highly trained home interviewers collect and encrypt data on laptops, use printed materials to prompt and verify responses, and verify prescription medicine use by examining container labels. The NHANES is a survey conducted annually by the National Center for Health Statistics (NCHS) housed within the Centers for Disease Control and Prevention (CDC) since 1960s. According to the CDC, the survey is a collection of physical examinations and interviews with a nationally representative sample of 5000 United States citizens. It is used to monitor the health and nutritional status of non-institutionalized individuals in the US (18). NHANES uses a representative sample of noninstitutionalized U.S. civilians, selected by a complex, multistage probability design. Briefly, participants were interviewed in their homes and subsequently examined in mobile examination centers (MEC) in 15 U.S. geographic locations. All participants provided informed consent prior to data collection. The survey uses stratified, multistage probability sampling to obtain nationally representative samples for each year; data from questionnaire and laboratory tests are released every 2 years (18).

Adults aged 21 years and older with non-missing values on nutrient intake from diet (which includes both food and drink) and supplements were included in our study. The final sample included 8,443 participants that corresponded to a weighted sample of 223,400,729 individuals for the years 2011–2012 and 232,006,739 for the years 2015–2016. The study is in compliance with the University at Buffalo Institutional Review Board policies. The subgroup analysis examined 952 patients who reported receiving Medicaid benefits, with 444 patients in 2011–12 and 508 in 2015–2016.

2.2 Nutrient intake measures

We hypothesize that after the ACA, more patients received nutritional counseling that in turn, led to better food choices and/or increased supplement intake to improve overall health and wellbeing. Nutrient intake from food was estimated based on two dietary recalls. All NHANES participants are eligible to participate in two 24-h dietary recall interviews performed first in the Mobile Examination Center and second telephonically within 3–10 days of the medical examination (19, 20). Nutrient intake from supplements was ascertained in the participant's home before physical examination. Dietary supplement information is collected by trained interviewers. An affirmative response to supplement use is followed by an examination of the supplement containers by the interviewer who enters the product name and strength into the Computer-Assisted Personal Interviewing (CAPI) system. This information is later reviewed by trained nutritionists at the NCHS to discern the exact product reported by participants and calculate daily average intake of nutrients based on the previous 30 days' food, supplement, and antacid intake.

Sufficient nutritional intake was determined based on the US recommended daily allowances (RDA) and other standards (19–23). Information was gathered on the following nutrients: calcium, folate (as dietary folate equivalent or, DFE), dietary fiber, lycopene, magnesium, potassium, selenium, lutein + zeaxanthin, vitamin B6, vitamin B12, vitamin C, vitamin D (D2 and D3), vitamin K, and zinc. These nutrients were chosen due to previous research indicating their utility in cancer prevention and immune health (23–36). RDA was chosen so as to make the study more translatable to the general public as well as to be translatable to the labeling as found on many supplements.

2.3 Participant characteristics

Nutritional intake can vary by individual personal characteristics and socio-economic status (5, 37–40). Demographic details of participants, such as age, gender, and race were provided in an interview. Social characteristics were also provided via interview and included: smoking status categorized as current, former, and never smoker; level of education categorized as ≤high school graduate, some college, and ≥ Bachelor's degree. Participants answered whether they were US citizens or not. Socioeconomic status (SES) was assessed in terms of income to poverty ratio (IPR), and was categorized as <100%, 100 to <200%, 200 to <300%, and ≥ 300%. Health insurance was categorized as Private, Medicare, Medicaid, Other and None. Body mass index (BMI) was measured as part of the anthropometric examination and was utilized to calculate obesity, defined as a BMI of greater than or equal to 30. The medical conditions questionnaire is asked in the home by a trained interviewer utilizing the CAPI system with questions stratified by gender (for conditions related to specific anatomy) and age. Participants were asked if they had ever been told they had cancer, arthritis, diabetes, congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), or hypertension.

2.4 Statistical analysis

All analyses were conducted using appropriate survey weights in SAS 9.4. Descriptive analyses of continuous variables included calculating the means (SD) and frequencies (%) of anthropometric, medical, and categorical sociodemographic characteristics of the participants. Propensity score (PS) matching was utilized to assist in controlling for any differences in the survey sample between 2011–2012 and 2015–2016 cohorts. Participants from each time period were matched on age, gender, race/ethnicity, level of education, income, comorbidities, and citizenship status. After PS matching, there were no statistically significant differences in the sample by the key covariates; the final sample for analysis included 7309 patients.

We used binary logistic regressions stratified and clustered by the NHANES survey strata (PSU). The ACA variable is a binary variable based on the NHANES cohort, since the ACA is a national policy. With respect to nutritional intake, the outcome variable was defined as whether or not an individual consumed a recommended daily amount (RDA), for each nutrient. We incorporated survey weights to extrapolate the survey results from the subsample of the NHANES participants who completed a 2-day dietary recall (SAS proc surveylogistic) to the national community population. Odds ratios (Post- vs. Pre-ACA) were adjusted for the participants age, sex, race, education, income to poverty ratio, comorbidities (arthritis, diabetes, CHF, COPD, HTN, obesity cancer), US citizenship, and smoker status to control for the independent association with these factors on nutritional intake (5, 37–40). Total nutrient intake combined the intake of both food and supplement. A regression analysis was run for each type of nutrient intake against total nutrient intake at significance $p < 0.05$.

We also evaluated nutrient consumption of patients with nutrition-related comorbidities that increased in prevalence during the time of the study (arthritis, diabetes, obesity and cancer, Table 1). Because cancer patients are at significant risk for malnutrition analyses were conducted on a subgroup of 719 cancer survivors from the

TABLE 1 Population characteristics before and after the ACA (n = 8,443), weighted results, by percent.

Characteristic	Pre-ACA (2011–2012) n = 223,400,729	Post-ACA (2015–2016) n = 232,006,739	value of p
Age	47.4 (0.9)	48.3 (0.7)	0.46
Sex			0.77
Male	48.2	48.6	
Female	51.8	51.4	
Race			0.93
Mexican American	8.2	8.7	
Other Hispanic	6.0	5.8	
NH White	67.0	65.3	
NH Black	11.4	10.7	
Other	7.3	9.5	
Education			0.82
≤High School Graduate	36.3	34.3	
Some College	31.7	33.5	
≥Bachelor's Degree	32.1	32.1	
Income to Poverty Ratio			0.06
<100% FPL	17.9	13.6	
100 to <200% FPL	20.7	19.0	
200 to <300% FPL	14.1	16.8	
> = 300% FPL	38.3	38.6	
Refused to answer	8.9	12.0	
Comorbidities			
Arthritis	23.6	28.3	0.04
Diabetes	9.3	11.5	0.03
CHF	2.6	2.4	0.58
COPD	7.5	3.3	<0.001
HTN	31.8	32.2	0.85
Obesity	34.6	39.8	0.03
Cancer	9.9	12.4	0.02
US Citizen	82.9	82.8	0.98
Regular Healthcare Provider	85.2	83.5	0.35
Smoker			0.37
Current	20.0	17.6	
Former	24.2	25.9	
Never	55.9	56.4	
Insurance			0.02
Private	60.9	61.0	
Medicare	7.5	9.0	
Medicaid	6.8	8.6	
Other	6.0	8.2	
None	18.7	13.2	
Dietary Supplements			0.05
No	46.8	41.5	
Yes	53.2	58.5	

CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; HTN, hypertension; FPL, federal poverty limit; NH, non-Hispanic.
Data presented as frequency (%) or mean (SD); p represents results of t-test or chi-square test of difference as applicable.

matched sample. The cancer survivors were individuals who reported being told they had cancer. Similar analyses were conducted on the subgroup of 952 Medicaid beneficiaries from the matched sample, because of the significant regulatory overhaul of the program due to the ACA. Descriptive statistics, mean and standard error were calculated for a sample of Medicaid patients included in the NHANES population surveyed. A test of differences was run examining survey respondents reported nutrient intake from dietary recall and nutrient intake from supplements.

3 Results

3.1 Characteristics of the study cohort

The mean age was similar to the general population (45.7 pre and 43.8 years post ACA, $p = 0.32$). The proportion of Medicaid recipients increased from 6.8% of the respondents prior to the ACA to 8.6% in the post-ACA cohort ($p = 0.02$). After the ACA, fewer individuals reported not having insurance coverage (18.7% vs. 13.2%, $p = 0.02$) mainly due to the increase in Medicaid expansion. Patient demographics did not change significantly over the study time period (Table 1). The study participants were on average 47.4 years old prior to the ACA and 48.3 years old after the ACA, predominantly Non-Hispanic White (67.0% vs. 65.3%), female (51.8 and 51.4%), and privately insured (60.9% vs. 61.0%). Most participants reported having a regular source of primary care (85.2% vs. 83.5%; $p = 0.35$).

The proportion of participants reporting using at least one dietary supplement increased from 53.2 to 58.5%, $p = 0.05$. Prevalence of several chronic diseases related to nutritional status also increased during this time (arthritis, diabetes, obesity, and cancer, $p < 0.05$), while prevalence of COPD decreased (7.5% vs. 3.3%, $p < 0.001$). After propensity score matching, there were no significant differences in the comorbidities and smoking status of the participants before and after the ACA.

3.2 Evaluation of nutrient intake

Table 2 presents the average total daily nutrient intake as percent of RDA by ACA status by intake source (food vs. dietary supplements). For several nutrients, the average intake exceeded the RDA before the ACA and remained high afterwards. For instance, the intake of vitamin D was above RDA before the ACA (103% RDA) and further increased to 141% RDA ($p = 0.01$) post-ACA as vitamin D supplementation increased from 70% pre-ACA to 77% post-ACA. Other nutrients with increased intake included calcium, folate, selenium, vitamins B6, B12, C, and K, and zinc, and were mainly obtained from food.

At the same time, intake of many core nutrients remained under the recommended amount. Many participants with nutrient intake below RDA from food, also did not use enough supplementation to fill the nutrient gap from food by reaching the nutrient-specific RDA goal neither pre- nor post-ACA. Potassium intake declined from 93% RDA pre-ACA to 87% RDA post-ACA ($p = 0.01$) and came primarily from food. Intake of dietary fiber, lycopene and lutein and zeaxanthin remained below 60% RDA before and after the ACA, with minimal supplementation.

3.3 Subgroup analysis

We conducted a multivariable adjusted regression analysis for a subgroup of Medicaid beneficiaries, a population subgroup that grew the most under the ACA (Table 3). Medicaid beneficiaries reported significantly more nutritional deficiencies than the

TABLE 2 Average daily nutrient intake from food and supplements by ACA status.

Nutrients	RDA	Pre-ACA			Post-ACA			$p^\wedge$
		Average DNI (mean, SE)	% of RDA	% from supplements	Average DNI (mean, SE)	% of RDA	% from supplements	
Vitamin D (D2 + D3) (mcg)	15–20 μg/d	15.4 (1.0)	103	70	20.7 (1.7)	141	77	0.01
Potassium (mg)	2600–3400 μg/d	2778.8 (39.5)	93	<1	2617.9 (46.4)	87	<1	0.01
Calcium (mg)	1000–1300 μg/d	1134.6 (22.7)	114	15	1077.9 (27.3)	109	13	0.12
Folate, DFE (mcg)	400 μg/d	784.3 (15.6)	196	28	741.4 (28.0)	187	30	0.19
Dietary fiber (gm)	21–38 g/d	18.3 (0.4)	58	<1	17.3 (0.5)	55	<1	0.11
Lycopene (mcg)	10,000	5485.7 (246.8)	55	2	5325.8 (256.1)	53	1	0.66
Magnesium (mg)	320–420 μg/d	339.1 (6.6)	92	9	337.9 (10.5)	91	10	0.92
Selenium (mcg)	55 μg/d	146.8 (16.1)	267	22	127.9 (2.3)	232	11	0.25
Lutein + zeaxanthin (mcg)	12,000	2093.8 (220.4)	17	18	1947.4 (131.8)	16	16	0.57
Vitamin B6 (mg)	1.3–1.7 mg/d	5.8 (0.4)	442	62	4.9 (0.6)	368	57	0.20
Vitamin B12 (mcg)	2.4 μg/d	67.3 (10.6)	2804	91	85.2 (10.8)	3675	93	0.25
Vitamin C (mg)	75–90 mg	175.2 (8.8)	212	51	169.5 (10.8)	208	55	0.69
Vitamin K (mcg)	90–120 μg/d	133.9 (9.2)	128	5	128.3 (4.9)	122	6	0.60
Zinc (mg)	8–11 μg/d	15.6 (0.3)	164	27	15.3 (0.4)	162	27	0.51

$p^\wedge$ indicates statistically significant change in nutrient intake of all NHANES participants pre and post ACA implementation.

TABLE 3 Medicaid status nutrient intake^.

		2011–2012				2015–2016			
	RDA	Total mean	Food mean	Suppl mean	% suppl	Total mean	Food mean	Suppl mean	% suppl
Calcium (n)	1000–1300 μg/d	989.25 (444)	897.72 (444)	302.96 (131)	30.21%	958.06 (508)	854.30 (508)	350.60 (146)	29.60%
Folate (n)	400 μg/d	623.42 (444)	496.38 (444)	591.41 (95)	21.40%	662.20 (508)	455.70 (508)	880.21 (113)	22.24%
Fiber (n)	21–38 g/d	15.09 (444)	15.08 (444)	0.46 (2)	0.35%	13.97 (508)	13.93 (508)	3.32 (6)	1.16%
Lycopene (n)		4804.55 (386)	4809.62 (382)	711.63 (18)	4.66%	4305.22 (444)	4290.50 (440)	504.23 (39)	8.78%
Magnesium (n)	320–420 μg/d	274.49 (444)	262.37 (444)	66.98 (83)	18.69%	277.18 (508)	249.02 (508)	142.33* (88)	17.32%
Potassium (n)	2600–3400 μg/d	2397.37 (444)	2391.90 (444)	50.42 (52)	11.71%	2245.63 (508)	2236.73 (508)	92.81* (58)	11.42%
Polyunsaturated Fatty Acids (n)	6–8 g/d	16.75 (444)	16.74 (444)	0.80 (8)	1.80%	16.92 (508)	16.90 (508)	0.68 (17)	3.35%
Selenium (n)	55 μg/d	106.69 (444)	100.74 (444)	40.86 (67)	15.09%	111.85 (508)	103.63 (508)	54.66 (80)	15.75%
Lutein + Zeaxanthin (n)		1230.09 (444)	1188.75 (444)	883.07 (21)	4.73%	1435.03 (508)	1383.02 (508)	708.72 (42)	8.27%
Vitamin B6 (n)	1.3–1.7 mg/d	4.32 (444)	1.94 (444)	11.19 (94)	21.17%	3.16 (508)	1.83 (508)	5.97 (110)	21.65%
Vitamin B12 (n)	2.4 μg/d	43.43 (444)	6.10 (444)	166.35 (101)	22.75%	40.01 (508)	5.42 (507)	135.12 (125)	24.61%
Vitamin C (n)	75–90 mg	136.08 (444)	80.37 (444)	227.69 (108)	24.32%	145.68 (508)	74.5 (508)	273.19 (121)	23.82%
Vitamin D (D2 + D3) (n)	15–20 μg/d	9.84 (443)	4.77 (442)	16.03 (127)	31.44%	14.11 (505)	4.71 (504)	30.77* (146)	30.44%
Vitamin K (n)	90–120 μg/d	92.63 (444)	89.22 (444)	25.24 (64)	14.41%	109.33 (508)	102.28 (508)	48.16* (73)	14.37%
Zinc (n)	90–120 μg/d	12.19 (444)	10.06 (444)	10.48 (86)	19.37%	12.76 (508)	9.59 (508)	14.92* (107)	21.06%

^Comparing nutrient intake from food consumption and use of supplements between 2011–2012 and 2015–2016.
*Statistically significant results for change in mean amount of supplementation $\alpha = 0.05$.

general population (for calcium, vitamin D and zinc). Similar to the general population, there was an increase in the number of people reporting dietary supplement intake after the ACA. Nutritional intake of micro and macro-nutrients among Medicaid beneficiaries significantly increased for five of the 15 core nutrients, mainly due to an increase in the use of nutritional supplements (Magnesium, Potassium, Vitamin D (D2 + D3), Vitamin K, and Zinc). Most notably intake of Vitamin D (D2 + D3) showed a statistically significant increase for overall increase in nutritional intake and increase in intake from supplementation, where respondents reported a 47.9% increase in Vitamin D supplement intake from 2011 to 2011 (16.03 μg/d) to 2015–2016 (30.77 μg/d) ($p < 0.05$). Similarly, the increase in total Magnesium intake pre- versus post-ACA, was primarily due to statistically significant increase in magnesium supplementation (66.98, 142.33, $p = <0.05$, respectively). Despite the increases, the total intake of Vitamin D and Magnesium still remained below current RDA recommendations at 15–20 μg/d and 320–420 μg/d, respectively. Considerably, intake for Vitamin K and Zinc increased post-ACA and levels were within RDA limits.

No significant changes pre-post ACA in nutrient intake and supplementation were detected in subgroups of patients with these disorders except for cancer (Table 4). After the ACA, beneficiaries with cancer history were significantly more likely to take adequate (RDA) amount of lycopene, but less likely to consume enough folate or vitamin B12. While the odds of consuming sufficient dosage of vitamin D did not change among patients with cancer after the ACA passage, the general population was more likely to have an intake of vitamin D at or above RDA recommendations (OR: 1.34; 95% CI [1.10–1.63]; $p = 0.005$).

4 Discussion

In this population-based study, we examined changes in individual nutritional intake that were enabled by the national health insurance reform as a part of the 2010 ACA. We demonstrated a significant increase in the use of some dietary supplements among the general population as well as for low-income populations post-ACA era, especially for vitamin D. Notwithstanding, many Americans continue to not meet the RDA for potassium, dietary fiber, and magnesium, despite improvements in insurance coverage and increased availability of no-cost nutrition counseling. Medicaid beneficiaries reported significantly more nutrient deficiencies than the general population (for calcium, vitamin D and zinc) but also demonstrated a greater increase in use of dietary supplements post-ACA (for calcium, folate, fiber, magnesium, potassium, vitamins D and K and zinc). Patients with a history of cancer demonstrated better intake of lycopene and lutein, nutrients known to have protective effects in cancer, but were less likely to consume enough of other core nutrients (folate and B12) (41).

Several studies have suggested a positive impact of the health insurance expansion on the use of preventive services among the highest risk populations, mainly due to the affordability of these services. Smoking cessation uptake was shown to be higher following the ACA than prior to its inception, and those enrolled in Medicaid were more likely to quit smoking than those with commercial insurance (OR 1.49; 95% CI 1.29, 1.73) (37). Moreover, despite current economic theory that suggests new access to health insurance can create *ex ante* moral hazard, Cotti and colleagues found that low-income individuals who benefitted from the public insurance expansion due to the ACA actually engaged in healthier health habits

TABLE 4 ACA status and odds of reaching RDA nutrient intake.

	General population (*n* = 7,308)			Cancer survivors (*n* = 719)		
Nutrient	Adjusted odds ratio*	95% CI	*p*	Adjusted odds ratio*	95% CI	*p*
Calcium	0.88	(0.73–1.06)	0.16	1.06	(0.65–1.73)	0.82
Folate, DFE	0.74	(0.60–0.91)	0.01	0.46	(0.30–0.73)	0.001
Dietary fiber	0.94	(0.75–1.19)	0.60	1.01	(0.54–1.90)	0.97
Lycopene	1.08	(0.88–1.34)	0.44	2.21	(1.09–4.49)	0.03
Magnesium	1.02	(0.83–1.26)	0.84	1.32	(0.79–2.22)	0.28
Potassium	0.76	(0.63–0.91)	0.005	1.07	(0.65–1.75)	0.79
Selenium	0.98	(0.75–1.27)	0.85	1.07	(0.58–2.00)	0.82
Lutein + zeaxanthin	0.78	(0.47–1.29)	0.32	0.82	(0.21–3.22)	0.77
Vitamin B6	0.86	(0.70–1.06)	0.16	0.96	(0.59–1.58)	0.88
Vitamin B12	0.77	(0.64–0.92)	0.01	0.54	(0.32–0.90)	0.02
Vitamin C	1.02	(0.82–1.26)	0.86	1.42	(0.85–2.36)	0.17
Vitamin D (D2 + D3)	1.34	(1.10–1.63)	0.005	1.69	(0.99–2.86)	0.052
Vitamin K	1.15	(0.94–1.40)	0.17	1.25	(0.73–2.14)	0.42
Zinc	0.83	(0.70–0.99)	0.04	0.75	(0.47–1.18)	0.21

*ORs (Post- vs Pre-ACA) adjusted for age, sex, race, education, income to poverty ratio, comorbidities (arthritis, diabetes, CHF, COPD, HTN, obesity cancer), US citizen, and smoker status. Total nutrient intake combines the intake of both food and supplement.

(38). A population-based observational study assessed the impact of the ACA on food insecurity and found that Medicaid expansion following ACA implementation was associated with a 2.2 percentage-point decline in very low food security (VLFS) (39). This study concluded that low-income families experienced less food insecurity because out-of-pocket health care expenses were reduced because of Medicaid expansion, thereby increasing expendable income to purchase food.

One possible explanation for the limited impact of the ACA on the general population nutrition status is a lack of patient knowledge surrounding benefits of coverage of available nutrition services (40). Additionally, primary care providers do find value in nutrition counseling but often feel poorly trained to discuss nutrition with their patients, and too short on time to engage with their patients (42, 43). Individuals obtained most of their nutrients from food, with the exception of vitamins C, D, B6 and B12 which came mostly from supplementation which is similar to a recent finding by Devarshi et al. (44). Patients with history of cancer are more likely to receive nutrition counseling than the general population as a part of their multidisciplinary cancer and survivorship care but may be more inclined to focus on cancer survival than general wellness (45, 46).

One potential explanation for the increased intake in vitamin D post-ACA is the increase in the number of reports of its association with various disease states and conditions that accidently happened during the same time as the ACA reform. Vitamin D has been studied and shown to prevent falls in older adult patients, reduce fracture risk in post-menopausal women and in individuals with osteoporosis (47–50). Similarly, the increased proportion of cancer patients with adequate lycopene intake may be reflective of its publicized role as an antioxidant for cancer, specifically prostate cancer (51, 52). With research studies and social media reports supporting the use of vitamin D and lycopene, clinicians may be more likely to recommend their intake in specific patient populations, as a result of having a more informed understanding of their benefits. In addition to clinicians, others involved in wellness programs, such as insurance companies and employers, may also play a role in educating their clients and employees about the benefits of adequate nutrition.

The reduction in potassium intake post-ACA, and the corresponding reduction in the number of individuals with sufficient intake of potassium, vitamin B12 and folate may be related to the reduction in consumption of foods enriched with these nutrients. According to the US National Institutes of Health, top sources of potassium among American adults are milk, potatoes, coffee, tea and other nonalcoholic beverages, but potassium can also be found in fruits and vegetables such as bananas. Folate is commonly found in fortified cereals, grains, bread and pasta, and well as dark green veggies including asparagus. Shan et al. studied food trends among American adults from the years 1999 to 2016, and found a decrease in consumption of low-quality carbohydrates, which included potatoes and beverages with added sugars (53). In studying trends in food intake, many higher income countries fall short on adequate fruit and vegetable intake (54). In the wake of increasing awareness of healthy eating, Americans may be curbing the intake of foods known as low-quality carbohydrates (such as potatoes, breads, and pasta) which are significant sources of potassium and folate, without adequately adding alternative sources, such as fruits and vegetables.

Data from the National Health and Nutrition Examination Survey, 2015–2018 reports that the percentage of adults who consumed any fruit on a given day has decreased over time, from 77.2% in 1999–2000 to 64.9% in 2017–2018 (55). Moreover, statistics indicate that the ability to access fresh produce is directly associated with income levels. In fact, NHANES continues to report that "the percentage of adults who consume any vegetables increased with increasing family income, from 92.5% of those from families with incomes less than 130% of FPL to 94.4% of those from families with incomes between 130 and 349% of FPL to 97.1% of those from families with incomes at or greater than

350% of FPL" (55). Therefore, programs like the ACA are needed to support families' ability to access nutritionally dense foods or access supplements such as multi-vitamins.

Vitamin B12 is commonly found in animal products such as meat, fish, poultry, milk and eggs. Unlike potassium and folate, the larger percentage of vitamin B12 intake in this study (>90%) was from supplementation. Our review of available over-the-counter dietary supplements indicated that amounts of vitamin B12 contained in many products does often exceed the RDA. This may explain the findings that intake of vitamin B12 was over 2800% RDA before and after the ACA. Our study also found many Americans already consume the RDA of B12 from food alone (Table 2).

One of the main effects of the ACA was Medicaid expansion. In 2015, 29 states had expanded eligibility for their Medicaid programs, per the ACA initiatives, resulting in a 13% increase in Medicaid enrollment. Moreover, Medicaid and its affiliated program, the State Children's Health Insurance Program (SCHIP), designed to provide insurance coverage for children whose families earn too much to qualify for Medicaid, expanded coverage by increasing the eligibility for pregnant and new mothers from 133% Federal Poverty Level to 138% (56). This resulted in decreases for the uninsurance rate for new mothers from 20.2 to 11.3% (2011, 2015), respectively, (57). This is consistent with the observed increase in the use of folate, mainly for pregnant people, and/or a multivitamin regimen as covered by Medicaid.

As food access issues in vulnerable communities continues to be a pervasive problem that is difficult to address, improving access to multivitamins or other nutrition supplements can be an alternative approach to ensuring appropriate nutrient intake for at-risk populations (58). However, shortages of primary care providers in these communities may impact the ability of individuals to receive nutrition counseling and gain access to prescription nutrient supplementation. In a 2017 report by the New England Journal of Medicine, Miller and Wherry reported that while Medicaid expansion increase coverage and access to care, post implementation, it was also associated with longer wait time for appointments (59). Further research is needed to determine the overall effectiveness of supplement use in this population to help achieve optimal nutrient intake, improve overall health, and reduce chronic disease, particularly in vulnerable populations where access to care and adequate nutrition remains an issue. This finding also highlights an opportunity for increasing access to nutrition intervention and counseling in community settings, especially in specific patient populations that stand to benefit even more from these interventions.

Our study has several limitations related to its cross-sectional design and the inherent biases associated with the use of patient self-reported data. Because the NHANES uses respondent panels that are updated every 2 years, we could not track the actual changes in individual behavior from prior to after the ACA. Instead, we used cross-sectional and longitudinal survey weights to extrapolate the results obtained from the two study cohorts to the US population. Furthermore, individual self-reported data on nutrient intake, primary care utilization and comorbid conditions could not be validated with direct tests and observations. Hence, we were limited in our ability to examine the role of access to healthcare services as a mediator of theassociation with ACA coverage expansion and the use of nutrition supplementation.

5 Conclusion

To our knowledge, this is the first study that examined the association of an expansion in a national health insurance on nutritional status of general population, with the special emphasis on the populations at greater risk for nutritional deficiencies – low-income individuals and patients with chronic conditions. Our findings suggest that the ACA expansion had a positive association with the population nutritional intake, mainly due to the increase in the use of dietary supplements but not food. Future studies should evaluate the long-term impact of the individuals ACA components (e.g., prevention programs, Medicaid expansion, prohibiting the denial of coverage to individuals with pre-existing conditions, among others) on the nutrient intake of the American population. More research is needed to inform accurate and culturally appropriate dissemination of public health messaging about the health benefits of adequate intake of key nutrients from food.

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

AS, TT, KM, and KN: study conceptualization and design. HK, AS, JN, and KN: data curation and formal analysis. HK, AS, TT, and KN: writing – original draft. KN, KM, and SM: funding acquisition. HK, AS, KN, PD, KM, and SM: writing – review & editing. All authors approved the final version of the manuscript.

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Conflict of interest

PD, KM, and SM were employed by Pharmavite.

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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Egg consumption and growth in children: a meta-analysis of interventional trials

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Introduction: Stunting and wasting are prevalent in low- and middle-income countries, putting children at risk for disease and disability. Eggs are a nutrient-rich food that can potentially facilitate growth.

Purpose: The aim of this meta-analysis was to evaluate the potential beneficial effect of egg supplementation on growth in children.

Methods: Following the PRISMA guidelines, PubMed and Healthline (Ovid) were systematically searched for interventional studies on egg supplementation for growth in children aged 6 months to 18 years, with no restrictions on date. Studies were evaluated for quality using Cochrane's GRADE technique. Data were pooled and reported as means and 95% confidence intervals.

Results: Seven studies reporting on 9 unique interventions in 3,575 male and female participants were included in the meta-analysis. Participants in the intervention groups experienced significantly greater increases in height/length (by 0.47 [0.13, 0.80] cm, $p < 0.01$) and weight (by 0.07 [0.01, 0.13] kg, $p = 0.03$) when compared to those in the control groups.

Conclusion: Eggs are an affordable, nutritious option for improving growth in children, though more studies with longer interventions are warranted.

Systematic review registration: PROSPERO (CRD42021289609: https://www.crd.york.ac.uk/prospero/).

KEYWORDS

stunting, wasting, children, growth, egg, supplement

Introduction

In low- and middle-income countries, stunting and wasting in children remain among the most serious and prevalent consequences of poor nutrition (1). Stunting in children 0–59 months of age can be moderate or severe—defined as a height-for-age Z-score (2) that is more than 2 and 3 standard deviations (3), respectively, below the median from the World Health Organization standards—and both forms have major health consequences for those affected (1). Stunting is associated with chronic undernutrition and exposure to diarrheal disease which exacerbates nutrient losses, and can lead to neonatal death, increased susceptibility to infectious disease, and poor motor and cognitive development (4, 5). Cognitive impairment related to stunting may persist in adolescence and adulthood, limiting academic attainment and eventually impeding economic productivity and societal progress (6).

While the causes of stunting are multifaceted and may begin *in utero,* diet quality during complementary feeding and childhood is a potential contributor (7–9). Lack of dietary diversity and under-consumption of animal source foods are associated with decreased linear growth in young children, and failure to rectify undernutrition is negatively associated with catch-up growth in older children and early adolescence (9–11). Current interventions for stunting both during the critical period of early growth and the later window for potential catch-up growth rely heavily on fortified foods and nutritional supplements, and very few have evaluated potential available and affordable whole food alternatives (12). Eggs—an animal source food rich in protein, essential fatty acids, choline, vitamin A, and vitamin B12 (13)—can improve the diet quality of children (14) and potentially facilitate growth, as has been demonstrated in both observational and experimental studies (15). For example, a deficiency of choline negatively impacts lean body mass growth in infants (14, 16, 17), whereas intake of high-quality animal protein is associated with increased growth trajectory in both weight and height (18). Moreover, a large observational study in the United States found that delayed egg introduction during infancy is associated with a lower mean height-for-age Z-score and a higher risk of stunting in 6-year-old children (19).

There is an increasing interest in evaluating the effect of locally available food sources for nutrition in children. Eggs, in particular, are relatively affordable compared to other animal source foods, and homestead egg production is a feasible option for both improving the diet of the whole family and providing a secondary source of income (13). However, at present, there are few interventional trials of egg consumption and linear growth in children of any age, with inconsistent results between studies. Leveraging the increased power available through meta-analysis, we aimed to evaluate the treatment effects of pediatric egg consumption on linear growth outcomes across the currently published interventional trials.

Methods

This review was conducted following the Preferred Reporting Items for Systematic Review and Meta-Analysis checklist (20). The protocol has been pre-registered with the PROSPERO database (registration number: CRD42021289609).

Data sources and searches

To identify interventional trials investigating the effect of egg consumption on growth in children, we systematically searched the databases PubMed and Healthline (Ovid) on 5 November 2021. We used the following search terms with no restrictions on language, date, type of study, or place of publication: "egg" AND "children" or "child" AND "growth" or "height" or "length" or "weight." Terms were chosen to ensure results would focus on absolute changes in height and weight, and not in shifts in the prevalence of stunting or wasting or in children's status from stunted or wasted to non-stunted or non-wasted, respectively. Additional studies were sought by cross-referencing the bibliography of the initially identified studies and through referral by involved contributors to other relevant literature.

Eligibility criteria

We included all interventional trials (randomized or not) in which provision of eggs alone, or eggs together with another food or supplement, was the primary intervention, and only if there was no secondary, behavioral intervention (e.g., a sanitation intervention, or dietary counseling) in conjunction with the primary, that would make it difficult to parse the sole effect of the dietary intervention. Studies were eligible if they included participants from the age of 6 months through the age of 18 years to capture children from the earliest period of complementary feeding, through the end of the late catch-up growth period of adolescence, with the intention to separate out results for young (<2 years) and older (>2 years). Studies were included if they reported outcomes of height (or length) and weight. Excluded studies were those that were observational and associational rather than experimental in nature, not written in the English language, and/or if they were secondary analyses of already included studies. Additionally, we excluded studies on egg-shell consumption, as we were primarily interested in the effect of the egg itself. We also excluded studies where increased egg consumption was a by-product of a community-based egg production intervention where egg intake was not the primary exposure and growth was not the primary outcome of interest.

Data extraction and quality assessment

We created a data extraction tool in which the following information was retrieved from identified studies: first author and year, study design, participant information (including sample size, age, and location), methodology (including intervention duration, dose, and frequency), outcomes measured, and growth-specific results (mean ± SD). Studies were evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) technique, and the overall evidence for each outcome was given a GRADE rating of either high, moderate, low, or very low. Risk of bias was assessed using the RoB-2 tool available through Cochrane's ReviewManager (RevMan) software. We evaluated the risk of bias along the following domains: Random Sequence Generation (selection bias), Allocation Concealment (selection bias), Blinding of Participants and Personnel (performance bias), Blinding of Outcome Assessment (detection bias), Incomplete Outcome Data (attrition bias), Selective Reporting (reporting bias), and Other Biases.

Statistical analysis

The continuous primary outcomes assessed were the change in height/length from baseline and the change in weight from baseline. Data for the analysis (means and SDs) were extracted from each article's

Abbreviations: CI, Confidence interval; HAZ, Height-for-age Z-score; GRADE, Grading of recommendations assessment, development and evaluation; PRISMA, Preferred reporting items for systematic review and meta-analysis; RCT, Randomized controlled trial; RevMan, Review manager; SD, Standard deviation; UHT, Ultra-high temperature.

text, tables, and figures. When the standard error was reported in the published paper, it was converted to SD by multiplying by the square root of the sample size. When none of these data were available in the published paper, they were obtained by contacting the authors of the primary studies. We pooled data using Cochrane's RevMan software and R statistical software for the subgroup analyses. Continuous outcomes were analyzed in RevMan and R using the mean difference with 95% confidence interval (12), and statistical significance was set at $p \leq 0.05$.

We evaluated statistical heterogeneity using Tau-squared, I-squared, and Chi-squared measures of heterogeneity. For our purposes, an I-squared of <25% and >75% signified low level and high level of heterogeneity, respectively. An I-squared between 25% and 75% was considered to be of moderate heterogeneity. If $p > 0.05$, it was assumed that no significant heterogeneity existed. A random-effects model was used for all analyses.

Additionally, a meta-regression subgroup analysis was performed by age, excluding the two interventions conducted in children over the age of 2 years. A third analysis was performed (growth rate-adjusted analysis), normalizing the changes in growth for the duration of the intervention of each study.

Results

Study identification and retrieval

Database searches through PubMed and Healthline (Ovid) resulted in 696 unique articles after removing duplicates; the PRISMA flow diagram is shown in Figure 1. An additional article was found by searching clinicaltrials.gov, resulting in a total of 697 articles to be reviewed. The titles and abstracts of these 697 unique articles were then reviewed by one member of the research team (EAL), resulting in 15 trials for full-text review. This process was confirmed by a second member of the research team (KSBL). EAL then reviewed the full text of these 15 trials, and KSBL confirmed any decisions. Any disagreements were resolved by a third member of the research team. Later in this process, one additional study (21) was identified during an oral presentation at a scientific conference (ICN Tokyo, December 2022), and results from the final published paper were included. This resulted in 7 articles and 9 unique interventions meeting the inclusion criteria (Figure 1).

Characteristics of included studies

Six of the 7 studies were randomized controlled trials (RCTs; Table 1). The one non-randomized intervention study used a comparator group from an observational cohort with participants who had similar characteristics (24). In total, 3,575 male and female participants were included. While Baum et al. (22) and Suta et al. (21) did not report stunting and wasting at baseline, roughly 30% of the children in the study by Bierut et al. (23) were stunted and fewer than 5% were wasted at the start of the intervention. In the study by Iannotti et al. (12), baseline stunting prevalence was 38%, and baseline underweight prevalence was 4% in the control group and 10% in the intervention group. In Mahfuz et al. (24), children were only included if they had a length-to-age Z-score < 1, and 47% in both the intervention and comparator groups had stunting (baseline rates of wasting were not reported). The baseline prevalence rates of stunting, underweight, and wasting in the study by Stewart et al. (7) were 14, 8, and 1%, respectively. In Zhao et al. (25), prevalence of stunting and wasting at baseline was not reported. The age of participants across all included studies ranged from 6 months to 14 years. Egg-alone interventions ranged from 1 to 2 eggs daily with an intervention duration of 6–8 months. Egg-plus interventions varied greatly and

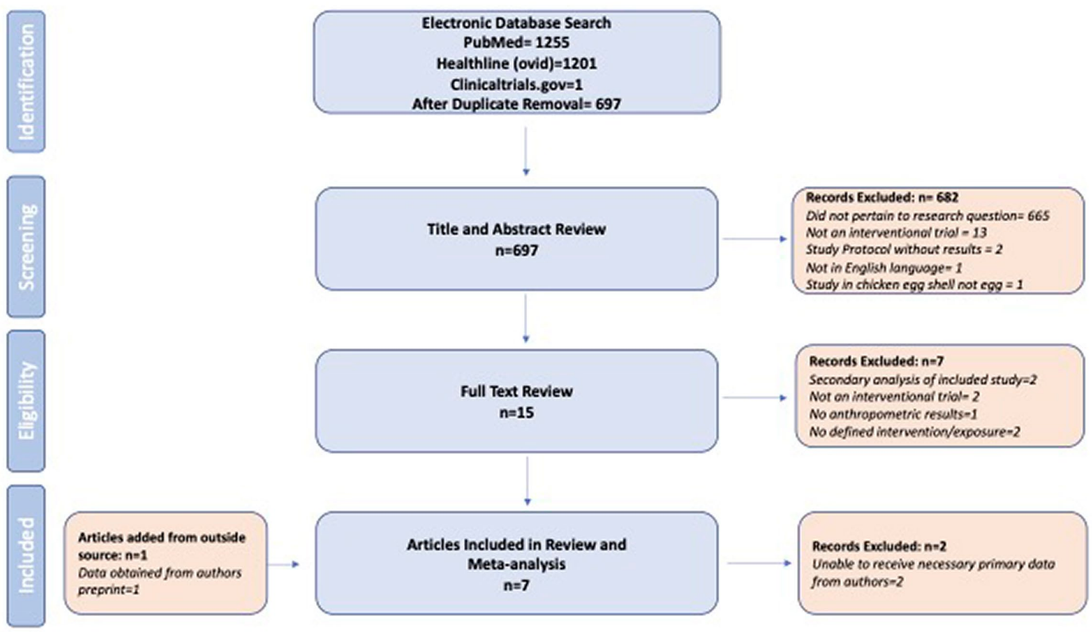

FIGURE 1
Identification and screening of included studies.

TABLE 1 Characteristics of included studies.

Author	Design	Country	Participants	*n*	Age	Arms	Duration
Baum et al. (22)	RCT	Uganda	Males and females	241	6–9 years	1 egg or 2 egg supplement vs. no supplement	5 times per week for 6 months
Bierut et al. (23)	RCT	Malawi	Males and females	275	9 months	Bovine colostrum (5.7 g) and dried whole egg powder (4.3 g) vs. isoenergetic amounts of unfortified corn/soy flour (15 g)	2 times per day for 3 months
Iannotti et al. (12)	RCT	Ecuador	Males and females	163	6–9 months	1 egg supplement vs. no supplement	1 time per day for 6 months
Mahfuz et al. (24)	Non-randomized intervention	Bangladesh	Males and females	646	12–18 months	1 boiled egg, 150 mL UHT milk, and 1 sachet micronutrient powder vs. no supplement	6 days per week for 60 days
Stewart et al. (7)	RCT	Malawi	Males and females	660	6–9 months	1 egg supplement vs. no supplement	1 time per day for 6 months
Suta et al. (21)	Cluster randomized trial	Thailand	Males and Females	791	8–14 years	Whole egg v. yolk free supplement vs. no supplement	10 per week for 35 weeks
Zhao et al. (25)	RCT	China	Males and females	955	6–13 years	200 g milk and 50 g braised egg vs. usual diet	1 time per day for 2 years

RCT, randomized controlled trial; UHT, ultra-high temperature.

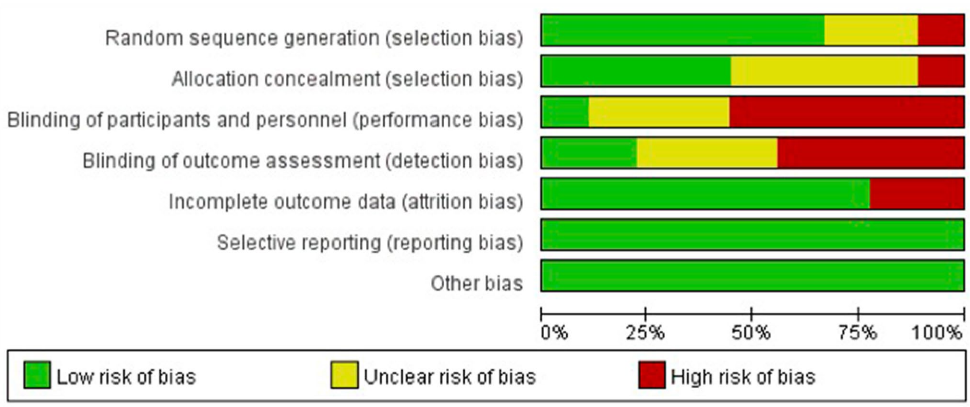

FIGURE 2
Risk of bias assessment.

included vitamin A, milk, micronutrient sachets, and bovine colostrum in addition to egg. These interventions ranged in duration from 2 months to 2 years. All of the included studies were conducted in low- and middle-income countries.

Quality assessment and risk of bias

The quality of the included studies as assessed by the GRADE criteria demonstrated high certainty of the evidence for the outcomes of both weight and height/length. The risk of bias of the included studies is shown in Figure 2. One study had a high risk of selection bias due to the non-randomized design (24). Four studies had high risk of performance bias as the participants were not blinded to the intervention, and three of the studies did not report whether participants and/or personnel were blinded, resulting in unclear risk of bias (7, 12, 21, 22, 24, 25). Another study had a high (25%) dropout rate overall, which also seemed to be different among the intervention arms (22). In the two-egg group, there were no dropouts; in the one-egg group, 7 participants did not complete the study (9%), and in the no-egg control group the number of participants who did not complete the study was 52 (52%) (22).

Effect of egg consumption on change in height/length

A meta-analysis of the 9 interventions (across the 7 studies) revealed that children in the egg supplementation groups had a greater increase in height when compared to those in the control groups by 0.47 cm (95% CI: 0.13, 0.80 cm, $p < 0.01$; Figure 3). Two comparisons (22, 25) favored the control group, but these differences were not statistically significant. There was high level of heterogeneity across interventions ($p < 0.00001$; $I^2 = 89.0\%$; Figure 3).

A subgroup analysis was also performed, according to the age of the participants In the included studies: one with participants over the age of 2 years (4 studies), and one with participants under the age of 2 years (4 studies). When analyzed by subgroup, there was no significant treatment effect in the older age group (*p* = *0.28*), but there was a significant benefit of egg supplementation in the younger age group by 0.43 cm (95% CI: 0.09, 0.77 cm, *p* = *0.01*). Both subgroup analyses were characterized by substantial heterogeneity (Figure 4).

Additionally, a third analysis was conducted on the rate of change, to account for any differences in intervention duration across studies. Rate of change for each study was calculated as: mean difference in outcome/duration of intervention. The results of this analysis for height were marginally beneficial in favor of egg supplementation (Figure 5).

Effect of egg consumption on change in body weight

The meta-analysis of the 9 interventions demonstrated that children in the egg supplementation groups had a greater increase in weight when compared to those in the control groups by 0.07 kg (95% CI: 0.01, 0.13 kg, *p* = *0.006*; Figure 6). Three comparisons (21, 22, 25) were in favor of the control group, but results were not statistically significant (Figure 6). There was moderate heterogeneity across interventions (*p* = *0.02*; I^2 = 57.0%).

A subgroup analysis by age was also performed. Body weight was not significantly affected by egg supplementation in children older than 2 years of age (*p* = *0.70*), but in the younger age group (≤2 years old), there was a significant beneficial effect by 0.23 kg (95% CI: 0.06, 0.40 kg, *p* < *0.01*). There was moderate heterogeneity in both subgroup analyses (Figure 7).

In a rate-adjusted analysis for weight, there were no significant differences between groups (Figure 8).

Discussion

This meta-analysis is the first to evaluate the effect of egg consumption, alone or as part of a multi-component supplementation, on growth in children. Overall, improved growth in terms of both weight and height was observed in the treatment groups when compared to the control groups. The overall benefit was an additional 0.43 cm in height and an additional 0.07 kg in weight. Furthermore, these beneficial changes were more pronounced in children younger than 2 years of age as opposed to those older than 2 years. These findings are consistent with the observation that earlier introduction of eggs during infancy is associated with better growth indicators in children 1–6 years old (19), and suggest that supplementation with eggs can potentially improve the nutritional status of young children in low- and middle-income countries. Similarly, they suggest an urgency for early intervention, as the results were less supportive of interventions after 2 years of age.

While the overall results from our study reveal a statistically significant beneficial effect on both children's height and weight with egg supplementation, it is doubtful if these gains will be significant clinically, particularly given the rapid growth that occurs in children under the age of 2 years. From ages 6–12 months, the rate of growth for infants in high-income settings is about 1 cm per month, or 6 cm over the course of 6 months (26). A difference of 0.4 cm, therefore, equates to an extra 2 weeks of linear growth over a period of about 6 months, which may not be significant clinically. Similarly, weight increased by 0.07 kg in the treatment group compared to the control group, whereas the average infant is expected to grow by about 0.1 kg per week in late infancy (26). However, there is evidence that the whole population of young children (≤ 3 years old) in low- and middle-income countries experiences faltering growth relative to the international standard; therefore, increases even of a small magnitude may have clinical relevance despite not meeting the international standards (27). For these reasons, it is possible that egg supplementation would yield clinically relevant benefits in low- and middle-income settings, and particularly for children with more compromised nutritional status.

Even more so, when these effects are compared to other meta-analyses of nutrition supplementation for growth in children, their clinical relevance becomes clearer. Two other meta-analyses—one on milk and milk product supplementation (for 3 to 24 months) in children aged 6–18 years, and another one on milk protein supplementation (for 1 week to 1 year) in children aged 9 months to 12 years—found that supplementation resulted in increased weight gains of 0.48 kg (95% CI: 0.19, 0.76 kg; *p* = *0.001*) and 0.42 kg (95% CI: 0.23, 0.61 kg; *p* < *0.001*), respectively (28, 29). However, only the milk

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Baum, J. I. 1egg 2017	2.476	0.681	89	2.68	0.92	56	14.3%	-0.20 [-0.48, 0.08]
Baum, J. I. 2eggs 2017	3.385	1.551	96	2.68	0.92	56	13.0%	0.70 [0.31, 1.10]
Bierut, T. 2021	3.4	1.3	137	2.9	1.3	138	14.0%	0.50 [0.19, 0.81]
Iannotti, L.L. 2017	9.31	2	80	7.22	1.62	83	10.9%	2.09 [1.53, 2.65]
Mahfuz, M. 2020	3.58	0.91	472	3.41	0.94	174	15.3%	0.17 [0.01, 0.33]
Stewart, C.P. 2019	6.98	1.28	331	6.82	1.31	329	15.0%	0.16 [-0.04, 0.36]
Suta, S. whole egg 2023	6.9	13.8	238	3.4	13.5	197	1.5%	3.50 [0.93, 6.07]
Suta, S. yolk-free egg 2023	3.7	13.6	200	3.4	13.5	197	1.4%	0.30 [-2.37, 2.97]
Zhao, R. 2021	6.18	1.91	538	6.19	1.88	417	14.6%	-0.01 [-0.25, 0.23]
Total (95% CI)			**2181**			**1647**	**100.0%**	**0.47 [0.13, 0.80]**

Heterogeneity: Tau² = 0.18; Chi² = 71.16, df = 8 (P < 0.00001); I² = 89%
Test for overall effect: Z = 2.75 (P = 0.006)

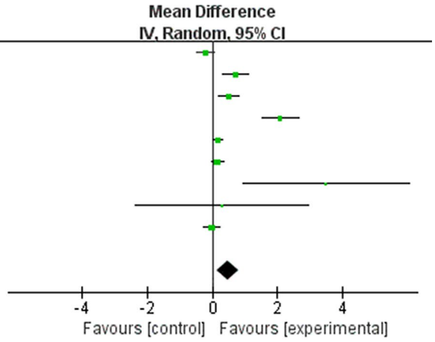

FIGURE 3
Effect of egg supplementation on height (cm).

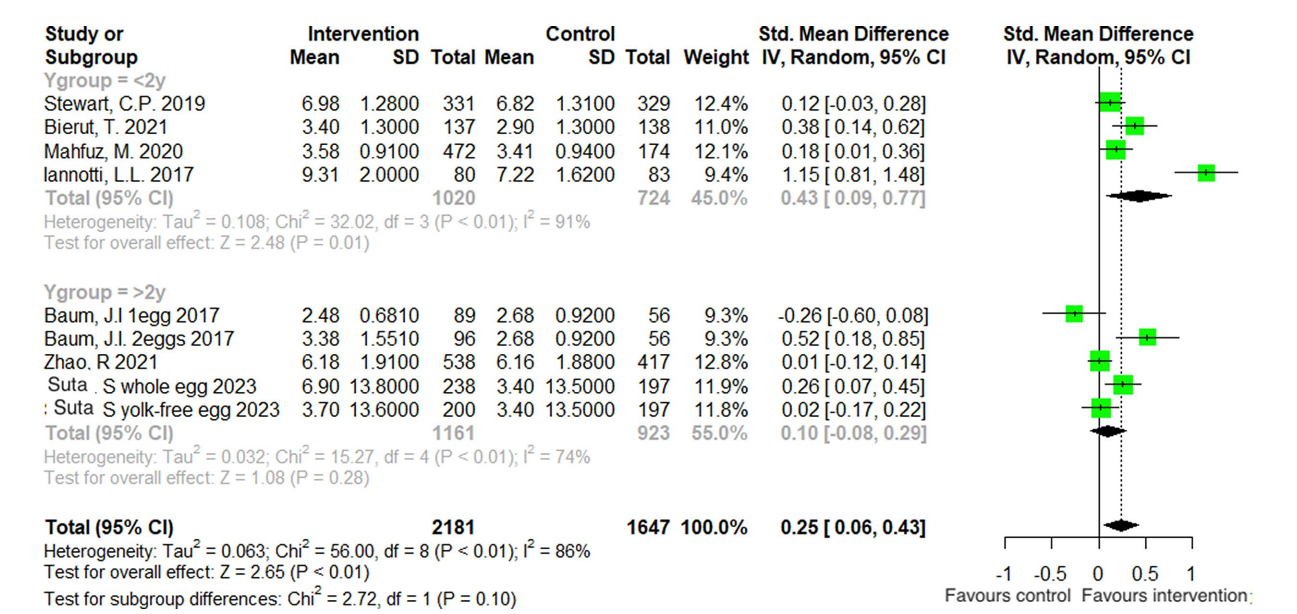

FIGURE 4
Effect of egg supplementation on height (cm)—analysis stratified by age.

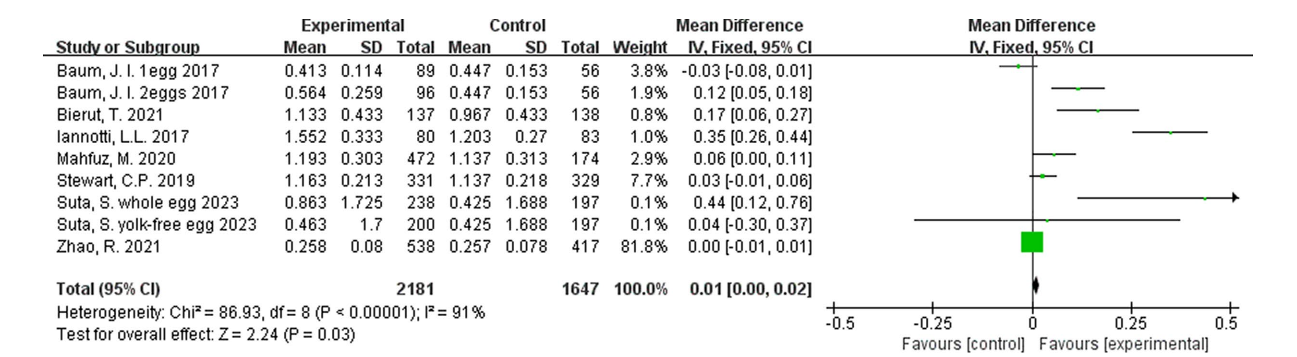

FIGURE 5
Effect of egg supplementation on height (cm/month)—analysis adjusted for intervention duration.

protein supplement produced significant results for height/length, with the treatment group benefitting from a 0.42 cm additional increase when compared to the control group (29). Importantly, the duration of supplementation (28), the age of children (28) and their nutritional status at baseline (29) were associated with the observed benefits in response to the intervention. In this sense, although the egg-associated gains found in our meta-analysis were modest, they were seen along both the axes of weight and height/length, perhaps indicating that eggs may offer better long-term nutrition and health outcomes for young children.

However, barriers remain for egg consumption by children in low- and middle-income countries. Availability and cost may play a role, as do cultural norms around food, such as which family members are prioritized to receive available food (30). While small-scale production efforts will likely not shift general population intakes, integrated education about chicken farming and nutrition has been shown to increase egg consumption at the community level, and may be a feasible approach to improving diet and growth outcomes in children (31, 32). It is also crucial that nutrition education and outreach not end at the conclusion of the study period—as evidenced by the growth faltering during follow-up after the active intervention in Ecuador (12).

There were some limitations to this work. Despite the affordability, accessibility, and efficacy of eggs as a nutrition supplement, experimental studies on their use in children in clinical trial settings are few. Furthermore, there is high heterogeneity among trials, particularly pertaining to results for height. To minimize this problem, subgroup analyses stratified by age were conducted but heterogeneity remained high. Importantly, all current research on the topic has been conducted in low- and middle-income countries. These regions often have the burden both of malnutrition and disease and infection also impacting growth, making it difficult to generalize results to higher income countries. It is conceivable that the sole presence of research conducted in low- and middle-income countries is due to publication bias, as areas with more stunting and wasting have a lower baseline

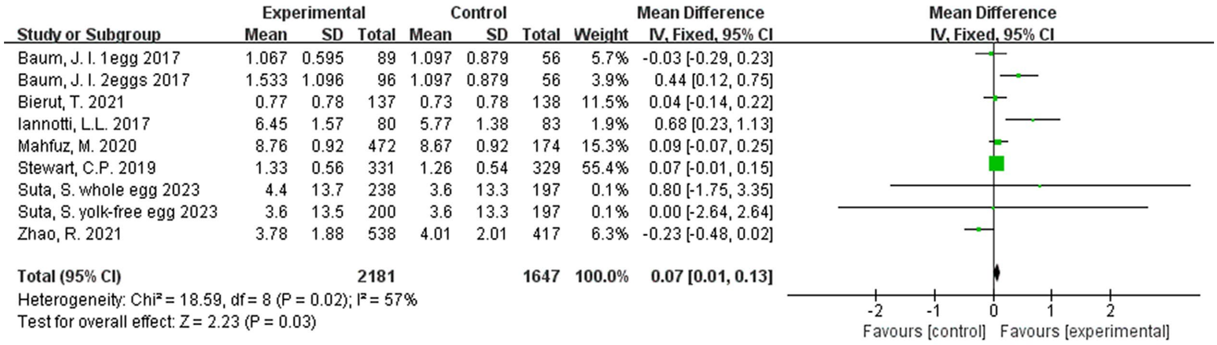

FIGURE 6
Effect of egg supplementation on weight (kg).

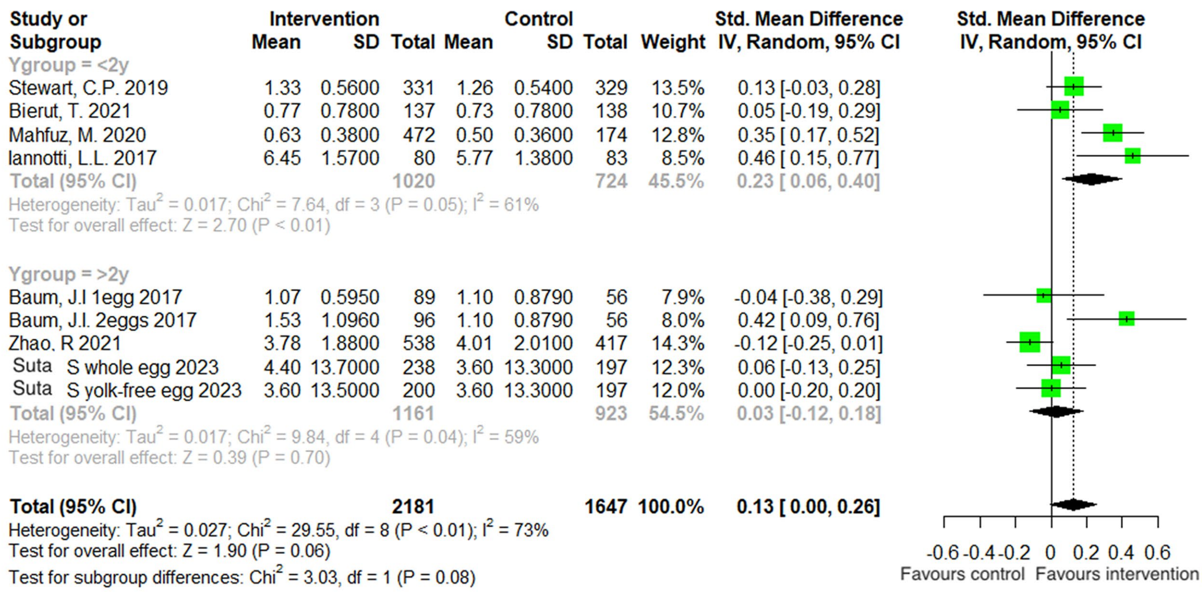

FIGURE 7
Effect of egg supplementation on weight (kg)—analysis stratified by age.

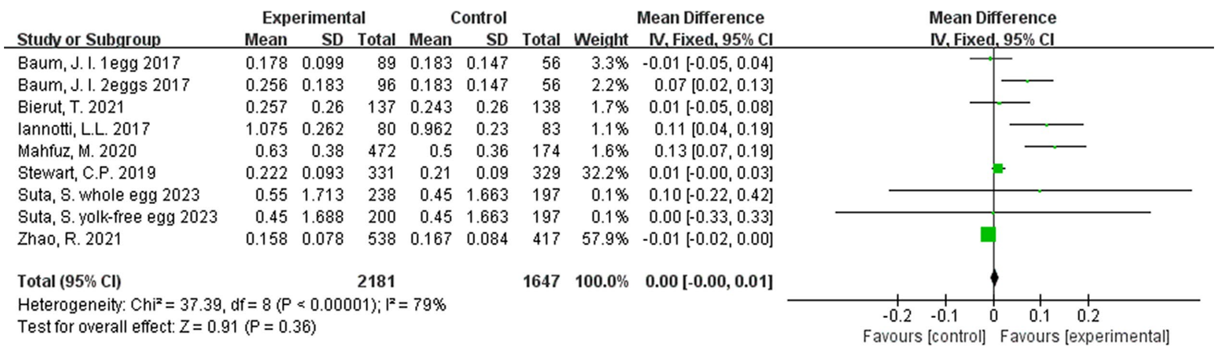

FIGURE 8
Effect of egg supplementation on weight (kg/month)—analysis adjusted for intervention duration.

from which to improve and are therefore more likely to generate significant results (29). Furthermore, it is hard to attribute the observed benefits to eggs *per se*, particularly since not all the interventions evaluated eggs alone; some evaluated eggs together with other supplements. In addition, some studies compared the active intervention against a control group that received an iso-energetic supplement (23), while most compared the active intervention against a control group that did not receive anything (7, 12, 22). It is thus possible that some of the beneficial effects of egg supplementation may have been due to the relative energy surplus. Likewise, total dietary energy intake was not reported in all studies and thus the lack of an effect of egg supplementation in some studies may have been masked by an overall dietary energy deficit and negative energy balance. Lastly, a limitation of our statistical analysis was that the control group in two studies (21, 22) was counted twice—once in comparison with the one-egg group, and once in comparison with the two-eggs group.

Conclusion

This meta-analysis found a modest benefit to children's growth by supplementing their diet with eggs—whether alone or together with other dietary components—for a period of 2–8 months. Whether longer periods of supplementation, or supplementation exclusively in those with stunting or wasting, will produce greater and clinically more meaningful effects remains to be examined in future studies. Larger RCTs evaluating egg consumption alone over longer periods of time are needed to precisely determine the effects of eggs on growth in children. Given that eggs are an easily accessible food in many low- and middle-income countries that can be locally produced, results of such an intervention could provide families with a realistic and manageable nutrition goal for their children.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: datasets are owned by the authors of the original articles included in the meta-analysis, and may be requested from them. Requests to access these datasets should be directed to eal254@cornell.edu.

Author contributions

EL: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. ZZ: Data curation, Formal analysis, Software, Visualization, Writing – review & editing. KB-L: Project administration, Validation, Writing – review & editing. FM: Conceptualization, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

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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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Healthy dietary practices and its' associated factors among adults of Nekemte dwellers, Oromia State, Western Ethiopia

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Background and purposes: Appropriate healthy dietary practices are essential for well-being. Adopting of healthy lifestyle remains challenging worldwide. Ethiopia has an unacceptably high burden of malnutrition like other least developed countries. However, healthy dietary practices and their associated factors were not conducted in Nekemte town. Hence, the study was designed to assess healthy dietary practices and associated factors among middle-aged adults in Nekemte town from January 15 to February 30, 2019.

Methods: A community-based cross-sectional study design was applied in Nekemte town. Primary data were gathered using a questionnaire from 266 adults and checked for normality. In both bivariate and multivariate logistic regression analyses the association and significance were determined at $P < 0.05$.

Results: The Magnitude of dieting practice was 73.31% (unhealthy) and 26.69% (healthy), respectively. Being low-income households ($P = 0.001$), not married ($p = 0.001$), had a daily meal frequency [AOR: 1.91, 95% CI: (1.04, 2.71), and had poor knowledge of healthy diet AOR: 3.87, 95% CI: (3.23, 5.65)] were associated with unhealthy diets.

Conclusion: The researchers identified unhealthy diet practices were widespread in the study samples of Nekemte populations. Hereafter, community-based lifestyle and Nutrition education through intensive participation of community leaders is highly recommended.

KEYWORDS

health diet practice, prevalence, associated factors, adulthood, Nekemte

1 Introduction

A healthy diet can be defined as a pattern of food intake that has beneficial effects on health or at least no harmful effects. Unhealthy diets connote countries with scarce resources and from the four major risk factors of non-communicable diseases, unhealthy diet has been strongly associated with these diseases (1, 2). Many studies have shown that unhealthy diet not only increases the risk of metabolic syndrome, but also the potential risk factor for diseases like osteoporosis (3).

People follow dietary practices for different reasons at different times, such as: malnutrition prevention globally, usual dieting, holidays, celebrations, and out-catering, and they are defined as amalgamations of foods (4). This food may be a healthy diet or an unhealthy diet that has a positive or negative impact on the human body. Poor diet practices are habitual in low-income countries, and of the four major risk factors for non-communicable diseases, an unhealthy diet has been related to those illnesses (1, 2). Different researchers argued that this scenario is not only risky for metabolic syndrome development but also a potential risk factor for diseases like osteoporosis (3).

Whatever the recommended healthy diet intake is there, it is still under discussion in several cases. Only 42.56% of the countries in the world have their own dietary guidelines in world (5). Likewise, Ethiopia is at the initial stage of formulating the policy.

Evidence-based studies show that globally, people use the dietary diversity score to measure and indicate a good proxy of dietary quality (6–8). it is known that people in low- and middle-income countries typically eat fewer food groups than their staple foods, resulting in a low dietary diversity score (6). This is an arithmetical indicator of poor diet quality (6, 9).

Regarding food items, about 75% of the Ethiopian diet is cereal-based monotonous feed (7, 10, 11). Other studies reveal that the prevalence of low and medium dietary diversity scores among Ethiopian populations was 60% and 40%, respectively (12, 13).

Non-communicable Diseases (NCDs) are rapidly increasing globally and emerging radically in East Africa among adults (14, 15). Diet adequacies were formulated for different age groups of adults, but findings identified that many adults still do not follow a healthy diet. It is noted that there has been no such investigation regarding the topic based on adults in west Ethiopia of a specific age. Thus, the researchers are interested in investigating the prevalence of healthy diet practices and associated factors among middle-aged adults in Nekemte Town from January 15 to February 30, 2019.

2 Methodology

2.1 Narration of the study area

This study was conducted in the Oromia Region, western Ethiopia, at the hub of western Ethiopian Towns (Nekemte Town) to predict study populations' healthy diet practices and their associated factors. The study is located 328 kilometers west of Addis Ababa.

2.2 Study design and period

A descriptive epidemiological study design, typically a community-based cross-sectional study was conducted to determine the status of dietary practices and their predictors among adults from January 15, 2019, to February 30, 2019.

2.3 Participants

All middle-aged (41–64 years) adults in Nekemte Town were selected as samples and adults unfit for selection criteria were not eligible currently for the research.

2.4 Sample size determinations

The sample size was determined by using the formula [n = [(Za/2)2*P (1−P)]/d2]. By considering the following assumptions: Za/2 = 1.96 at 95% confidence interval, a margin of error of 5%, and the most common prevalent is the component of metabolic syndrome among apparently healthy Ethiopian adults (with a proportion of 19.6% of central obesity) (16) which is; the final sample size was 266.

2.5 Sampling techniques

A probability sampling design was implemented for study participants. From six communities (administratively small sub-cities; locally termed Ganda or Kebele), one community was randomly selected by Systematic random sampling technique and the other one purposively. To ensure the relevance of the data, a third of the kebele must be selected. Additionally, another community was assigned that is not adjacent to the former Ganda or Kebele but has a similar socio-economic status. A simple random sampling method was applied to select study participants.

2.6 Data collection instruments

The data collection tool used well-structured Food Frequency Questionnaires. The FANTA and FAO (17, 18) a 7-day food-frequency questionnaire was used to assess dietary diversity score with twelve food groups. Questions contain socio-demographic and health diet mocks. The questionnaire can be implemented at the household or individual level, according to the purpose of the study. The HDDS indicator provides a glimpse of a household's ability to access food as well as its socioeconomic status. Guiding Framework, Retrieved October 21, 2017, Method of Construction, the following 12 food groups are used to calculate the HDDS indicator: A. Cereals B. Root and tubers C. Vegetables D. Fruits E. Meat, poultry, offal F. Eggs G. Fish and seafood H. Pulses/legumes/nuts I. Milk and milk products J. Oil/fats K. Sugar/honey L. Miscellaneous; Each food group is assigned a score of 1 (if consumed) or 0 (if not consumed). The household score will range from 0–12 and is equal to the total number of food groups consumed by the household: Sum (A + to + L). The average household dietary diversity score for the population of study can be calculated as follows: Sum (HDDS)/Total number of households surveyed (5, 17, 18). Trained data collectors (five BSc Nurses and two MSc/MPH in Nutrition), including researchers, collected the information sequentially.

2.7 Reliability and validity test

The respondents were requested for their time prior to the main study or beta test. According to Mugenda and Mugenda (19), the reliability pre-test sample size can be between 1% and 10% of the total sample. Thus, 5% of the total sample was used as a pilot study to ensure reliability. A pilot test was carried out to evaluate the completeness, precision, accuracy, and clarity of the questionnaires; this ensured the reliability of the data collection instruments used (19). After the amendment of the final questionnaire, the researcher explained the purpose of the research and sought permission from the institution to carry out the actual research. The final questionnaires were distributed to the respondents with the help of research assistants. This enhanced the speed of data collection. Each completed questionnaire was treated as a unique case and a sequential number was given to each. Filling out the questionnaire took approximately 10 min. Prior to running the regression model, the existence of homoscedasticity, multi-collinearity, and normality assumptions was checked. A multivariate logistic model was used to isolate independent predictors of healthy dietary practices. The collected data was edited and entered into the Statistical Package for the Social Sciences (SPSS version 24) software to enable the carrying out of the analysis.

2.8 Data processing and analysis

The developed instrument for dietary practices was used to assess it (20). To assess dietary intake, 24-h multi-step recall was performed with FAO methodology, at least one food group in 7 days of a week. The collected data points were cascaded based on standards. The data were first checked for completeness and consistency, and cleaned for outliers and missing values. Adults' mean dietary score from information gathered from respondents. Prior to running the regression model, the existence of homoscedasticity, multi-collinearity, and normality assumptions was checked. The researcher tested the model using the variance inflation factor (VIF). The predictors should be free of multicollinearity problems, most studies argue that if the mean VIF is less than 10, the model has no problem with multicollinearity.

The data was described using IBM software, (SPSS version 24). The findings were presented with frequency, percentage, and descriptive summaries used to explain the number of study participants in the analysis. Multivariable logistic regression analysis was performed to calculate the association between risk factors and significance level at a *P*-value of 0.05.

2.9 Ethical review and consent form

To conduct the study, ethical consideration was approved and taken from the Food and Nutrition Research Institute at Jimma University, Institutional Review Board (IRB) of the Institute of Health (Reference Number: IHRPGY/596/2019).

Prior to starting the study, an informed consent form was taken from the study participants. For illiterate respondents, the questionnaires were translated into their native language to understand the purpose.

3 Results

3.1 Subject characteristics

Of the 266 samples completed for the gender distribution, the majority of the respondents (186, or 69.93%) had unhealthy diet practices, and 62.78% were female. Among male participants, 71 (71.72%) had unhealthy diet practices. Findings revealed that 146 (54.89%) of the participants had a low income of 1.25 USD/44.45 ETB, and 209 (78.57) adopted unhealthy dietary habits. Similar to nearly three-fourths of participants, 187 (70.30%) of the adults were illiterate, and of the total illiterate participants, 134 (71.66%) had an unhealthy diet (**Table 1**).

3.1.1 The prevalence of healthy dietary practices

The overall prevalence of dietary practices assessed using dietary diversity score, for sure, indicated that 195 (73.31%) and 71 (26.69%) of respondents adopted unhealthy and healthy diets, respectively (**Figure 1**).

3.2 Factors associated with dietary foods

On bivariate logistic regression analysis: marital status, education, meal frequency, and income of participants demonstrate a relationship with the adoption of healthy foods among middle-aged Nekemte populations. Adults having low-income households, illiteracy, and meal frequency less than 3 times per day were significantly associated with unhealthy diet practices [AOR: 1.59, 95% CI: (1.37, 3.21), AOR: 3.20, 95% CI: (2.04, 5.98), AOR: 1.91,

TABLE 1 Socio-demographic of participants with distribution dietary practices, Nekemte (*n* = 266).

Variable	Categories	Dietary practices, n (%)	
		Unhealthy	Healthy
Sex	Female	115(68.86)	52(31.14)
	Male	71(71.72)	28(28.28)
	Total	186(69.93)	80(30.07)
Age group in years	41–48	119(82.10)	26(17.90)
	49–56	52(67.53)	25(32.47)
	57–64	29(65.90)	15(34.10)
	Total	200(75.19)	66(24.81)
Income			
(USD/person/day)	<1.25 USD	110(75.34)	36(24.66)
	>1.25 USD	99(82.50)	21(17.50)
	Total	209(78.57)	57(21.43)
Marital status	Not married	69(78.41)	19(21.59)
	Married	127(71.35)	51(28.65)
	Total	196(73.68)	70(26.32)
Educational status	Literate	51(64.56)	28(35.44)
	Illiterate	134(71.66)	53(28.34)
	Total	185(69.55)	81(30.45)

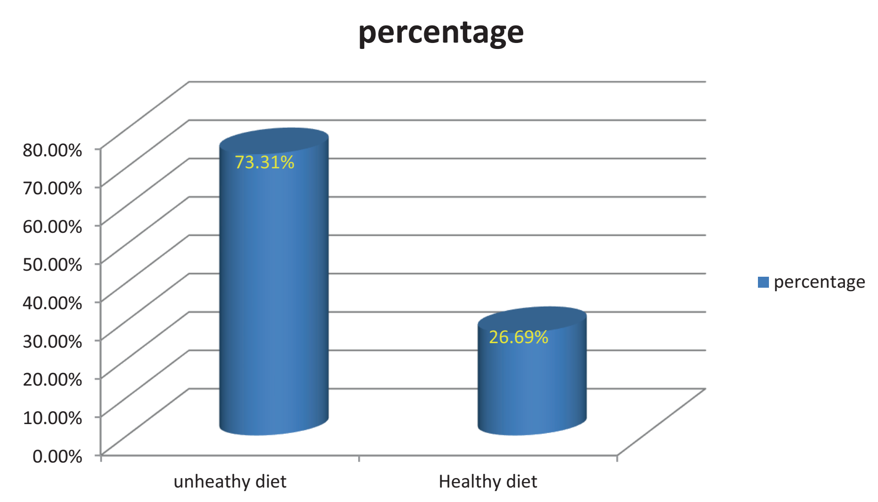

FIGURE 1
The prevalence of healthy diet consumed by respondents in Nekemte town (*n* = 266), 2019.

95% CI: (1.04, 2.71), respectively]. The odds of having an unhealthy diet were almost three times (AOR = 3.87, 95% CI: 3.23, 5.65) higher for adults with poor knowledge of healthy diet compared to participants who did have that knowledge (Table 2).

4 Discussions

This community-based study found that 73.31% of middle-aged adults adopted an unhealthy diet, which indicates a high prevalence. Similarly, 183 (68.80%) of the adults had three or fewer meal frequency consumption patterns per day.

According to Darmon and Drewnowski (21), the findings postulated that individuals with lower socio-economic status adapt and adopt unhealthy diets when compared to those with a higher one among adults in Australia (21). Likewise, the current study revealed that the healthy diet of participants was significantly associated (*p* 0.001) with the daily income of adults. This research outcome was also confirmed by studies done on Dietary intakes among US adults (22) and in Australia (23). Also, a study in the UK agreed that participants from households reporting lower financial or food security (since the start of the COVID-19 pandemic in the UK in February 2020) had poorer diets in some respects than participants from other households (24).

TABLE 2 A multivariate logistic analysis findings of factors associated with a healthy diet (*n* = 266), 2019.

		Diet practice				
Variables	Categories	Unhealthy	Healthy	COR	AOR(95% CI)	*P*-Value
Sex	Female	115	52	0.87(0.25,0.97)	0.54(0.48, 0.87)	0.868
	Male	71	28	1	1	1
Income						
USD/person/day	<1.25	110	36	0.65(0.39,0.86)	1.59(1.37, 3.21)	0.001**
	>1.25	99	21	1	1	1
Education	Illiterate	134	53	1.39(1.05,2.97)	1.20(1.04,5.98)	0.047*
	Literate	51	28	1	1	1
Marital status	Unmarried	69	19	1.46(1.13,4.85)	0.59(0.52, 0.98)	0.001**
	Married	127	51	1	1	1
Urban farming	Yes	5	17	0.07(0.05,0.76)	0.40(0.32, 0.58)	0.576
	No	203	45	1	1	1
Meal frequency	<3/day	148	35	22.19(19.25,26.97)	1.91(1.04,2.71)	0.000*
	≥3/day	13	68	1	1	1
Knowledge on a healthy diet	Poor	123	44	4.68(1.89,7.57)	3.87(3.23,5.65)	0.001**
	Good	37	62	1	1	1

Significances considered at $^{*}p < 0.05$, $^{**}p \leq 0.001$.

Having poor knowledge of healthy diets was strongly associated with having an unhealthy diet [AOR: 3.87, 95% CI: (3.23, 5.65)]. Similar to this finding, having good perceptions and valuable knowledge regarding the healthy diet concept is critically necessary for allowing people to make the "right life" choices. Another systematic review indeed suggests that nutrition knowledge is one of the factors that are most consistently related to a healthy diet (25).

Healthy food access is significant for improving population health (26). However; we found that many populations adopt unhealthy diets, which are highly prevalent at the study site. Independent variables showed a significant relationship with dependent ones among adults. Finally, this research shows that, in addition to confounding, the distortion of the association between diet and risk factors cannot be generalized unless entire populations adapt and adopt healthy diets.

This study has many strengths, but it also comes with different limitations. The study was limited by a smaller sample size, biophysical and biomarker characteristics of respondents were not considered. Besides that, cross-sectional studies have limitations. This study plays a crucial role in policy reviews, putting direction for implementers' work on awareness creation for the adoption of a healthy diet and food security issues that boldly need great attention to measure the food quantity consumed with frequency. Besides, this study revealed that adult diet throughout life is masked, so future research perspectives will study inculcating rural and urban populations in a nationwide context using the evidence.

5 Conclusion

This research revealed that the prevalence of unhealthy diet practices was high (73.31%). And, majority of the participants had < 3 times the average meal frequency in a single day, and predicting variables were also associated with a healthy diet. The WHO recommends that people should eat a combination of different foods, including staple foods, legumes, vegetables, fruit, and animal source foods. On the contrary, almost all adults living in Nekemte Town practice cereal-based monotonous food. In recommendations, awareness creation about the adoption of a healthy diet, and food security issues we boldly need to pay attention to measuring the food quantity consumed with frequency. Besides, future research needs to study inculcating rural and urban populations nationwide.

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

To conduct the study, ethical consideration was approved and taken from the Food and Nutrition Research Institute at Jimma University, Institutional Review Board (IRB) of the Institute of Health (Reference Number: IHRPGY/596/2019). Prior to starting the study, an informed consent form was taken from the study participants. For illiterate respondents, the questionnaires were translated into their native language to understand the purpose.

Author contributions

AA: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing—original draft, Writing—review and editing. DT: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review and editing. TB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review and editing.

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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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Tea intake and total body bone mineral density of all ages: a Mendelian randomization analysis

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Background: There is increasing evidence indicating that tea intake affects bone mineral density levels; however, the causality between tea intake and bone mineral density is inconclusive. This study aimed to assess the causal relationship between tea intake and total body bone mineral density (TB-BMD) through two-sample Mendelian randomization (MR) analysis.

Methods: We conducted a two-sample MR approach to estimate the potential causal effects of tea intake on TB-BMD at all ages in a European population. The analyses were performed using summary statistics obtained for single-nucleotide polymorphisms (SNPs), identified from a genome-wide association meta-analysis of tea intake (N = up to 447,485 individuals) and from the GEnetic Factors for OSteoporosis (GEFOS) Consortium's genome-wide association meta-analysis (N = up to 56,284 individuals), with baseline data collected in 2018 and populations derived from the European ancestry. The association between each SNP and TB-BMD was weighted by its association with tea intake, and estimates were combined mainly using an inverse-variance weighted meta-analysis. In addition, we explored the potential causal effects between green tea intake, herbal tea intake, and TB-BMD.

Results: The MR analysis revealed that genetically determined tea intake exerts a causal impact on TB-BMD, with an odds ratio (OR) of 1.204 (95% CI: 1.062–1.366, $p = 0.004$), especially in the age group of 45–60 years (OR = 1.360, 95% CI: 1.088–1.700, $p = 0.007$). No horizontal pleiotropy and heterogeneity were observed. However, there was no causal effect of tea intake on TB-BMD in the age groups of 0–15, 15–30, 30–45, and over 60 years. In the subgroup analysis, when green tea intake was regarded as the exposure factor, no salient associations were found between green tea consumption and TB-BMD (IVW $p = 0.368$). Similarly, there was also no causal association between herbal tea intake and TB-BMD (IVW $p = 0.264$).

Conclusion: The findings of this study support the evidence that tea consumption increases bone density and reduces the risk of osteoporosis in the age group of 45–60 years within the European population.

KEYWORDS

tea intake, total body bone mineral density (TB-BMD), causality, Mendelian randomization (MR) analysis, genome-wide association study (GWAS)

Introduction

Bone mineral density (BMD) serves not only as an indicator of bone strength but also as a crucial measure for assessing osteoporosis. With the global population aging, the risk of osteoporosis is on the rise each year, particularly among the elderly and postmenopausal women. This has emerged as a significant public health concern, leading to an increased societal and economic burden (1). Osteoporosis exhibits a strong correlation with gender and age, influenced by factors such as race, height, body mass index, and unhealthy lifestyle choices (such as smoking, drinking, and coffee consumption) (2–6). It is noteworthy that the relationship between tea consumption and bone mineral density, and its potential role in osteoporosis, has been a topic often misunderstood. Previously, there was a misconception that drinking tea could lead to calcium loss and subsequently contribute to osteoporosis. This belief stems from the idea that caffeine in tea might hinder calcium absorption in the digestive tract and increase calcium excretion through urine (7–9). Additionally, the oxalates present in tea were thought to bind with calcium ions, resulting in a gradual loss of calcium from bones, thereby elevating the risk of fractures (10). Previous animal experiments and clinical studies showed a positive correlation between caffeine intake, particularly from coffee, and calcium loss, increasing the risk of osteoporosis and bone fractures (11–13). However, it is crucial to note that tea, unlike coffee, contains a more complex composition beyond just caffeine, and its impact on bone density may differ. Recently, an expanding body of observational research indicates that tea consumption does not contribute to calcium loss or a reduction in bone density (14, 15). Several studies have highlighted the potential benefits of tea in effectively enhancing bone density and preventing osteoporosis. However, it is challenging to establish conclusive evidence based solely on traditional observational studies. The causal relationship between tea intake and its impact on bone density remains unclear.

Mendelian randomization (MR) serves as an invaluable epidemiological tool, leveraging genetic variations linked to exposure factors to investigate the relationships between these genetic variants and outcomes, such as disease occurrence or mortality. Its core principle involves using genetic data as a means to effectively probe causal connections between a specific exposure and a particular outcome. Consequently, associations uncovered through MR are less prone to reverse causation and are less likely to be influenced by confounding factors. Moreover, MR, to a certain extent, addresses the limitations inherent in traditional randomized controlled trials (RCTs) and observational studies. In this study, a two-sample MR analysis was performed to explore the potential causal impact of tea intake on TB-BMD across all age groups, utilizing genetic data sourced from the GWAS database.

Methods

Study design and data source

In the MR analysis, it is crucial that the SNPs utilized as instrumental variables (IVs) satisfy three indispensable criteria. First, they must exhibit a strong association with the exposure variable (tea intake). Second, the selected SNPs should be independent of potential confounding factors. Finally, the instrumental variables should exert influence on the outcomes (total body bone mineral density, TB-BMD) solely through exposure without operating through alternative pathways (Figure 1).

We extracted reliable data sources based on the most comprehensive GWAS to explore the causal correlation between exposure and outcomes. Since all data were opened previously, and corresponding ethical review and informed consent had been obtained, we no longer needed any additional ethical approval.

Specifically, summary data pertaining to tea intake and TB-BMD across all age groups were sourced from the IEU OpenGWAS project.[1] The data on tea intake was sourced from the United Kingdom Biobank (MRC-IEU), comprising a substantial sample size of 447,485 participants. Notably, the United Kingdom Biobank constitutes a cohort study encompassing individuals aged 40–69 years in the United Kingdom (16). Furthermore, the data on green tea and herbal tea intake were also extracted from the Medical Research Council Integrative Epidemiology Unit (MRC-IEU). The TB-BMD dataset, focusing on TB-BMD, was derived from a meta-analysis encompassing 30 genome-wide association studies (GWASs) and a total of 56,284 samples. These samples span a diverse age range, including 11,807 individuals aged 0–15 years, 4,180 individuals aged 15–30 years, 10,062 individuals aged 30–45 years, 18,805 individuals aged 45–60 years, and 22,504 individuals aged over 60 years (17) (Table 1). The TB-BMD serves as a reliable metric for evaluating osteoporosis and predicting fractures. To identify participants with osteopenia or osteoporosis, BMD *t*-scores were employed, adhering to the criteria set by the World Health Organization (18). Osteopenia was defined by a *t*-score ranging from −1 to −2.5, while osteoporosis was characterized by a *t*-score falling below −2.5 (18). Typically, dual-energy X-ray absorptiometry (DXA, Hologic Inc, Waltham, MA) was utilized to measure the total body bone density in grams per square centimeter (g/cm^2). However, for pediatric individuals aged 0–15 years, the measurement method involves total body less head (TBLH) (17). In an effort to mitigate potential biases and minimize confounding factors, all subjects in this study belonged to the European ancestry group.

Selection and validation of instrumental variables

An essential step was taken to meticulously select eligible IVs for tea intake based on the GWAS datasets. Specifically, the chosen single-nucleotide polymorphisms (SNPs) were required not only to exhibit a robust association with tea intake at a genome-wide significant level of $p < 5 \times 10^{-8}$ but also to undergo a clumping process, preventing biased results due to linkage disequilibrium (LD). The presence of SNPs in linkage equilibrium was detailed in the study conducted by Wu et al. (19). The criteria for the clumping procedure were $R^2 = 0.001$ and window size = 10,000 kb. Moreover, we employed the PhenoScanner database[2] to comprehensively scan for genetic variants linked to potential confounding factors. To address potential bias stemming from weak IVs, the F-statistic formula was employed for SNP selection, allowing the identification of SNPs with robust

1 https://gwas.mrcieu.ac.uk/

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

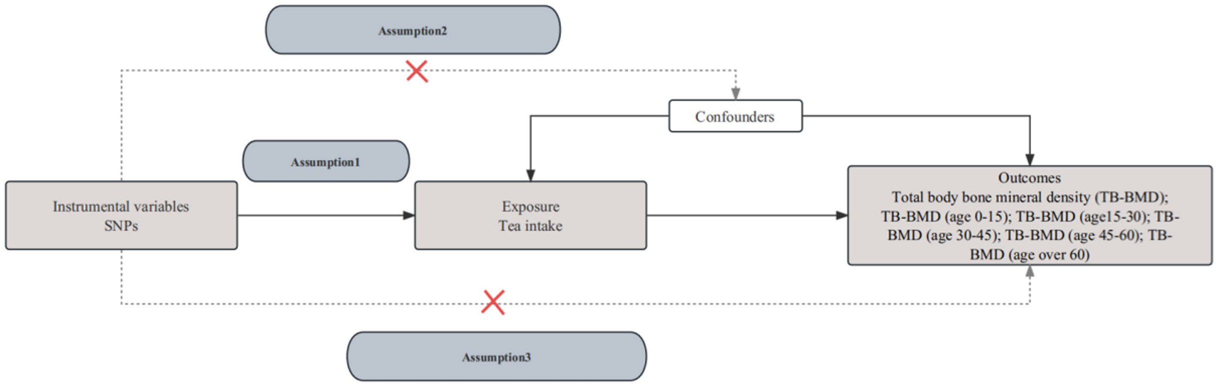

FIGURE 1
The flowchart of the Mendelian randomization assumptions.

TABLE 1 Summary of GWAS in this study.

Exposure or outcomes	Consortia	Sample size	Number of SNPs	Ancestry	Year
Tea intake	MRC-IEU	447,485	9,851,867	European	2018
Green tea intake	MRC-IEU	64,949	9,851,867	European	2018
Herbal tea intake	MRC-IEU	64,949	9,851,867	European	2018
Total body bone mineral density	GEFOS	56,284	16,162,733	European	2018
Total body bone mineral density (age 0–15)	GEFOS	11,807	9,351,693	European	2018
Total body bone mineral density (age 15–30)	GEFOS	4,180	8,509,502	European	2018
Total body bone mineral density (age 30–45)	GEFOS	10,062	9,656,698	European	2018
Total body bone mineral density (age 45–60)	GEFOS	18,805	10,304,110	European	2018
Total body bone mineral density (age over 60)	GEFOS	22,504	11,932,096	European	2018

GWAS, Genome-wide association study; SNP, Single-nucleotide polymorphism; and GEFOS, Genetic factors for osteoporosis.

statistical power (20, 21). Subsequently, we extracted outcome data associated with the retained SNPs. Finally, to ensure the consistency of effect alleles between the exposure and outcome datasets, we harmonized the datasets by excluding palindromic and ambiguous SNPs with non-concordant alleles.

Statistical analysis

The causal relationship between genetically predicted tea intake and TB-BMD was assessed through a two-sample MR analysis employing the Two-Sample MR package (22, 23). Various methods, such as MR-Egger, weighted median, inverse-variance weighted (IVW), simple mode, and weighted mode, were employed in the MR analysis to assess the relationship between tea consumption and TB-BMD across all ages. The IVW method, widely adopted in MR analysis, offers both fixed-effects and random-effects versions. As a meta-analytical technique, IVW combines the Wald estimates of causal effects for individual IVs, thereby providing comprehensive effect estimates of the exposure's impact on the outcome (24). To enhance the robustness of the results, both IVW and MR-Egger assessments were utilized to evaluate the presence of heterogeneity. Heterogeneity was examined to discern variations among the IVs using Cochran's Q statistic. A significance level of $p < 0.05$ indicated heterogeneity, prompting the application of the random-effects model for subsequent analyses; otherwise, the fixed-effects model was employed (25–27). Furthermore, the MR-Egger regression approach and the MR pleiotropy residual sum and outlier (MR-PRESSO) method were employed to identify and address pleiotropy effects (28). In the MR-Egger regression test, a significance level of $p < 0.05$ indicated the presence of pleiotropy (29), while in the MR-PRESSO test, outliers were initially identified, followed by horizontal multiple-effects outlier correction. The subsequent assessment determined whether there was a significant difference in causal effects after removing the outliers (27). To further ensure the reliability of the analysis, a "leave-one-out" sensitivity analysis was conducted to explore the potential impact of individual SNPs on introducing bias and influencing the overall causal effect (19).

All analyses were carried out using two-sample MR (22) and MR-PRESSO (30) packages in software R (version 4.3.1).

Results

After excluding SNPs with LD associated with tea intake based on parameters r^2 and kb, conducting a search for surrogate SNPs, and retrieving SNPs linked to potential confounders through the PhenoScanner database, we identified 41 qualified SNPs selected as IVs for tea intake. Subsequently, we aligned the outcome data with the exposure SNPs, selecting corresponding SNPs associated with the exposure SNPs as IVs for further MR analyses (Supplementary Tables S1–S7). In our study, all IVs exhibited F-statistics exceeding 10, indicating minimal potential bias from weak IVs.

Figure 2 illustrates the MR outcomes investigating the causal connection between genetically predicted tea intake and TB-BMD across all age groups (0–15, 15–30, 30–45, 45–60, and over 60 years) using different methods. The IVW method, as the primary analytical approach, revealed a robust causal relationship between genetically predicted tea intake and TB-BMD (OR 1.204, 95% CI 1.062–1.366, $p = 0.004$), especially in the age group of 45–60 years (OR 1.360, 95% CI 1.088–1.700, $p = 0.007$). However, no causal relationship was observed between tea intake and TB-BMD in the age groups of 0–15, 15–30, 30–45, and 60 years and above. The MR-Egger, weighted mode, simple mode, and weighted median approaches consistently demonstrated concordant outcomes.

Significant heterogeneity was not observed between tea intake and TB-BMD ($p > 0.05$), and this lack of heterogeneity persisted within the age group of 45–60 years. The IVW and MR-Egger methods were employed to check for heterogeneity, and Table 2 presents Cochran's Q and p values, respectively. The MR-Egger regression analysis did not reveal any evident directional pleiotropy (intercept = 0.003, $p = 0.347$; Figure 3), including among individuals aged 45–60 years (intercept = 0.001, $p = 0.831$) (Supplementary Figure S2). Moreover, the leave-one-out method analyzed single SNP risk, indicating that the association between tea intake and TB-BMD (including the age group 45–60) was not driven by any single SNP (Supplementary Figures S1, S2). The single SNP risk evaluation did not change significantly, reinforcing the robustness of the MR analysis. Additionally, the forest plot and the funnel plot are displayed in Supplementary Figures S1, S2. However, no causality was identified between tea consumption and TB-BMD across different age groups (0–15, 15–30, 30–45, and over 60 years) (Table 2; Supplementary Figures S3–S6).

Furthermore, Tables 3, 4 present the MR results for the causal relationship between green tea consumption and TM-BMD, and herbal tea consumption and TB-BMD, respectively. The findings consistently indicate the absence of a causal relationship between green tea or herbal tea consumption and TB-BMD.

Discussion

We conducted comprehensive two-sample MR research to explore the causal effect of tea intake on BMD using GWAS summary data. All results showed that individuals with a genetic inclination toward consuming higher amounts of tea had a higher likelihood of experiencing increased BMD (OR = 1.204, $p < 0.05$). The findings remained consistent and robust even after conducting a thorough series of sensitivity analyses. Our research explored the causal relationship between tea consumption and bone density, proving that drinking tea does not affect calcium absorption or cause osteoporosis. On the contrary, it increases bone density and reduces the risk of osteoporosis, debunking the misconception that "drinking tea leads to osteoporosis."

As predicted, our findings substantiated the results from several previous population-based observational studies and prospective studies, presenting that drinking tea contributes to the prevention of osteoporosis and an increase in BMD. Research on the relationship between tea consumption and bone density has yielded controversial findings. In 2017, Zhang et al. conducted a meta-analysis, revealing that tea consumption might enhance bone density and mitigate bone loss [odds ratio (OR): 0.66; 95% confidence interval (CI), 0.47–0.94; $p = 0.02$], particularly in areas such as the lumbar spine, hips, femoral neck, femoral trochanter, and femoral greater trochanter (all p values <0.05), indicating its potential in osteoporosis prevention (31). A 10-year tracking study in Sweden involving over 30,000 individuals suggested that consuming fewer than or up to four cups of tea daily exhibited no significant correlation with osteoporotic fractures among women (32). Another study assessed postmenopausal women in two Asian developing countries (Iran and India) for osteoporosis risk factors, indicating that consuming seven or more cups of tea per day was a significant protective factor against decreased bone density, ultimately reducing the risk of fractures (OR: 0.3; CI: 0.1–0.5) (33). Furthermore, research conducted by scholars at Texas Tech University in the United States proposed that long-term green tea consumption not only refrains from diminishing bone density but also may actually contribute to its increase (34).

These studies collectively point out that tea, due to its antioxidative, anti-inflammatory, bone-forming, and bone-resorption-inhibiting effects, can regulate bone metabolism, thereby preventing the onset of osteoporosis. This is particularly true for the main components of tea, the catechin compounds. After menopause, women experience a sharp decline in estrogen levels, which makes them susceptible to osteoporosis fractures. However, polyphenolic substances in tea can modulate skeletal health by exerting estrogen-like activities, enhancing osteoblast activity, and suppressing osteoclast-mediated bone resorption (35). According to Korean scholars, an investigation of data from 3,530 postmenopausal women between 2008 and 2011 indicated that women who consumed 1–3 cups of green tea daily exhibited significantly lower rates of osteopenia and osteoporosis compared to those who either did not consume green tea or consumed less than one cup daily (OR 1.81 and 1.85, 95% CI, 1.20–2.71; and 1.23–2.77) (36). Moreover, a 5-year prospective study involving 1,027 elderly women aged 7,085 years in Western Australia demonstrated that tea drinkers had a 2.8% higher total hip bone density compared to non-tea drinkers ($p < 0.05$) (37).

Based on the previous relevant reports overseas, some studies conducted in China have also confirmed the beneficial effects of tea consumption on increasing bone density and reducing the risk of fractures. A prospective study involving 453,625 adults found that tea drinkers had a significantly lower risk of fractures compared to non-tea drinkers (HR: 0.88; 95% CI: 0.83, 0.93) (38). Professor Huang further discovered that tea consumption was a significant independent predictor of bone density ($\beta = 0.068$, $p < 0.05$) (39), particularly among postmenopausal women.

Methods	SE	OR (95%CI)	N	P
TB−BMD				
MR Egger	0.147	1.062 (0.796, 1.416)	40	0.685
Weighted median	0.092	1.179 (0.984, 1.412)	40	0.074
IVW	0.064	1.204 (1.062, 1.366)	40	0.004
Simple mode	0.179	1.329 (0.937, 1.886)	40	0.119
Weighted mode	0.099	1.183 (0.975, 1.436)	40	0.097
TB−BMD (age 0−15)				
MR Egger	0.303	0.836 (0.462, 1.514)	41	0.558
Weighted median	0.187	1.114 (0.771, 1.608)	41	0.565
IVW	0.131	1.204 (0.931, 1.556)	41	0.157
Simple mode	0.335	0.968 (0.502, 1.868)	41	0.923
Weighted mode	0.215	1.089 (0.714, 1.660)	41	0.694
TB−BMD (age15−30)				
MR Egger	0.566	1.155 (0.381, 3.505)	41	0.8
Weighted median	0.349	1.280 (0.646, 2.536)	41	0.479
IVW	0.234	0.993 (0.628, 1.572)	41	0.977
Simple mode	0.783	1.211 (0.261, 5.614)	41	0.808
Weighted mode	0.559	1.345 (0.449, 4.028)	41	0.599
TB−BMD (age 30−45)				
MR Egger	0.42	1.479 (0.649, 3.367)	41	0.357
Weighted median	0.222	1.470 (0.951, 2.274)	41	0.083
IVW	0.179	1.183 (0.834, 1.680)	41	0.346
Simple mode	0.407	1.538 (0.693, 3.415)	41	0.297
Weighted mode	0.28	1.657 (0.958, 2.867)	41	0.079
TB−BMD (age 45−60)				
MR Egger	0.262	1.293 (0.774, 2.162)	41	0.333
Weighted median	0.151	1.431 (1.064, 1.925)	41	0.018
IVW	0.114	1.360 (1.088, 1.700)	41	0.007
Simple mode	0.297	1.390 (0.776, 2.488)	41	0.275
Weighted mode	0.186	1.390 (0.965, 2.002)	41	0.085
TB−BMD (age over 60)				
MR Egger	0.287	1.085 (0.618, 1.904)	41	0.778
Weighted median	0.151	0.986 (0.734, 1.324)	41	0.923
IVW	0.124	0.957 (0.750, 1.220)	41	0.72
Simple mode	0.255	0.837 (0.508, 1.380)	41	0.489
Weighted mode	0.164	0.941 (0.683, 1.297)	41	0.714

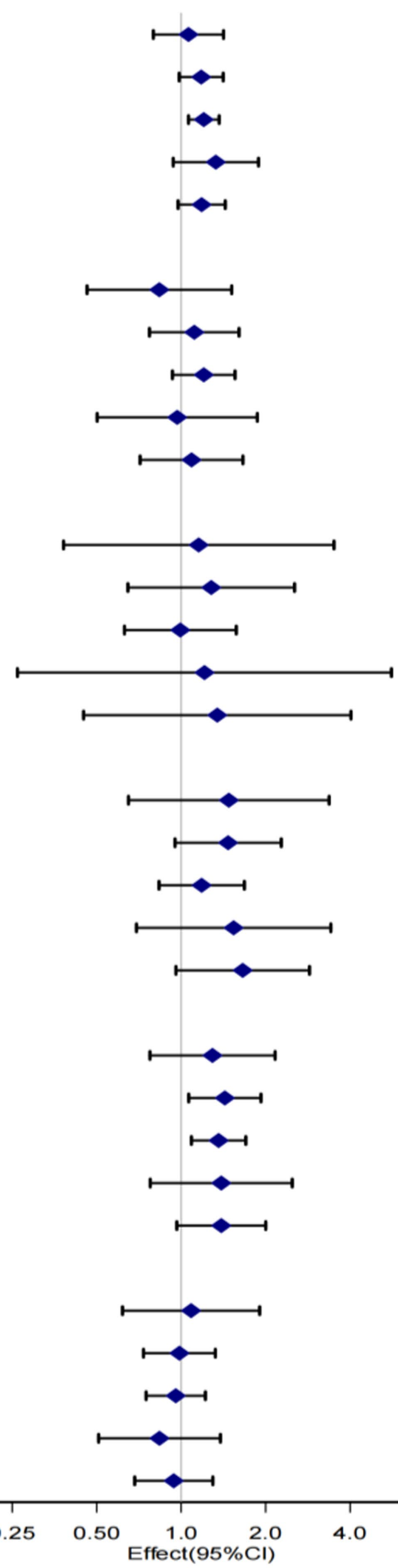

FIGURE 2
MR analysis of the causality of tea intake on TB-BMD at all ages. TB-BMD, Total body bone mineral density; IVW, Inverse-variance weighted; SNP, Single-nucleotide polymorphism; OR, Odds ratio; and CI, confidence interval.

TABLE 2 Heterogeneity test of the causal association between tea intake and TB-BMD in all ages.

Exposure	Outcome	MR-Egger		IVW	
		Cochran's Q	p	Cochran's Q	p
Tea intake	TB-BMD	47.532	0.115	48.696	0.115
Tea intake	TB-BMD (age 0–15)	37.799	0.433	39.610	0.398
Tea intake	TB-BMD (age 15–30)	41.145	0.335	41.238	0.373
Tea intake	TB-BMD (age 30–45)	54.608	0.031	55.119	0.036
Tea intake	TB-BMD (age 45–60)	44.237	0.193	44.292	0.223
Tea intake	TB-BMD (age over 60)	66.162	0.003	66.578	0.004

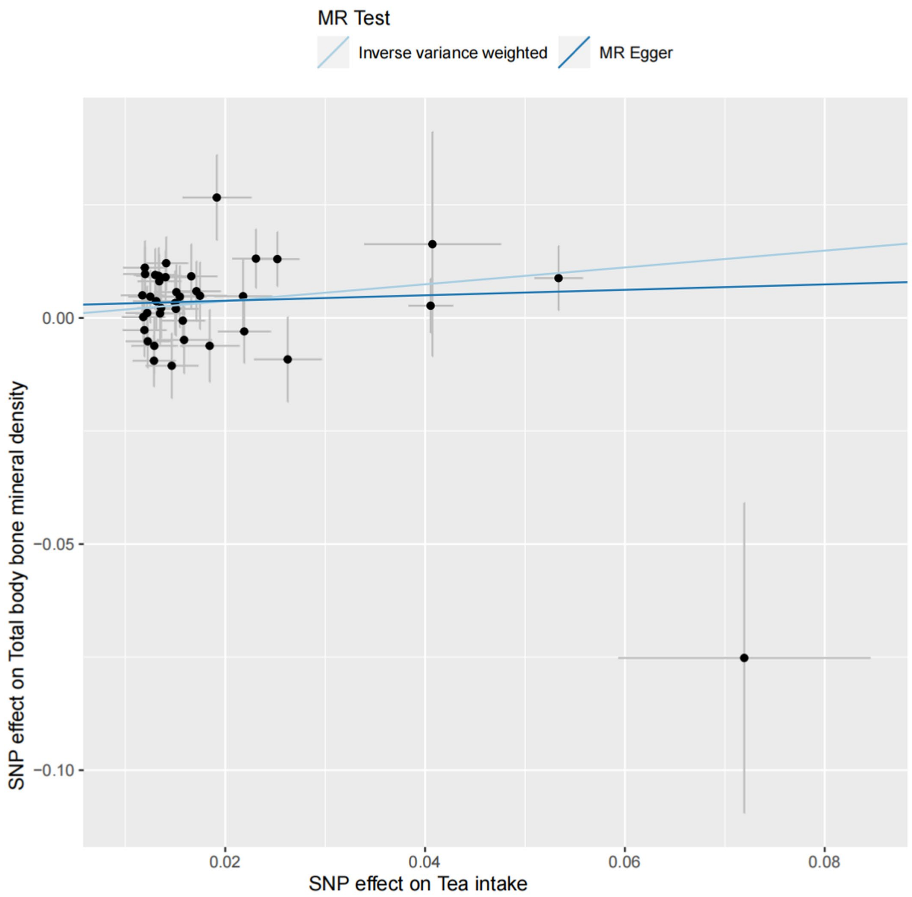

FIGURE 3
The scatter plot of the causal effect of tea intake on TB-BMD. Analyses were conducted using inverse-variance weighted and MR-Egger methods. The slope of each line corresponds to the causal estimates for two methods.

TABLE 3 MR analysis of the causality of green tea intake on TB-BMD.

Exposure	Outcome	Number of SNPs	Methods	OR (95%CI)	SE	p
Green tea intake	TB-BMD	21	MR Egger	1.000 (0.992,1.008)	0.004	0.955
			Weighted median	0.999(0.993,1.005)	0.003	0.725
			Inverse-variance weighted	0.998 (0.994,1.002)	0.002	0.368
			Simple mode	1.001 (0.991,1.011)	0.005	0.811
			Weighted mode	1.001 (0.992,1.010)	0.005	0.891

TB-BMD, Total body bone mineral density; SNP, Single-nucleotide polymorphism; OR, Odds ratio; and CI, Confidence interval.

TABLE 4 MR analysis of the causality of herbal tea intake on TB-BMD.

Exposure	Outcome	Number of SNPs	Methods	OR (95%CI)	SE	p
Herbal tea intake	TB-BMD	19	MR Egger	1.002 (0.980,1.025)	0.011	0.837
			Weighted median	0.998 (0.992,1.004)	0.003	0.496
			Inverse-variance weighted	0.998 (0.993,1.002)	0.002	0.264
			Simple mode	0.996 (0.985,1.007)	0.006	0.481
			Weighted mode	0.997 (0.987,1.007)	0.005	0.562

TB-BMD, Total body bone mineral density; SNP, Single-nucleotide polymorphism; OR, Odds ratio; and CI, Confidence interval.

Long-term moderate tea drinking was found to be beneficial for skeletal health in postmenopausal women. However, the effect of tea drinking on bone health in men appears to be less significant, and the quantity of tea consumed has no apparent impact on skeletal health (40). In recent years, a study conducted by a team from Zhejiang University on postmenopausal women revealed significant differences in the impact of tea consumption on bone density between pre- and post-menopausal stages. Pre-menopausal tea drinkers showed significant increases in both total and regional bone density, with a more pronounced effect observed among those who consumed tea at least four times a week (41). Additionally, an epidemiological survey demonstrated a positive correlation between tea consumption and spine bone density, but with a non-linear increase as the duration of tea drinking increased (42).

There are several strengths in conducting the MR analysis. First, MR analysis effectively reduces the potential biases such as confounding factors and reverse causality. Second, utilizing SNPs associated with exposure enhances the precision of estimating potential causal relationships. Additionally, multiple evaluation methods and rigorous sensitivity analyses ensure the robustness of the results. Moreover, the direction of causal relationships in genetic correlations is deterministic. However, our study also has some limitations. First, the outcomes of MR may be influenced by different ethnicities, as heterogeneous populations can introduce biased effect estimates. This study is limited to individuals of European ancestry, and genetic variations among different ethnicities, countries, and regions may affect causal relationships. Second, MR studies cannot completely rule out hidden and unknown confounding factors, and future research should employ novel approaches and larger sample sizes to confirm the causal relationship between tea consumption and BMD. Finally, the MR analysis only provides statistical evidence for causal associations, and potential causal relationships should be comprehensively explored in conjunction with biological mechanisms.

Conclusion

In conclusion, a genetic predisposition to tea intake was linked to increased BMD. As a result, moderate tea consumption in daily life does not warrant concern regarding calcium loss and osteoporosis. However, to validate the accuracy of these findings and to achieve a deeper understanding of the underlying pathophysiological mechanisms, further research employing advanced methods, larger GWAS datasets, and more comprehensive investigations is imperative.

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.

Author contributions

CX: Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft. YT: Investigation, Methodology, Writing – original draft. WN: Conceptualization, Writing – review & editing.

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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.2024.1289730/full#supplementary-material

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Creating healthy eating and active environments in early learning settings: protocol of the CHEERS eHealth intervention study

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Background: Early childhood educators through their daily interactions with children, play a central role in shaping young children's health behaviors. Given their influential role, early childhood educators are often targeted in interventions aiming at enhancing their nutrition and physical activity practices.

Methods: This paper presents the design of the CHEERS eHealth program to improve nutrition and physical activity practices within Early Childhood Education and Care (ECEC) centers. The study has a longitudinal quasi-experimental design with recruitment of ECECs across Alberta Canada. ECEC intervention group educators complete 12 weekly online nutrition and physical activity modules and participate in weekly communities of practice sessions to discuss practical applications within their centers. Outcome assessments are scheduled at baseline (T1), mid-point at 5 months (T2), and end of program after 10 months (T3). Outcome measures include the Creating Healthy Eating and Active Environments survey (CHEERS), Mindful Eating Questionnaire (MEQ), Canadian Behavior, Attitude and Nutrition Knowledge Survey (C-BANKS 2.0), Physical Literacy Knowledge, Attitude, Self-Efficacy, and Behavior (PLKASB-ECE), the Environment and Policy Assessment and Observation (EPAO) derived variables, and an objective measure of children's physical activity using ActiGraph GT3X accelerometers. Linear mixed model analyses will be used to evaluate the effectiveness of the intervention. Qualitative assessments comprise exit interviews and open-response questions embedded within the educational modules.

Results: Preliminary baseline data from the 2019 cohort indicate no statistically significant differences between the intervention and control groups for the primary outcome variables, except age. Educators' personal nutrition-related knowledge, attitude and behaviors were positively associated with their self-assessments of the nutrition environment and practices in ECECs. A significant correlation was observed between educators' self-reported physical activity practices and observed activity practices. The CHEERS survey Food Served subscale showed a positive correlation with the objective measures of EPAO-Foods Provided and Nutrition Policy subdomains.

Discussion: We propose that this eHealth intervention would be an effective scaling up approach to enhancing the nutrition and physical activity environments of ECECs by fostering improved nutrition and physical activity-related knowledge, attitudes, and adherence to best practices which will potentially lead to improved outcomes for children in their care.

KEYWORDS

early childhood education and care, nutrition, physical activity, online intervention, eHealth, educators, Health Promotion

1 Introduction

Early childhood experiences and the environment in which they occur, can shape and influence cognitive and behavioral outcomes in later life (1). Research supports the strong link between early childhood development and adult health (2). Lifestyle habits such as eating and physical activity habits are examples of behaviors that take root during early life stages and persist into adulthood (3, 4). Poor dietary patterns and sedentary behavior during childhood are associated with increased risk of adult obesity, type II diabetes, cardiovascular disease, dyslipidemia and hypertension (5–8). On the other hand, healthy behaviors have been shown to have protective effects (9–11). What is more, good health in early childhood positively impacts cognitive functioning and learning capacities, setting the stage for academic success and better future prospects (12, 13). The period of early childhood is thus a critical time to build foundations for healthy behaviors.

In Canada, research shows that the diet quality of children and youth is poor with suboptimal fruit and vegetable intake (14, 15). In addition, 13.8% of total energy consumption comes from free sugar including added sugars and sugars naturally present in honey, syrups, fruits juices and concentrates (16), exceeding the World Health Organization's recommended limit of 10% (17). Children's behaviors, including health behaviors, have been hypothesized to be influenced by layers of their environment (18). The microenvironment is the layer closest to the child and has the strongest and earliest influence on a child's development and behaviors (18). Early learning settings are part of this microenvironment and are considered among the first and primary settings in which health behaviors are developed and nurtured (19). These settings play a significant role in shaping children's lifelong habits related to eating and physical activity (20). Participation in early learning and childcare in Canada has witnessed a surge over the past two decades with approximately 60% of children enrolled in some form of early childhood education (21). Children attending full-time day care centers can spend up to 6 h or more per day in these facilities (22) and are consequently assumed to get a significant portion of their nutritional needs in those centers (23). Hence, early childhood settings are critical venues for fostering healthy eating and activity behaviors of young children in Canada.

Early childhood educators are an important part of early learning settings and play a major role in shaping children's health behaviors (24). Early childhood educators can influence children's eating and activity behaviors in many ways. They serve as role models, control access to food, provide opportunities for movement, and support children's self-regulation skills (24–27). Early childhood educators' personal knowledge and beliefs with regards to nutrition has been found to impact their feeding practices within care settings (28). Misconceptions and inaccuracies in personal knowledge and a lack of training can negatively impact the child care nutrition environment (28–30). On the other hand, research has shown that training opportunities for educators on evidence-based guidelines has the potential to enhance their practices leading to improved health outcomes for children (31–33). Health interventions targeting nutrition and physical activity in early learning settings are increasingly acknowledged for their effectiveness in promoting healthy behaviors early in life (34) aligning with the objectives of Sustainable Development Goal 3 which aims to ensure health and wellbeing for all (35).

The high level of reach and availability of the internet has made it a promising channel to scale up and deliver behavioral change health interventions (36–38). Online or eHealth interventions have gained traction as they enable participant engagement anytime-anywhere, provide repetitive access opportunities, and a wider reach at a lower cost (39–41). Web-based interventions in Early Childhood Education and Care (ECEC) settings have been shown to be highly acceptable and effective in improving nutrition knowledge and practices (42, 43). Within the Canadian context, eHealth interventions in ECEC settings remain limited (44, 45), particularly those designed to support child care educators align with best practice nutrition and physical activity guidelines. Given the available evidence supporting the need of a comprehensive intervention within the ECEC setting along with the effectiveness and sustainability of online modalities, the purpose of this paper is to describe the design of a virtual eHealth nutrition and physical activity intervention aimed at improving the knowledge, beliefs and adherence to best practices of early childhood educators in Alberta, Canada.

2 Materials and methods

2.1 Study design

The *"CHEERS HEAPful of FUN: raising healthy Albertans"* (CHEERS program) is a health and wellness virtual educational support program that aims to improve the knowledge and skills of early childhood educators and support the implementation of evidence-informed best nutrition and physical activity practices within ECEC settings. The study follows a quasi-experimental design and uses the Social Cognitive Theory for behavior change as a foundation (46). The Social Cognitive Model posits the mutual influence and interplay between behaviors, personal factors and the environment, also known as "*Reciprocal Determinism*" (46, 47). Health behaviors are influenced by personal factors and the physical and social environment, and in turn, individuals can influence their environment through behavior (46). This study integrates key constructs of the Social Cognitive Theory and addresses within-person influences such as educators' knowledge and beliefs to encourage the adoption of best nutrition and activity practices which in turn can promote healthier ECEC environments. The Social Cognitive Theory was used as a guiding model for the development of the CHEERS materials. The modules were designed to improve educators' skills and knowledge, aligning with the Social Cognitive Theory constructs, and provide essential information, empowering educators to implement best practices. Another key application of the Social Cognitive Theory pertains to the construct of self-efficacy. Within the Communities of Practice sessions that follow the modules, educators engage in a social negotiation to interpret and integrate the knowledge gained from the modules within their work as early childhood educators to reshape their efficacy beliefs of implementing best practices.

2.2 Participants and recruitment

2.2.1 Childcare centers and educators

Participant recruitment was implemented in the province of Alberta, Canada between 2019 and 2022. Children's Services, Early Childhood Development Branch from the Alberta government, provided the research team with the full list of ECEC programs. ECEC centers were randomly selected for recruitment using postal codes to stratify the selection of ECEC centers from large urban population centers (population >100,000), medium population centers (30,000–99,000), small population centers (1000–29,999), and rural areas (population <1000) throughout the province (48). Starting in 2019, center directors were invited to participate through phone correspondence and provided with general information on the study. Center directors had the option to be in the intervention or control group based on their assessment of both available time and capacity to participate. Centers that agree to participate receive an email package with instructions, a consent form, links to surveys, and contact information of a trained research associate to answer potential questions. Inclusion criteria include: (1) licensed facility-based centers (2) providing care for a minimum of 15 children aged 2–5 years, (3) access to a computer and internet connection and (4) not currently enrolled in any other intervention to improve nutrition and activity practices. Exclusion criteria include (1) unlicensed ECEC centers, and (2) family day home or after school care program. Each participating center was requested to identify a minimum of three staff members to be included in the study, with a preference to include two educators and the center director or manager.

2.2.2 Children

Children were recruited to objectively measure physical activity and sedentary levels from participating ECEC centers. Children were recruited from the classroom of participating educators consisting of children aged between 2 and 5 years. Inclusion criteria include: (1) prior written consent from a parent or guardian to participate; (2) be aged between 2 to 5 years; and (3) be enrolled full-time at the center. Center staff were asked to distribute informational flyers and consent forms to parents via center communication methods with parents. Flyers provided parents with information about the study, what children would be doing as part of the study, and phone numbers of research staff. Parents/guardians provided written consent and answered demographic questions related to birth date and sex when returning the consent forms. Completed consent forms were collected by research staff for child participation and children provided verbal assent during data collection.

2.3 Development of materials

The online educational modules were developed in 2019 by a team of content expert developers in nutrition (early childhood dietitians in provincial health authority), physical activity (Be Fit For Life Center), wellness (university faculty member wellness specialist) and early childhood (university faculty member in early childhood) and used evidence-informed best practices to ensure high-quality content. These modules were then sent for review by content expert reviewers, identified from each of the specialties, and revised as necessary prior to implementation. Modules were further reviewed by an Early Childhood Educator university faculty member to ensure the messaging was respectfully communicated.

2.4 Intervention procedure

The CHEERS program is a quasi-experimental study with repeated measures that started in 2019 and will continue until 2023. Each year the program is repeated and involves a new cohort with repeated measurements at baseline, 5 and 10 months from baseline. During this period, ECEC centers in the intervention group participate in a series of activities. Firstly, they complete a set of online surveys hosted on Qualtrics. In addition to the surveys, the participants in the intervention group complete a 12-week set of online professional development modules and attend virtual weekly Communities of Practice sessions. The 5-month measurement served as a mid-point assessment and timed after the completion of the online modules and online Communities of Practice sessions. The 10-month measurement, a post intervention follow-up, aimed to evaluate the sustained and long-term impact of the intervention.

2.4.1 Online modules

The modules cover topics on healthy eating patterns and the eating environment, physical activity and literacy, self-care for educators, parents as partners, and the importance of sleep for children. The full list of modules can be found in **Table 1**. The modules are hosted on Articulate 360 (Articulate Global Inc) with the content authoring tool Rise, which offers several embedding features and pre-built interactions such as card sorting, flashcards, knowledge checks, and click-through processes. The modules include clear learning objectives, instructional videos, links to useful resources and embedded reflection questions that can be accessed through computer or mobile devices. Participants in the intervention group are asked to complete one module per week, with each module taking 1 to 2 h to complete. An example module can be seen in the Supplementary material. Each module requires participants to submit their name and email address as an exit response, which is used to track module completion. This allows the research team to send a reminder email to only those participants who have not yet completed the material. This approach ensures participants have the opportunity to complete the modules before moving to the next phase of the program.

2.4.2 Communities of practice

Participants engage in two different Communities of Practice throughout the intervention. First while completing the weekly educational modules participants attend online Communities of Practice which provide opportunities to reflect on the practical applications of the module topics with other directors and educators participating in the program. Second, after completing the 12-week modules, participants attend online Communities of Practice meetings with the other participants located at their center. These meetings are facilitated by Health Promotion Leaders who are experts in nutrition, public health, or physical activity. During the post-educational Communities of Practice meetings participants: co-collaborate on strategies to integrate the learning into programming within their respective centers; share challenges they are facing and strategies to overcome them; and are provided with additional resources from the Health Promotion Leaders as needed to support change within their centers. These Communities of Practice meetings take place over a period of 5 months with a session scheduled each month.

2.4.3 CHEERS challenge

Finally, participants are invited to take part in the "CHEERS Challenge" where they are encouraged to utilize the resources gained through the program and mobilize the knowledge they learned to other staff in the center or with parents.

2.4.4 Incentives

Participants in the control group receive an honorarium of $25 for completing surveys at baseline and then again at 10 months. Intervention participants are compensated for their time in the program with a knowledge grant during the 12-week education modules ($300) and the after 5-month Health Promotion Leader communities of practice component ($300). Participants are invited to a wrap up session at the end of the program to share preliminary study findings and celebrate the community.

2.4.5 Control group

Participants in the control group are instructed to continue with their usual practice, with the control group only filling surveys at baseline and end of program with no access to the intervention components.

2.5 Data collection and outcome measures

This study follows a mixed-method approach to evaluate the impact of the program in changing practices at the level of ECEC centers. Questionnaires are administered online using Qualtrics platform that is compatible across mobiles and computers. Surveys are administered at three time points: baseline (T1), after 5 months (T2), and after 10 months at the end of the program (T3). Outcome measures are summarized in Table 2.

TABLE 1 Module topics.

Module 1: Physical literacy–activity for health	Module 7: Nutrition–mealtime experiences
Module 2: Nutrition–what is on the plate?	Module 8: Physical literacy–free play
Module 3: Self-care: you first!	Module 9: Physical literacy–environments matter
Module 4: Nutrition–the feeding relationship	Module 10: Nutrition–curriculum connections
Module 5: Self-care–managing stress	Module 11: Parents as partners
Module 6: Self-care strategies	Module 12: Sleep and health

2.5.1 Quantitative assessments

2.5.1.1 CHEERS

The CHEERS survey is a community-based educator-administered self-audit survey designed to offer ECEC centers an assessment of their center's healthy eating and physical activity environment (49). Each of the center's participating educators complete the CHEERS tool which includes 59 items and measures four constructs: Food Served- n = 23 items, (e.g., My childcare center serves vegetables and fruit prepared with little or no added fat, sugar or salt), Healthy Eating Environment- n = 18 items, (e.g., My childcare center provides children with an assigned area, with few distractions, to sit and eat), Healthy Eating Program Planning- n = 6 items, (e.g., My childcare staff members attend professional development on nutrition education), and Physical Activity Environment- n = 12 items, (e.g., My childcare center has indoor space available for physical activity). Items are measured using a seven-point scale with response options ranging from 1 = "never" to 7 = "always." The four subscale scores are calculated using an average of the items in the grouping. The CHEERS score is calculated as the average of the four subscales with higher scores reflecting adherence with optimal practices in nutrition and physical activity. The CHEERS survey provides ECEC centers with an assessment of their eating and activity environments and provides a personalized report that identifies one area of strength and one area for improvement in each of the four measured domains. The development of the CHEERS tool followed a structured content validation process with a final Flesch–Kincaid readability grade 8.1 (50). It has also been assessed within the educator community and shows evidence of good internal consistency (Cronbach's α = 0.91), intra-rater reliability (ICC = 0.81), inter-rater reliability (ICC = 0.59), and concurrent validity with direct observation (ICC = 0.65) (51) as well as online administration (ICC = 0.86) (52).

2.5.1.2 Mindful Eating Questionnaire (MEQ)

The Mindful Eating Questionnaire is a 28-item self-reported scale that measures mindful eating behaviors (53). Each of the

TABLE 2 List of outcome measures.

	Method	Baseline (T1)		Mid-point (T2: 5 months from baseline)		End (T3: 10 months from baseline)	
		IV	CTRL	IV	CTRL	IV	CTRL
ECEC measures							
CHEERS (self-reported assessment of ECEC nutrition and physical activity environment)	Online	X	X	X		X	X
PLKASB-ECE (self-reported measure of educators' knowledge, attitude, self-efficacy and behaviors related to physical literacy in ECECs environment)	Online	X	X	X		X	X
EPAO (observational tool of nutrition and physical activity environments in ECECs)	Observational	X	X			X	X
Educator measures							
MEQ (self-reported mindful eating behaviors of educators)	Online	X	X	X		X	X
C-BANKS 2.0 (self-reported dietary knowledge, attitude and behaviors of educators)	Online	X	X			X	X
Self-reflection questions	Online	X					
Exit interviews	Online					X	
Child measure							
Physical activity	ActiGraph Accelerometer	X	X			X	X

IV, intervention; CTRL, control.

center's participating educators complete the MEQ measures which include five domains: disinhibition, awareness, external cues, emotional response and distraction. It uses a four-point Likert scale with responses ranging from 1 (*never/rarely*) to 4 (*always/usually*), where higher scores reflect greater mindful eating. Each subscale score is calculated as the means of items, excluding the "not-applicable" responses. The total score is calculated as the mean of the five subscales with a higher score reflecting more mindful eating.

2.5.1.3 Canadian Behavior, Attitude and Nutrition Knowledge Survey (C-BANKS 2.0)

The C-BANKS 2.0 is a survey designed to measure nutrition-related knowledge, attitude, and behavior in the Canadian population (54). Each of the center's participating educators complete the C-BANKS 2.0 which is comprised of 60 items and measures three constructs: Knowledge (20 items), Attitude (5 items) and Behavior (35 items). The C-BANKS 2.0 has been assessed for reliability and validity in Canadian adults (54). Knowledge items are scored as correct (1) or incorrect (0) and summed to create a total score. Attitude items use a seven-point Likert scale ranging from 1 (*strongly disagree*) to 7 (*strongly agree*) and a total attitude score is calculated as the mean of items where a higher score reflects more positive attitudes. Behavior items employ a seven-point Likert scale ranging from 1 (*never*) to 7 (*always*) and the total score is calculated using the average of the items. The total C-BANKS 2.0 score is calculated as the average of the three subscales.

2.5.1.4 Physical Literacy Knowledge, Attitude, Self-efficacy, and Behavior (PLKASB-ECE) questionnaire for early childhood educators

Physical literacy is the motivation, confidence, physical competence, and knowledge and understanding to enable all individuals to value and take responsibility to engage in physical activities for life (55). The PLKASB-ECE is a self-administered instrument that measures early childhood educators' knowledge, attitude, self-efficacy, and behaviors related to physical literacy in childcare environments. Each of the center's participating educators complete the PLKASB-ECE which consists of 6 subscales: Knowledge ($n = 7$) measuring educators' physical literacy knowledge; Perception of Knowledge ($n = 2$) measuring educators' perception of their understanding related to physical literacy concepts, Self-Efficacy ($n = 3$) measuring educator's self-efficacy toward developing physical literacy skills in the early learning environment, Attitude ($n = 3$) measuring educators' attitudes regarding physical literacy and health outcomes; Environment Behaviors ($n = 3$) measuring educators' perception of their physical literacy promotion practices in the ECE environment; and Personal Behaviors ($n = 3$) measuring perception of personal physical activity behaviors. Knowledge items are scored correct (1) or incorrect (0) and summed to derive the total score with a higher score indicating higher knowledge of physical literacy concepts. All perception, self-efficacy, attitudinal, and behavior items are scored on a seven-point Likert scale from 1 (*strongly disagree*) to 7 (*strongly agree*) and averaged into a mean score for each subscale (56).

2.5.1.5 Environment and Policy Assessment and Observation- EPAO

The EPAO instrument is used to objectively assess the nutrition and physical activity environment of participating ECEC centers. A single EPAO assessment is completed per center which consists of a day-long observation of practices within childcare facilities and includes a document review of the centers' policies by a member of the research team (57). Trained Health Promotion Leaders (HPLs) visit consenting centers twice per year (Fall and Spring of every year) to complete the EPAO on a day agreed upon between directors and the HPL conducting the observation. Observations of nutrition and physical activity (PA) practices typically take place in the participating educators' classroom, consisting of children ages 2–5 years. The nutrition related sections of the EPAO assess the overall nutrition environment and compliance with best nutrition practices. This study utilized the following EPAO nutrition-derived variables: Foods Provided, Feeding Environment, Feeding Practices and Nutrition Policy. The physical activity portion of the EPAO measures the following subdomains: outdoor and indoor play, educator's physical activity practices and professional development, screen time and physical activity policy. In this study, the EPAO-overall PA score was used and calculated by summing all subdomains.

2.5.1.6 Accelerometers

ActiGraph GT3X accelerometers are used to objectively measure physical activity and sedentary levels of a subset of children from the participating ECEC centers. ActiGraph accelerometers have consistently been shown to provide reliable and valid estimates of physical activity in preschool children (58, 59). Educators are trained how to correctly attach and remove accelerometers by research staff. Children wear the accelerometers on the right hip using an elastic waist belt. Prior to placing the accelerometers, children's assent is also obtained. Children wear the accelerometers for 7 days while they are at the ECEC centers. Children are fitted when they arrived at the child care center and accelerometers are removed at the end of each day. In this study, activity counts are set to 15-s Epochs. A minimum wear-time of 250 min/day for 4 days is required for a valid day. ECEC educators are trained to take children's weight and height and record all wear-time related information in a daily log. Height is measured to the nearest 0.1 cm using a portable stadiometer (Seca 213 stadiometer) and weight is measured to the nearest 0.1 kg using an electronic scale. Using Pate et al. (60) proposed cut-off points, PA intensity is categorized into: sedentary PA: <200 counts/15 s, light PA: 200-419 counts/15 s, moderate PA: ≥420 counts/15 s and vigorous PA: ≥842 counts/15 s.

2.5.2 Qualitative assessments

2.5.2.1 Exit interviews

Following the completion of the 10-month intervention, educators and directors from the intervention group are invited to have 30-min semi-structured interviews conducted by HPLs via Google Meet. The interviews aim to assess participants' experiences with the online modules and community sessions, barriers encountered, potential areas for improvement, and changes that occurred in their centers since joining the CHEERS program. The interviews are digitally recorded and subsequently transcribed into written form for further analysis.

2.5.2.2 Modules reflective questions

The 12 online modules contain open-response questions at the end of each educational module. The questions are completed within the SurveyMonkey platform and are intended to stimulate participants' reflective practice and enhance their overall learning experience.

2.6 Statistical analysis

2.6.1 Intervention evaluation

We hypothesize that the CHEERS program will be effective in promoting positive changes in educator's knowledge, beliefs, and practices leading to significant improvements in ECEC center nutrition and physical activity environments. Linear mixed model (LMM) analyses will be used to analyze the intervention effects on educator's outcome measures. A three-level model with repeated measurements as first level, educators as second level and ECECs as third level will be used.

A qualitative thematic analysis using the NVivo 10 program will be conducted to explore participants' perspective, satisfaction and evaluation of the CHEERS intervention, their use of online modules, their experiences, changes in knowledge, attitude, food provision and practices and barriers and enablers influencing adherence to best nutrition and physical activity practices.

2.6.2 Baseline descriptive characteristic and associations

In the current paper, we present baseline characteristics for the intervention and control groups. Additionally, we explore correlations between the CHEERS self-assessment survey and corresponding EPAO subdomains at baseline. We also assess the association between early childhood educator's baseline personal nutrition knowledge, beliefs, and behavior with the corresponding CHEERS nutrition composite. All analyses are done using SPSS, version 28 (SPSS Inc., Chicago, Illinois) and statistical significance was set at a p-value < 0.05. A descriptive analysis was performed using means and standard deviations for continuous variables, whereas numbers and percentages were used for categorical variables. T-tests or chi-square tests were conducted to compare baseline demographic characteristics between the intervention and control groups. Fisher's exact test was also used when the expected frequency was less than 5. Bivariate analyses examining the association between CHEERS and the C-BANKS 2.0 and EPAO subdomains were carried out using Pearson or Spearman correlation tests for non-normal data.

3 Results

3.1 Demographic profile by group

In 2019, 144 centers were invited to participate, of those 83 center directors (58%) agreed to allow recruitment of educators in their centers. Once the intervention started, five centers withdrew from the study due to three main reasons, change in center director, closure of center, and center staff left the profession resulting in 78 centers total. The intervention group comprised 138 participants (50 centers) and the control group 70 (28 centers). The baseline assessments of the first year (2019) revealed a study population 96.2% female with an average age of 40.05 ± 11.67 years. The majority were educators making up 62% of participants followed by directors accounting for 30.3% of the sample, 1.4% were cooks and 6.3% owners. Most participants were Level 3 which represents an educational completion of a 2-year diploma or university degree (44.4%) while 15.9% were Level 1 which represents the completion of the minimum requirement of the Child Care orientation program. The majority of the ECEC programs were private (70.7%) and provided food (91.4%). At baseline, with the exception of participant age, there were no significant differences in demographic characteristics between intervention and control groups (**Table 3**).

3.2 Association of educators' personal nutrition-related knowledge, attitude, and behavior (C-BANKS 2.0 scores) with CHEERS nutrition subdomains

As shown in **Table 4**, C-BANKS 2.0 and CHEERS total scores were significantly correlated, $r = 0.156$, $p = 0.049$. Specifically, the dietary behavior subscale of the C-BANKS 2.0 was significantly correlated with all of CHEERS nutrition subdomains (Food Served, Healthy Eating Environment and Healthy Program Planning) ($p < 0.005$). In other words, educators' personal dietary behavior was significantly correlated with the nutrition environment and practices in the centers. Additionally, educators' nutrition knowledge and attitudes were significantly positively correlated with their dietary behaviors. Educators with greater knowledge and a more positive attitude had significantly healthier dietary behaviors ($p < 0.005$).

3.3 Association of CHEERS components with corresponding EPAO subdomains

The Physical Activity Environment (PAE) subscale of CHEERS was significantly correlated with the overall EPAO-PA score, $r = 0.462$, $p = 0.035$ (**Table 5**). This means, educators' self-assessment of their center's physical activity as measured by the CHEERS survey was significantly correlated with the physical activity environment and practices as objectively observed using the EPAO. The Food Served (FS) subscale of CHEERS was significantly correlated with the EPAO-Foods Provided subdomain, $r = 0.549$, $p = 0.010$, and with the EPAO-Nutrition Policy subdomain, $r = 0.438$, $p = 0.047$. In other words, the EPAO's objective measurements of the food provided and the centers' nutrition policy were significantly correlated with educators' self-reported scores on food served. No significant associations were found between the self-reported Healthy Eating Environment scores from the CHEERS survey and the EPAO's objective assessments of feeding environment and practices ($p > 0.005$).

4 Discussion

This article describes the protocol of the CHEERS eHealth intervention program which aims to encourage environmental changes of early learning settings at a wide geographical scale (provincial scope). We propose that the intervention would be an effective scaling up public health innovation due to its unique approach of utilizing eHealth strategies to improve the nutrition and physical activity environments of ECECs through staff education and community building to increase knowledge, attitudes, and adherence to best practices. This paper also provides baseline characteristics of the participants who took part in the CHEERS program in the first year and compares self-reported and observed eating and activity practices. In addition, the relation of educators' own nutrition knowledge, attitudes and behaviors with self-reported evaluations of nutrition environments is examined.

Findings of the current study add to the existing research demonstrating the influence of educators' own nutrition behaviors on food practices at the ECEC level (61). Our results indicate that educators with higher nutrition knowledge and positive attitudes toward healthy eating were more likely to engage in personal healthy dietary behaviors. Furthermore, the healthy dietary behavior of educators was positively correlated with better nutrition practices at the ECEC level. Educators who adopt healthier eating habits were more likely to provide supportive food environments and feeding practices to children in their care. Our finding that educators' own dietary behaviors are associated with better mealtime practices and environment is supported by several theories including the Social Cognitive Theory (46) and the Ecological Model (18). Those theories underline the influence of important caregivers' behaviors on promoting a child's healthy habits through mechanisms such as normative practices, social support and role modeling. We did not find direct significant correlations between nutrition knowledge and attitudes with the feeding environment. However, our results suggest that educators' knowledge and nutrition beliefs may influence personal dietary practices which can in turn contribute to the creation of better food environments and feeding practices at the daycare level. Future research should explore the indirect association between early childhood educator's own nutrition knowledge and attitudes with feeding practices by investigating the potential mediating effect of personal dietary behaviors.

Objective measurement of the physical activity environment as measured by the EPAO-PA subscale was significantly associated with self-reports of physical activity environment and practices. The Physical Activity Environment subscale of the CHEERS survey measures a similar concept to the EPAO, in that they both evaluate daily physical activity opportunities, play equipment use, staff practices, professional development, and physical activity policy. The strong positive correlation between the CHEERS Physical Activity Environment subscale with the EPAO-PA subdomain suggests that the CHEERS self-report measure is closely aligned with the observational measure and provides further evidence of concurrent validity for the CHEERS survey assessing physical activity practices in child care centers (62, 63).

Similarly, the results of our study reveal a strong positive correlation between the Food Served subscale of the CHEERS and the objectively measured EPAO-Foods Provided and Nutrition

TABLE 3 Characteristics of ECECs and ECEC educators.

Characteristics	All N (%) (*n* = 208)	IV (*n* = 138)	CTRL (*n* = 70)	*p*-value
Number of children enrolled per center				
Mean (± SD)	46.17 ± 34.40	47.40 ± 37.16	44.17 ± 29.93	0.694[a]
Auspice				
Non-profit	61 (29.3)	40 (29)	21 (30)	0.879[b]
For-profit	147 (70.7)	98 (71)	49 (70)	
Food provision				
Center provides food	191 (91.4)	124 (89.2)	67 (95.7)	0.114[b]
Parent provides food	18 (8.6)	15 (10.8)	3 (4.3)	
Education level of ECEC educators				
Level 1 (orientation course)	33 (15.9)	23 (16.7)	10 (14.5)	0.340[b]
Level 2 (1-year certificate)	28 (13.5)	18 (13)	10 (14.5)	
Level 3 (2-year diploma)	92 (44.4)	66 (47.8)	26 (37.7)	
University degree	54 (26.1)	31 (22.5)	23 (33.3)	
Age of ECEC educators	40.05 ± 11.67	41.49 ± 11.85	37.20 ± 10.82	0.012[a]
Gender				
Male	6 (3.8)	6 (5.8)	0 (0)	0.097[c]
Female	151 (96.2)	98 (94.2)	53 (100)	
Position				
Director	63 (30.3)	39 (28.3)	24 (34.3)	0.753[c]
Educator	129 (62)	89 (64.5)	40 (57.1)	
Cook/chef	3 (1.4)	2 (1.4)	1 (1.4)	
Owner/operator	13 (6.3)	8 (5.8)	5 (7.1)	

[a]*p*-value for the Independent Samples *T*-test.
[b]*p*-value for the chi-square test.
[c]*p*-value for Fisher's exact test.
SD, standard deviation; N, sample size; IV, intervention; CTRL, control.

TABLE 4 Spearman Correlations between CHEERS and C-BANKS 2.0 for the total sample (*N* = 160) [Adapted from Lafave et al. (54)].

Correlations							
					C-BANKS 2.0		
	CHEERS–NS	FS	HEE	HEPP	Knowledge	Attitude	Behavior
C-BANKS	0.156*						
Knowledge	0.031	0.029	0.017	0.005	–		
Attitude	0.007	0.050	0.037	0.041	0.232*	–	
Behavior	0.428*	0.348*	0.412*	0.383*	0.256*	0.294*	–

*Correlation is significant at the 0.05 level (2-tailed).
CHEERS NS, CHEERS nutrition score; FS, food served; HEE, healthy eating environment; HEPP, healthy eating program planning.

Policy subdomains. The latter can be explained by the two tools measuring similar constructs. For instance, the Food Served subscale of the CHEERS asks about fruit and vegetable provision, dark leafy greens, high fiber food, and fatty foods which are also captured in the EPAO-Foods Provided subdomain. Additionally, the document review of the nutrition policy in the EPAO includes an evaluation section that assesses the menu and foods and beverages served at the daycare center corresponding closely with the constructs measured in the Food Served subscale of CHEERS. The alignment between the self-report data and observational measurements provides further evidence of concurrent validity for the CHEERS audit tool.

We anticipated significant correlations between the Healthy Eating Environment Subscale of CHEERS and the EPAO-Feeding Environment and Feeding Practices, as they measure similar aspects such as "*using food as reward/bribe,*" "*encouraging children to try new foods,*" "*engaging in pleasant conversations during meal time,*" "*allowing children to decide what and how much to eat.*" However, no significant associations were observed. The lack of correlation could be attributed to various factors. One explanation may be that CHEERS serves as an audit tool for educators to self-assess their practices and create action plans accordingly. As in all self-assessment tools, educators may have a tendency to overreport favorable behaviors (64). It is also plausible that

TABLE 5 Correlations between CHEERS and EPAO subdomains ($N = 21$).

Correlations				
	CHEERS			
EPAO	FS	HEE	HEPP	PAE
Foods provided	0.549[a]*	–	–	–
Feeding environment	–	0.283[a]	0.283[a]	–
Feeding practices	–	0.035[a]	0.19[a]	–
Nutrition policy	0.438[b]*	0.186[b]	0.329[b]	–
Physical activity score	–	–	–	0.462[b]*

[a]Coefficient for the Pearson correlation.
[b]Coefficient for the Spearman correlation.
*Correlation is significant at the 0.05 level (2-tailed).
FS, food served; HEE, healthy eating environment; HEPP, healthy eating program planning; PAE, physical activity environment.

educators initially perceive their feeding practices as optimal based on their subjective evaluation. However, as they engage in the intervention and complete the different trainings to gain a deeper understanding of what really constitutes optimal nutrition practices and recommended guidelines, it is possible that educators may reassess and evaluate their practices differently by the end of the intervention. Overall, the observed discrepancies highlight the need for the intervention to address best nutrition practices and to examine changes in the scores of both assessment tools before and after the intervention.

4.1 Strengths and limitations

The quasi-experimental design of our study can be viewed as a limitation. However, including centers based on their willingness and capacity to implement the intervention provides a more realistic insight into how such programs might be embraced in real-world day care settings. Additionally, no significant baseline differences were observed between intervention and control group, except for age, which is controlled for in all analyses. Another limitation of this study is that most data were self-reported and the possibility of educators reporting a more favorable picture of their practices cannot be ruled out. However, to balance this we also used an objective observational measure (EPAO). Furthermore, although ECEC centers were randomly selected, the study's scope was limited to ECEC centers in the province of Alberta which limits the generalizability of the findings. Additionally, the majority of participants (94.6%) were women which brings into question the representativeness of the study sample. However, this aligns with the gender distribution in the ECEC workforce as per the latest statistics (96%) (65).

One of the strengths of this intervention is the use of both quantitative and qualitative research methods for the implementation of the intervention and analysis of results. This triangulation of data will contribute to a more comprehensive evaluation of the intervention, its impact and future maintenance.

5 Conclusion

To our knowledge the CHEERS program is the first eHealth intervention tool in Canada that comprehensively addresses both the nutrition and physical activity social and physical environment in ECECs. The development of the CHEERS program was based on evidence and theory-driven methodology and shows great potential of being a practical and sustainable scaling up approach to promoting healthier early childhood education and care settings.

Data availability statement

The datasets presented in this article are not readily available because the datasets generated during and/or analyzed during the current study are not publicly available due to participants' privacy under the REB approval. Requests to access the datasets should be directed to LL, llafave@mtroyal.ca.

Ethics statement

The studies involving humans were approved by the Human Research Ethics Board at Mount Royal University (no. 101768). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LL: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing. JH: Data curation, Formal Analysis, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review and editing. AW: Conceptualization, Data curation, Investigation, Methodology, Resources, Validation, Writing – review and editing. CM: Conceptualization, Data curation, Investigation, Methodology, Resources, Validation, Writing – review and editing.

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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.2024.1337873/full#supplementary-material

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Exploring the health benefits of traditionally fermented wax gourd: flavor substances, probiotics, and impact on gut microbiota

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Background: The fermented wax gourd, often referred to as "smelly wax gourd," is a traditional food that undergoes natural fermentation. It's a staple in eastern China and is recognized as Ningbo's "city-fermented food." Characterized by its distinct putrid flavor and soft texture, its safety, nutritional aspects, and sensory attributes have not been extensively studied.

Methods: In this research, the microbial community and flavor components of fermented wax gourd during its traditional fermentation were analyzed. The safety and impact on the gut microbiota were also assessed by administering it to healthy and pseudo-germ-free mice.

Results: The findings revealed that organic acids primarily contribute to the gourd's flavor during fermentation. The aroma reminiscent of fruits is due to 2-methyl-butyric acid, while butyric, pentanoic, caproic, and octanoic acids are responsible for their characteristic smelly taste. In the fermentation of traditional foods, the abundance of substances and open fermentation contribute to the diversity of microorganisms in the system, and the reproduction and metabolism of microorganisms drive the fermentation of foods. From the results of this study, the flavor peaks on the 10th day of fermentation. Predominant microbes include *Lactobacillus fermentum*, *Streptococcus equinus*, *Fusobacterium perfoetens*, *Weissella confusa*, and *Lactobacillus plantarum*. Notably, *Lactobacillus* was the most abundant probiotic in the early fermentation stages. The "smelly" taste of smelly wax gourd was mainly derived from butyric acid, valeric acid, caproic acid, caprylic acid, p-methylphenol and other compounds, and the abundance of *Caldicoprobacter algeriensis*, *Mariniphaga anaerophila*, *Streptococcus equi* and *Lactobacillus* were significantly correlated with 4 of the above 5 acids. These four bacteria may contribute more to the "smelly" taste of smelly wax gourd. In the study, compared with the control group (CONT), the abundance of *Helicobacter ganmani*, *H. Chanicola*, *Lactobacillus animalis*, *Lactobacillus gadi* and *Lactobacillus reuteri* decreased in mice groups treated with anti-biological pretreatment followed by gavage of smelly wax gourd (A.SWG) and the smelly wax gourd (SWG) groups. Conversely, *Muribaculum intestinale*, *Prevotellamassilia timonensis*, *Alistipes putredinis*, *Kineothrix alysoides* and *Clostridium indolis*' abundance of increases. Mice that underwent fecal microbiota transplantation (FMT) exhibited a higher abundance of probiotics like *Bifidobacterium animalis*, *Bifidobacterium pseudolongum*, *Lactobacillus johnsonii*, and others compared to the fecal culture microbiota transplantation

(CMT) group. However, the CMT group had a higher presence of fermented and Royce lactobacilli.

Implication: Consuming fermented wax gourd can enhance the presence of beneficial probiotics and reduce pathogenic *Helicobacter* sp. in the mouse gut. Both *Lactobacillus* sp. and *Bifidobacterium* sp. showed increased abundance post fecal microbiota and fecal culture microbiota transplantation.

KEYWORDS

fermented wax gourd, microbial diversity, flavor substance, gut microbes, traditional food

1 Introduction

The consumer demand for fermented vegetables with high nutritional value and probiotic microorganisms is increasing, also due to the consequence of the growing trend of vegetarianism and the increasing prevalence of cow's milk allergy (Martins et al., 2013; Kumar et al., 2015; Flom and Sicherer, 2019; Okoye et al., 2023). Several famous traditional fermented vegetables are globally consumed and widely investigated, such as kimchi (Cheigh et al., 1994; Kim et al., 2021), Sichuan pickle (Rao et al., 2018) and sauerkraut (Beganović et al., 2015).

Fermented wax gourd (*yan-dong-gua*) also is a traditional fermented vegetable and famous cuisine in eastern China, dating back to the Song dynasty in Chinese history (A.D. 960–1279) (Wu et al., 2016b). Wax gourd, *Benincasa hispida*, consists of water 96.1 g, energy 54 kJ (13 kcal), protein 0.4 g, fat 0.2 g, carbohydrate 3.0 g (dietary fiber 2.9 g) per 100 g according to the data from the United States Department of Agriculture (Lim, 2012). The wax gourd has been consumed for many centuries for culinary and folk medical purposes as its health-promoting activities, such as alleviating cough and diabetes mellitus, stimulating urination, reducing body fat and blood triglycerides, and anti-obesity effects (Kang and Kwon, 2003; Ajuru and Nmom, 2017). Historically, fermented wax gourd served as a source of nutrients in winter when fresh vegetables were scarce due to its preserving a nutritive value of wax gourd, creating a desirable sensory and creamy texture property, and proving a particular safety food by proper homemade fermentation (Wu et al., 2016a). It is still popular and served in most restaurants in Ningbo, recognized as "the city fermented food" of Ningbo (one of the cities in east China). A fermented wax gourd is still homemade or restaurant-made naturally by adding salt (approximately 5–10%) and old fermented wax gourd solution as a starter at the beginning of the fermentation process and then through fermenting under anaerobic conditions for 10–20 days (Lan et al., 2012; Wu Z.-F. et al., 2015).

Unlike the famous and well-studied fermented vegetable (e.g., kimchi), the fermented wax gourd was only locally well-known, possibly due to the unique flavor and the lack of safety evaluation of this spontaneous fermentation, which is hard to bring modern consumer interests. Nonetheless, fermented gourds also offer the advantages of traditional vegetarian fermentation. On the one hand, fermented gourds prolong their shelf life, reduce their size and facilitate transportation (Ayed et al., 2020). On the other hand, during fermentation, the high enzymatic activity of lactic acid breaks down. It detoxifies many compounds (e.g., phenolic compounds, colorants, mycotoxins, pesticides, etc.) that are present in a variety of raw materials and, due to their high bioactivity, produces several biologically active substances (e.g., volatile fatty acids) (Park et al., 2014). It has been shown that lactic acid (LA) fermentation can reduce the risk of foodborne poisoning and health problems and that gram-negative bacteria's growth is typically inhibited in the early stages of fermentation (Ogrodowczyk and Drabinska, 2021).

The microorganism plays an essential role in the flavor (Sidira et al., 2016; Xu et al., 2018), hygiene (Byakika et al., 2019), and functions of fermented foods during the process of fermentation (Choi et al., 2012; Ashraf and Shah, 2014; Wastyk et al., 2021; Diez-Ozaeta and Astiazaran, 2022). Lactic acid bacteria (LAB), such as *Weissella* spp. and *Lactobacillus* spp., were predominant bacteria in the ready-to-eat fermented wax gourd (Lan et al., 2009; Zhao et al., 2014).

In previous reports, the species of yeasts in pickled wax gourd, as well as the volatility compounds, were identified by 5.8 S rDNA-ITS (Internal Transcribed Spacer) sequencing and GC-MS (Gas Chromatography-Mass Spectrometry), respectively (Lin et al., 2023); and the microorganism of Pickled wax gourd was cultured with the medium in order to investigate the number and species of bacteria and yeast in pickled wax gourd (Wu Z.-F. et al., 2015). However, none of the previous studies can fully show the changes in microbial communities in the fermentation process of smelly wax gourd. Therefore, using 16S rDNA and ITS rDNA sequencing combination can investigate the succession of all microorganisms in the fermentation process of smelly wax gourd and subsequently clearly analyze the relationship between flavor components and microorganisms.

Numerous studies have revealed that gut microbiota plays a pivotal role in human health and disease. On the one hand, gut microbiota improves energy absorption and nutrients (Power et al., 2014). On the other hand, gut microbiota is closely associated with regulating human diseases, such as obesity, hypertension, metabolic syndrome, colitis, diabetes, etc. (Yoshimoto et al., 2013; Monk et al., 2016; Pevsner-Fischer et al., 2017; Chen et al., 2018). As previous reports, *Lactobacillus* isolated from kimchi could inhibit atopic dermatitis in NC/Nga mice, proving that Kimchi had an anti-obesity effect in diet-induced obese mice (Won et al., 2011; Park et al., 2012; Cui et al., 2015; Kim et al., 2020). However, studies of the interaction effect between fermented foods and gut microbiota are very limited so far, as well as smelly wax gourd. Therefore, it is necessary and feasible to investigate it.

In this study, high-throughput sequencing was used to investigate the link between the formation of volatiles and microorganisms during the fermentation of wax gourd, and also to explore the effects of these substances on the gut microbiota of mice, as well as the effects of FMT and cultured microbiota transplantation (CMT). It is expected to introduce a new perspective to the study of intestinal microbiota and provide a valuable theoretical basis for the study of fermented foods.

2 Materials and methods

2.1 Materials

The stinky winter melon was produced at the San Oxi Jia Cai market in Ningbo, Zhejiang Province, China. The standard methyl butyrate (0.898 g/mL, 25°C) was purchased from Shanghai Aladdin Biochemical Technology Co. of China. E.ZN.A. Fecal DNA kits were purchased from OMEGA (Norcross, GA, United States). In animal experiments, 25 specific pathogen-free (SPF)-grade 4 weeks-old male ICR mice (20 ± 2 g, Licence No. 1710300006) were obtained from Hangzhou Experimental Animal Center (Hangzhou, Zhejiang, China).

2.2 HS-SPME-GC-MS analysis

2.2.1 Internal standard, sample preparation and micro-extraction conditions

The winter melon salt embryos were immersed in the odour brine and sealed for fermentation at room temperature. The whole fermentation process lasted for 15 days. Samples were taken quickly from the center of the jar at the time points of 0 day (D0), 5 days (D5), 10 d (D10) and 15 days (D15) of fermentation, respectively, and mixed in the ratio of 1:1 (w:v), and homogenates were made in a high-speed tissue masher to be analyzed in the subsequent analysis. The standard of 0.898 g/mL methyl butyrate was prepared into a solution with a concentration of 89.8 mg/L, and stored in the refrigerator at −4°C for spare parts (Li et al., 2021).

Five grams of the homogenate sample was weighed into a 15 mL SPME headspace vial, 20 μL of methyl butyrate standard solution was added and the cap was quickly closed, and the extraction was carried out by inserting a 100 μm PDMS fibre tip in a water bath at 60°C for 45 min, and then inserting the extraction tip into the gas-phase injection port and resolving the sample for 3 min at 230°C after completion of the extraction (Yang et al., 2022).

2.2.2 GC-MS conditions

GC-MS analyzed the smelly wax gourd using GC-MS 7890A/5977A (Agilent Technologies Inc., Palo Alto, CA, United States), equipped with a silica capillary column (Vocol, 60 m × 0.32 mm × 1.8 μm thickness, Supelco, Inc., Bellefonte, PA, United States). The initial oven temperature was set at 32°C and kept warm for 5 min. The temperature was then increased to 45°C at a rate of 3°C/min and kept warm for 1 min for the first stage, then increased to 230°C at a rate of 5°C/min and kept warm for 20 min. The mass spectral ionization mode was EI, with a spectral scanning range of 45–550 *m*/*z*. The mass spectral transfer line temperature was 230°C, and the ion source temperature was 220°C. Volatile flavor compounds were analyzed semi-quantitatively by comparison to the chromatographic peak areas of internal standards.

2.3 Animal experimental design

2.3.1 Ethics statement

All experimental procedures and animal care adhered to the guidelines set by the "Experimental Animal Care and Use Guide" from the Experimental Animal Center of Ningbo University, which is affiliated with the Experimental Animal Sharing Service Platform of Zhejiang Province. The animal study received approval from the same center, under the approval number SYXK (Zhejiang 20080110).

2.3.2 Grouping and feeding

After 1 week of adaptation, the 15 4 weeks-old ICR male mice (weighing approximately 20 ± 2 g) were randomly divided into 3 groups, A.SWG group, control group (CONT) and SWG group (5 mice per group). To establish a pseudo-germ-free mouse model (Heimesaat et al., 2011; Brun et al., 2013), mice in A.SWG group first received 1 week of antibiotic pretreatment (comprising vancomycin 50 mg/kg, neomycin 100 mg/kg, metronidazole 100 mg/kg, and ampicillin 100 mg/kg; 100 μL per mouse per 12 h) by oral gavage and then they were given the smelly wax gourd mixture (20 g/kg/day) for 4 weeks (Haug et al., 2011). Mice in the SWG group were given saline for one week, then they were gavaged the smelly wax gourd mixture (20 g/kg/day) for 4 weeks. While CONT group mice were given the same amount of saline by gavage for 5 weeks.

Based on the established experiment (the smelly wax gourd treatment), the microbiota transplantation was carried out. After 4 weeks of intervention, daily collection of their feces under aseptic conditions was initiated, and a portion of the feces was used for gavage colonization into recipient mice, while another portion of the feces was cultured in anaerobic medium and then gavage colonized into recipient mice. The specific operation method is as follows:

Ten ICR mice were randomly divided into two groups with 5 mice in each group. One group received fecal microbiota transplantation (FMT) from the SWG group by gavage for 4 weeks (200 mg/day, 200 μL per mouse). The other group received culture microbiota transplantation (CMT) from the SWG group by gavage for 4 weeks (200 mg/day, 200 μL per mouse).

2.3.2.1 Fecal microbiota transplantation

The feces were resuspended in sterile saline at 100 mg/mL, and the solution was strongly mixed with Vortex-Genie for 10 s, and then centrifuged at 1,200 rpm for 3 min. The supernatant was collected and gavage into recipient mice. This process is guaranteed to be completed within 10 min to prevent changes in bacterial composition.

2.3.2.2 Fecal culture microbiota transplantation

The feces were cultured in the anaerobic medium at 100 mg/mL for 3 days and stored at −80°C. The feces were removed before each gavage and centrifuged at 1,200 rpm for 3 min. Then the culture medium was removed and an equal amount of normal saline was added. The solution was mixed vigorously for 10 s using a desktop vortex meter, and the supernatant was collected and gavage into recipient mice.

2.4 Total DNA extraction, PCR, and sequencing

Use the E.ZN.A. ® Fecal DNA kits extracted DNA from samples according to the manufacturer's instructions, and the number and concentration of extracted DNA was measured by Thermo NanoDrop 2000 C (Thermo Fisher Scientific, MA, United States). The reagent, designed to uncover DNA from trace amounts of sample, is effective for the preparation of the DNA of most bacteria. Nuclear-free water was used for blank. Total DNA were eluted in 50 μL elution buffer, stored at −80°C, and subjected to PCR testing by LC Sciences Co., Ltd. in Zhejiang (Hangzhou China). The V3–V4 region of the 16S rRNA gene was amplified with slightly modified versions of primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5'-GGACTACHVGGGTWTCTAAT-3′). The 5′ ends of the primers were tagged with specific barcode per sample and universal sequencing primers. The total volume of PCR amplification was 25 μL reaction mixture, containing 25 ng template DNA, 12.5 μL PCR Premix Ex Taq TM Hot Start Version (12.5 μL) (Takara Biotechnology Co., Ltd., Dalian, Liaoning, China), each primer 0.1 μM, PCR-grade water adjusted volume. PCR conditions for amplified nucleus 16S fragment: 98°C initial denaturation 30 s, 98°C denaturation 10 s, 54°C annealing 30 s, 72°C stretching 45 s, final stretching 10 min, total 35 cycles (Zhang et al., 2018).

The PCR products were verified using 2% agarose gel electrophoresis and purified with a QIAquick PCR Purification Kit. Then purified PCR products were used to establish the amplicon library and the amplicon library sequenced by MiSeq Illumina 2×300 paired-end sequencing on an Illumina MiSeq platform (Illumina, San Diego, CA, United States) by LC Sciences Co., Ltd. (Hangzhou, Zhejiang, China).

2.5 Data analysis

As previously described (Lu et al., 2017), QIIME (version 1.8.0) (Caporaso et al., 2010), and PEAR (version 0.9.6) (Zhang et al., 2014) were used to filter the raw data as follows: (1) merging the reads that were overlapped by more than 10 bp, and removing the reads that could not be merged; (2) data belonging to each sample were identified through the barcode sequence; (3) when the average quality score on the 10 bp sliding window was below 20, the reads were truncated; and (4) the low-complexity sequences were also discarded by PRINDEQ (version 0.20.4) (Schmieder and Edwards, 2011). Then, Uchime (version 4.2.40) (Edgar et al., 2011) was used to identify and discard the chimera sequences. Uclust (version 1.1.579) assigns sequences with similarity ≥97% to the same Operational Taxonomic Unit (OTU) (Edgar, 2010). Representative sequences were chosen for each OTU, and taxonomic data were then assigned to each representative sequence using the RDP (Ribosomal Database Project) classifier (Wang et al., 2007). The OTUs abundance information is normalized using the sequence number criterion corresponding to the sample with the lowest sequence; the diversity complexity of a single sample species is analyzed using the four indices of Alpha diversity Chao 1, Observed species, Shannon, and Simpson, All indices in the sample were calculated using mothur (version 1.30.1) (Schloss et al., 2009). Beta diversity analysis was used to assess differences in sample species complexity. Calculation of beta diversity using Principal Coordinate (PCoA) and FastTree cluster analysis (2.1.3) (Price et al., 2010) Muscle (version 3.8.31) (Edgar, 2004), and the vegan package in R (version 3.2).

Values that align with a healthy distribution, determined using the standard deviation of the average, were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. Data not fitting this distribution were analyzed using the Mann–Whitney test. A p-value of less than 0.05 indicates a statistically significant difference. Bar charts were created using GraphPad Prism8. To identify significant differences in the relative abundance of microbial groups between groups at the species level, we employed the Linear Discriminant Analysis Effect Size (LEfSe) method.

2.6 Accession numbers

The following sequences have been archived in the NCBI Sequence Read Archive database. Those associated with accession number PRJNA1030292 pertain to the microbial diversity observed during cucurbit fermentation. Meanwhile, data linked to accession number PRJNA1030303 are representative of the intestinal microorganisms in mice subjected to an odorous cucurbit-based diet.

3 Results

3.1 Changes of flavor substances and microbiota of smelly wax gourd

3.1.1 Volatile compounds of smelly wax gourd

The volatile compounds present in the smelly wax gourd were analyzed using GC-MS, as detailed in Supplementary Table S1. This analysis identified a total of 86 volatile constituents across four different samples. These constituents comprised 15 acids, 20 alcohols, 8 aldehydes, 5 ketones, 7 phenols, 16 hydrocarbons, 4 esters, 2 benzenes, and 9 other compounds. Interestingly, the number of volatile compounds first decreased and then increased during the fermentation process.

Throughout fermentation, alcohol and phenol concentrations decreased until the fifth day. Conversely, the concentrations of acids, aldehydes, ketones, and hydrocarbons declined until the 10th day. Acids consistently had the highest concentration. During fermentation, the proportion of acid in the total flavor substances exhibited a parabolic trend, with percentages of 88.42 ± 0.35%, 87.21 ± 0.03%, 90.33 ± 0.02%, and 87.12 ± 0.28% at different stages. Butanoic acid was always the most prevalent acid. Alcohol content displayed an inverted parabolic trend with fermentation time, registering at 5.3 ± 0.34%, 5.11 ± 0.03%, 1.55 ± 0.02%, and 2.47 ± 0.28%. Among aldehydes, benzaldehyde, 2,5-dimethyl- consistently had the highest concentration at each fermentation stage, with values of 1.11 ± 0.19%, 1.92 ± 0.01%, 1.45 ± 0.09%, and 0.98 ± 0.09%. Notably, on the 10th day of fermentation, compounds like L-lactic acid (6.60 ± 0.01%), 2-methyl-2-Butanedioic acid (0.48 ± 0.01%), and 3-Ethylcyclopentanone (0.77 ± 0.05%) emerged. Benzene derivatives, specifically p-xylene and ethylbenzene, were only detected on the 10th and 15th days of fermentation. Further details can be found in Figure 1 and Supplementary Table S1.

3.1.2 Diversity of microbiota during the fermentation of wax gourd

The alpha diversity of the smelly wax gourd index at different fermentation times was shown in Table 1. The coverage rate of each sample reached 1.00, indicating that the sequences in the sample library were measured. At the early stage of fermentation (0 day), the OTU number, Chao1, Shannon, and Simpson indices were highest, which were 251, 257.21, 4.71, and 0.88, respectively, indicating that the community richness and diversity of smelly wax gourd at 0d was the highest. On the contrary, 5 days was the lowest.

Beta diversity reflects diversity differences among samples. The overall structure of the microbiota at four fermentation periods of smelly wax gourd was analyzed by weighted UniFrac PCoA. As shown in Figure 2, the microbial structure of smelly wax gourd at 5 days and 10 days fermentation was close, while compared with fermentation at 0 day, fermentation at 15 days had the most significant difference in microflora structure, indicating the structure of the microbial community changes during the fermentation of smelly wax gourd.

3.1.3 Microbial shifts based on taxon-based analysis

As shown in Figure 3, at the phylum level, the bacteria community of the smelly wax gourd was mainly composed of Firmicutes, Proteobacteria, Bacteroidetes, Fusobacteria, Synergistetes, and Spirochaetes. The six phyla accounted for 91.48, 93.78, 94.50, and 92.37% of the entire sequence of each group in four fermentation periods, respectively. Firmicutes were the most abundant phylum in all samples, accounting for more than 67% of the total sequence of each group. Their abundance increased with the prolonging of fermentation time, reaching the highest (79.02%) at D10 of fermentation, then decreased to 70.92%, while the abundance of Proteobacteria increased to 7.96% at D15.

At the species level, *Lactobacillus fermentum*, *Streptococcus equinus*, *Fusobacterium perfoetens*, *Weissella confuse*, and *Lactobacillus plantarum* were the dominant bacteria during the fermentation process of the wax gourd. The abundance of *Lactobacillus fermentum* was the highest (37.28%) at the D0 stage, then its abundance decreased to 22.46, 14.55, and 7.55% at D5, D10, and D15, respectively. The abundance of *Streptococcus equinus* increased significantly from 16.46% (D0) to 42.30% (D5) and then decreased from 46.98% (D10) to 21.76% (D15). The abundance of *Lactobacillus plantarum* was less than 7% in the first three fermentation stages but increased significantly to 27.68% in the D15 stage (Figure 4 and Supplementary Table S2).

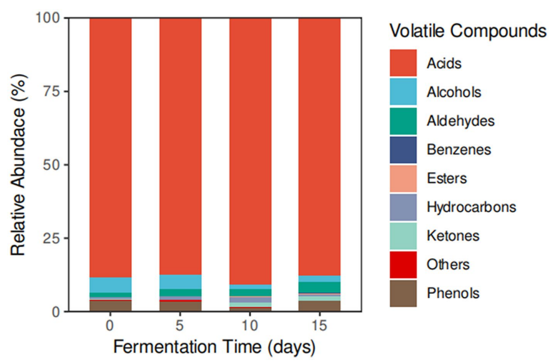

FIGURE 1
Differences in flavor content during the fermentation cycle.

3.1.4 The correlation between the bacteria and the flavor substance of smelly wax gourd

Based on the correlation analysis method, the correlation diagram of microbial species and volatile substance composition of smelly wax gourd was constructed (Figure 5). Among them, *Streptococcus equinus* and *Weissella confusa* were positively correlated with organic acid. In contrast, *Lactococcus lactis*, *Pediococcus pentosaceus*, *Aminobacterium colombiense*, *M. anaerophila*, *C. algeriensis*, *Oceanimonas smirnovii*, and *Halocella cellulosilytica* were positively correlated with organic acid, which was the dominant flavor substance in the smelly wax gourd.

Furthermore, significant correlations were observed between specific bacteria and various organic acids. Three bacteria showed significant positive correlations with acetic acid, while 11 exhibited negative correlations. Similarly, three bacteria displayed notable positive correlations with butyric acid, with 10 demonstrating negative associations. Additionally, 13 bacteria showed a significant positive correlation with lactic acid and seven showed a negative correlation. Similarly, two bacteria exhibited strong positive correlations with octanoic acid, whereas six had negative correlations. Two bacteria also displayed significant positive correlations with pentanoic acid, with 12 exhibiting negative associations, and two bacteria showed significant positive correlations with hexanoic acid, while 12 had negative correlations. Finally, five bacteria were notably positively correlated with 2,3-butanediol, while 18 showed negative correlations (Figure 5).

3.2 Effects of smelly wax gourd diet on gut microbiota in mice

3.2.1 Diversity of gut microbiota in mice

After 5 weeks of feeding, we sequenced fecal samples to elucidate the contribution of the smelly wax gourd treatment, fecal microbiota transplantation, and culture microbiota transplantation on the gut microbiota structure. The Chao1 observed species, Shannon, and Simpson indices were all alpha diversity indexes. Thereinto, the Chao1 and observed species indices represent community richness while others represent community diversity. In this study, the smelly wax gourd treatment increased community richness and diversity in mice, and the community richness and diversity in the SWG group were higher than that in A.SWG group (Table 2).

Beta diversity reflects diversity differences among samples. Weighted UniFrac PCoA analyzed the gut microbiota's overall structure in three mice groups. As shown in Figure 6, the smelly wax gourd treatment caused the deviation of fecal microflora from the CONT group, and the deviation from the control group was furthest in the SWG group compared with the A.SWG group, indicating that diet has some effect on the gut microbiota compared to antibiotic treatment.

TABLE 1 Microbial α-diversity index in the fermentation of wax gourd.

Fermented time	Cover	OTU	Chao1	Shannon	Simpson
0 day	1.00	251	257.21	4.71	0.88
5 days	1.00	181	182.03	3.96	0.82
10 days	1.00	229	235.92	4.05	0.81
15 days	1.00	196	197.31	4.63	0.88

3.2.2 Microbial shifts based on taxon-based analysis

Based on the principle of relative abundance greater than 2%, a total of 10 dominant species were selected from the three groups, among which *Helicobacter ganmani* and *H. canicola* belong to Proteobacteria, *Muribaculum intestinale*, *Prevotellamassilia timonensis*, and *Alistipes putredinis* belong to Bacteroidetes, *Lactobacillus animalis*, *Kineothrix alysoides*, *Lactobacillus reuteri*, and *Clostridium indolis* belong to the Firmicutes, compared with the CONT group, the abundance of *H. ganmani*, *H. canicola*, *L. animalis*, *Lactobacillus gasseri*, and *L. reuteri* decreased in the SWG group. In contrast, the abundance of *M. intestinale*, *P. timonensis*, *A. putredinis*, *K. alysoides*, and *C. indolis* increased. The variation trend of the A.SWG group was consistent with that of the SWG group. In addition, the supplement of smelly wax gourd led to the disappearance of *H. ganmani*, and *H. canicola* (Figure 7 and Supplementary Table S3).

LEfSe analysis identified other representative species of each group as biomarkers to distinguish different groups. According to our LDA of the effective size, the abundances of many taxa were significantly different among the CONT, SWG, and A.SWG groups (LDA score >3.0; Figure 8). There were 55 biomarkers for species levels, including 16 species with high abundance in the CONT group, 24 species with high abundance in the SWG group, and 15 species with high abundance in the A.SWG group. Supplement of smelly wax gourd led to the disappearance of *Bacteroides vulgatus*, *B. finegoldii*, *H. canicola*, *H. ganmani*, *Lyticum flagellatum* and *Mailhella massiliensis*, but also adding some new species, including *Clostridium perfringens*, *M. intestini*, *Caecibacterium sporoformans*, *C. oryzae*, *Culturomica massiliensis*, *H. hepaticus*, *P. merdae*, *Ruminococcus flavefaciens*, *R-lactaris*, *Sphingopyxis terrae*, *R. champanellensis* and *R. albus*. In addition, a supplement of smelly wax gourd significantly decreased the abundance of *L. gasseri* (Supplementary Table S4).

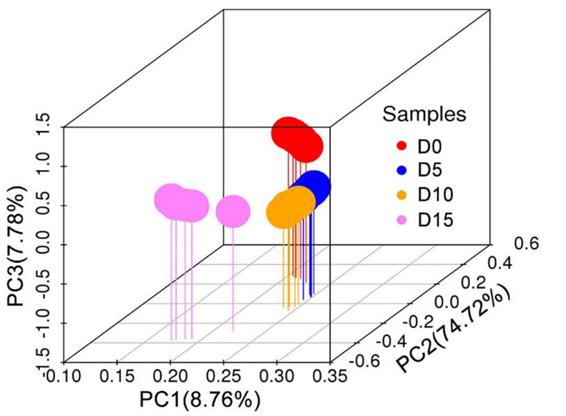

FIGURE 2
Weighted UniFrac PCoA analysis of microbiota.

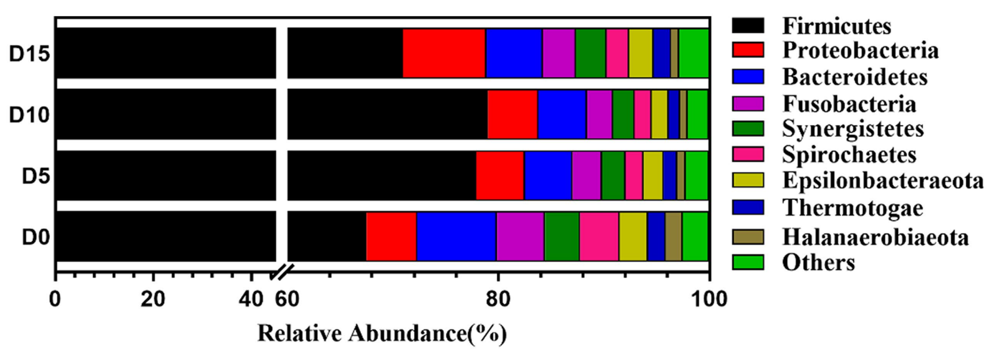

FIGURE 3
Changes on the phylum level of microbiota.

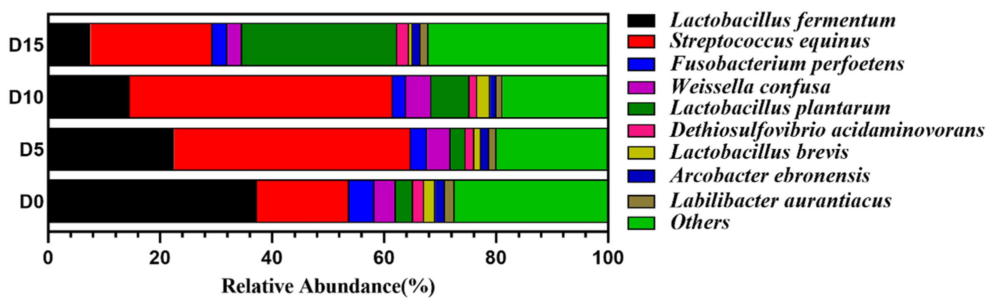

FIGURE 4
Changes on the species level of microbiota.

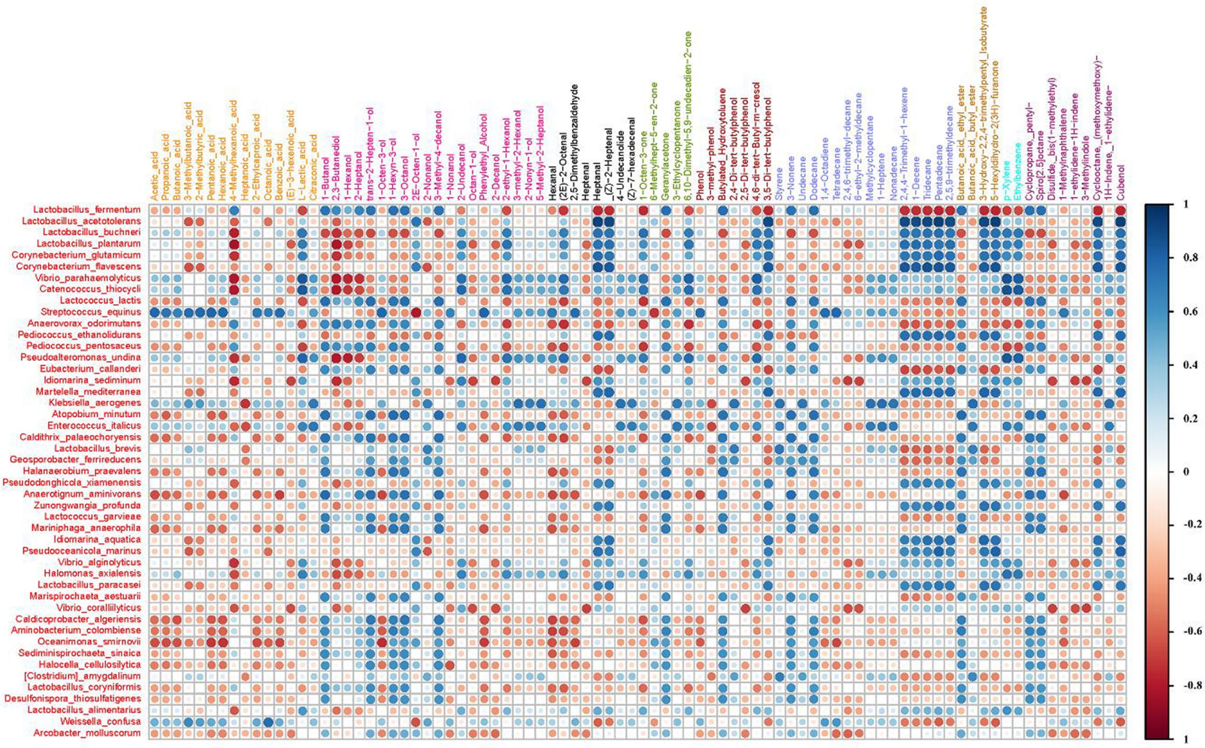

FIGURE 5
The corrplot of 47 species and 86 volatile substances. Line: species, column: volatile substances, blue is positive correlation, red is negative correlation. The larger the bubble, the greater the correlation, and vice versa.

TABLE 2 Alpha diversity index of the mouse microbiota.

Groups	Coverage	Observed	Chao1	Shannon	Simpson
CONT	0.98	411.67	610.84	5.14	0.89
SWG	0.98	684.00	894.41	7.52	0.99
A.SWG	0.98	521.67	719.05	5.80	0.83

3.3 Effects of FMT and CMT on gut microbiota in mice

3.3.1 Diversity of gut microbiota in mice

The Alpha diversity coverage of the two groups achieved 0.98, indicating that sample sequencing results can reflect the real situation of samples. The diversity results showed that the CMT group's Observed index and Chao1 index were lower than the FMT group's. In contrast, the Shannon index and Simpson index were similar, indicating that the difference between the two transplantation methods was reflected in the bacterial abundance, while that of the FMT group was higher (Table 3).

As shown in Figure 9, the gut microbiota of FMT and CMT was a deviation from each other.

3.3.2 Microbial shifts based on taxon-based analysis

Based on the principle of relative abundance greater than 2%, a total of 10 dominant species were selected from the three groups, among which *M. intestinale*, *Prevotellamassilia timonensis*, *Prevotella dentalis*, *Bacteroides acidifaciens*, and *Bacteroides stercorirosoris* belong to Bacteroidetes, *L. animalis*, *L. gasseri*, *Faecalibaculum rodentium*, and *L. reuteri* belong to the Firmicutes, *Helicobacter typhlonius* belong to Proteobacteria. The abundance of *L. animalis* in the CMT group was 37.17%, was higher than that in the FMT group. The abundance of *M. intestinale* was higher in the CMT group (16.19%), while the abundance of *L. gasseri* was higher in the FMT group (11.68%). In addition, the abundance of *P. dentalis*, *Bacteroides acidifaciens*, *F. rodentium*, and *Bacteroides stercorirosoris* in the FMT group was higher than that in the CMT group. In comparison the abundance of *L. reuteri*, *P. timonensis*, and *H. typhlonius* in the FMT group was lower than that in the CMT group (Figure 10 and Supplementary Table S5).

LEfSe analysis (LDA score >3.0) based on the gut microbiota of the three groups of mice showed that there were 10 biomarkers at the species level, which showed that the abundance of *P. dentalis*, *P. merdae*, *M. schaedleri*, *H. canicola*, *F. rodentium*, and *B. stercorirosoris* was higher in the FMT group. However, the abundance of *Prevotella oralis*, *H. hepatae*, *Enterorhabdus caecimuris*, and *Adlercreutzia muris* was higher in the CMT group, among which *P. dentalis*, *P. merdae*, and *B. stercorirosoris* were not detected in the CMT group (Figure 11 and Supplementary Table S6).

4 Discussion

The flavor composition of fermented food is multifaceted. First of all, the flavor should derived from various flavoring substances contained in the raw vegetables themselves, which are still retained in the raw materials after fermentation, such as various intermediate products of the physiological metabolism of vegetables, as well as the trace volatile flavor substances contained in the vegetables themselves, which have important contributions to flavor. Secondly, the flavor should be derived from microbial fermentation. Various microorganisms in the system decompose the fermentable carbohydrates, proteins, and other substances in the raw materials to supply their growth and produce many substances with unique flavors. Thirdly, the formation of the flavor of kimchi is the result of the above factors. For example, organic acids contained in the raw materials may combine with alcohols produced by microorganisms to form aromatic esters (Kim et al., 2012).

Butyric acid, also known as caseic acid, has the odor of rotten butter, and other unpleasant odors, such as caproic acid and caprylic acid, form the odor of smelly wax gourd. In contrast 2-methyl butyric acid exists in the aroma components of various fruits, playing a particular role in enhancing aroma. The concentration of 2, 3-butanediol, hexanol, 1-nonyl alcohol, and octyl alcohol was the highest at the D5 phase. The concentration of acetaldehyde and anti-2-octenal was the highest at the D10 phase. These alcohols and aldehydes had high concentrations in pickled wax gourd and pickled amaranth stem (Wu Z.-F. et al., 2015; Wu et al., 2016b). Among the phenolic substances, p-methyl phenol has the highest concentration in the D5 phase, while phenol has the highest concentration in the D10 phase. P-methyl phenol has a unique odor, while phenol has the phenolic odor of plastic and rubber, which enhances the odor of brine. Phenols are also the main aroma components of Wang Zhihe stinky tofu (Liu et al., 2012). The peculiar smell of bamboo shoots during the fermentation of traditional bamboo shoots in Taiwan was caused by p-methyl phenol (Steinhaus and Schieberle, 2005; Selvaraj et al., 2013; Saito et al., 2018). Tyrosine is bamboo shoots' primary free amino acid, which can be decomposed into p-methyl phenol (Tang et al., 2018). Since amaranth root and bamboo shoot are both high-fiber plants, it is speculated that p-methyl phenol may come from fermentation products of amaranth root. The structural formula of tyrosine contains phenol, so it is speculated that phenol may come

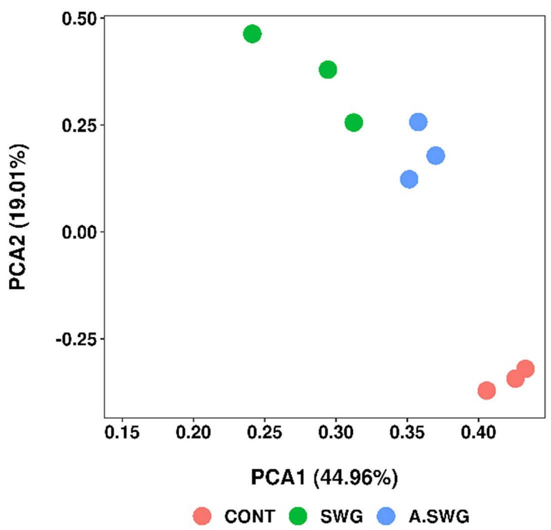

FIGURE 6
Weighted UniFrac PCoA analysis of gut microbiota.

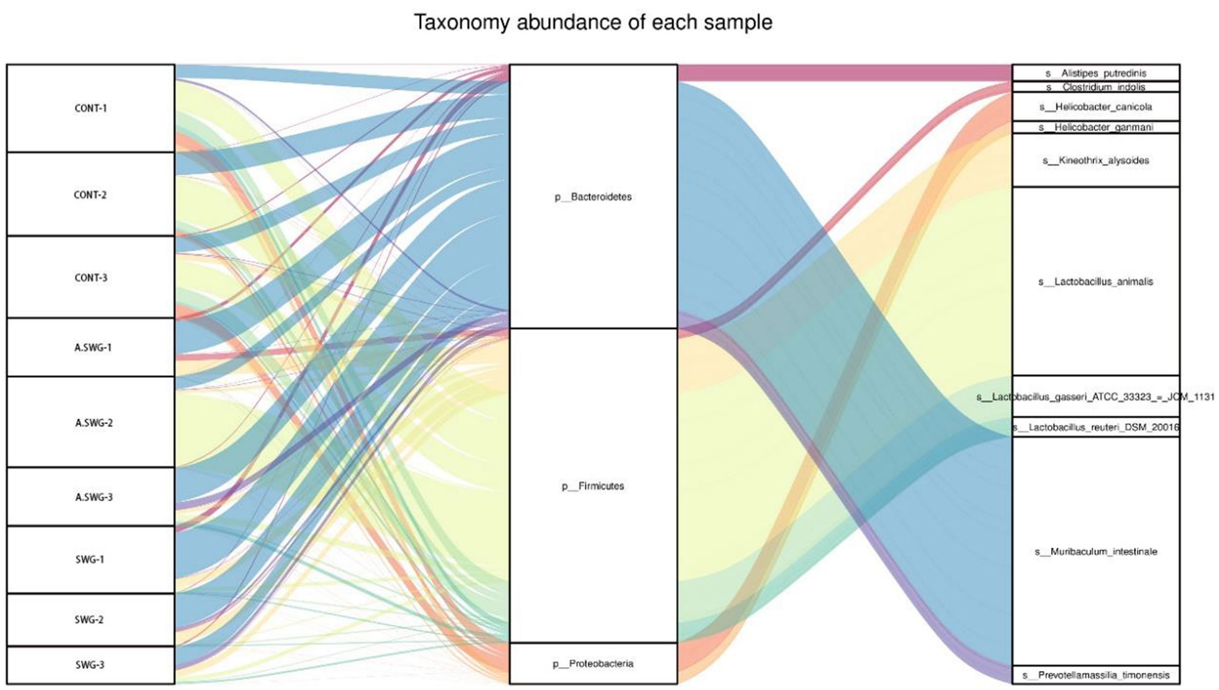

FIGURE 7
Variation trend on the phylum and species level of rectum microbiota.

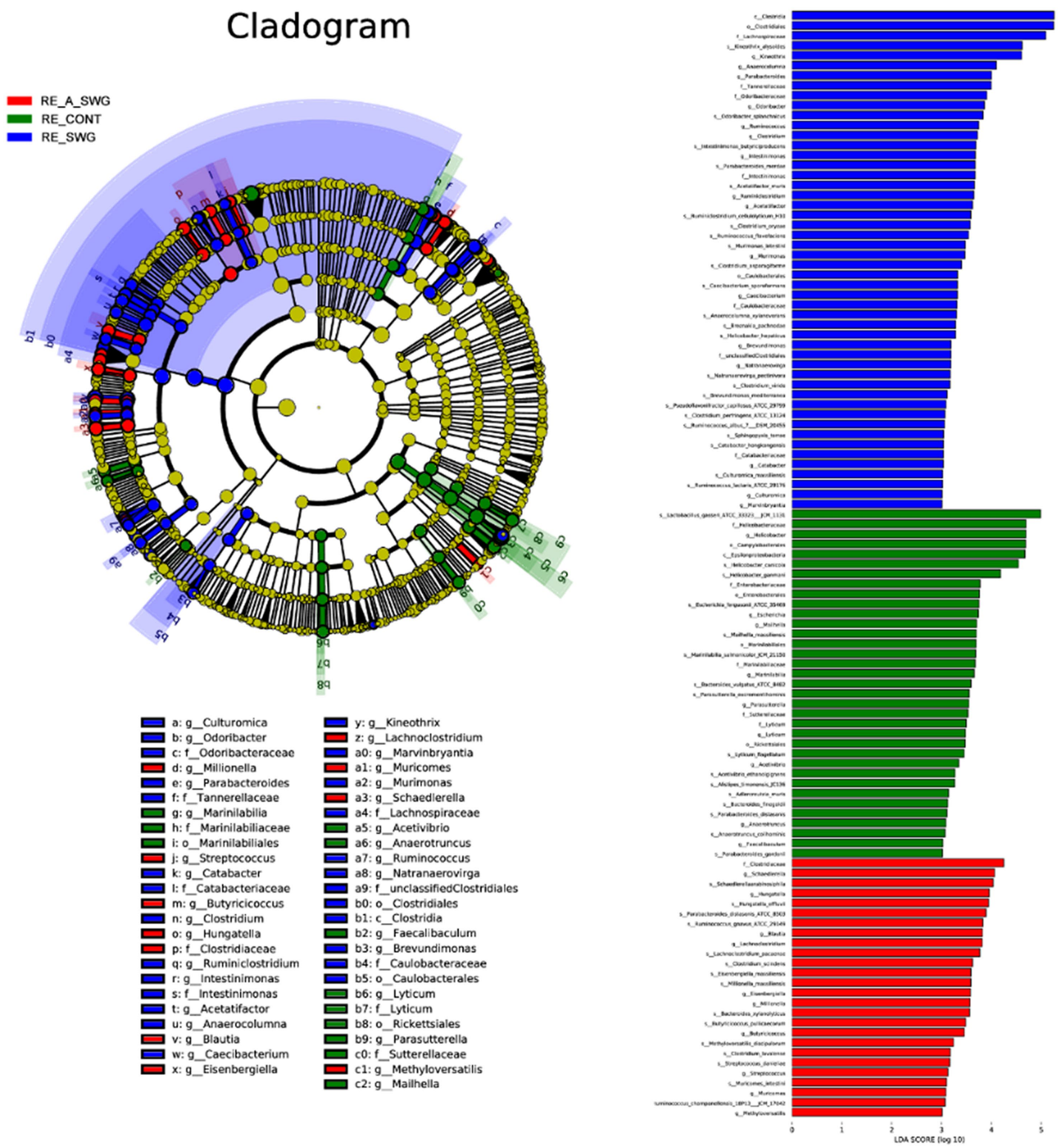

FIGURE 8
LEfSe analysis of gut microbiota.

TABLE 3 The modulation of the gut microbiota structure in mice.

Groups	Coverage	Observed	Chao1	Shannon	Simpson
FMT	0.98	416.33	653.12	4.72	0.83
CMT	0.98	379.00	571.72	4.71	0.83

from the decomposition of tyrosine, and there may be a specific correlation between phenol and p-methyl phenol (Doerner et al., 2009a,b; Franchi et al., 2018; Saito et al., 2018). In addition, volatile compounds such as methylcyclopentane, 1,4-octadiene, and butyl butyrate were also detected. To sum up, the flavoring substances in the D10 stage were the most abundant, and there were no harmful volatile substances, therefore, this may be considered the best time to consume it.

Lactobacillus is a gram-positive and anaerobic fermentation bacterium in various fermented foods (Holzapfel et al., 2001). In many cases, these *Lactobacillus* were also used as starter cultures for industrial and artisanal foods because they help maintain the flavor and texture of fermented foods. Their primary function is to convert the fermentation of sugars in raw materials into lactic acid. The production of antimicrobial peptides, exopolysaccharides, and a variety of other metabolites are their important properties isolated *L. fermentum* with potential probiotics from traditional dairy products (Ross et al., 2002; Tamime, 2002; Bao et al., 2010). The study found that *L. fermentum* has a high tolerance to simulated gastric intestinal fluid and bile salt, and broad antibacterial activity, with the most significant potential of probiotics. In addition, *L. plantarum* is versatile, widely found in the fermentation of dairy products, meat, and many vegetables, and is capable of surviving gastric transport and colonizing the intestines of humans and other mammals (de Vries et al., 2006). Of the hundreds of reports in the 20th century about the safe use of *L. plantarum*, only a few suggested that certain *L. plantarum* might be involved in infection.

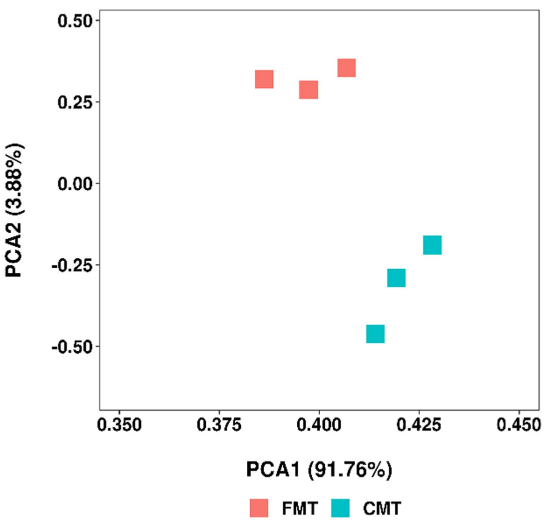

FIGURE 9
Weighted UniFrac PCoA analysis of gut microbiota.

Lactobacillus acetotolerans, *Corynebacterium flavescens*, and *Pseudooceanicola marinus* had high positive correlation coefficients with decanolactone, cubenol and pentadecane. *Lactobacillus acidophilus* and *Corynebacterium flavus* had high positive correlation coefficients with 2-Heptenaldehyde, decene, 2,4,4-trimethyl-1-hexene, 2,5,9-trimethyl-decane, 3, 5-tert-butylphenol, 2,2,4-trimethyl-1,3-pentanediol mono-isobutyrate, Cyclooctane, heptanal, and tridecane. The negative correlation coefficient was higher between *L. plantarum* and 2, 3-butanediol and 4-methyl-acetic acid. The "smelly" taste of the smelly wax gourd was mainly derived from the odor of butyric acid, valeric acid, caproic acid, octanoic acid, p-methyl phenol, and other compounds. At the same time, the abundance of *C. algeriensis*, *M. anaerophila*, *S. equinus*, and *W. confuse* was significantly correlated with 4 of the above 5 acids, indicating that these 4 bacteria might contribute more to the "smelly" taste of the smelly wax gourd.

It has been reported that *L. gasseri* in the upper intestine can play a role in blood glucose regulation by affecting the ACSL3-dependent fatty acid sensing pathway (Bauer et al., 2018), *L. reuteri* has been reported to exist naturally in the intestines of almost all vertebrates and mammals. It has a strong adhesion ability to the intestinal mucosa, which can improve the distribution of intestinal flora, antagonize the colonization of harmful bacteria, and avoid intestinal diseases (Yokota et al., 2018). In this study, the abundance of *L. gasseri* and *L. reuteri* were decreased in SWG and A.SWG groups. Supplementation with smelly wax gourd caused the disappearance of *H. ganmani* and *H. canicola*. In addition, the abundance of *Ruminococcus* was increased in SWG and A.SWG groups, which could produce short-chain fatty acids. Fecal microbiota transplantation increased the abundance of short-chain fatty acid bacteria such as *B. acidoides*, *Bifidobacteria*, and

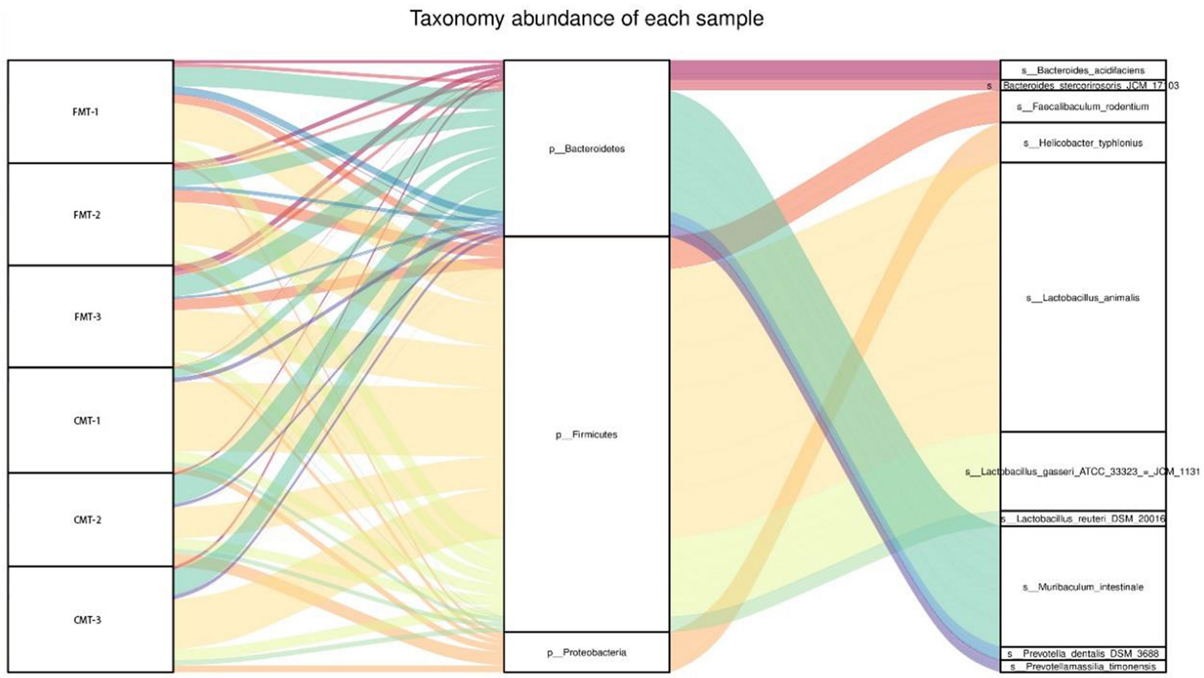

FIGURE 10
Variation trend on the phylum and species level of rectum microbiota.

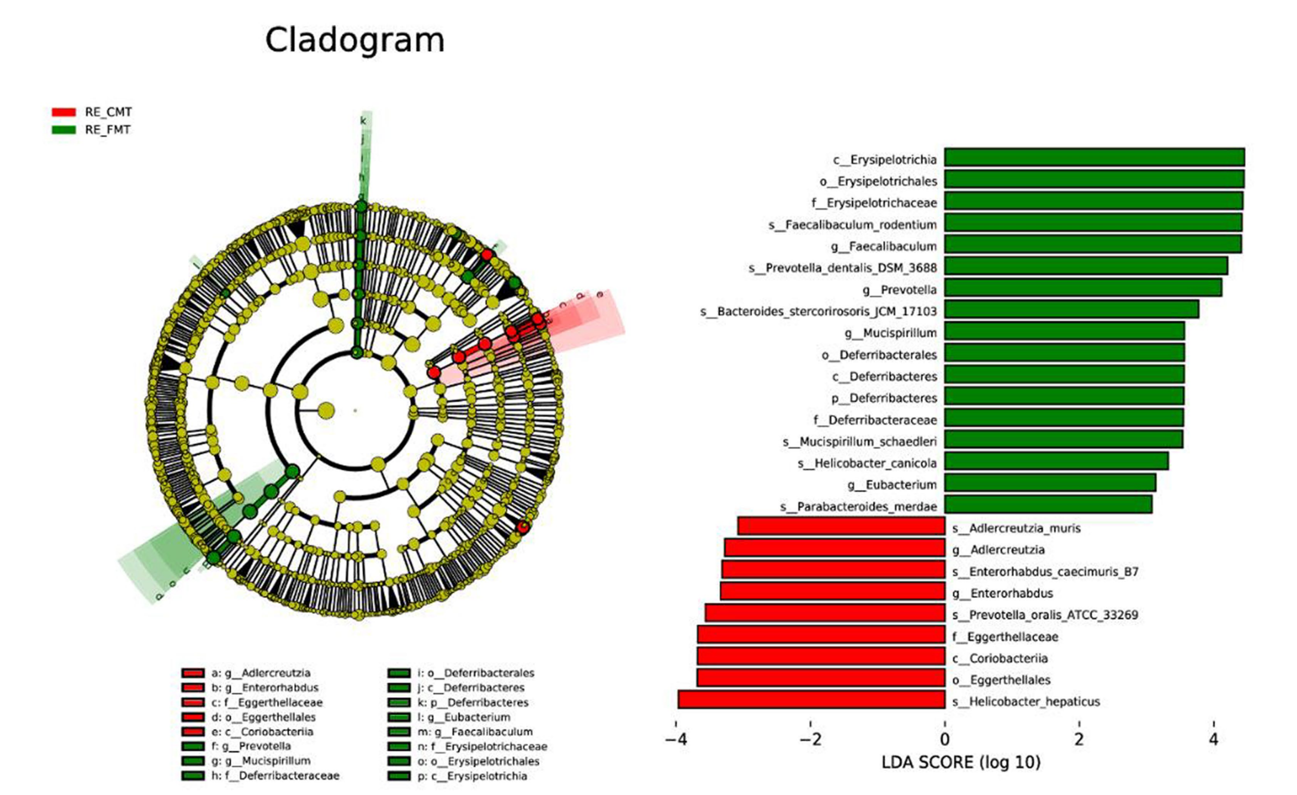

FIGURE 11
LEfSe analysis of gut microbiome.

Faecalibaculum. In contrast some *Helicobacter* such as *H. typhlonius*, *H. canicola*, and *H. ganmani*, were present in both the FMT and CMT groups. In addition, compared with the CMT group, the abundance of *L. gasseri*, *F. rodentium*, *B. acidoides*, and *B. stercorirosoris* in the FMT group was higher, while the abundance of *L. animalis*, *M. intestinale*, *L. reuteri*, and *H. typhlonius* was lower.

5 Conclusion

In our study of stinky winter melon fermentation, it was found that the main flavor compounds were acids, with 2-methylbutyric acid providing the melon with an attractive fruity aroma. The peak abundance and best flavor were observed at 10 days of fermentation (D10), with key microorganisms including *Lactobacillus fermentum*, *Streptococcus equinus*, *Clostridium perfringens*, *Mariniphaga anaerophila*, and *Lactobacillus brevis*. *Lactobacillus* displayed potential probiotic effects and was most abundant at the initial stage (D0) of fermentation, while *Streptococcus equinus*, *Mariniphaga anaerophila*, and *Caldicoprobacter algeriensis* were linked to butyrate production. *Caldicoprobacter algeriensis* notably correlated with butyric, valeric, capric, and caproic acids. For optimal consumption, stinky winter melon fermented for 10 days preserved rich flavor compounds while minimizing the loss of lactic acid bacteria. Additionally, our investigation into fecal bacteria transplantation enhanced the diversity of intestinal flora in mice, particularly in the fecal transplantation group, showcasing the potential benefits of this approach and revealing distinct microbial genera such as *Lactobacillus*, *Bifidobacterium*, *Mycobacterium*, and *Spirochetes* compared to fecal culture transplantation. These findings offer insights into the culinary and probiotic potential of stinky winter melon and highlight the promise of fecal transplantation for promoting gut microbiota diversity.

Data availability statement

The data presented in the study are deposited in the NCBI repository, accession number PRJNA1030292 and PRJNA1030303.

Ethics statement

The animal study was approved by Laboratory Animal Center of Zhejiang Academy of Medical Sciences (Hangzhou, Zhejiang). The certificate number of MICE is SYXK Chol 2014-0001, No. 1710300006. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

NW: Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. WB: Conceptualization, Data curation, Methodology, Writing – original draft. MG: Methodology, Writing – review & editing. JX:

Conceptualization, Funding acquisition, Writing – review & editing. JH: Conceptualization, Methodology, Writing – review & editing. CL: Conceptualization, Methodology, Writing – review & editing. TM: Conceptualization, Methodology, Writing – review & editing. JZ: Conceptualization, Data curation, Writing – review & editing. WZ: Methodology, Writing – review & editing. XS: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

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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/fsufs.2024.1314537/full#supplementary-material

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Factors associated with food security in Depok City, Indonesia during the COVID-19 pandemic: a cross-sectional study

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Introduction: The COVID-19 pandemic has considerably impacted food insecurity. The study aimed to assess the impact of the COVID-19 pandemic on food security and its related factors.

Methods: The study employed a cross-sectional design, utilizing face-to-face interviews to collect data from 140 selected households using Simple Random Sampling. The study assessed the independent variables: socio-economic characteristics including parental age, maternal education, employment status, family type, number of biological children, family income, and food expenditure, with the food security level as the dependent variable. Family food security was determined through a modified version of the United States Family Food Security Survey Module-USDA, utilizing 15 of the original 18 questions. Binary Logistic Regression Analysis was employed to identify the dominant factor related to household food insecurity.

Results and discussion: A significant proportion of families (66.4%) experienced food insecurity, where severe hunger was the most prevalent problem (25%). Parental age and employment, maternal education, family composition, number of biological children, income, total food expenditure, government social assistance, the impact of COVID-19 on the family, and percentage of food consumption to total spending ($p < 0.001$) significantly influenced family food security levels. The Binary Logistic Regression Analysis revealed that mothers over the age of 44 had a 9.9-fold increased likelihood of experiencing food insecurity compared to those under 35. Mothers with lower levels of education exhibited a 6.9-fold higher probability of food insecurity than those with moderate education. Families incorporating non-food expenses demonstrated a 23-fold greater risk of food insecurity than those without such expenditures. Families who received government social assistance were more at risk of food insecurity than those without such support. Thus, government social assistance played a critical role as a primary determinant factor for food security during the pandemic, with a probability of 45 times.

Conclusion: Food security levels were influenced by various factors, including parental age and employment, maternal education, family composition, number of children, income, total food expenditure, government social assistance, and the impact of COVID-19 on the family. Government social assistance was essential during COVID-19 to enhance food security.

KEYWORDS

COVID-19, food security, food insecurity, determinants, government social assistance

1 Introduction

The COVID-19 pandemic caused high food price inflation in all countries at the retail level. It reflects prolonged supply disruptions caused by social distancing measures, currency devaluation, and other factors (Bairagi et al., 2022; Muller et al., 2022). The increasing levels of food insecurity pose a threat to previous developmental achievements. Furthermore, the spikes in agricultural commodity prices were due to high demand, weather uncertainties, macroeconomic conditions, and disruptions in the supply chain. As retail prices and income declined, the family was compelled to reduce the quantity and quality of food consumption, which impacted food insecurity (FAO, 2011).

The specific challenge faced in metropolitan areas during the pandemic was food insecurity due to increased food prices (47.1%), reduced market availability (41.4%), and also challenges in accessing traditional foods (Shafiee et al., 2023). In March 2020, household food insecurity in the United States increased to 38% from 11% in 2018 (Wolfson and Leung, 2020). The global impact of COVID-19 on food security in developing countries, including Indonesia, has been significant, and in Tehran Province, Iran, households' food security initially improved during the early stages of the pandemic (Charvadeh et al., 2021; Movahed et al., 2022). During the COVID-19 pandemic, the food security situation of rural households has worsened. There have been changes in the consumption of certain food groups (Movahed et al., 2022).

Indonesia's food security in 2022 scored 60.2 on the Global Food Security Index (GFSI), an improvement from 59.2 in 2021. Despite the increase, it ranked 69th out of 113 countries, falling below the global average of 62.2 and the Asia-Pacific average of 63.4 (Rizaty, 2022). The GFSI evaluates four distinct factors, including accessibility, availability, quality and safety, natural resources, and resilience. The slide from the Food Security Index reveals the adverse impact of COVID-19 on Indonesia's food security (Global Food Security Index, 2022). The impact signs are characterized by obstacles in the import–export sector, difficulties in obtaining local food supplies, and halts in economic activities. The challenge of attaining sustainable development objective one, prioritizing poverty and hunger alleviation, is intertwined with the food security dilemma. The populace's food purchasing has persistently declined, emphasizing the gravity of the situation. The complexity of this issue necessitates coordinated resolution efforts from all stakeholders, as indicated by relevant studies analyzing the interplay between COVID-19 and Indonesian food security. The insufficient savings to offset income loss during this period increased the number of individuals who are indebted to grocery stores and food vendors (Widyaningsih et al., 2022). Additionally, only 19% of families reported having sufficient nutrition, while 35% indicated a frequent reduction in food intake due to financial constraints (UNDP, Prospera, SMERU UNICEF, 2021). Furthermore, LIPI's et al. (2020) study revealed that 35.9% of respondents experienced food insecurity, with 23.84% facing food insecurity without hunger, 10.14% dealing with moderate hunger, and 1.95% experiencing acute hunger (Prakoso, 2020). Additionally, Fitriah et al. (2021) found that the COVID-19 pandemic worsened Indonesia's already challenging food affordability, availability, and price stability.

The achievement of food security in Indonesia has routinely encountered numerous obstacles, as expounded in the Decree of the Head of the Food Security Agency Number 82/KPTS/RC.110/J/10/2020 (Ministry of Agriculture of Food Security Agency, 2021). These challenges encompass regions with restricted access to food, high rates of poverty, a significant portion of income is spent on food, limited access to electricity and clean water, low levels of education, an elevated risk of underweight and malnutrition, as well as high levels of stunted growth in young children and reduced life expectancy. The COVID-19 pandemic has compounded food security issues in certain regions. Indonesian Law Number 18/2012 stipulates attaining food security through meeting the state's and its population's food needs. This requirement necessitates the availability of sufficient, safe, diverse, nutritious, just, and affordable food while also honoring the population's religious, philosophical, and cultural practices. Food security is achieved when individuals have physical and economic access to adequate food to lead productive and healthy lives. It is impacted by several factors that fall into food availability, accessibility, and utilization (FAO, 2009; Setyorini et al., 2022).

Social assistance through food and cash is one way to increase food security during the pandemic. In Indonesia, the Ministry of Agriculture has created four strategies regarding food security (Maharani, 2016). These strategies entail enhancing production capacity, diversifying local food sources, strengthening food reserves and logistics systems, and promoting contemporary agriculture techniques. Several programs were implemented to achieve the objectives, including intelligent farming, screen houses to increase horticultural commodity production beyond the usual growing season, farmer corporations' establishments, and food estates. The pandemic has led to many innovations in the food industry, including urban farming and integrated agriculture practices.

Depok, a semi-urban city situated as a buffer zone in DKI Jakarta Province, has faced considerable food insecurity. This issue has impacted 65% of households at various levels (Syafiq et al., 2022). Regarding similar circumstances, a significant majority (61.8%) have also undergone comparable challenges. Family income has been crucial in maintaining food security during the pandemic (Hidayah and Fikawati, 2021). The economic welfare of households has significantly declined, resulting in reduced earnings and an inability to fulfill necessities (Syahreza and Manaf, 2021). Furthermore, a notable association was found between food security and stunting rates throughout the pandemic (Sugiyanti et al., 2023). Additional research is needed on food security in Depok City, focusing on the socio-demographic characteristics, the impact of non-food spending on food security, government/private/NGO social assistance, the effects of COVID-19 on families, and the proportion of food expenditure to total income.

The research on factors affecting food security during COVID-19 in Depok City still needs to be completed (Hidayah and Fikawati, 2021; Syafiq et al., 2022). The COVID-19 pandemic is affecting households' food security in Depok City due to worsening employment and income conditions. The two previous studies did not examine other factors that affect food security, such as government social assistance, living arrangements with family members, the impact of COVID-19 on families, the age of parents, the number of children, and the percentage of total expenditures spent on food. Hence, a study on the factors affecting household food security during the pandemic in Depok City can add to the existing research literature. The present findings can assist in comprehending the needs of urban populations during the COVID-19 epidemic. This understanding can facilitate the planning and implementation of preventive measures for these populations, such as government social assistance and protection. The objective of this study was to assess the factors associated with food security among selected families in Depok City during the COVID-19 pandemic.

2 Materials and methods

2.1 Study design

This cross-sectional study assessed 140 individuals from eight villages: Rangkapan Jaya Baru, Rangkapan Jaya Lama, Mampang, Beji, Sukamaju Baru, Depok, Sukatani, Bojongsari Baru, and Sawangan. Initially, Depok Jaya was chosen as the place to study, but then Depok Jaya was not included in the study due to many COVID-19 cases during the assessment period. Ethical clearance was acquired from the Health Research Ethics Commission of Agency for Health Research, Indonesian Ministry of Health (Number: LB.02.01/2.KE.374/2021), and all participants provided informed consent before the study in early July 2021.

2.2 Population and sample

The study population consisted of individual residing in eight villages in Depok City, West Java Province, Indonesia. Simple Random Sampling was used to select respondents who met the inclusion criteria, including households from high, middle, and low socio-economic backgrounds, permanent residency in the villages, and having at least one biological child under five or adolescent. One hundred forty respondents fully participated in this study, and a sample size was determined using proportional estimation with relative precision (Lwanga and Lemeshow, 1991).

$$n = Z^2 1-\alpha/2 \, \frac{(1-P)}{€2P}$$

In the formula, n represents the sample size, $Z_{1-\alpha/2}$ represents the error rate of 1.64 at a 90% Confidence Level, and € represents the relative precision at 0.10. P is the prevalence of household food insecurity in Depok City at 65% (Syafiq et al., 2022). After inputting the values into Lemeshow's formula, the sample size was estimated to be 140 individuals. The number of respondents selected from each village is drawn in Table 1.

2.3 Study variables

The study analyzed multiple independent variables, including the socio-demographic characteristics (parental age, maternal education, parent employment, cohabitation with family members, number of biological children, and family income), total food consumption during the COVID-19 pandemic; the relationship between non-food and food expenditure; decrease in income; social assistance from government, private or NGO sources; and the percentage of food consumption relative to total expenditure. The study focused on food security within families, with data collected through face-to-face interviews conducted by trained enumerators at respondents' residences on 15–30 July 2021. To ensure the safety and confidentiality of participants during face-to-face interviews, we obtained signed informed consent, trained enumerators, and explained the confidentiality before interviews to the respondents.

2.4 Data analysis

The univariate analysis presented the frequency distribution of both dependent and independent variables to describe and clarify the self-characterization of all variables by the respondents. Categorical data were illustrated through tables and pie/diagram figures, whereas numerical data were represented using mean, standard deviation, minimum, and maximum values. The Chi-Square test was to examine the association between socio-demographic characteristics, reducing total income during a pandemic, social assistance from the government, private or NGO sources, COVID-19 impact on family income, and the percentage of food expenditure to total expenditure and the households' food security with significance set at $p < 0.05$. The Odds Ratio (OR) was calculated with a 95% Confidence Interval (CI) to evaluate the relationship between food security and other independent variables.

The Binary Logistic Regression Test was used to identify the significant predictors of food insecurity at a household level. It is useful when the dependent variable is dichotomous, like food secure and food insecure (Hosmer et al., 2013). To determine the respondents' significant concerns about food availability during a pandemic, we used a four-point Likert scale. If the Likert mean score of an item is lower than 2.50, then the respondents have a minor concern about the particular item (Wanjohi and Syokau, 2021).

Food security level is defined by calculating the scores for each answer from the 15 statements in the United States Family Food Security Survey Module-USDA. Responses labeled frequent,

TABLE 1 Frequency distribution of respondents by area.

Villages	Frequency	
	n (person)	%
Bojongsari	25	17.9
Sawangan	31	22.1
Pancoran Mas	47	33.6
Beji	14	10.0
Tapos	23	16.4
Total	140	100.0

occasional, do not know, and never were assigned a score of 1. Household food security was categorized into four levels: food security (score of 0), food insecurity without hunger (scores of 1–2), food insecurity with mild hunger (scores of 3–7), and food insecurity with severe hunger (scores of 8–15). We put the code for food insecurity with 0 and food security with 1 in the Binary Logistic Regression Test. Food security is defined as meeting the physiological needs for growth and public health standards of regions, communities, or households at all times. Food insecurity is defined as when they do not have regular access to sufficient safe and nutritious food for average growth, development, and an active, healthy life (FAO, 2009). The instrument of the present study comprised 15 questions, which were modified from the 18 items of the United States Family Food Security Survey Module-USDA. We used 15 questions due to the combination of Child Stage 1 and Child Stage 2 into Child Stage. The purpose of the modification was to enhance the clarity of Child Stage statements for households.

3 Results

3.1 Demographic characteristic

The demographic characteristics of female participants are described in Table 2. A significant portion of mothers were over 49, while those between 35 and 49 and above were almost equally represented. The average age of mothers was 38.5 ± 8.7 years, with the youngest being 21 and the oldest 74. On the other hand, most fathers were between 35 and 49 years old, with an average of 41.1 + 9.9 years, a maximum age of 59, and a minimum age of 21. Over 75% of respondents had achieved intermediate final education, exceeding 12 years of study. Most mothers were unemployed, while most fathers were employed in the private sector. More than half of the respondents lived with their spouses and children; most mothers had two biological children. Half of all respondents reported a family income below US$ 193.5, with monthly expenses ranging from US$ 64.5 to 193.5. A number of individuals suffered decreased revenue due to the COVID-19 pandemic. The Depok City Government provided most respondents with social assistance through essential items and monetary support (Table 2).

3.2 Food availability and food insecurity

Table 3 displays eight statements regarding food availability during the COVID-19. A majority of respondents express vital concern about running out of food. Some respondents faced various challenges, including the inability to buy nutritionally balanced food, limited funds to feed children, food shortages due to affordability issues, weight loss due to insufficient food, and children eating less than three meals daily (Table 2). When asked about the food availability at the household level during the pandemic, all respondents gave an average score above 2.5 on a four-point Likert scale. This indicates that respondents were greatly concerned about food availability during the pandemic (Table 3). The proportion of families experiencing mild food insecurity compared to those without hunger is drawn in Table 4. There were 66.4% of households with various levels of food insecurity during the COVID-19 pandemic. Most households experienced severe food insecurity, the most prevalent category (Table 4).

Table 5 displays the discrepancies in levels of food security depending on socio-demographic characteristics, monthly family spending, social support from government/private/NGO, and the percentage of food consumption compared to the total expenses. A greater parental age was associated with increased family food insecurity, while a higher rate of family food insecurity influenced a lower maternal education level. Unemployed mothers experienced a higher incidence of food insecurity than those employed. In comparison, fathers in labor and online motorcycle taxiing occupations had greater food security than self-employed fathers. Additionally, larger families with more than three biological children tended to possess greater food security. Additionally, individuals with a monthly income below 3 million and, a total monthly food expenditure of less than 1 million, experiencing a reduction in monthly revenue, receiving social assistance from government, private, or NGO sources, and those significantly impacted by COVID-19 had higher levels of food insecurity. Furthermore, households with a lower percentage of food consumption than total expenditure reported elevated levels of food insecurity (Table 5).

The Binary Logistic Regression Analysis shows the maternal age, mothers' educational level, government social assistance, and the percentage of food consumption to total expenditure influenced by the household's food security. Social assistance was the most impactful variable related to family food insecurity, with a probability of 45 times (Table 6). Maternal age, maternal education, government social assistance, and the percentage of food expenditure to total expenditure strongly affected household food security.

4 Discussion

The objective of the study aimed to assess the impact of the COVID-19 pandemic on food security and its related factors. The initial step assessed the food security situation of urban households. The results indicated that more than half of the total households had food insecurity, with the most significant proportion being severe. This finding aligns with similar studies on COVID-19 (Hermawati et al., 2022; Syafiq et al., 2022; Dewi et al., 2023). COVID-19 has significantly impacted family incomes, as demonstrated by the marked disparity in family income and food security. Food insecurity could have severe and long-lasting health consequences (Leddy et al., 2020). During COVID-19, the proportion of food-insecure families was twice as high as that of food-resilient families. Food insecurity was prevalent in several countries, with rates reaching 27% in Australia (Kent et al., 2022), California (Escobar et al., 2021), Nepal (Singh et al., 2021), and Bangladesh (Shuvo et al., 2022). Poverty and low income were the significant factors for food insecurity, significantly contributing to malnutrition and poverty in middle and lower-income communities. Food insecurity levels were assessed as low, moderate, or high based on individual perceptions and food purchases, according to studies by Elsahoryi et al. (2020) and Kakaei et al. (2022). Surveyed individuals reported that the primary impact of COVID-19 was a reduction in income. Most families received government-sponsored aid in food and money, resulting in decreased energy intake or reduced food consumption concerning overall expenses.

TABLE 2 Sociodemographic characteristic.

Variable	*n*	%	Mean ± DS	Min– Max
Mother's age group (years old)			38.5 ± 8.7	21–74
20–34	53	10.7		
35–49	72	33.6		
>49	15	34.3		
Father's age group (years old)			41.1 ± 9.9	21–59
20–34	42	30.0		
35–49	68	48.6		
>49	30	21.4		
Mother's final education				
Low (less than 12 years of schooling)	52	37.1		
Medium (more than 12 years of schooling)	88	63.9		
Working status of mother				
Yes	55	39.3		
No	85	60.7		
Main job of father				
Freelance worker/online motorcycle	38	27.1		
Entrepreneur/trader	43	30.7		
Private employee	59	42.2		
Living with				
Husband, wife, children	97	69.3		
Husband/wife	19	13.6		
Husband, wife, children, other family	24	17.1		
Number of biological children (person)				
1	38	27.1		
2	64	45.7		
≥ 3	38	27.2		
Total family income (US$)				
≤193.5	81	57.9		
>193.5	59	42.1		
Total food expenditure (US$)				
<64.5	35	25.0		
64.5–193.5	71	50.7		
>193.5	34	24.3		
Social assistance from government				
Yes	97	69.3		
No	43	30.7		
Reducing total family income				
Yes	102	72.9		
No	38	27.1		
Yes	111	79.3		
No	29	20.7		
Percentage of food expenditure to total expenditure				
High (>60% of total expenditure)	102	72.9		
Low (<60% of total expenditure)	38	27.1		

DS, Deviation Standard.

TABLE 3 The statement of family food availability during the pandemic.

Statement	Always (= 1)		Often (=2)		Sometimes (= 3)		Never (=4)		Mean ± SD
	n	%	*n*	%	*n*	%	*n*	%	
Worried that the family's food will run out	8	5.7	38	27.1	47	33.6	47	33.6	2.9 ± 0.9
Cannot afford food during COVID-19	0	0.0	18	12.9	34	24.3	88	62.9	3.5 ± 0.7
Not able to provide balanced nutritional food	2	1.4	18	12.9	49	36.0	71	50.7	3.4 ± 0.7
	0	0.0	20	14.3	43	30.7	77	55.0	3.4 ± 0.7
Only able to provide small funds for children	0	0.0	11	7.9	28	20.0	101	72.1	3.6 ± 0.6
Had ever not eaten enough because we can	1	0.7	6	4.3	18	12.9	115	82.1	3.8 + 0.6
not provide enough food for children	5	3.6	7	6.0	22	15.7	106	75.7	3.6 ± 0.7

TABLE 4 The household food security level.

Level	*n*	%
Insecure (severe, mild degrees, and without hunger):	93	66.4
Severe	35	25.0
Mild	30	21.4
Without hunger	28	20.0
Secure	47	33.6

The main findings of the present study are socio-demographic factors linked to family food insecurity, which are parental age, employment status, maternal education, number of biological children, family composition, income, total food expenses, government social aid, and the proportion of food expenditure to total income. These results are consistent with previous studies conducted in Tulungagung and Yogyakarta. The determinants of food security in Tulungagung include income, employment status, and type of social assistance, as noted by Dewi et al. (2023). Similarly, Hermawati et al. (2022) found that factors influencing food security in Yogyakarta were employment status, family income, and number of dependents. Engel's law states that increased income directly leads to increased food consumption. In this context, more than 75% of the participants had a food consumption ratio to non-food spending of less than 60%, resulting in their categorization as food insecure (Cirera and Masset, 2010).

Older parents were more likely to experience increased food insecurity, as indicated by the positive correlation between parental age and food insecurity. This correlation corresponds with a study conducted in several regions of Indonesia (Syafiq et al., 2022). Additionally, younger respondents (under 31 years) were twice as likely to experience food insecurity as their older counterparts, as supported by previous studies (Shuvo et al., 2022). Age correlated firmly with economic conditions within the family, as the more senior participants possessed more secure and enduring employment alongside a stable job status, which differed significantly from their younger counterparts (Abdullah et al., 2019). The difference in age-related food insecurity between the two studies may be due to the age groups in the previous analysis, which faced substantial job loss and income reduction, mainly in the younger productive age (21–35 years) and middle adulthood (36–50 years; Shuvo et al., 2022).

The parent's employment status significantly affected family food insecurity. Employed parents were found to have a lower proportion of family food insecurity when compared to those who were unemployed. This suggests that employment results in a more favorable income, facilitating food purchases for families. This observation is consistent with other studies conducted by Tabrizi et al. (2018), Cordero-Ahiman et al. (2020), Movahed et al. (2022), Suresh et al. (2022), and Syafiq et al. (2022). Families experiencing job loss are more likely to face food insecurity, have insufficient food, and experience a decline in child nutrition (Farrington, 2021).

Lower levels of maternal education were significantly influenced by higher rates of food insecurity during COVID-19. This finding hindered access to nutritional knowledge, hindering efforts to maintain a healthy diet, which is crucial for boosting immunity and coping with difficult circumstances (Aman and Masood, 2020). Mothers' educational attainment also played a vital role in shaping judgments regarding food intake, highlighting their responsibility to establish and administer the food budget for the entire family (Astuti and Sulistyowati, 2013). Mothers with higher education are more aware of family health and nutrition, which is crucial for ensuring food security (Quandt et al., 2004).

The number of biological children influenced the food security in the present study, which agrees with the results of the studies by Ramesh et al. (2010) and Safarpour et al. (2014). Food insecurity was more prevalent among children in larger families, particularly those with three or more children. The greater the number of children, the greater the food that must be provided. Failure to meet these needs can lead to food insecurity (Nord, 2010).

Family income and family size or dependents were also affected by food security during COVID-19 in the current study. "Family dependents" refers to members who rely financially on the household, including employed and unemployed biological or non-biological relatives (Hanum, 2018). Households with more dependents tended to have higher expenditures, affecting food and non-food security (Hermawati et al., 2022). The Central Statistics Agency classified dependents into three groups: small families with 1–3 individuals, medium-sized families with 4–6 individuals, and large families with

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TABLE 5 Differences in the proportion of household food security levels based on socio-demographic characteristics, monthly family expenditure, social assistance from the government, and the percentage of food expenditure to total expenditure.

Variable	Food insecure		Food secure		Total	*p*	OR	95% OR
	n	%	*n*	%				
Mother's age group (years old)								
<35	24	45.3	29	54.7	53		1	
35–44	42	76.4	13	23.6	55	*0.001	3.90	1.71–8.90
>44	27	84.4	5	15.6	32		6.53	2.18–19.54
Father's age group (years old)								
<35	15	35.7	27	64.3	42		1	
35–44	35	76.1	11	23.9	46	*0.001	5.73	2.27–14.46
>44	43	82.7	9	17.3	52		8.60	3.31–22.78
Mother's final education								
Academy/University	5	27.8	13	72.2	18		1	
Graduated from senior high school/equal	48	69.6	21	30.4	69	*0.001	5.94	1.88–18.80
Graduated from junior high school/equal	40	75.5	13	24.5	53		8.00	2.39–26.73
Working status of mother								
Yes	30	545	25	45.5	55		1	
No	63	74.1	22	25.9	85	*0.017	2.39	1.16–4.90
The main job of the father								
Employee	12	35.3	22	64.7	34		1	
Entrepreneur/trader	50	73.5	18	26.5	68	*0.001	5.09	2.10–12.35
Freelance worker/online motorcycle	31	81.6	7	18.4	38		8.12	2.76–23.92
Living with								
Husband, wife, children	72	62.1	44	37.9	116	*0.016	1	
Husband, wife, children, other family	21	87.5	3	12.5	24		4.28	1.21–15.18
Number of biological children (pers.)								
One	18	47.4	20	52,6	38		1	
Two	44	68.8	20	31.3	64	0.006	2.44	1.07–5.59
Three	31	81.6	7	18.4	38		4.92	1.74–13.90
Monthly total family income								
More than US$ 193.5	31	52.5	28	47.5	59	0.003	1	1.43–6.08
Less than/equal US$ 193.5	62	76.5	19	23.5	81		2.95	
Reducing total family income during pandemic								
No	13	34.2	25	65.8	38	0.001	1	3.08–15.87
Yes	80	78.4	22	21.6	102		6.99	
Getting social assistance from government during pandemic								
No	11	25.6	32	74.4	43	*0.001	1	6.61–38.29
Yes	82	84.5	15	15.5	97		15.90	
COVID-19 has an impact on family income								
No	9	31.0	20	69.0	29	*0.001	1	
Yes	84	75.7	27	24.3	111		6.91	2.82–16.98
Percentage of food expenditure to total expenditure								
Low (< 60% of total expenditure)	16	42.1	22	57.9	38	*0.001	1	2.82–16.98
High (>60% of total expenditure)	77	75.5	25	24.5	102		4.24	1.93–9.30

*$p < 0.05$ at significant level.

TABLE 6 Selected factors influencing household food security.

	B	S.E.	Wald	*P*	Ratio
Mother's age group (years old)					
35–44 vs. <35	0.757	0.784	0.933	0.334	2.132
>44 vs. <35	2.300***	0.871	6.981	0.008	9.977
Mother's final education					
Graduated from senior high school or more vs. graduated from junior high school or below	1.940**	0.832	5.442	0.020	6.958
Getting social assistance from the government					
Yes vs. no	3.808***	0.739	26.569	0.000	45.051
Percentage of food expenditure to total expenditure					
More than 60% vs. less than 60%	2.097***	0.776	7.299	0.007	8.142
Constant	−4.995***	1.158	18.593	0.000	0.007

Coefficient of Omnibus Mode = 122.23 with $p = 0.001$, −2LL = 66.49, Cox and Snell R Square = 0.551 and Nagelkerhe R Square = 0.765. ** $p < 0.05$. *** $p < 0.01$. Food security = − 4.995 + 1.940 (Mother's final education) + 3.808 (getting social assistance from the government) + 2.097 (percentage of food expenditure to total expenditure).

more than six individuals (Central Bureau of Statistic, 2000). Most respondents were medium-sized, consisting of parents with more than three biological children who cohabited with spouses, children, and other family members. The number of family dependents affects the level of food expenditure and household consumption patterns. The larger the household, the higher the likelihood of experiencing food insecurity, as it requires more money to meet the daily needs of additional individuals, including food and other necessities (Hanum, 2018). Higher-income households can allocate more funds toward food after fulfilling other financial obligations (Ashgar and Muhammad, 2013).

The food consumption to total expenditure ratio is positively influenced by food insecurity. Higher food expenditures were associated with reduced food security. Families experiencing food insecurity allocated more than 60% of their spending to food, while food-secure families allocated less than 60%. These findings align with previous research on family food security in Klaten Regency (Amaliyah and Handayani, 2011) and Langsa City, Aceh Province (Azharina et al., 2021). Households who spend more on food compared to other needs are at a higher risk of experiencing food insecurity. This situation often affects low-income families. These households tend to prioritize purchasing affordable food over nutritious options. Consequently, their expenditure on food is more significant than other items (Herdiansyah et al., 2024).

Social assistance from the government, private sector, or non-governmental organizations was a determinative factor in increasing family food security by 3.8 times compared to those lacking support. This assistance typically included essential food items such as biscuits, milk, rice, eggs, and cash. These findings are consistent with similar investigations (Lawal et al., 2022; Dewi et al., 2023; Lee et al., 2023). Social assistance in food, cash, and necessities can improve family food security amid the COVID-19 crisis (Diouf et al., 2022). As a result, this research recommends offering social assistance in the form of nutritious food, cash, and fundamental necessities for 6 months during COVID-19, especially for low-income families. This assistance should include items like milk, biscuits, and rice. Collaboration between the government, private sector, and NGOs is necessary to improve family food security and ensure adequate daily living assistance. Social assistance can reduce extreme poverty and improve food security while increasing household resilience during times of crisis (FAO, 2013).

4.1 Limitation

Future studies must address several limitations in the present study. First, the study included participants from all socio-economic classes. Still, it has not been possible to determine the proportion of food security among those from low socio-economic groups. Second, the sample was limited to only 9 out of 63 villages in Depok, which may have limited the ability to represent food security on a city-wide level accurately. Third, the assessment of the relationship between energy, protein intake, and food insecurity among family members was impeded due to a need for more data on macronutrient intake. Fourthly, respondents' perceptions were evaluated through a questionnaire comprising 15 food security questions (never, do not know, often, and sometimes), which may introduce subjectivity. Fifth, recall bias can occur in the study when the data is collected retrospectively or after the event. However, the study has specific strengths worth highlighting. First, it was affected by the previous research on the factors affecting family food security in Depok City during the COVID-19 pandemic. Second, the primary data collection was carried out through structured interviews during home visits, which helped identify the socio-economic conditions contributing to food insecurity.

5 Conclusion

According to the findings of this study, urban households experienced mild-to-severe food insecurity during the pandemic. The risk of food insecurity was strongly influenced by various factors such as socioeconomic characteristics: parental age, employment status, maternal education, number of biological children, family size, income, food consumption, and government social assistance. In this context; government social assistance played a prominent role in enhancing food security. Households with government social assistance are 45 times more likely to have food security than households without government social assistance. Therefore, government, private organizations, and local and international NGOs should provide social aid to enhance urban households' food security during the COVID-19 pandemic, especially for those who lost their jobs or income. Furthermore, it is recommended to research the impact of social protection on the food security of low-income households affected by COVID-19 in Depok City. Future studies should consider mild, moderate, and severe food insecurity factors. It is essential to examine how macro and micro-nutrient intake affects the nutritional status of undernourished children due to COVID-19.

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 studies involving humans were approved by the Health Research Ethics Commission of Agency for Health Research, Indonesian Ministry of Health. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

FF: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

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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.

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Reflections of well-being: navigating body image, chronic energy deficiency, and nutritional intake among urban and rural adolescents

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Background: Adolescent growth and development is a period of very specific nutritional problems. As a result of poor growth and development, 36.3% of adolescents in Indonesia are at risk of developing CED. The purpose of this study was to determine the description of body image, the incidence of Chronic Energy Deficiency (CED), and nutritional intake in adolescents in urban and rural areas.

Methods: This study used a descriptive quantitative design with a cross-sectional study conducted in Bandung and Sumedang on 387 adolescents aged 13–15 years. The instruments used in this study were body image questionnaire Figure Rating Scale (FRS) method, 2 × 24-h food recall, and anthropometry for Measuring mid upper arm circumference (MUAC).

Results: Results of this study showed that more than half of adolescents in urban (54.0%) and rural areas (61.7%) were at risk of CED, had negative body image perceptions in urban (69.1%) and rural areas (62.3%), and underconsumption of macronutrients in both urban and rural adolescents.

Conclusion: Most adolescents in urban and rural areas still consume less energy, carbohydrates, and protein. Perceived body image and nutrient intake contribute to the incidence of CED in adolescents.

KEYWORDS

adolescents, body image, chronic energy deficiency (CED), nutritional intake, rural, urban

1 Introduction

The growth period in adolescents is a time of very specific nutritional problems. The growth and development of puberty, linear growth, and the occurrence of neurodevelopmental changes are strongly influenced by nutritional intake that meets the needs of adolescents (1). Adequate intake of nutrients such as carbohydrates, protein, iron, calcium and other micronutrients will increase in adolescence and is strongly influenced by diet. Not fulfilling nutrients will hinder optimal growth and development and cause nutritional problems in adolescents (2). Nutritional problems often occur due to unbalanced nutrient intake and can cause chronic energy deficiency (CED) and anemia due to iron deficiency (3).

Chronic energy deficiency (CED) is a condition in which an adolescent girl or woman suffers from nutritional deficiencies (energy and protein) that occur over a long period of time or even years (4). A person is diagnosed as being at risk of developing CED if the mid upper arm circumference (MUAC) is <23.5 cm. Adolescents who experience CED are often caused by low macronutrient intake, especially energy and protein, which can also contribute to low micronutrient intake. In the case of adolescent girls who suffer from CED, if not treated properly, it will be sustained and lead to low academic performance. Malnutrition is a major contributing factor to low academic performance and school enrollment (5). In addition, CED also affects pregnancy which can trigger other diseases such as anemia, premature labor, birth of low-weight babies (LBW), giving birth to stunted children, and even risking death (6).

The prevalence of chronic energy deficiency (CED) among adolescent girls (aged 15–19 years) in Indonesia in 2020 has increased from 2018, which was 33.5 to 36.3% (7). Based on this prevalence, an estimated 7.5 million or 3–4 out of 10 adolescent girls in Indonesia suffer from anemia. Another impact of CED on adolescents is the inhibition of growth and development, cognitive abilities, and susceptibility to infectious diseases (8). There are several factors that influence the occurrence of CED in adolescents, direct and indirect factors. Direct factors are infectious diseases and age while indirect factors are knowledge of nutrition and physical activity of adolescents (9). In developing countries, the main influencing factor is infectious diseases caused by poor diet and nutritional intake.

In adolescence, there is a growing awareness of appearance (body image) depending on the opinions of others and the environment. Dissatisfaction with their appearance occurs if their body shape looks fatter or feels too thin than usual, causing adolescents to be more likely to restrict their diet (10, 11). Body image in adolescents greatly affects their diet, including the selection of food ingredients, meal frequency, and nutritional intake (12). This study aims to see how the description of body image, CED, and nutritional intake in adolescents in urban and rural areas.

Health problems in the adolescent phase need more attention due to the many factors that can affect their well-being. The increasing prevalence of adolescent CED in Indonesia indicates the need for more serious handling to prevent long-term health problems. At this age, adolescents begin to choose the food they consume and pay attention to their body shape according to their self-perception and social environment (13). Adolescents who have a negative body perception are likely to make the wrong diet and lead to nutritional deficiencies (14). The aims of this study is to:

1. Analyze how body image perceptions among adolescents in urban and rural areas
2. Analyze the nutritional status and prevalence of CED among adolescents in urban and rural areas
3. Analyze the macronutrient intake of adolescents in urban and rural areas

2 Materials and methods

2.1 Study population

The population in this study were junior high school students in 5 schools in urban and 5 schools in rural areas. Research location were determined based on criteria and school clusters in each urban and rural areas. To represent the heterogenization of the respondents, the schools were determined based on economic status, school location, and type of school, either public or private school. Economic status was considered as one of the criteria because it can affect the diet of adults and also affect the diet of their children. Based on the research, this may indicate that wealthier people can consume a healthier and more diverse food which affects nutritional status. The sampling process used random sampling techniques, with the inclusion criteria of adolescents aged 13–15 years and willing to participate in the entire research program by filling out informed consent. This sampling was determined using the Slovin formula with an error rate of 5%. Based on the sampling results and the actual situation in the location, 387 students were obtained as respondents in this study (Figure 1).

2.2 Policies and publication ethics

The research was conducted in August–September 2023, and has a publication ethics code with Number LB.01.03/6/317/2023 from Ministry of Health of the Republic of Indonesia Directorate General of Health Workers of Mataram Health Polytechnic.

2.3 Measurement and definition

The design of this study was descriptive quantitative research with a cross-sectional approach with a research focus to determine the description of body image, CED, and macronutrient intake in adolescents in urban and rural areas. The research instruments used in this study were body image questionnaire Figure Rating Scale (FRS) method, 2 × 24-h food recall, and anthropometry to determine age, nutritional status and MUAC. The data results were tested statistically to describe the frequency of each variable. Here are the research steps in this study:

2.3.1 Preparation of the research instruments

This research uses various types of instruments that can cover the required data results.

2.3.1.1 Figure rating scale

Figure Rating Scale (FRS) is a psychological assessment instrument used to measure perceptions of body beauty and body image, and can be used in research related to body image, self-esteem, and disorder. This instrument consists of illustrative images of various forms of male and female body sizes equipped with 11 questions related to ideal body perceptions. In this study, the instrument was used to assess how adolescents aged 13–15 years perceived their body image.

2.3.1.2 Food recall 2 × 24 h

Food recall is an assessment instrument used to collect information through interviews about food consumed by individuals during a certain period. In this study, the food recall instrument contains meal times, food and drinks consumed, including the amount and type of food. This instrument is used to see how nutritional intake, especially macronutrients, in

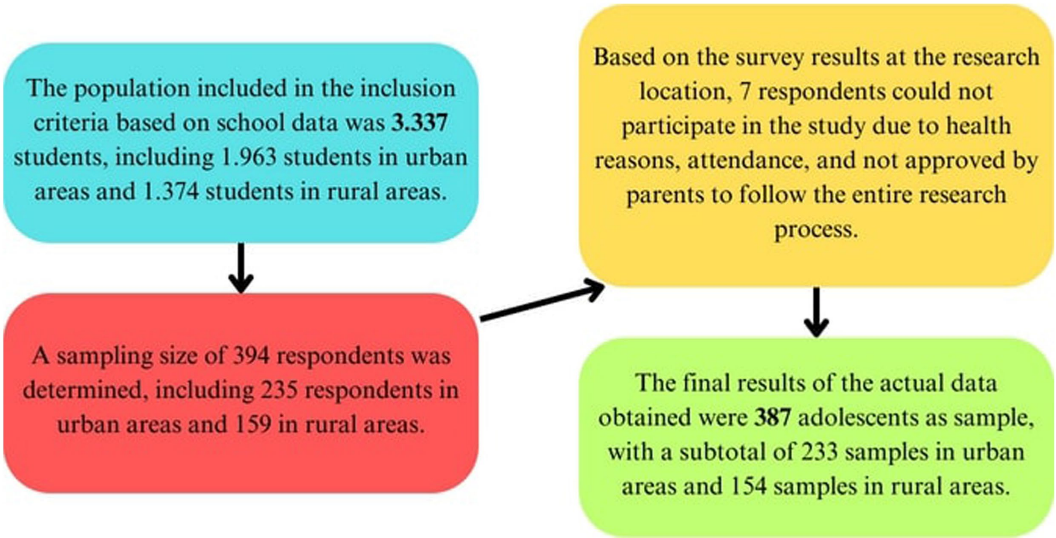

FIGURE 1
Research population and sample.

adolescents aged 13–15 years. This study used a 2 × 24 h food recall instrument on weekdays and weekends, in order to represent the overall nutritional intake and the resulting data is more qualified.

2.3.1.3 Anthropometry

This instrument was prepared to determine the nutritional status of respondents including gender, age, weight, height, and mid upper arm circumference (MUAC). MUAC is one of the anthropometric indicators used to assess the nutritional status of chronic energy deficiency (CED) in adolescents. The equipment used to measure anthropometry is a SAGA stadiometer to measure height, Omron HBF-375 Bioelectrical Impedance Analysis (BIA) to measure body composition, especially body weight, and an automatic meter to measure upper arm circumference.

2.3.2 Instrument trials

The results of the instrument preparation carried out by the research team were then tested on adolescents aged 13–15 years. The instrument testing process was conducted with the aim of knowing how long it takes to do the interview process and anthropometry to respondents, as well as to find out whether the instrument compiled has represented the data needed by the research team. The instrument trial was conducted in one school with 8 adolescents who became trial samples. The results of the instrument trial was the need to make improvements to the interview procedure and anthropometric measurement process, because during the trial it took quite a long time and was less efficient. As the instrument was compiled based on various previous studies, the instrument was in accordance with the aims of this study.

2.3.3 Cooperation with the schools

Based on the research location that had been determined, the research team met with the school and conveyed the purpose of the research. The research team conveyed the entire research process and methods used, discussed the place and time of data collection, and the necessary administration. The permit documents were fully completed by the research team so that the cooperation process went well.

2.3.4 Providing informed consent

This research requires informed consent approved by the parents of adolescents who become research samples. The informed consent was provided and delivered to the students through the school office. Students who become samples are required to bring the informed consent during the data collection process so that it is confirmed that they can take part in the entire research process.

2.3.5 Data collection process

Data collection was carried out by enumerators who had a background in nutrition education and had participated in a training or trial process before data collection, so that they were able to comprehend and master the used instruments. The instrument used was in paper form and also digital using google form. All students who became samples were asked to fill out the Figure Rating Scale (FRS) instrument via their smartphones, followed by the anthropometric process and food recall interviews directly with each sample. The food recall interview process is carried out twice according to the data needed.

2.3.6 Data cleaning process

The data that has been collected was inputted using Microsoft excel and a cleaning process was conducted to find out if there were any errors or miss-entries during the data collection process. This also aims to facilitate the analysis process.

2.3.7 Data analysis process

The data analysis process was carried out by professional assistance analyzed using IBM SPSS Statistics 27 software. Data analysis includes the frequency of age, gender, nutritional status, body image perception classification, and the average value of macronutrient consumption (median) of adolescents aged 13–15 years.

3 Results

3.1 Characteristics and nutritional status

Adolescence is a transitional period from childhood to adulthood, starting from the age of 10–13 years and ending at the age of 18–22 years (15). Table 1 shows that in urban areas, the percentage of research respondents were male (48.5%) and female (51.5%), while in rural areas the research respondents were male (46.8%) and female (53.2%). Urban areas respondents aged 13 years (36.9%), 14 years (42.1%), 15 years (21.0%) and in rural areas respondents aged 13 years (48.7%), 14 years (41.6%), 15 years (9.7%). Comparison based on the results of data analysis, more than half of the female respondents were 14 years old in urban areas and 13 years old in rural areas. The classification of economic status was determined by referring to the average income in urban and rural areas. Based on economic status, adolescent parents in urban areas had an income of ≤3,500,000 (50.6%) and in rural areas (62.3%). Table 1 shows that the percentage of adolescents at risk of developing CED is higher in rural areas (61.7%) and urban areas (54.0%).

3.2 Body image perception

Body image perception was classified into two categories, namely positive and negative perceptions. Positive body image is associated with bodily satisfaction and acceptance, whereas negative body image is associated with unhappiness and the desire for one's body to change (16). Based on Table 2, adolescents in urban areas had a negative body image (69.1%) and a positive body image (30.9%). In rural areas, adolescents who have a negative body image (62.3%) and positive (37.7%). The results of this study are in line with the research of Fitria et al. (17), stating that as many as 73.63% of adolescents in Yogyakarta have a negative body image. In contrast to research in Italy, men and women showed a good perception of their bodies, and showed little tendency to overestimate one's weight status ($p = 0.073$) (18).

TABLE 1 Sociodemographic characteristics and nutritional status ($n = 387$).

Variable	Urban (n = 233)	Rural (n = 154)
	n (%)	n (%)
Gender		
Male	113 (48.5)	72 (46.8)
Female	120 (51.5)	82 (53.2)
Age		
13 years	86 (36.9)	75 (48.7)
14 years	98 (42.1)	64 (41.6)
15 years	49 (21.0)	15 (9.7)
Economic status		
> IDR3,500,000	115 (49.4)	58 (37.7)
≤ IDR3,500,000	118 (50.6)	96 (62.3)
Nutritional status		
Normal	107 (45.9)	59 (38.3)
CED (<23,5 cm)	126 (54.0)	95 (61.7)

3.3 Nutritional intake

The nutrient intakes analyzed in this study were macronutrients including energy, protein, fat and carbohydrate intake. Nutritional Adequacy Rate (NAR) was classified based on the provisions set by the Indonesian minister of health, in the regulation of the minister of health of the Republic of Indonesia No. 28 of 2019. Adolescent boys aged 13–15 must consume sufficient energy (2,400 kcal), protein (70 g), fat (80 g) and carbohydrates (350 g). Adolescent girls aged 13–15 should consume sufficient energy (2050 kcal), protein (65 g), fat (70 g) and carbohydrates (300 g). Adolescent are considered fulfilled if they reach the NAR. Based on Table 3, the energy intake in urban areas was found to be less than the requirement based on the NAR, with a percentage of less (89.3%) and adequate (10.7%). As well as, in rural areas, adolescents' energy intake was categorized as less (80.5%) and adequate (19.5%). Protein intake of adolescents in urban areas was categorized as less (87.6%), adequate (12.4%), and in rural with percentages of less (82.5%) and sufficient (17.5%). Fat intake was categorized as deficient with percentages of adolescents in urban (69.0%) and rural areas (79.9%). The consumption of carbohydrate intake of adolescents was also categorized as insufficient with percentages in urban (91.8%) and rural areas (85.7%).

TABLE 2 Body image perception ($n = 387$).

Body image perception	Urban (n = 233)	Rural (n = 154)
	n (%)	n (%)
Positive	72 (30.9)	58 (37.7)
Negative	161 (69.1)	96 (62.3)

TABLE 3 Macronutrients intake ($N = 387$).

Macronutrients Intake	Urban (n = 233)	Rural (n = 154)
	n (%)	n (%)
Energy		
Less	208 (89.3)	124 (80.5)
Adequate	25 (10.7)	30 (19.5)
Protein		
Less	204 (87.6)	127 (82.5)
Adequate	29 (12.4)	27 (17.5)
Fat		
Less	161 (69.0)	123 (79.9)
Adequate	72 (31.0)	31 (20.1)
Carbohydrate		
Less	214 (91.8)	132 (85.7)
Adequate	19 (8.2)	22 (14.3)

TABLE 4 Correlation between variables (*N = 387*).

	Nutritional status					
	Urban (*n* = 233)			Rural (*n* = 154)		
	CED n (%)	Normal n (%)	Sig.	CED n (%)	Normal n (%)	Sig.
Body image perception						
Positive	25 (75.7)	8 (24.3)	0.007	18 (66.6)	9 (33.4)	0.558
Negative	101 (50.5)	99 (49.5)		77 (60.6)	50 (39.4)	
Macronutrients intake						
Energy						
Less	113 (54.3)	95 (45.7)	0.993	77 (62)	47 (38)	0.386
Adequate	13 (52)	12 (48)		18 (60)	12 (40)	
Protein						
Less	109 (53.4)	95 (46.6)	0.178	78 (61.4)	49 (38.6)	0.51
Adequate	17 (58.6)	12 (41.4)		17 (63)	10 (37)	
Fat						
Less	93 (57.7)	68 (42.3)	0.214	82 (66.6)	41 (33.4)	0.675
Adequate	33 (45.8)	39 (54.2)		13 (41.9)	18 (58.1)	
Carbohydrate						
Less	118 (55.1)	96 (44.9)	0.123	77 (58.3)	55 (41.7)	0.25
Adequate	6 (31.5)	13 (68.5)		18 (81.8)	4 (18.2)	

3.4 Correlation between nutritional status and influencing variables

According to Table 4, result showed there was no significant correlation between the variables. It was found that adolescents in urban areas who had negative body image perceptions had the highest percentage of CED (50.5%). In rural areas, adolescents who had a negative body image and were diagnosed with CED had a higher percentage compared to adolescents in urban areas (60.6%). Despite the lack of correlation between the variables, the data in Table 4 showed that in both urban and rural areas, adolescents who had negative body image perceptions and also consumed less macronutrients were significantly more affected by CED.

4 Discussion

Adolescence is a transitional period from childhood to adulthood, starting from the age of 10–13 years and ending at the age of 18–22 years (15). Adolescence is a critical developmental period regarding the perception of body shape or size (19). Based on the results of the analysis, more than half of the female respondents were 14 years old in urban areas and 13 years old in rural areas.

Mid Upper Arm Circumference (MUAC) is a measurement of arm circumference midway between the tip of the elbow and the tip of the shoulder that has the potential to offer a simple alternative for assessing nutrition in adolescents (20). According to the Indonesian Ministry of Health, the size of LILA at risk of suffering from SEZ is <23.5 cm and Body Mass Index (BMI) <18.5 kg/m2 (2, 21). CED is defined as a chronic nutritional problem that results from low energy intake over many years (22). Seen in Table 1, the percentage of adolescents at risk of developing CED in rural areas is greater, in urban areas (54.0%) and rural areas (61.7%). If defined further, this figure is quite higher than the research conducted by Rahmadi, stating that as many as 44.9% of adolescents in rural areas were at risk of experiencing CED (2). Research by Wardhani, also stated that as many as 55.0% of adolescents in urban areas were at risk of being CED (23). According to Purba et al., CED can be caused by various factors such as lack of energy intake, protein intake and also family income (24) (Figure 1).

Body image refers to the way individuals see and feel about their own physical appearance. As adolescents' bodies undergo many changes during growth, perceptions of body image can have a significant impact on their mental and emotional well-being (25). In this study, adolescents in both urban and rural areas had negative perceptions of their bodies. In Indonesia, adolescents' body image perceptions are often influenced by the prevailing culture and social norms. Indonesian culture has diverse views on beauty and body idealization that can influence how adolescents see themselves. In addition, physiological, cognitive and sociocultural factors regarding ideal body size can also influence body image perceptions (26, 27). Adolescents may feel pressure to achieve body standards that are considered ideal by society, sometimes in unhealthy ways such as excessive dieting (28).

In the digital age, adolescents are exposed to "perfect" body images through social media platforms (29). This can create feelings of dissatisfaction with their physical appearance and increase the desire to achieve often unrealistic standards (1). In addition, family, peers and social media contribute to body shape or size dissatisfaction (30). Perceived body image and nutritional status have a strong relationship in a person's physical and psychological health (27, 31). Body image refers to the way individuals see and feel about their physical appearance, while nutritional status reflects body health based on nutrient intake and weight. These two aspects influence each other and have a significant impact on each other. Negative body image perceptions can affect a person's nutritional status. A person who is dissatisfied with their physical appearance may tend to take extreme measures, such as unhealthy dieting, to achieve unrealistic beauty standards. This can negatively affect their nutrient intake and nutritional status. In Hoseini's study, it was stated that there was a significant relationship ($p = 0.023$) between perceived body image and healthy eating patterns (32). In addition, eating disorders such as anorexia nervosa and bulimia nervosa are often closely related to negative body image perceptions. Individuals with these eating disorders tend to have deep dissatisfaction with their physical appearance and attempt to control their weight in unhealthy ways, which can lead to serious nutritional problems (33).

Based on the results of the data analysis, it can be concluded that adolescents' consumption of macronutrients is still relatively low compared to the established RDAs. In urban areas, adolescents tend to consume less macronutrients, especially energy and carbohydrate. Whereas in rural areas, adolescents tend to consume less fat intake compared to the intake of other nutrients. In line with the research of Vilhar et al., only 75% of adolescents consume carbohydrates and protein, 44% consume fat and only 10% of adolescents whose consumption is close to the recommended nutritional needs (34).

Adolescents will experience many changes, for example in physical and body changes there is an increase in muscle mass, and an increase in fat tissue in the body, as well as hormonal changes. These changes have an impact on their increasing nutritional needs. The occurrence of an imbalance between nutritional needs and intake will cause nutritional problems, both overnutrition and undernutrition problems (24). Adolescent growth and development requires complete macro and micronutrients along with increased physical activity (35). Lack of consumption of nutrient intake, especially energy and protein intake, can increase the risk of developing CED nutritional problems in adolescents (34).

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 studies involving humans were approved by Mataram Health Polytechnic, Directorate General of Health Personnel, Ministry of Health of the Republic of Indonesia (LB.01.03/6/317/2023). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

CY: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. DR: Conceptualization, Data curation, Investigation, Writing – original draft. MM: Conceptualization, Data curation, Formal analysis, Software, Writing – review & editing. DS: Investigation, Writing – original draft.

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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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Fresh Moringa Stenopetala leaves consumption and its determinants among pregnant women in southern Ethiopia

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Background: A woman's health and nutritional status has significant impact on her pregnancy situation. However, many pregnant women are undernourished. Moringa stenopetala is a plant consumed worldwide in various forms, and its consumption showed a reduction in the incidence of malnutrition. Although Moringa stenopetala is rich in essential macro- and micronutrients, there is little evidence on the proportion and determinants of fresh Moringa stenopetala leaf intake among pregnant women. The objective of this study was to fill this gap in the littérature and provide a baseline evidence for further research or intervention by investigation the proportion and determinants of fresh Moringa stenopetala leaf intake among pregnant women in the Gamo zone, south Ethiopian region.

Methods: A community-based cross-sectional study was conducted among 623 randomly selected pregnant women using a pre-tested and structured questionnaire via a face-to-face interview. The consumption pattern was assessed based on a self-reported dietary history over the last 30 days before data collection. Multivariable logistic regression model was fitted using STATA version 14. An adjusted odds ratio with a 95% confidence interval was reported to show an association between the dependent and independent variables with level of statistical significance at a *p*-value of <0.05.

Results: The proportion of fresh Moringa stenopetala leaves intake among pregnant women was 49.60% (95% CI: 45.67, 53.52%). The déterminants of fresh moringa leaf intake were being below 24 years old (AOR: 2.92; 95% CI: 1.51, 5.63), rural résidence (AOR: 1.97; 95% CI: 1.10, 3.50), antenatal care attendance (AOR: 2.08; 95% CI: 1.03, 4.21), history of contraceptive use (AOR: 1.88; 95% CI: 1.03, 3.55), and having a good knowledge about the importance of moringa Stenopetala (AOR: 9.76; 95% CI: 5.30, 17.95).

Conclusion: The study showed that almost half of the pregnant women consumed fresh Moringa stenopetala leaves. Women's age, place of residence, prenatal care, history of contraceptive use, and knowledge of the benefits of Moringa Stenopetala were positively associated with the consumption of fresh Moringa Stenopetala leaves. Therefore, health authorities and stakeholders involved in maternal and child health need to target older women, and urban residents and promote the benefits of consumption by strengthening uptake of maternal health services and raising awareness about Moringa Stenopetela. Future studies involving large scale and longitudinal designs evidence are required to further validate the findings so that this nutritious diet can be promoted widely among pregnant women in the study area and Ethiopia at large.

KEYWORDS

Moringa stenopetala, pregnant women, Gamo zone, southern Ethiopia, consumption

Introduction

Women have unique nutritional needs throughout their lifespan, especially during pre-pregnancy and pregnancy periods. Access to nutritious, safe, affordable, and sustainable food is crucial for women's survival, health and well-being. Pregnancy also increases women's need for protein, vitamins and minerals (1).

Despite this, 154 million (10%) and 520 million (33%) women worldwide were underweight and anemic, respectively, in 2020 (2). The overall pooled prevalence of malnutrition during pregnancy was 23.5% in Africa (3) and 29.07% in Ethiopia in 2021 (4). Inadequate nutritional intake is common among pregnant women due to food taboos, food insecurity, infectious diseases, and inadequate care (5–9). Malnutrition during pregnancy increases the incidence of low birth weight, preterm birth, poor APGAR (appearance, pulse, grimacing, activity, and respiration) scores, stillbirth, neonatal death, and postpartum hemorrhage, leading to poor pregnancy outcomes (10, 11). In contrast, better nutrition during pregnancy is essential to promote fetal health, survival, growth, and development. It is also a key strategy to reduce the burden of malnutrition, incidence of non - communicable diseases, and maternal and child mortality (12–18). However, most pregnant women in Ethiopia have poor and inadequate nutritional practices, with a total prevalence of 67.7% was malnourished (7, 19). Therefore, improving maternal nutritional status is an urgent priority.

Moringa Stenopetala (MS) is a fast-growing drought-resistant tropical tree that originated in India (20). This is commonly referred to as the "miracle tree," (21) "never-dying tree," (22) and "mother's best friend" (23). It has different names in different localities, such as "Nébéday" in Senegal, "Yevu-ti" in Ghana and Togo, "Malunggay" in the Philippines (24), "Mlonge" in Kenya and Tanzania, "Zakalanda" in Zimbabwe, horseradish tree in the United States (24), horseradish or ben oil tree in the United Kingdom, "drumstick tree" in India (21, 22), and "Shiferaw" in Ethiopia (25). It also has different vernacular names in southern Ethiopia, such as "Aleko" or "Aluko" or "Haleko" in Gamo and Wolaita, "Kallanki" in Bena, "Telahu" in Tsemay, "Haleko" or "Shelchada" in Konso, and "Haleko" in Burje and Derashe (25).

Studies showed MS is safe for pregnant women and has a variety of nutritional and health benefits including reducing the risk of malnutrition, low birth weight, and anemia. Consuming MS during pregnancy may also help prevent DNA damage caused by stress and adverse pregnancy outcomes and improve breast milk supply. Additionally, MS has been shown to have anti-inflammatory and antioxidant effects that help fight diseases (26–33). Evidence also showed that MS leaves are rich in flavonoids, calcium, and antioxidants, making them suitable for consumption by pregnant women. In addition, MS leaf extract nanoparticles are reported to reduce anxiety levels among pregnant women with high blood pressure, and affected their blood pressure by increasing serotonin levels (34).

A country-wide study in Ethiopia found that 100 g of MS leaves contained 28.44% protein, 0.7% fat, 38.49% carbohydrate, 11.62% fiber, 54.85 mg iron, 1,918 mg calcium, 2.16 mg zinc, 2,094 mg potassium, 214.10 mg sodium, 28.49 mg vitamin C, 12.95 mg vitamin A, and other important nutrients (25). The finding indicate that MS species found in Ethiopia are rich in nutrients and their consumption helps in obtaining important macro- and micronutrients (25, 35–37). Current evidence in Ethiopia suggests that rising food prices will affect the intake of nutritious foods (38). MS leaves are a great way to fill this gap as they are rich in macro- and micronutrients and cheap as well.

Studies on consumption of MS species showed that it was widespread practice: 63% in India (39), 81% in Africa (39), 87.2% in Togo (40),and 90% in South Africa (41) but it has been underutilized (36%) in East Shoa Zones of Oromia,Ethiopia (42). Age, education level, marital status, occupation, household wealth status, awareness level, knowledge of plant biology, gender, and family size (43, 44) were factors associated with MS species consumption in the general population. However, such studies on the extent and determinants of MS use are lack among pregnant women. Although MS is widely cultivated in Ethiopia and has been indicated as having nutritional and health benefits for pregnant women, little is known about the level of consumption and factors affecting this during pregnancy. There are few studies on the nutritional, medicinal, and water purification properties of MS, and its effect on maternal hemoglobin levels, but there is little scientific evidence supporting MS consumption in pregnant women (25, 45). Therefore, this study aimed to investigate the level of consumption of fresh MS leaves, and factors associated with it among pregnant women in Gamo zone, southern Ethiopia.

Materials and methods

Study setting

The study was conducted in the Arba Minch Zuria and Chencha Districts, Gamo Zone, southern Ethiopia. It is located 434 Km and 443 km south of Addis Ababa (the capital city of Ethiopia) respectively (46). Based on projection made from the 2007 Ethiopian population and housing census the population residing in the two districts summed up to 353,019 in 2021/22, and the expected number of pregnant women was estimated to be 12,214. The area has two hospitals, nine health centers, sixty-three health posts, and a number of private health facilities at different levels of service standards that collectively provide curative, preventive, and rehabilitative services for the population (45).In the study area, fresh MS leaves are commonly prepared and consumed in the form of 'Kurkufa', and 'Fosossie' (through directly cooking a corn flour or its wraps with the leaves), and 'Kita'(in the form ofa flat bread locally prepared from various cereals including

Abbreviations: ANC, Antenatal care; AOR, Adjusted odds ratio; APGAR score, Appearance, Pulse, Grimace, Activity, and Respiration; CI, Confidence interval; FAO, Food and Agriculture Organization of the United Nation; GA, Gestational age; MS, Moringa stenopetala; MUAC, Mid Upper Arm Circumference; NICU, Newborn intensive care unit; OR, Odds ratio; PCA, Principal components analysis.

corn, wheat, barley or 'Teff'), and 'Haleko' (directly cooking the MS leaves in a separate dish like spinach and/or in the form of soup).

Study design and period

A community-based cross-sectional study was conducted from May 8th to June 20, 2022.

Population of the study

The source populations were all pregnant women living in Arba Minch Zuria and Chencha district, and the study populations were pregnant women in selected kebeles (*smallest administrative unit in Ethiopia*) in Arba Minch Zuria and Chencha district.

Eligibility

Inclusion criteria

All pregnant women who were in between 20 and 26 weeks of gestation at the time of recruitment and permanently residing in Arba Minch Zuria and Chencha district were included in the study.

Exclusion criteria

Women who did not give consent to participate in the study and who were seriously ill or unable to give information for the interview were excluded from the study. Women were considered as seriously ill when they had a health condition that made them unable to speak and carried a high risk of mortality and were more generally not able to perform activities and daily living and were excessively straining their care givers (47).

Sample size and sampling procedure

The sample size required to assess fresh MS consumption habits in pregnant women was estimated using the sample size estimation formula for single population proportions using Epiinfo version 7.2.3.1. As there are no previous studies on the proportion of fresh MS leaf consumption habits among pregnant women, we assumed a proportion of fresh MS leaf consumption during pregnancy (50%) (48, 49), and a confidence level of 95%, a margin of error of 5%. Considering the design effect of 1.5 for compensation of potential error due to the multistage cluster sampling and estimated non-response rate of 10%, the final sample size was 633.6(634).

A multistage cluster sampling method was used to recruit study participants into the study. From zone two districts were included in the survey. First, 10 kebeles from each of the districts were selected by lottery method. The list of pregnant women was taken from family folders available at the health posts, and samples were proportionally allocated to each of the selected kebeles based on the number of pregnant women with gestational age between 20 and 26 weeks. Finally, the required pregnant women were selected using simple random sampling technique from the sampling frame of pregnant women prepared for each kebele.

Study variable

The dependent variable was consumption of fresh MS leaves and the independent variables were socio-economic and demographic factors (age of mother, Place of residence, Educational Status), health and obstetric related factors (Gravidity, Number of <5 children, Pre-Contraceptive use, ANC attendance), nutritional factors (Changed dietary intake, Dietary diversity, Food aversion,) and knowledge about MS.

Operational definitions

MS consumers: women who consumed fresh moringa leaves at least once in the last 30 days (one month) before the data collection date regardless of amount or frequency were classified as consumers; otherwise they were regarded as non-consumers.

Knowledge about MS was assessed using questions consisting of 10-items, and women with knowledge scores equal to or above the mean score were classified as having good knowledge, and those who scored below the mean score were classified as having poor knowledge.

Measurement

Women's nutritional status was assessed using the mid-upper arm circumference (MUAC). This was measured using a flexible, non-stretchable standard tape at the mid-point between the tips of the ulna and the acromion and olecranon processes on the shoulder blade. The MUAC measurement was made on the right arm to the nearest 0.1 cm. Women with MUAC ≥23 centimeters were regarded as well nourished, and those with MUAC <23 centimeters were regarded as undernourished (7).

Minimum dietary diversity (MDD-W): Data were collected using a 24-h dietary recall method according to Food and Agriculture Organizations' (FAO) 2016 guideline. For each of the 10 food groups, a woman was asked what they ate 24 h before the data collection time with a score of 1 for yes and 0 for no. Scores were calculated by counting the number of food groups. Finally, women with a score of 5 or more out of 10 were categorized as having adequate dietary diversity; otherwise they were regarded as having inadequate dietary diversity (50).

Pregnancy was determined based on women's self-report of the first day of their last menstrual period (LMP), and gestational age was calculated based on the LMP and the date of data collection (51).

Data collection instrument

A pre-tested, interviewer-administered, and structured questionnaire, developed in English, and translated into the local language was used to collect data. Diet-related factors were assessed using a validated 24-h food frequency questionnaire (50). Household socioeconomic status was assessed by permanent household assets using a questionnaire adapted from the Ethiopian demographic and health survey household wealth index assessment tool (5).

Data collection techniques

Data were collected through face-to-face interview using a mobile phone-based application that allows filling of information electronically in both online and offline means and transfer of data to an online server created for this purpose (52). Ten nurses and two public health professionals were assigned to collect and supervise the data, respectively. Before data collection, the data collection teams were trained for 2 days on interview techniques and questionnaire content.

Data processing and analysis

Data were checked daily online for completeness, consistency, and missing values. Once data collection was completed, the raw data were downloaded and exported to STATA statistical software version 14.0 for analysis. Principal component analysis (PCA) was conducted to determine participants' wealth status and assess women's knowledge on the importance of consuming fresh MS leaves. Three of the knowledge items ["Can consuming M prevent malnutrition?" "Is MS used as medicine?," and "Does MS increase breast milk production?"] were removed because of commonalities less than 0.5 (0.49, 0.44, and 0.467, respectively). PCA revealed three factors explaining 85.3% of the total variance, and the factor score of the first factor explaining the maximum variance was used to classify women's knowledge on the importance of consuming fresh MS leaves. Reliability analysis also showed acceptable internal consistency (Cronbach's alpha = 0.85). Descriptive statistics including frequencies, percentages, means, and standard deviations were used to describe the characteristics of the study participants. For all explanatory variables, bivariate analysis was performed to assess the presence or absence of association with the outcome variable, and variables with p-values less than 0.25 in the bivariate analysis were included in the multivariable logistic regression analysis model. Adjusted odds ratio with its 95% CI and p-values < 0.05, respectively, were used to determine the degree of association and statistical significance. The results are presented in tables, figures, and texts.

Ethics approval and consent to participate

Ethical approval and permission were granted by the Institutional Review Board of Jimma University Institute of Health (reference number: THRPG 1/469/2022). Written approval was obtained from the Gamo zone health department, and health offices of Arba Minch zuria and Chencha districts. Before participation, all participants provided written informed consent as approved by the ethics committee, and all procedures were performed in accordance with the relevant guidelines and regulations of the Declaration of Helsinki on ethical principles for medical research involving human subjects. Data collectors obtained written consent by reading the consent form to participants before the interview and proceeded to interview only after confirming that women were willing to participate in the study. We tried to reduce social desirability bias by conducting interviews with participants in a private place at their homes while at the same time ensuring protection of participants' privacy, and confidentiality throughout the data collection process.

Results

Socio-demographic and economic characteristics of the study participants

A total of 623 pregnant women participated in the study giving a response rate of 98.3%. The mean age the participants was 25 years (SD ± 0.17), 500 (80.26%) of the participants were formally unemployed women who were housewives. Over half (51.69%) and two-third (68.86%) respectively were from rural areas and had less than five family members (Table 1).

Health and obstetric related characteristics of the study participant

Most of the study participants had less than five pregnancies (91.81%), and deliveries (93.54%).Nearly three-fourth of the participants (72.39%) had history of antenatal attendance (Table 2).

Dietary related characteristics of the study participants

Three hundred nine (49.60, 95% CI, 45.67, 53.52%) of the pregnant women consumed fresh MS leaves in the form of "Kurkufa," 'fossosie', and 'kita" along with 'haleko', a local dish made from fresh MS leaves. Nearly one-quarter of the pregnant women (23.27%) were malnourished, and nearly half (46.07%) did not eat the recommended number of meals during this pregnancy. Only 13 participants (2.09%) skipped meals during the current pregnancy (Table 3).

Women's knowledge on moringa stenopetala leaf consumption

Two hundred thirty-nine (38.36%) of the study participants had good knowledge about the importance of consuming fresh MS leaves. Participants were most likely to know about the edible parts of the MS tree (57.14%), followed by the importance of consuming MS for the pregnant mother and her fetus (55.22%). However, what they least knew was that MS can be consumed in powder form (1.12%), followed by its use in treating diabetes (11.08%) (Figure 1).

Frequency of fresh moringa stenopetala leaf consumption

Approximately half of women who consumed fresh MS leaves (44.34%) consumed it three or more times a day, and 178 women (77.4%) used it as a food source (Figure 2).

TABLE 1 Socio-demographic characteristics of pregnant women in Gamo zone, Southern Ethiopia, 2022.

Variables	Frequency	Percent
Age of respondent		
Below 24	273	43.82
24 to 34	328	52.65
Above 34	22	3.53
Place of residence		
Rural	322	51.69
Urban	301	48.31
Religion		
Muslim	02	0.32
Orthodox	138	22.15
Protestant	483	77.53
Marital status		
Married	621	99.68
Other	02	0.32
Maternal educational level		
No formal education	119	19.10
Grade 1 to 4	112	17.98
Grade 5 to 8	159	25.52
Grade 9 and10	146	23.43
Grade 11 and 12	34	5.46
College and above	53	8.51
Husband educational level		
No formal education	85	13.69
Grade 1 to 4	251	40.42
Grade 5 to 8	221	35.59
Grade 9 and 10	64	10.31
Grade 11 and 12	85	13.69
College and above	251	40.42
Occupation of the women		
Governmental worker	24	3.85
Merchant	82	13.16
Student/Farmer	17	2.73
House wife	500	80.26
Family size		
Less 5	429	68.86
5 and more	194	31.14
House hold head		
Male	597	95.83
Female	26	4.17
Wealth index		
Low	199	31.94
Middle	201	32.26
High	223	35.79

TABLE 2 Obstetrics and health-related characteristics of pregnant women in Gamo zone, southern Ethiopia, 2022.

Variables	Frequency	Percent
Age at first pregnancy		
Less or equal to 18 years	110	17.66
19 to 24 years	416	66.77
More than 24 years	97	15.57
Number of pregnancy		
One to four	572	91.81
Five and more	51	8.19
Number of delivery		
One to four	391	93.54
Five and more	27	6.46
History of abortion		
No	546	87.64
Yes	77	12.36
Number of under 5 children		
One	252	65.12
Two	135	34.88
Pregnancy status		
Unplanned	210	33.71
Planned	413	66.29
ANC visit		
No	172	27.61
Yes	451	72.39
GA at first ANC visit		
Less than 16wks	220	48.78
More than or equal to 16wks	231	51.22
Had Nausea/ vomiting		
No	294	47.19
Yes	329	52.81
Pre- pregnancy contraceptive use		
No	293	47.03
Yes	330	52.97
Had health insurance		
No	352	56.50
Yes	271	43.50
Distance to nearest health center		
Less than 5 km	470	75.44
More than 5 km	153	24.56

Factors associated with fresh moringa stenopetala leaf consumption

Of the 12 candidate variables for multivariable logistic regression analysis, seven associated with MS leaf intake during pregnancy. Age, place of residence, attendance to antenatal care, use of contraception before pregnancy, increased number of frequency, and amount of food intake, consumption of five or more food groups within the past 24 h, and women's knowledge of the importance of consuming fresh MS

TABLE 3 Dietary related characteristics of pregnant women in Gamo zone, Southern Ethiopia, 2022.

Variables	Frequency	Percent
Maternal nutritional status		
Under nourished	145	23.27
Well nourished	478	76.73
Meal frequency per day		
Two	13	2.09
Three	274	43.98
Four	249	39.97
Five	87	13.96
Increased the frequency and amount of dietary intake		
No	393	63.08
Yes	230	36.92
Dietary diversity for women		
No	347	55.70
Yes	276	44.30
Food aversion		
No	566	90.85
Yes	57	9.15
Getting nutritional counseling		
No	353	56.66
Yes	270	43.34
Fresh moringa leaf consumption		
Not consumed	314	50.40
Consumed	309	49.60

leaves during pregnancy. The odds of fresh MS leaves intake was higher in women aged <24 years [AOR = 2.92, 95%CI (1.51, 5.63)], and in rural residents [AOR = 1.97, 95% CI (1.10, 3.50)] compared with their age and residence counterparts, respectively. Visited health care facility for ANC [AOR = 2.08, 95% CI (1.03, 4.21)], increased the frequency and quantity of food intake during pregnancy [AOR = 2.89, 95% CI (1.54, 5.41)], consumed five or more food groups in the last 24 h [AOR = 2.33, 95% CI (1.33, 4.08)], using contraceptives before pregnancy [AOR = 1.88, 95% CI (1.03, 3.55)], and had good knowledge on the importance of MS consumption [AOR = 9.76, 95% CI (5.30, 17.95)] were positively associated factors (Table 4).

Discussion

Pregnancy is a critical period during which significant physiological and biochemical changes occur in the mother and the developing fetus (53). Maternal diet before and during pregnancy is a key factor in the health of the mother as well as the child and can have a lasting impact on the child's health and future development. MS is a nutritious plant grown in tropical regions of the developing world. All parts of the tree can be used in a variety of beneficial ways, but the leaves are a particularly good source of vitamins and other nutrients. Gram for gram, fresh moringa leaves have been reported to contain seven times more vitamin C than oranges, four times more vitamin A than carrots, three times more iron than spinach, four times more calcium than milk, and three times more vitamin D than cereals. They also contain more potassium than bananas and twice as much protein as yogurt (35). Micronutrient and vitamin intake is an important measure to promote maternal and child nutrition, health, and well-being, and should be continued throughout pregnancy, regardless of the nutritional status of the mother, especially in low- and

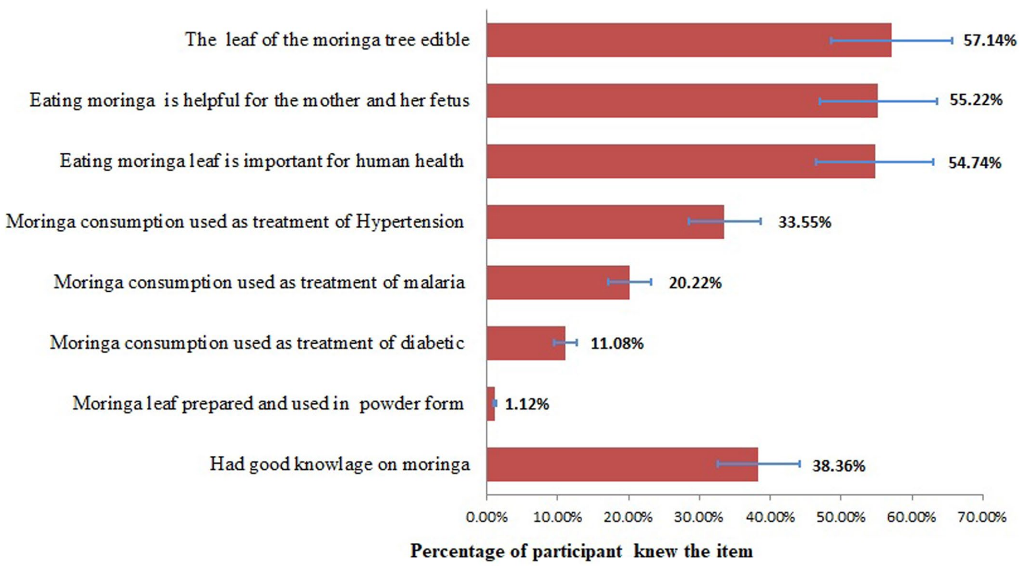

FIGURE 1
Knowledge of pregnant women on the importance of moringa in Gamo zone, Southern Ethiopia, 2022.

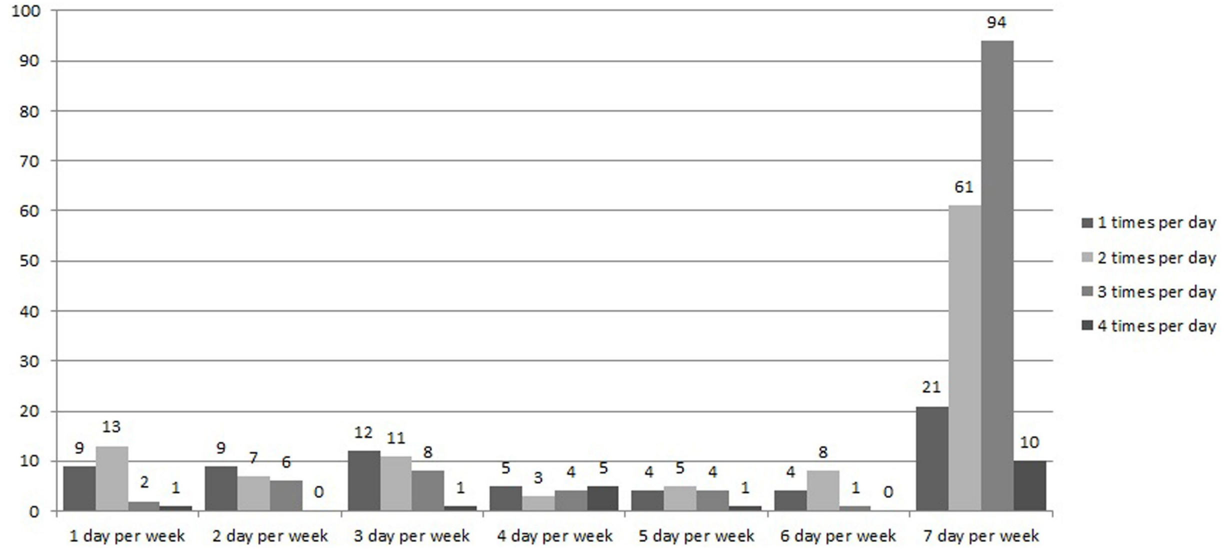

FIGURE 2
Frequency of fresh moringa stenopetala leaves consumption among pregnant women in Gamo zone, Southern Ethiopia, 2022.

middle-income countries. Moringa stenopetala leaves contain both micronutrients and vitamins, but consumption is still low in Ethiopia. In this study, we attempted to investigate the intake of fresh MS leaves and factors associated with the consumption among pregnant women in the Gamo zone of southern Ethiopia. The intake rate of fresh MS leaves among pregnant women was 49.60%. This is higher than previous studies in adults in central Ethiopia and Africa (39, 42), but lower than studies in Mauritius, Togo, and India (39, 40, 44). A possible explanation for this difference is probably the difference in culture, food habits, and awareness of the study participants. Pregnant women under 24 years of age were three times more likely to consume fresh MS leaves than those aged 24–34 years. This may be due to globalization, increased access to various media (54), and "education for all" (55) helping to improve dietary habits among young people. This contradicts a study conducted in Mauritius (44). This difference may be due to differences in age and food culture. Our study shows that rural residents are twice more likely to consume fresh MS leaves than urban residents. Similar results were reported in a study in Mauritius (44). A possible reason for this is the ease of growing and obtaining the crop in rural areas, increasing the likelihood of consuming fresh MS. Furthermore, rural residents are less likely to utilize modern healthcare due to lack of awareness and accessibility. Thus, they might rely on traditional medicine which promotes the consumption of MS leaves for its medicinal benefits (56). Pregnant women who visited health facilities for ANC were twice as likely to eat fresh MS leaves compared to non-visitors. The fact that ANC service is a good opportunity to provide pregnant women with advice on proper dietary habits (57) might increase their knowledge on healthy diet during pregnancy, which acts as a fuel for the intake of nutritious foods such as MS (58, 59).

Participants who increased their food intake during pregnancy were three times more likely to eat fresh MS leaves compared to participants who did not increase their food intake during pregnancy. Similarly, women who consumed five or more food groups were approximately twice as likely to consume fresh MS leaves compared to their counterparts. This can be explained by the fact that an increase in the frequency and amount of food intake can be used to increase the possibility of dietary diversity (58, 60). This could be because an increase in the frequency, amount, and food groups of meals could contribute to an increase in the consumption of more diverse foods including fresh MS leaves. Pregnant women with good knowledge about the importance of MS leaves were 10 times more likely to eat MS leaves compared to those with a lack of knowledge. This is in line with studies conducted in Mauritius (44) and Ethiopia (61) and could be because basic knowledge about nutrition can guide food choices (62). Women who used contraceptives before pregnancy were twice as likely to consume fresh MS leaves compared to those who did not. This may be because extended stays in health facilities promote health-promoting behaviors and the consumption of various food groups, increasing the likelihood of consuming fresh MS leaves (58). More than half of the pregnant women in the study area did not eat fresh MS leaves during pregnancy, and the majority of them did not have sufficient knowledge about the importance of MS leaves. The implication of these findings is that improved knowledge about the importance of fresh MS leaves consumption during pregnancy through increased utilization of health services, nutritional advice, and information dissemination could increase the consumption of fresh MS leaves, thereby allowing pregnant women to benefit from the nutritional content of MS.

Strength and limitations

The study was conducted at the community level in home gardens that provide an amenity to address a sensitive issue and is intended to be generalizable. There is a chance of recall bias on responses about dietary diversity and MS leaves consumption patterns particularly because of our participants background of education and culture. However, we have considered data from the most recent month before

TABLE 4 Factors associated with fresh moringa leaf consumption during pregnancy in Gamo zone, southern Ethiopia, 2022.

Variable	Consumer *n* = 309	Non-consumer *n* = 314	COR (95% CI)	AOR (95% CI)
Age in years				
Less 24	166 (53.72)	107 (34.08)	2.19 (1.58, 3.04)**	2.92 (1.51, 5.63)**
24 to 34	136 (44.01)	192 (61.15)	1	1
Above 34	7 (2.27)	15 (4.78)	0.66 (0.26,1.66)	1.02 (0.28, 3.76)
Place of residence				
Rural	185 (59.87)	137 (43.63)	1.93 (1.40, 2.65)**	1.97 (1.10, 3.50)*
Urban	124 (40.13)	177 (56.37)	1	1
ANC follow up				
No	57 (18.45)	115 (36.62)	1	1
Yes	252 (81.55)	199 (63.38)	2.55 (1.76, 3.69)**	2.08 (1.03, 4.21)*
Pre- contraceptive use				
No	126 (40.78)	167 (53.18)	1	1
Yes	183 (59.22)	147 (46.82)	1.65 (1.20, 2.26)**	1.88 (1.03, 3.55)*
Increased the frequency and amount of dietary intake				
No	138 (44.66)	255 (81.21)	1	1
Yes	171 (55.34)	59(18.79)	5.35 (3.73, 7.68)**	2.89 (1.54, 5.41)**
Dietary diversity				
No	139 (44.98)	208 (66.24)	1	1
Yes	170 (55.02)	106 (33.76)	2.40 (1.73, 3.31)**	2.33 (1.33, 4.08)**
Knowledge on moringa				
Poor knowledge	113 (36.57)	271 (86.31)	1	1
Good knowledge	196 (63.43)	43 (13.69)	10.93 (7.35,16.24)**	9.76 (5.30, 17.95)**

** $p < 0.01$; *p-value <0.05.

the survey period which is deemed to minimize recall bias regarding consumption habits. Added on this, the cross-sectional nature of the data might affect temporality and strength of the evidence reported from this study (63).

Conclusion

The study found that only half of the pregnant women consumed fresh MS leaves during pregnancy. Age, place of residence, utilization of health services (ANC visits and contraceptive use), knowledge about the importance of consuming MS leaves, higher frequency and amount of food intake, and consumption of more than five food groups were independently associated with consumption of fresh MS leaves. Therefore, the relevant authorities should promote the utilization of this wonderful gift to increase knowledge and consumption habits. Furthermore, the above factors associated with MS leaf consumption should be taken into consideration in the efforts. Policymakers should invest more and pay attention to the production of "high quality" food from plants like MS. Further research is needed to determine the amount of fresh MS leaves consumed per meal (per serving) and other relevant factors that may prevent this group from consuming this wonderful plant.

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 humans were approved by the Institutional Review Board (of) Institute of Health, Jimma University, with reference number Ref. No. THRPG 1/469/2022. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

ZD: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. GD: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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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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Nutritional knowledge and practice among antiretroviral therapy user adults in Bule Hora hospital, southern Oromia, Ethiopia

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Introduction: Nutrition is the necessary basis for life, health, and human development over the entire lifespan. Poor nutritional knowledge, poor nutritional practices, and malnutrition among HIV-positive adults can contribute to accelerating the progression of Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) and related diseases. Therefore, this study aimed to assess the dietary knowledge, practices and associated factors of HIV-positive adults participating in antiretroviral therapy (ART) at Bule Hora Hospital, West Guji Zone, South Oromia, Ethiopia.

Methods: A cross-sectional institutional study was conducted among 418 HIV-positive adults by systematic sampling technique. Semi-structured questionnaires were used for data collection and analyzed with SPSS version 21.0. Logistic regression analyses were used to identify factors associated with dependent variables using adjusted odds ratio (AOR), with 95% CI (confidence interval) at $p < 0.05$.

Results: The result of this study showed that the prevalence of poor nutritional knowledge and poor nutritional practices among (HIV) positive adults was 74.9 and 69.1%, respectively. In the multivariate analysis, adult age (AOR = 2.37, 95% CI: 1.30, 4.32), marital status (AOR = 2.46, 95% CI: 1.29, 4, 69), educational level (AOR = 1.83, 95% CI: 1.01, 3.30) and occupational status (AOR = 0.55, 95% CI: 0.25, 0.94) were significantly associated with the nutritional knowledge. Educational level (AOR = 2.58, 95% CI: 1.48, 4.50), monthly income (AOR = 2.80, 95% CI: 1.68, 4.69), and adult occupational status (AOR = 0.48, 95% CI: 0.26, 0.89) were also significantly associated with the level of dietary practice.

Conclusion: It was concluded that the respondents' nutritional knowledge and practices in the city of Bule Hora were poor compared to other national findings. The identified factors related to nutritional knowledge and practices were educational level, monthly income, adult occupation, and marital status of respondents in the study area. Therefore, each concerned agency should address the above gaps in nutritional knowledge and practices of HIV-positive adults in the study area.

KEYWORDS

nutritional knowledge, adults, associated factors, dietary practice, antiretroviral therapy, Ethiopia

1 Introduction

Human immunodeficiency virus (HIV) continues to be a significant public health concern worldwide, resulting in the loss of 40.4 million lives to date, while transmission persists in every country across the globe (1). Globally, 15 nations are responsible for almost three-quarters of the total population living with HIV (2). It is of utmost importance to guarantee that individuals living with HIV in these countries are able to access essential HIV treatment services. Over the past 20 years, there has been undeniable evidence of significant achievements in reducing the negative impacts of HIV, such as morbidity, mortality, transmission, and stigma (3).

According to the Joint United Nations Program on HIV in 2016, there were approximately 36.7 million individuals living with HIV/Acquired immunodeficiency syndrome (AIDS) worldwide (4). Out of this number, around 19.5 million people had access to antiretroviral therapy (ART). The African region continues to be the most heavily impacted, with 19.4 million individuals living with HIV/AIDS, including 11.4 million who have access to ART.

Ethiopia has one of the world's highest rates of malnutrition and it is frequently observed in HIV-positive adults in Ethiopia who are in advanced stages of the disease, experiencing anemia, diarrhea, and have a Clusters of differentiation 4 (CD4) count below 200 cells/mm^3 (5). The intertwining of malnutrition and human immunodeficiency virus (HIV) creates a relentless cycle that is further exacerbated in countries with low and middle-income levels. HIV/AIDS patients necessitate an additional 10% of energy when they are asymptomatic and 20–30% more when they experience symptoms, in contrast to uninfected individuals (6). Nevertheless, the presence of food insecurity and malnutrition adversely impacts the dietary consumption and quality life of these individuals (7). Malnutrition alone can have a detrimental impact on the cluster of differentiation-four (CD4+) T cells, resulting in an impaired B-cell response. Approximately 35 million people live with HIV/AIDS in the world (8).

Proper nutrition plays a crucial role in maintaining long-term health and overall well-being. Research indicates that individuals with HIV who consistently consume nutritious meals in appropriate portions are able to improve their tolerance to HIV medications, manage a healthy weight, and experience an enhanced sense of well-being (9). Good nutrition could be therefore improve the quality of life of people living with HIV/AIDS.

The 2011 Ethiopia Demographic and Health Survey (EDHS) showed that the urban prevalence of HIV/AIDS was 4.2% and in the rural it was 0.6% (10). The EDHS also further reported that HIV prevalence varies by region, ranging from 0.9% in Southern Nations, Nationalities, and Peoples' Region (SNNPR) to 6.5% in Gambella regions of Ethiopia. For examples in 2005, Gambella, Addis Ababa, and Harari regions had the highest prevalence rates of 6.0, 4.7, and 3.5%, respectively (11). Conversely, the 2016 survey showed that Gambella regional state had the highest prevalence rate at 4.8%, followed by Addis Ababa at 3.4%.

Improper nutritional knowledge and practice management is a serious problem among people living with HIV/AIDS. Inadequate nutritional knowledge and practice of people with HIV infection lead to cause improper management of disease and lower immunological status as well as accelerates the progression of HIV/AIDS-related disease (12). In India, about 52% of HIV-positive patients had poor nutritional knowledge whereas 72% had poor nutritional practice (13). According to the study conducted in Ethiopia, about 25.8 and 3.2% of HIV-positive adults had poor nutritional knowledge and poor dietary practices, respectively (14).

Various factors influence the nutritional and dietary habits of individuals living with HIV. Among them, the occurrence of gastrointestinal symptoms, familiarity with the concept of good nutrition, and possessing adequate knowledge about nutrition were found to be influential factors in determining dietary practices (14). The study conducted in Ghana also reported that about 9.1% of the participants had inadequate knowledge about nutrition (15). In Nigeria, it was reported that 11.7% of women living with HIV/AIDS had low scores in nutritional knowledge (16). Another study conducted by Anand and Puri (17) revealed that 12.0% of people living with HIV from New Delhi, India had poor understanding of nutrition.

Despite progress made in the past 10 years, there is still a lack of representation of young adults in studies aimed at enhancing HIV prevention and treatment. Even though communities in the study area face disadvantages in comparison to those residing in other regions of Ethiopia, there is a lack of health and nutrition information available. Knowledge and practice of HIV-positive adults' (>19 ages). Hence, the purpose of this research was to assess the nutritional knowledge, practices, and influencing factors in HIV-positive patients undergoing antiretroviral treatment at Bule Hora Hospital in Southern Oromia, Ethiopia.

2 Materials and methods

2.1 Description of the study area

The research was carried out at the Bule Hora Hospital Clinic, situated in the Oromia region of Ethiopia. This particular study is positioned in the West Guji Zonal Town, which is located to the south of Oromia and also south of the capital city, Addis Ababa. The distance between Bule Hora and Addis Ababa is approximately 470 km. The city of Bule Hora has a population of around 14,1,579 and is equipped with a health center and a hospital to cater to the healthcare needs of the residents and surrounding areas. In addition to serving the local population, this hospital also provides medical services to individuals from neighboring zones such as Gedao zone, including Gedab, Corso, and Chelelektu districts. Bule Hora Hospital currently has a total of 1,650 people living with HIV (PLHIV), all of whom are receiving antiretroviral therapy (ART) and being closely monitored.

2.2 Study design and period

A structured and pre-tested questionnaire was used to conduct an institutional cross-sectional study design, which aimed to evaluate the nutritional knowledge and practices of ART users who are HIV-positive adults aged over 19 years at Bule Hora Hospital. This study was carried out at Bule Hora Hospital in West Guji City, Oromia Regional State, Ethiopia, between September 23, 2019 and January 10, 2020.

2.3 Source and study population

All PLWHA, and ART user adults aged greater than 19 years old who were on follow-up in Bule Hora hospital were used a source population while those all PLWHA and ART user adults who were selected using systematic sampling method were used as study population.

2.4 Inclusion and exclusion criteria

Patients who had already started ART and were older than 19 years were included in the study area. Patients who are critically ill and unable to communicate, HIV cases who have not yet started ART, pregnant women, and non-voluntaries were excluded from the study.

2.5 Sample size determination

The minimum sample size of the study was calculated using the single population proportion formula with the following assumptions:

$$n = \frac{Z^2.p.q}{d^2}$$

Where: $q = 1 - P$, $n =$ a minimum number of sample size, $p = 45\%$ (0.45; proportion of undernutrition among adult HIV-positive individuals) in the study area from a similar survey (18), $d =$ margin of error = 5%. Therefore, by considering 10% of non-response rate, the final sample size used for this study was 380.

2.6 Sampling procedures

The targeted sampling procedure was used to select the HIV-positive adult patients of Bule Hora Hospital to submit their responses to the questionnaire provided. Since the patients were arranged systematically, the systematic sampling method was used for this study. This sampling interval was explained using the formula: $K = N/n$, where: $K =$ the sampling interval used to select every K^{th} item/subject from the sampling frame. $N =$ total population size of patients with HIV in Bule Hora Hospital = 1,650, n = sample size ($n = 418$ patients). Therefore $K = 1650/418 = \sim 4$. Systematic sampling technique was used to select every 4th patient from the total sample frame of 1,650 HIV-positive adults who had follow up at the clinic to compose the 418 patients as the sample size. The data collection was carried out on a daily basis from September 2019 to January 2020 until the necessary sample for the study was obtained.

2.7 Study variables

The dependent variables of this study included dietary knowledge and practices of HIV-positive adults. The independent variables also included socioeconomic and demographic factors; gender, age, marital status, education level, family size, adult occupation, monthly income, ethnicity, place of residence, and religion.

2.8 Data collection method

The items of the questionnaire were developed from similar studies and adapted depending on the field of study. The details of nutritional knowledge and practices among adult ART users (>19 years) were collected by distributing structured questionnaires to respondents from the targeted study area. The data collection technique was performed using a semi-structured questionnaire to obtain all the required information. These questionnaires were developed in English and then translated into Afan Oromo, the local language, and back into English to ensure consistency using fluent individuals and pre-tested at nearby health center (Tore Health Center) on 5% of the total number of adults selected. The questionnaire determined information on socio-demographic characteristics (age, marital status, place of residence, level of education, family size, income, professional position, religion, and ethnicity); Nutritional knowledge, and practice of HIV-positive adults in the study area. Data collectors and supervisors received intensive training for two days on the purpose of the study, questionnaire, data collection methods, process of matching study participants, and ethics of the study during data collection.

To ensure the quality of the data, the English version of the questionnaire was carefully translated to the local language which was 'Afaan Oromoo' version and back to English by language translators to check for consistency. The data collectors received extensive training on how to use the questionnaires and information was collected under the close supervision of trained supervisors and principal investigators to obtain reliable and valid data. At the end of each day, the collected data were checked by principal investigator for completeness and consistency.

2.8.1 Nutritional knowledge

Data on nutritional knowledge were collected using a semi-structured questionnaire from HIV-positive adults undergoing antiretroviral therapy in the study area. The questionnaires were designed on nutritional knowledge, containing 12 questions to categorize their knowledge into good and poor nutritional knowledge depending on their answer. Therefore, for every correct answer, the score is one (1) point and for every wrong answer, the score zero (0) point. Then, an individual could score a minimum of zero and a maximum of 12 points. Thus, the respondents who scored less than six (0–5.99) out of 12 questions had poor nutritional knowledge and those who scored greater than six (6–12) had good nutritional knowledge.

2.8.2 Dietary practice

The dietary practice questionnaires were designed to assess the nutritional practices of HIV-positive adult patients (>19 years) attending an antiretroviral therapy (ART) clinic in the study area. There were 11 questions with positive and negative answers. Therefore, for every correct answer, the score is one (1) point and for every wrong answer, the score zero (0) point. Then, an individual could score a minimum of zero and a maximum of 11 points. Thus, the respondents who scored less than half (0–5.499) out of 11 questions had poor dietary practices and those patients who scored equal to or greater than half (5.5–11) had good dietary practices.

2.9 Ethical consideration

The ethical approval was obtained from the Research Review Board at Wollega University. A formal letter of cooperation was then requested from Wollega University to Bule Hora Hospital. Then a letter of support was written to the hospital's ART Clinic office. Customer response was anonymous and the data collectors informed customers that they had the full right to withdraw from the study or to refuse to participate. A consent form was also attached to the questionnaire to obtain each individual's permission.

2.10 Statistical analysis

The raw data collected through questionnaire survey, interview, and direct observation were first checked for completeness, coded, and entered into the computer using the Version 21 Statistical Package for Social Sciences (SPSS) for further analysis. Data on adult socio-demographic characteristics were summarized using descriptive statistics such as frequency, mean and percentage. In addition, Logistic regression analysis was also employed to see the association between dependent and independent variables at a p value less than 0.05. Finally, adjusted odds ratio (AOR) with a 95% confidence level was reported.

3 Result and discussion

3.1 Socio-demographic characteristics of the respondents

Four hundred and eighteen HIV-positive adults were sampled for this study and all completed the questionnaires with a 100% response rate. In this study, 45 and 55% of participants were male and female, respectively. Moreover, 35.9% of the respondents were married, 28.5% widowed, 23.7% divorced, and 12.0% were single (Table 1). In addition, about 36.8% of the study participants had no formal education. Concerning the residence, more than half participants were lived in urban area.

TABLE 1 Socio-demographic characteristics of respondents.

Variables	Category	Frequency	Percent
Sex	Male	188	45.0%
	Female	230	55.0%
Ages of adult	20–30	180	43.06%
	31–40	159	38.0%
	>40	79	18.9
	Mean age	33 ± 1.6	
Marital status	Single	50	12.0%
	Married	150	35.9%
	Divorced	99	23.7%
	Windowed	119	28.5%
Educational level	No formal education	154	36.8%
	Completed Primary	166	39.7%
	Tenth completed and above	98	23.4%
Place of residence	Urban	259	52.0%
	Rural	159	38.0%
Family size	≤ 3	253	60.5
	4–6	111	26.6
	≥7	54	12.9
Average monthly income	1,000–2000	168	40.2%
	2001–3,000	150	35.9%
	≥ 3,001	100	23.9%
Religion	Orthodox	125	29.9%
	Protestant	171	40.9%
	Wakefata	83	19.9%
	Muslim	39	9.3%
Adults occupation	Farmer/housewife	70	16.7%
	Merchant	106	25.4%
	Governmental employee	110	26.3%
	Daily laborers and others	132	31.6%
Ethnic group	Oromo	257	61.5%
	Gedao	97	23.2%
	Burji, Amhara and others	64	15.3%

3.2 Nutritional knowledge of respondents

According to the finding of this study, 53.1% of the respondents knew that fish is as a source of protein while 28.2% of the participants did not. A similar study was conducted in Nigeria, which shows that about 55.9% of adult HIV/AIDS patients undergoing ART considered fish as a protein source (12). This can be explained by the fact that the level of education and health information increases the nutritional knowledge of the respondents.

In this study, almost half of 49.5% respondents had information about high-protein foods that build and repair body tissues while 36.6% had no information. This study result is lower than the study conducted in Ethiopia, 73.7% (14) and Nigeria, 80.6% (16) and in Ethiopia, which show which show proteins are used to build and repair body tissues. This difference could be due to a lack of information, sample size and seasonality of food production in the study area.

According to the results of the study, more than half of 56.9%of the respondents knew carbohydrates and lipids as sources of energy and almost a third of 31.1% did not know. A similar study was conducted in the city of Bahir Dar, north-western Ethiopia which shows that 71.1% of respondents knew that carbohydrates and fats are energy-giving nutrients for adults who were suffered from HIV/AIDS (14).

Furthermore, the findings indicate that nearly 72.5% of the participants were aware of the significance of maintaining a well-balanced diet to prevent infections, whereas 17.2% of them lacked this knowledge (Table 2). The result of the study is comparable with the previous studies conducted in Nigeria, which shows that 70.1% of the respondents knew the importance of a balanced diet in preventing infection (16). The study finding is also similar with the study conducted in Ethiopia, which reports that about 77.2% of the adults on antiretroviral therapy knew that balance diet can be used as prevention of infection (14).

According to the finding of this study, almost half of the 47.1% respondents knew about the iron source and 43.8% of them did not. The finding of the study is higher than the study conducted in western Oromia region, Ethiopia, which shows that 19.3% of pregnant mothers knew the iron source (19). This could be because HIV-positive adults are in contact with health workers at least once a month to receive medication and nutrition related information to get iron rich foods and increase the production of red blood cells.

Moreover, the result of the study revealed that almost three-quarters of 74.9% of the respondents had poor nutritional knowledge and a quarter of 25.1%of the had good nutritional knowledge in the study area. The finding is relatively higher than that of the study conducted at Bahir Dar city, Ethiopia which shows that 21.7% of adults on ART had good knowledge of nutrition (14). A similar study conducted in Nigeria also found that 23.5% of women living with HIV/AIDS had a good knowledge of nutrition (16). However, this finding is lower than the study conducted in Uganda, which shows that 88% of women living with HIV/AIDS had a good nutritional knowledge (20). Similarly, about 70.9% of the people living with HIV in Ghan (15) and 67% of study subjects in Swaziland (21). had good nutritional knowledge, which are higher than the current study finding. The difference in results may be due to health and nutrition information, health services, socio-demographic factors, sample size and data collection period.

3.3 The nutritional practice of respondents in the study area

Table 3 shows that more than half 54.3% of the participants ate breakfast every day while 32.1% of them did not. Besides, the majority, 66.5% respondents ate their meal <3 times, while 33.5% of them ate greater than 3 times in the previous 24 h (Table 3). The finding of this study is supported by a study conducted in Uganda (20), which reports that 78.2% of women with HIV/AIDS eat their meals <3 times in the previous 24 h. The result of the study also revealed that almost three-quarters of 72.5% of the participants had milk and dairy products while 17.2% of them did not use milk and milk products in the study area. This study finding was relatively higher than the study conducted in Nigeria, which shows that the majority of respondents (66.3%) used milk and dairy products (16).

Regarding preparing a balanced diet, almost half of the 49.0% said preparing a balanced meal is time-consuming while more than a third of the 40.7% said it is not time-consuming. The result of the study, supported by the study conducted in Nigeria (16), which reported that 40.1% of women living with HIV/AIDS agreed that preparing a balanced meal is not time-consuming.

The results of this study revealed that the majority of 82.5% of the respondents took medication after a meal, while 12.2% did not take any medication after a meal. This finding is agreed with a study conducted in India (13), which shows that 95.3% of HIV-infected women on ART treatment take medication after a meal.

Similarly, this study identified that almost nearest to half 49.5% of participants did not use snacks. These results disagreed with research carried out in India, which suggested that 30 % (30%) of the women practiced consuming snacks/fruits between their main meals (13). This difference might be because of the time of study and lack of nutritional and health information.

The result of the study showed that more than half 52.2% of the participants did not practice eating food during the period of illness. Besides, more than three fourth 78.7% of respondents were drunk 8 glasses of water per day and 14.4% of them did not. The result of this study is similar to the study conducted in India which shows that more than three fourth (76%) of HIV-infected women with ART treatment practice 8 glasses of water per day (13).

Regarding washing hands and utensils before and after preparing food, almost three-quarters of participants 72.7% answered that they

TABLE 2 Nutritional knowledge of respondents in the study area.

Variables/statements	Response			
		Yes, n (%)	No, n(%)	Do not know, n (%)
Knowledge about fish as sources of protein		222 (53.1)	118 (28.2)	78 (18.7)
Knowledge about the six component of food groups	F	193 (46.2)	162 (38.8)	63 (15.1)
Knowledge about food sources of carbohydrate		147 (35.2)	204 (48.8)	67 (16.0)
Knowledge about protein-rich food builds and repairs body tissues		207 (49.5)	153 (36.6)	58 (13.9)
Knowledge about carbohydrate and lipid as energy providers		238 (56.9)	130 (31.1)	50 (12.0)
Knowledge about balanced diet as preventing infection		303 (72.5)	72 (17.5)	43 (10.3)
Knowledge about the benefit of dietary diversity to HIV-positive patients		183 (43.8)	182 (43.5)	52 (12.4)
Knowledge about the source of iron		197 (47.1)	183 (43.8)	38 (9.1)
Knowledge about the source of vitamin- E		189 (45.2)	196 (46.9)	33 (7.9)
Knowledge about water as a nutrient		246 (58.9)	122 (29.2)	50 (12.0)
Knowledge about fruit and vegetable rich in vitamins and minerals		222 (53.1)	159 (38.0)	37 (8.9)
Knowledge about bananas as a control of diarrhea in HIV patients		236 (56.5)	165 (39.5)	17 (4.1)
Overall levels of nutritional knowledge	Good	105 (25.1%)		
	Poor	313 (74.9%)		

TABLE 3 Nutritional practice of respondents in the study area.

Variables		Frequency (n)	Percent (%)
Eating breakfast every day	Yes	227	54.3%
	No	134	32.1%
	Do not know	57	13.6%
Number of meals consumed in the preceding 24 h	< 3	278	66.5%
	≥3	140	33.5%
Eating milk and milk product	Yes	303	72.5%
	No	72	17.2%
	Do not know	43	10.3%
Preparing a balanced meal is not time consume	Yes	170	40.7%
	No	205	49.0%
	Do not know	43	10.3%
Washing fruit and vegetables before consumption	Yes	295	70.6%
	No	94	22.5%
	Do not know	29	6.9%
Taking medicine after a meal	Yes	345	82.5%
	No	51	12.2%
	Do not know	22	5.3%
Eating snacks between the main meal	Yes	207	49.5%
	No	194	46.4%
	Do not know	17	4.1%
Eating food during the period of illness	Yes	169	40.4%
	No	218	52.2%
	Do not know	31	7.4%
Drinking at least 8 glasses of water per day	Yes	329	77.8%
	No	60	14.8%
	Do not know	31	7.4%
Washing hands and utensils before & after the preparation of the food	Yes	304	72.7%
	No	76	18.2%
	Do not know	38	9.1%
Number of food groups consumed in the preceding 24 h	< 6	251	60.0%
	≥ 6	167	40.0%
Overall levels of nutritional practice	Good 129 30.9		
	Poor 289 69.1		

wash their hands and utensils, while very few of them 18.2% did not (Table 3).

The result of the study also revealed that the majority of 69.1% of the participants had poor dietary practices, while 30.9% of them had good dietary practices in the study area (Table 3). This result is relatively higher than the studies conducted in different regions of Ethiopia (14, 22–24). But the study finding is lower than the study conducted in Nigeria (16), which shows that 65.1% of women living with HIV/AIDS had good dietary practices. Another similar study was reported from the southern part of Ethiopia, which shows that about 60.1% of patients had inadequate dietary diversity (25).

Furthermore, this finding is lower than the study conducted in Nigeria (16), which shows that 65.1% of women living with HIV/AIDS had good nutritional practices. This study is similar with the study conducted in Switzerland, which shows that about 51% of pregnant and lactating women living with HIV/AIDS in had good nutritional practices (21). The study conducted in India showed that 28% of HIV-infected women had good nutritional practices (13).

3.4 Factors associated with the nutritional knowledge of respondents

In the multivariate analysis after controlling for possible confounders: adult age (AOR = 2.37, 95% CI: 1.30, 4.32), marital status (AOR = 2.46, 95% CI: 1.29, 4 0.69), an education level (AOR = 1.83, 95% CI: 1.01, 3.30) and occupation of adults (AOR = 0.49 and 0.55,

TABLE 4 Factors associated with the nutritional knowledge of respondents in the study area.

Variables	Frequency (n)	Percent (%)	COR(95%CI)	*p* value	AOR(95%CI)	*p* value
Age of adults						
20–30	180	43.1	1		1	
31–40	159	38.0%	2.95 (2.04,4.26) *	0.001	1.46 (0.82,2.58)	0.23
>40	79	18.9%	4.48 (3.00,6.70)*	0.02	2.37 (1.30,4.32)*	0.01
Marital status						
Single	50	12.0%	1		1	
Married	150	35.9%	4.00 (2.00,8.00)*	0.001	1.55 (0.70,3.45)	0.34
Divorced	99	23.7%	2.85 (1.98,4.10)*	0.02	1.40 (0.84,2.34)	0.31
Widowed	119	28.5%	5.19 (3.04,8.86)*	0.023	2.46 (1.29,4.69)*	0.014
Level of educational						
No formal education	154	36.8%	1		1	
Completed primary	166	39.7%	4.13 (2.77,6.16)*	0.001	1.83 (1.01,3.30)*	0.02
Tenth complete & above	98	23.4%	2.77 (1.96,3.91) *	0.01	1.32 (0.77,2.28)	0.56
Average monthly income						
1,000–2000	168	40.2%	1		1	
2001–3,000	150	35.9%	3.20 (2.24,4.56)*	0.04	1.41 (0.82,2.43)	0.057
≥3,001	100	23.9%	3.55 (2.41,5.22)*	0.36	1.56 (0.88,2.75)	0.56
Adults' occupational status						
Farmer/housewife only	70	15.6%	1		1	
Merchant	106	25.8%	2.04 (1.24,3.37)*	0.012	0.49 (0.25,0.94) *	0.001
Governmental employee	110	26.3%	2.31 (1.53,3.50)*	0.034	0.55 (0.31,0.99) *	0.002
Daily laborer	132	32.3%	3.40 (2.18, 5.31)*	0.001	0.91 (0.51,1.64)	0.061

*$p < 0.05$; statistically significant, 1; Reference, COR, Crude odds ratio; AOR, Adjusted odds ratio; ETB, Ethiopian Birr.

95% CI: 0.25, 0.94 and 0.31, 0.99) were factors significantly associated with the nutritional knowledge in the study area (Table 4).

Accordingly, the age of the participants was one of the factors associated with the nutritional knowledge of HIV-positive adults in the study area. Thus, study participants whose age group was over (>40 years) were 2.37 times more likely to be knowledgeable than those whose age group was 15–20 years old (AOR = 2.37, 95% CI: 1.30, 4.32). This finding is supported by the studies conducted in Nigeria (16, 26). This could be because as the participants get older, so does their nutritional knowledge.

Moreover, HIV-positive widowed adults were 2.46 times more likely to be knowledgeable about nutrition than single adults (AOR = 2.46, 95% CI: 1.29, 4.69). The result of this study is similar to that of the study conducted in Nigeria (16). This could be due to a lack of partnerships to receive nutritional information and a lack of accessible education.

Additionally, the educational status of HIV-positive adults was significantly associated with nutritional knowledge in this study area. Respondents who completed elementary school were 1.83 times more likely to have knowledge than those who had no formal education (AOR = 1.83, 95% CI: 1.01, 3.30). This finding is supported by the study conducted in Nigeria, where women living with HIV/AIDS with at least secondary education were more likely to have nutritional knowledge than women with no education (16). Other studies conducted in Malaysia and Nigeria found that people with higher levels of education had better nutrition knowledge (26, 27).This could be because HIV-positive adults living in higher socioeconomic status had the opportunity to purchase and use various electronic devices important for improving nutrition education.

The study finding also reported that participants who were merchants and civil servants were 51 and 45% less likely to be nutritionally literate than those who were day laborers, respectively (AOR = 0.49 and 0.55, 95% CI: 0 0.25, 0.94 and 0.31, 0.99) field of study. This study was supported by research conducted Nigeria (12).

3.5 Factors associated with the nutritional practice of respondents

Although in the multivariate analysis, after controlling for possible confounders: educational level (AOR = 2.58, 95% CI: 1.48, 4.50), average monthly income (AOR = 2.80, 95% CI: 1.68, 4.69), and adult occupation (AOR = 0.48, 95% CI: 0.26, 0.89) were significantly associated with the level of dietary practice in the study area (Table 5).

This finding was supported by the study conducted in Uganda, which shows that nutritional education and good nutritional practices were found to have a positive correlation. Their results were confirmed that well-structured dietary advice helps raise awareness of the importance of nutrition (20). Dietary advice not only improves food intake but also promotes dietary diversity (28). As nutritional knowledge increases, people living with HIV/AIDS tend to eat more meals than the traditional three meals in a day (29).

Another important factor influencing the dietary practices of HIV-positive adults was occupational status, according to this study.

TABLE 5 Factors associated with the nutritional practice of respondents in the study area.

Variables	Frequency (n)	Percent(%)	COR(95%CI)	*p* value	AOR(95%CI)	*p* value
Educational level						
Uneducated	154	36.8	1		1	
Completed Primary	166	39.7	3.53 (2.41, 5.17)*	0.01	2.58 (1.48, 4.50)*	0.001
Tenth complete & above	98	23.4	1.72 (1.26,2.36)*	0.21	1.17 (0.70, 1.95)	0.23
Family size						
≤3	253	60.5	1		1	
4–6	111	26.6	2.09 (1.60, 2.71)*	0.56	0.82 (0.50, 1.37)	0.45
>=7	54	12.9	1.92 (1.30, 2.84)*	0.43	0.69 (0.38, 1.24)	0.79
Monthly income in ETB						
1,000–2000	168	40.2	1		1	
2001–3,000	150	35.9	3.10 (2.18, 4.41)*	0.001	2.80 (1.68, 4.69)*	0.01
≥3,001	100	23.9	2.19 (1.55, 3.09)*	0.04	1.89 (1.14, 3.14)*	0.02
Occupational status of adults						
Farmer or housewife	70	16.7	1		1	
Merchant	106	25.4	1.19 (0.74, 1.90)*	0.021	0.48 (0.26, 0.89)*	0.03
Governmental employed	110	26.3	2.79 (1.81, 4.29)*	0.76	1.28 (0.73, 2.26)	0.34
Daily laborers and others	132	31.6	2.24 (1.49, 3.35)	0.56	1.10 (0.64, 1.88)	0.12

*$p < 0.05$; statistically significant, 1; Reference, COR, Crude odds ratio; AOR, Adjusted odds ratio; ETB, Ethiopian Birr.

Respondents whose professional position is a merchant were 52% less likely to practice dietary practice (AOR = 0.48, 95% CI: 0.26, 0.89) in the study area are. This finding was supported by the study conducted in Swaziland (21). This could be because these HIV-positive adults with a commercial occupation may not have time for dietary practices (food intake), buying or selling things, and spending many hours outside the home to earn the income they may be earning in great tiredness. While HIV-positive adults who have only been housewives may have enough time to eat.

Monthly income, as a proxy indicator of socioeconomic status, is strongly associated with access to adequate food intake/nutrition security. The current study finding is agreed with studies conducted in different regions of Ethiopia (24, 25). This could be because a higher average monthly income plays an important role in sourcing the groceries and purchasing the nutritional diet and has also prompted respondents to discover new things in terms of nutritional practices.

4 Conclusion

The result of this study revealed that participants in the study area had poor nutritional knowledge and poor dietary practices compared to other studies results. Variables such as age, marital status, educational level, and average monthly income were factors associated with nutritional knowledge. Educational level, family size, average monthly income, and adult occupation were also factors significantly associated with dietary practice. Hence, it is imperative for healthcare professionals in hospitals to impart nutritional education to HIV-positive adults, thereby enhancing their understanding of nutrition. Specialized programs and interventions focusing on nutrition should be implemented to enhance the nutritional knowledge of HIV-positive adults in the region. Furthermore, it is essential for the organization to support ART patients, particularly HIV-positive adults, in improving their dietary habits and overall well-being. Each party involved should strive to bridge the existing gaps in nutritional knowledge, practices, and status among HIV-positive adults in the specified area.

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 proposal was accepted and approved by the department, with ethical approval obtained from the Research Review Board at Wollega University. A formal letter of cooperation was then requested from Wollega University to BHH; then a letter of support was written to the hospital's ART Clinic office. Customer response was anonymous and the data collectors informed customers that they had the full right to withdraw from the study or to refuse to participate. A consent form was also attached to the questionnaire to obtain each individual's permission.

Author contributions

HFG: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing original draft, Writing review & editing. TY: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Supervision, Visualization, Writing original draft, Writing review & editing.

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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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Knowledge, attitudes, and practices of oil and salt intake and related influencing factors in Southwestern China

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Objects: Excessive oil and salt consumption is a public health issue, notably in China where intakes surpass WHO guidelines. The present study aims to examine the knowledge, attitudes, and practices of Southwestern China residents regarding oil and salt and explore the influencing factors.

Methods: This study used convenience sampling to collect data from 7,367 participants aged 18–75 in the Sichuan, Chongqing, Yunnan, and Guizhou regions of China via on-site face-to-face surveys. Descriptive statistics and generalized linear models were used to analyses knowledge, attitudes, and practices about oil and salt intake and their influencing factors among residents of Southwestern China.

Results: In Southwestern China, residents of Guizhou Province exhibited poor KAP regarding oil and salt. There were urban–rural differences in Yunnan, Sichuan, and Chongqing, and residents living in towns and cities were the favored factors for KAP scores. Groups engaged in self-employment/sales and freelance were risk factors for KAP score. Individuals with higher education was a favorable factor for KAP score. In Yunnan, Sichuan, and Chongqing groups with preference of salty tastes were favorable factors in KAP score. Diabetic patients were more likely to score low on oil and salt-related KAP performance.

Conclusion: In Southwestern China, residents of Guizhou Province displayed poor results in their KAP regarding oil and salt. The region of the province, ethnicity, urban and rural residence, education, taste preference, and prevalence of chronic diseases were the influencing factors of oil and salt-related KAP scores.

KEYWORDS

intake of oil and salt, knowledge, attitude, practice, Southwestern China

Introduction

Edible salt (NaCl) and cooking oil play a key role in enhancing the taste and texture of food and are major components of food and important flavoring agents. However long-term adherence to a high-fat, high-salt diet has been identified as a risk factor for the onset and progression of chronic diseases (1).

A meta-analysis of research on salt intake and cardiovascular disease found that every 5 g increase in daily salt intake was associated with a 17% increase in the risk of total cardiovascular disease and a 23% increase in the risk of stroke (2). He et al. (3) discovered that reducing salt intake modestly led to a 20% reduction in the risk of cardiovascular disease and 73 fewer cases of stroke. Prolonged consumption of a high-fat diet not only contributes to the obesity epidemic but also to the progression of a variety of metabolic diseases (4, 5), including type 2 diabetes, atherosclerosis, hypertension, and stroke (6, 7). Long-term high-salt and high-oil intake will also change the composition of the intestinal microbiota, which in turn affects the phenotype and function of CD4 T-cells in the intestinal tract (8). This phenomenon leads to increased susceptibility to infections inside and outside of the intestinal tract, thereby increasing the risk of developing chronic autoimmune diseases (9).

The Dietary Guidelines for Chinese Residents (2022 Edition) suggest that the daily salt intake of adults should be no more than 5 g, and cooking oil intake should be 25–30 g (10). According to the Report on Nutrition and Chronic Disease Situation of Chinese Residents (2020), China's dietary fat energy supply ratio has continued to increase, and the intake of edible oil and edible salt is higher than the recommended value (11). According to the survey data, the China's *per capita* daily intake of cooking salt is as high as 11 g/d (12), and the *per capita* daily intake of cooking oil is 41.8 g/d (13), which is higher than the recommended intake of the World Health Organization (14). The primary way for intake of salt and cooking oil in China is distinct from that of prepared foods in other developed countries, with most of the dosage being added during the cooking process according to personal taste preferences (15, 16). The Southwest region (Sichuan, Chongqing, Guizhou, and Yunnan), under the influence of regional, climate, and ethnic characteristics, has maintained a diet with emphasis on taste for a long time, leading to the problem of excessive intake of oil and salt in this region (17). The prevalence of chronic diseases is higher in Southwestern China than in the central and coastal regions (18). Meanwhile, a study has shown that the age-standardized cancer and coronary heart disease rates increased most substantially in Southwestern China from 2007 to 2016 (19).

Knowledge, attitudes, and practices (KAP) are key elements in facilitating individual behavioral changes by improving cognitive understanding and shaping beliefs and attitudes that lead to behavioral change (20). Haron et al. (21) reported that although the vast majority of study participants had positive attitude toward healthy salt intake, their knowledge and practice of healthy salt intake were not at a satisfactory level. In a study of urinary sodium excretion among healthcare workers in Malaysia, individual KAP regarding salt intake and health-related issues influenced salt intake (22). A study in Shandong, China showed that people with unfavorable attitudes toward sodium reduction were less likely to reduce their sodium intake (23). In research on oil and salt intake in the Iranian population, only 32% were aware of the dangers of excessive consumption of animal oils, and urban households had significantly higher levels of knowledge about oil and salt than rural households (24). In another study on dietary fat intake among adolescents, only 47.7% of students, 48.2% of parents were aware that frying is not a healthy method of food preparation (25).

Previous works focused on the disease risks of excessive oil and salt intake and examined pathological mechanisms involved in the development of various diseases. However, research on public perceptions of oil and salt consumption and related factors is still relatively limited, especially in the southwestern region of China, which has a long history of excessive oil and salt diets. Differences in KAP have been studied to help in the construction and implementation of educational strategies and public policies that promote targeted behavior change (26). Collection, analysis, and evaluation of KAP related to oil and salt intake in the population are important to construct effective salt and oil reduction strategies. Therefore, the purpose of the study is to investigate the knowledge, attitudes, and practices of residents in Southwestern China who have long existed previous works focused on the disease risks of excessive oil and salt intake and examined pathological mechanisms involved in the development of various diseases. However, research on public perceptions of oil and salt consumption and related factors is still relatively limited, especially in the southwestern region of China, which has a long history of excessive oil and salt diets eating habits, and to explore the influencing factors.

Methods

Study design and sample

This study adopted a cross-sectional design and conducted from February to May 2021 in Yunnan, Guizhou, and Sichuan provinces and Chongqing Municipality in Southwestern China. We recruited investigators from six colleges and universities selected from four provinces in Southwestern China. After screening and uniform training, 252 investigators conducted face-to-face field surveys using paper-based questionnaires in households and communities in each district.

The inclusion criteria for this study were as follows: (a) age of 18–75 years, (b) local resident at least 3 years, and (c) ability to understand the contents of the questionnaire and fill in carefully. Uncooperative and cognitively impaired individuals were excluded. Based on the fact that the awareness rate of dietary nutrition among Chinese adult residents surveyed in 2015 was 21.1% and considering sampling errors and invalid questionnaires, the sample size was calculated to be 6,960. A total of 8,535 residents participated. After excluding outliers and missing values, 7,367 participants were included in the analysis. All participants were informed about the study and provided consent before filling out the scale anonymously. This study has been approved by the Ethics Committee of Chongqing Medical University (approval number: 2021041).

Data collection

Data were obtained from the Questionnaire of dietary knowledge, attitudes, and practices of residents Southwestern China in the Dietary

Practices and Dietary Culture Survey of Chinese Nutrition Society. The Cronbach's α coefficient was calculated to be 0.825. In conducting the questionnaire survey, the participants were screened strictly according to the inclusion and exclusion criteria, and the investigators received unified training and passed the assessment.

The questionnaire consists of two parts: socio-demographic characteristics and basic knowledge of KAP for intake of oil and salt. Sociodemographic characteristics include (1) gender (male/female), (2) age, (3) height (self-reported), (4) weight (self-reported), (5) ethnicity (Han/minority), (6) residence (rural/urban), (7) region (Guizhou Province/Yunnan Province/Sichuan Province/Chongqing City), (8) long-term residents (yes/no), (9) occupation (animal husbandry and fishery/student/self-employed individual/sales/freelance), (10) education level (primary school and below/junior high school/senior high school/secondary technical school/junior college and above), (11) taste preference (light/sour/sweet/spicy/salty), (12) diagnosis of high blood pressure (yes/no), (13) diagnosis of diabetes (yes/no), and (14) diagnosis of gout (yes/no).

The KAP for intake of oil and salt by residents Southwestern China includes the following aspects: knowledge part: (1) guideline recommended daily intake of edible oil, (2) recommended daily salt intake, and (3) diet is the most direct and closely related to hypertension; attitudes part: (1) when cooking, I used vegetable oil instead of some animal oil (such as lard) and (2) condiments other than salt, I added extra salt intake; practices part: (1) how often you ate smoked products, (2) how often you eat cured products, (3) how often you eat hot pot each month, (4) in your daily diet, you consciously reduced the intake of cooking oil, and (5) made a conscious effort to reduce salt intake in your daily diet.

Data processing

The age categories are delineated as follows: 18–25 years as one category, 26–45 years as another category, and >45 years as a third category. Body mass index (BMI) was calculated based on the self-reported height and weight of the survey respondents (weight/height^2) and was classified as underweight ($\leq$18.5 kg/m^2), normal (18.5 kg/m^2 $\leq$ BMI < 24 kg/m^2), overweight (24 kg/m^2 $\leq$ BMI < 28 kg/m^2), and obesity (BMI $\geq$ 28 kg/m^2) (27). Occupations were divided into five groups (animal husbandry and fishery, student, self-employed individual/sales, freelance, and employee). Education level was divided into low (primary school and below/junior high school), medium (senior high school/secondary technical school), and high (junior college and above). Taste preferences were divided into five categories (light/sour/sweet/spicy/salty).

Ten questions about the oil and salt intake were asked from residents Southwestern China: three questions about knowledge, two questions about attitudes, and five questions about practices. In this context, a correct response in the knowledge section was assigned a value of 1 point, while an incorrect response received 0. Attitudes and practices were assessed using a five-point Likert scale. Within the attitudes section, each question was rated as follows: "Strongly agree" received 5 points, "Agree" received 4 points, "Not sure" received 3 points, "Disagree" received 2 points, and "Strongly disagree" received 1 point. In the practices section, each question was scored as follows: "Never" at 1 point, "Occasionally" at 2 points, "Sometimes" at 3 points, "Often" at 4 points, and "Always" at 4 points; "Occasionally" was assigned 2 points, "Sometimes" 3 points, "Often" 4 points, and "Every day" 5 points; "$\leq$ 1 time" was scored at 5 points, "2–3 times" at 4 points, "4–5 times" at 3 points, "6–7 times" at 2 points, and "$\geq$8 times" at 1 point. The score was computed and standardized on a 100-point scale.

Statistical analysis

Statistical analysis used frequencies and proportions (%) to describe categorical variables and mean ± standard deviation (SD) to describe continuous variables. The study employed a chi-square test for categorical variables and analysis of variance (ANOVA) for continuous variables to demonstrate variations in scores and responses for knowledge, attitudes, and practices across the four southwestern provinces and cities. T-test and ANOVA were utilized to evaluate differences in socio-demographic characteristics on KAP scores. Generalized linear models were used to assess the association between socio-demographic characteristics and KAP scores. Data were entered on EpiData software and analyzed with STATA version 17.0. Statistical significance was determined using a p-value of less than 0.05 (two-tailed).

Results

Basic demographic features

Of the 8,535 respondents who agreed to participate in the questionnaire survey, 7,367 study subjects were included after the implementation of the inclusion–exclusion criteria. The detailed basic demographic characteristics are tabulated in Table 1. The average age of the participants was 35.48 ± 14.41 years, with a high percentage of Han Chinese at 88.24% and the minority at 11.76%. The proportion of participants who lived in rural areas was 38.51%, and that in urban areas was 61.49%. The higher share of students in the occupational distribution was 22.37%. Taste preferences peaked at 41.39% for light and were nearly equal at 41.29% for spicy.

Knowledge, attitudes, practices responses

In terms of KAP scores, Guizhou scored lower than Sichuan and Chongqing in the total KAP score and the knowledge section, and the difference was significant (Table 2). Guizhou received the lowest total KAP score (65.80 ± 8.17) compared with other provinces and cities, with a statistically significant difference (p < 0.001). In terms of knowledge component scores, Guizhou province scored lower (30.75 ± 31.68) than the other provinces and cities (p < 0.001). In the attitudes component score, the score of Guizhou province (69.49 ± 13.68) was lower than those of Sichuan and Yunnan, with a statistically significant difference (p < 0.001). Among the practices component scores, Guizhou province scored significantly lower than the other provinces and cities in the total KAP score (p < 0.001). In fact, it scored significantly lower than Sichuan and Chongqing (p < 0.001).

TABLE 1 Distribution of basic demographic characteristics in four provinces and cities in Southwest China (n = 7,367).

Factor	Total	Yunnan	Guizhou	Sichuan	Chongqing	p-value
N	7,367	1,290	2,101	1,415	2,561	
Gender						0.18
Male	3,374 (48.80%)	586 (45.43%)	1,004 (47.79%)	640 (45.23%)	1,144 (44.67%)	
Female	3,993 (54.20%)	704 (54.57%)	1,097 (52.21%)	775 (54.77%)	1,417 (55.33%)	
Age (years)	35.48 (14.41)	36.94 (12.80)	33.87 (13.56)	35.49 (14.65)	36.07 (15.56)	<0.001
BMI	22.06 (2.96)	22.26 (2.90)	22.03 (3.04)	21.88 (2.86)	22.09 (2.97)	0.008
Nationality						<0.001
Han	6,501 (88.24%)	1,026 (79.53%)	1,714 (81.58%)	1,377 (97.31%)	2,384 (93.09%)	
Minority	866 (11.76%)	264 (20.47%)	387 (18.42%)	38 (2.69%)	177 (6.91%)	
Residence						<0.001
Urban	4,530 (61.49%)	794 (61.55%)	1,223 (58.21%)	859 (60.71%)	1,654 (64.58%)	
Rural	2,837 (38.51%)	496 (38.45%)	878 (41.79%)	556 (39.29%)	907 (35.42%)	
Long-term residents						<0.001
No	1,520 (20.63%)	275 (21.32%)	468 (22.28%)	358 (25.30%)	419 (16.36%)	
Yes	5,847 (79.37%)	1,015 (78.68%)	1,633 (77.72%)	1,057 (74.70%)	2,142 (83.64%)	
Occupation						<0.001
Animal husbandry and fishery	1,300 (17.65%)	193 (14.96%)	408 (19.42%)	237 (16.75%)	462 (18.04%)	
Student	1,648 (22.37%)	168 (13.02%)	408 (19.42%)	305 (21.55%)	767 (29.95%)	
Self-employed individual/Sales	1,711 (23.23%)	372 (28.84%)	591 (28.13%)	290 (20.49%)	458 (17.88%)	
Freelance	1,189 (16.14%)	191 (14.81%)	316 (15.04%)	232 (16.40%)	450 (17.57%)	
Employee	1,519 (20.62%)	366 (28.37%)	378 (17.99%)	351 (24.81%)	424 (16.56%)	
Education						<0.001
Low	2,275 (30.88%)	345 (26.74%)	742 (35.35%)	401 (28.34%)	787 (30.73%)	
Medium	1,470 (19.95%)	294 (22.79%)	418 (19.90%)	280 (19.79%)	478 (18.66%)	
High	3,622 (49.17%)	651 (50.47%)	941 (44.79%)	734 (51.87%)	1,296 (50.61%)	
Flavor preferences						<0.001
Light	3,044 (41.32%)	438 (33.95%)	803 (38.22%)	640 (45.23%)	1,163 (45.41%)	
Sour	341 (4.63%)	102 (7.91%)	107 (5.09%)	49 (3.46%)	83 (3.24%)	
Sweet	359 (4.87%)	77 (5.97%)	108 (5.14%)	50 (3.53%)	124 (4.84%)	
Spicy	3,042 (41.29%)	567 (43.95%)	937 (44.60%)	574 (40.57%)	964 (37.64%)	
Salty	581 (7.89%)	106 (8.22%)	146 (6.95%)	102 (7.21%)	227 (8.86%)	
Hypertension						0.008
No	6,841 (92.86%)	1,169 (90.62%)	1,959 (93.43%)	1,322 (93.24%)	2,391 (93.36%)	
Yes	526 (7.14%)	121 (9.38%)	142 (6.57%)	93 (6.76%)	170 (6.64%)	
Hyperlipidemia						<0.001
No	7,081 (96.12%)	1,208 (93.64%)	2,043 (97.24%)	1,372 (96.96%)	2,458 (95.98%)	
Yes	286 (3.88%)	82 (6.36%)	58 (2.76%)	43 (3.04%)	103 (4.02%)	
Diabetes						
No	7,182 (97.49%)	1,253 (97.13%)	2,068 (98.43%)	1,385 (97.88%)	2,476 (96.68%)	
Yes	185 (2.51%)	37 (2.87%)	33 (1.57%)	30 (2.12%)	85 (3.32%)	0.001
Gout						
No	7,148 (97.13%)	1,250 (96.90%)	2,035 (96.86%)	1,379 (97.46%)	2,484 (96.99%)	
Yes	219 (2.97%)	40 (3.10%)	66 (3.14%)	36 (2.54%)	77 (3.01%)	0.75

Data are presented as mean (SD) for continuous measures, and n (%) for categorical measure.

TABLE 2 Knowledge, attitude, and behavior scores of four provinces in Southwest China.

Factor	Total	Yunnan	Guizhou	Sichuan	Chongqing	F/x2	*p*-value
Mean±SD							
KAP total score	67.62 (8.19)	67.15 (7. 98)[b]	65.80 (8. 17)[a]	68.75 (7. 78)[c]	68.72 (8. 23)[c]	62.09	<0.001
Knowledge	35.86 (32.77)	42.14 (32. 89)[c]	30.75 (31. 68)[a]	40.40 (34. 13)[c]	34.37 (31. 99)[b]	44.45	<0.001
Attitude	70.44 (13.88)	71.42 (13. 84)[b]	69.49 (13. 68)[a]	71.30 (13. 84)[b]	70.26 (14. 04)[ab]	7.39	<0.001
Practice	70.30 (10.39)	68.44 (9. 90)[a]	68.53 (10. 43)[a]	71.14 (9. 79)[b]	72.23 (10. 52)[c]	68.45	<0.001

Mean ± SD, Average ± Standard Deviation; In two-by-two comparisons, a difference containing different letters indicates a statistically significant difference, while a difference containing the same letter indicates no statistically significant difference.

Single-factor analysis

The study found statistically significant differences ($p < 0.05$) in demographic characteristics such as province, gender, age, BMI, ethnicity, place of residence, occupation, education, taste preference, and prevalence of chronic diseases (hypertension, hyperlipidemia, diabetes mellitus, and gout) based on the results of the univariate analysis in Table 3.

Multi-variable analysis

Multifactorial analysis was conducted using multiple linear regression models, and subgroup analyses were carried out after dividing the Southwest region by province. Guizhou province had significantly lower scores for KAP ($\beta = -1.40$; 95%CI: −1.96 to −0.84) (Table 4). Furthermore, low scores were obtained for minorities ($\beta = -0.65$; 95%CI: −1.23 to −0.07) (Table 4). Moreover, groups involved in self-employment and sales occupations ($\beta = -1.56$; 95%CI: −2.17 to −0.94) as well as freelance ($\beta = -0.95$; 95%CI: −1.59 to −0.31) were at a higher risk for KAP scores (Table 4). The higher education level of the group was a favorable factor for the KAP score ($\beta = 1.16$; 95%CI: 0.56–1.75) (Table 4). Diabetic patients were highly likely to score low on oil and salt-related KAP performance ($\beta = -1.31$; 95%CI: −2.51 to −0.12) (Table 4). Significant variations in KAP scores were found between urban and rural areas in Yunnan ($\beta = 0.59$; 95%CI: 0.19–0.98), Sichuan ($\beta = 0.84$; 95%CI: 0.43–1.26), and Chongqing ($\beta = 0.85$; 95%CI: 0.44–1.25), with residents living in urban areas having higher KAP scores (Table 5). In Yunnan ($\beta = 1.33$; 95%CI: 0.61–2.05), Sichuan ($\beta = 1.14$; 95%CI: 0.42–1.85), and Chongqing ($\beta = 1.24$; 95%CI: 0.52–1.96), groups that favored salty tastes had favorable KAP scores (Table 5).

Discussion

Our cross-sectional survey investigated the current status of oil and salt intake knowledge, attitudes, and practices of residents in Southwestern China and their influencing factors. Overall, our study showed that Guizhou Province among the four southwestern provinces performed poorly in terms of KAP scores for oil and salt intake, which were not only subject to regional differences in terms of provinces but were also associated with ethnicity, urban/rural residence, occupation, education level, taste preference, and chronic disease prevalence. In the results of the provincial subgroup analysis, we found urban–rural differences in Yunnan, Sichuan, and Chongqing, but not in Guizhou. Among taste preferences, salty was a favorable factor for KAP scores.

Compared with the attitudes and practices dimensions, the score of the knowledge dimension of oil and salt intake is relatively low in Southwestern China. The possible reason may be that although residents pay attention to a light diet, they do not know the specific usage limits. This reflects the deficiency in the dissemination of limited-quantity knowledge. Given that most of the cooking behaviors with Chinese characteristics involve adding oil and salt by oneself during the cooking process, quantification is somewhat difficult (28). Therefore, publicity and education can be carried out in the form of teaching the usage methods of salt-restriction spoons and oil-restriction pots as well as the calculation methods of salt and oil contents on food labels. Guizhou Province exhibited inadequate performance in terms of KAP aspects about oil and salt intake. According to a survey conducted on the understanding of Chinese dietary guidelines, participants from Guizhou Province exhibited relatively lower familiarity with the dietary guidelines for individuals in China, consistent with the present findings (29). Investment in health policy planning and health education may vary from place to place due to different levels of economic development (30). Guizhou, as an economically underdeveloped area, has a relatively weak investment in primary health care (31). A pertinent study demonstrates that the standard of fundamental public health services in Guizhou Province is subpar, and substantial impediments exist in the progress of basic public health services (32). Hence, Guizhou Province exhibits a relatively low level of overall KAP, which may reflect the need for well-directed interventions aimed at enhancing dietary habits among its residents. In the future, Guizhou Province must enhance its efforts to promote the “three reduction and three health” (reduce salt, reduce oil, reduce sugar and healthy mouth, healthy weight, healthy bones) (33) and other relevant fundamental public health services compared with other states.

Education beyond high school is linked with high KAP scores. Further education allows individuals to enhance varied skills, such as augmented learning efficiency, cognitive and problem-solving skills, and better self-regulation (34). Additionally, individuals with higher levels of education frequently exhibit greater self-care awareness and a stronger inclination to obtain dietary knowledge (35). Therefore, individuals with higher levels of education are more inclined to comprehend nutritional information and emphasize the health hazards of excessive oil and salt consumption for self-regulation, consistent with past research (36, 37). Conversely, the health awareness of less educated individuals was comparatively weaker because of their constrained capacity to understand and value health-related

TABLE 3 Analysis of KAP scores for different socio-demographic characteristics.

Factor	Standardized KAP total score (Mean ± SD)	F/t	*p*-value
Province		62.09	<0.001
Yunnan	67.15 (7. 98)[b]		
Guizhou	65.80 (8.17)[a]		
Sichuan	68.75 (7.78)[c]		
Chongqing	68.72 (8. 23)[c]		
Gender		−2.87	<0.001
Male	67.32 (8.46)		
Female	67.87 (7.94)		
Age		25.36	<0.001
18–25 years	68.57 (8.53)[b]		
26–45 years	67.06 (8.12)[a]		
>45 years	67.26 (7.75)[a]		
BMI		15.76	<0.001
Underweight	68.27 (8.27)[b]		
Normal	67.86 (8.11)[b]		
Overweight or obesity	66.72 (8.27)[a]		
Nationality		4.09	<0.001
Han	67.76 (8.16)		
Minority	66.55 (8.32)		
Residence		−4.93	<0.001
Village	67.02 (8.35)		
Urban	67.99 (8.06)		
Long-term residents		−0.60	0.547
Yes	67.59 (8.21)		
No	67.73 (8.10)		
Occupation		41.96	<0.001
Animal husbandry and fishery	67.38 (7.86)[b]		
Student	69.45 (8.28)[c]		
Self-employed individual/Sales	66.09 (8.19)[a]		
Freelance	66.77 (8.21)[ab]		
Employee	68.22 (7.91)[b]		
Education		56.45	<0.001
Low	66.54 (8.09)[a]		
Medium	66.78 (8.23)[a]		
High	68.64 (8.11)[b]		
Flavor preferences		5.36	<0.001
Light	67.38 (7.89)[a]		
Sour	66.57 (8.39)[a]		
Sweet	67.23 (8.97)[a]		
Spicy	67.81 (8.17)[ab]		
Salty	68.73 (9.01)[b]		
Hypertension		−2.73	0.006
No	67.67 (8.19)		

(Continued)

TABLE 3 (Continued)

Factor	Standardized KAP total score (Mean ± SD)	F/t	*p*-value
Yes	66.68 (8.09)		
Hyperlipidemia		2.01	0.044
No	67.66 (8.16)		
Yes	66.66 (8.90)		
Diabetes		3.05	0.002
No	67.66 (8.15)		
Yes	65.80 (9.19)		
Gout		2.89	0.004
No	67.67 (8.13)		
Yes	66.04 (9.84)		

Mean ± SD, Average ± Standard Deviation; In two-by-two comparisons, a difference containing different letters indicates a statistically significant difference, while a difference containing the same letter indicates no statistically significant difference.

information (38). When implementing health education initiatives, approaches should be customized according to the specific attributes of the target audience. For instance, a more vivid, intuitive, and understandable form of education should be used when targeting groups with lower education levels.

The urban–rural differences observed in Sichuan, Chongqing, and Yunnan were consistent with previous research. A previous China Health and Nutrition Survey (CHNS) reported inferior performance in diet-related KAP in rural areas compared with urban regions (39). Rural–urban disparities in dietary patterns were observed in studies conducted by Gao et al. and He et al. (40, 41). Limited access to health education resources could be a reason for these disparities. Rural areas may face greater obstacles than urban areas in obtaining health information and consulting with expert healthcare professionals due to factors, such as geographic transportation (42). Furthermore, rural inhabitants show lower proficiency in accessing health information online, particularly concerning high-speed Internet access, in contrast to their urban counterparts (43). This capacity restraint may serve as a significant obstruction to the access and utilization of health-related information by rural residents.

This study found that people with a preference for salty taste have a relatively good performance in terms of KAP regarding oil and salt intake. However, as of now, there is no research directly confirming this finding. Based on this, we conduct the following discussions and speculations. Taste has long been thought to play a crucial role in eating behavior (44). Studies have shown that individuals tend to prefer certain taste, leading to higher consumption of such food (45, 46). Numerous studies also indicate that an excessive oil and salt diet is significantly associated with an increased incidence of chronic diseases (47–49). Moreover, high-salt and high-fat diets may interact through different mechanisms and jointly promote the development of hypertension and hyperlipidemia (50, 51). Therefore, people who have long maintained a high-oil and high-salt diet are likely to be aware of the disease risks that this eating habit may bring. Maintaining dietary regulation goals helps people resist food temptations and achieve self-control intention (52, 53). Based on the above viewpoints, we can reasonably speculate that among people with a preference for salty taste, if someone is fully aware of the risks of excessive oil and salt intake and takes active measures to limit oil and salt intake, then they are more likely to obtain better KAP scores in terms of oil and salt intake. This is because the awareness of risks will prompt them to actively acquire more knowledge about healthy eating, and then pay more attention to reasonably controlling salt intake in terms of attitude, and finally achieve a reduction in salt intake in practical behavior. In addition, family factors are also key points that cannot be ignored. Family members may monitor and manage individuals with a penchant for salty foods, thereby enhancing knowledge of healthy eating and promoting healthy eating behavior. A previous study pointed out that cooking classes are crucial to the influence of housemakers in promoting healthy eating, which can help reduce the level of oil and salt intake of the whole family (54). Educating family members about the dangers of excessive salt intake through a range of means, including primary healthcare providers and home cooks, can significantly enhance knowledge and ingrained behavior (55).

The reasons for the relatively low KAP scores regarding oil and salt intake among diabetic patients are discussed below. First, diabetic patients may place greater emphasis on controlling sugar intake in their daily diet but lack sufficient awareness of the impact of salt and fat intake. Furthermore, research has found common misconceptions in the dietary habits of diabetic patients, such as the belief that plant-based oils can be consumed without restriction, which may negatively affect their dietary management and overall health (56). Additionally, some studies have pointed out that low-sodium diets might increase the risk of adverse cardiovascular events in diabetic patients (57). Moreover, a study on dietary factors in diabetic patients found that the majority of respondents were unable to adhere to diabetes dietary recommendations due to incomes below the basic wage level (58). Base on this, the following measures could be taken to improve the situation. First, providing accurate dietary information and guidance can help diabetic patients correct misconceptions about oil and salt intake, clearly informing them of the appropriate consumption levels of various types of fats and the proper way to maintain a low-sodium diet, thereby correcting their misunderstandings. Secondly, considering patients' economic conditions and personal preferences, personalized dietary advice should be provided. For patients with lower incomes, recommending affordable food choices and cooking methods that meet the dietary requirements for diabetes can help

TABLE 4 Relationship between socio-demographic characteristics and overall KAP scores of the four provinces in the Southwest region (β, 95%CI).

Factor	Southwestern China
	Standardized KAP total score
Province	
Yunnan	0
Guizhou	**−1.40 (−1.96, −0.84)*****
Sichuan	**1.19 (0.57, 1.81)*****
Chongqing	**1.07 (0.52, 1.62)*****
Gender	
Male	0
Female	0.29 (−0.09, 0.66)
Age	
18–25 years	0
26–45 years	−0.09 (−0.69, 0.51)
>45 years	0.44 (−0.26, 1.14)
BMI	
Underweight	0
Normal	0.04 (−0.58, 0.65)
Overweight or obesity	−0.64 (−1.35, 0.06)
Nationality	
Han	0
Minority	**−0.65 (−1.23, −0.07)***
Residence	
Village	0
Urban	**0.80 (0.40, 1.20)*****
Occupation	
Animal husbandry and fishery	0
Student	0.69 (−0.14, 1.51)
Self-employed individual/Sales	**−1.56 (−2.17, −0.94)*****
Freelance	**−0.95 (−1.59, −0.31)****
Employee	−0.34 (−1.05, 0.38)
Education	
Low	0
Medium	−0.07 (−0.49, 0.64)
High	**1.16 (0.56, 1.75)*****
Flavor preferences	
Light	0
Sour	−0.81 (−1.72, 0.09)
Sweet	−0.40 (−1.29, 0.48)
Spicy	0.26 (−0.16, 0.68)
Salty	**1.29 (0.58, 2.00)*****
Hypertension	
No	0
Yes	−0.31 (−1.08, 0.47)
Hypertension	
No	0

(Continued)

TABLE 4 (Continued)

Factor	Southwestern China
	Standardized KAP total score
Yes	−0.58 (−1.57, 0.42)
Diabetes	
No	0
Yes	**−1.31 (−2.51, −0.12)***
Gout	
No	0
Yes	−0.98 (−2.07, 0.11)
Constant	66.70 (65.64, 67.76)

β, regression coefficient; CI, confidence interval; $*p<0.05$, $**p<0.01$, $***p<0.001$. Bolded values denote statistically significant data at the $p<0.05$ level.

them make healthier oil and salt intake choices while ensuring balanced nutrition within a limited budget.

In the present study, independent operators, sales personnel, and freelance practitioners had lower scores in KAP related to oil and salt intake due to the increased work pressure and uncertainty in their work hours. Research suggests that young individuals opt for fast and convenient food, such as takeout or pre-packaged meals, due to their uncertain work schedules (59). Maintaining longer working hours has been suggested to result in a lack of time for self-care and health maintenance in this population (60). This phenomenon could be a reason for their inability to pay adequate attention to healthy eating and nutritional knowledge. Irregular working hours may contribute to their preference for convenience foods and takeaways, which are generally high in fats and salts. Compared with conventional employees, those with more flexible working arrangements may experience a lack of social support from a fixed workplace. In a relatively fixed workplace environment, organizations may cover some healthcare services while supporting employees to assess their health status and take responsibility for their health by promoting a healthy lifestyle (61, 62). For self-employed individuals, salespeople, and freelance practitioners, the absence of this mechanism can lead to a sense of isolation when developing and maintaining healthy eating habits, ultimately hindering their access to healthcare and health education. In the future, this population should be reached out to optimize the allocation and utilization of health education resources.

Southwestern China is home to many ethnic minority groups, representing almost half of the country's ethnic minority population (63). These diverse groups have unique dietary behavior and habits. Research suggests that ethnic minority populations are in a critical stage of nutritional transition and are generally less educated and health-conscious (64). Ethnic minorities face challenges when establishing or sustaining healthy eating routines during nutritional transition. This finding might explain the lower KAP scores among this group.

Strengths and limitations

This initial cross-sectional analysis in Southwestern China employs a population survey to explore KAP regarding oil and salt intake. The strength of this study is its concentration in Southwestern China, where high oil and salt dietary habits are common. It investigates the current status of KAP of oil and salt intake among residents in this area, providing valuable support for the further development of health education initiatives aiming to reduce salt, sugar, and oil consumption. Additionally, the study provides a foundation for the advancement of quantitative measures related to oil and salt intake. However, this study has certain limitations. The survey did not incorporate the Tibet region, which is classified within the Southwest area of China's administrative planning. The reasons are due to transportation influences and other factors related to the COVID-19 outbreak. The data situation in the Tibetan region should be bolstered to accurately portray the cognizance of oil and salt intake in the southwest region of China. The utilization of convenience sampling significantly limits the representativeness of the findings in the population. Third, despite the use of face-to-face questionnaires by the investigator, all responses were self-reported by the participants, potentially leading to recall bias. Fourth, the cross-sectional research design does not allow for the inference of causal relationships.

Conclusion and policy recommendations

This study primarily aims to assess the current level of KAP related to oil and salt intake among residents in Southwestern China and explore influencing factors. Based on the findings, in Guizhou Province, residents exhibited the worst performance in terms of the KAP about oil and salt. The influencing factors of KAP scores related to oil and salt included the region in the province, ethnicity, rural or urban residence, level of education, and taste preferences. Due to differences in economic development levels and dietary habits among various provinces and cities, residents in different regions have different levels of awareness of oil and salt. Future health education programs should be adjusted according to the unique circumstances of each region. The government needs to promote relevant professionals to carry out dietary health education and conduct publicity in forms such as teaching the usage methods of salt-restriction spoons and oil-restriction pots and the calculation methods of salt and oil contents on food labels. At the same time, opportunities for rural residents to access modern communication technologies and experts should be increased because rural areas have relatively limited access to knowledge about oil and salt. For companies or communities where employees have irregular working hours, accessible nutritional health resources and information should

TABLE 5 Relationship between socio-demographic characteristics and KAP scores by province in four southwestern provinces (Yunnan, Guizhou, Sichuan, and Chongqing) (β, 95%CI).

Factor	Standardized KAP total score			
	Yunnan	Guizhou	Sichuan	Chongqing
Gender				
Male	—	—	—	0
Female	—	—	—	0.33 (−0.05, 0.72)
Age				
18–25 years	—	0	0	0
26–45 years	—	0.22 (−0.38, 0.81)	0.09 (−0.51, 0.69)	0.17 (−0.43, 0.77)
>45 years	—	**1.00 (0.32, 1.69)****	0.61 (−0.07, 1.28)	**0.81 (0.12, 1.50)***
BMI				
Underweight	0	—	—	0
Normal	−0.17 (−0.78, 0.44)	—	—	0.11 (−0.51, 0.73)
Overweight or obesity	**−1.04 (−1.72, −0.36)****	—	—	−0.63 (−1.34, 0.08)
Nationality				
Han	—	—	—	0
Minority	—	—	—	**−1.25 (−1.82, −0.67)*****
Residence				
Village	0	—	0	0
Urban	**0.59 (0.19, 0.98)****	—	**0.84 (0.43, 1.26)*****	**0.85 (0.44, 1.25)*****
Long-term residents				
Yes	—	—	0	—
No	—	—	0.04 (−0.44, 0.51)	—
Occupation				
Animal husbandry and fishery	—	0	0	0
Student	—	**1.41 (0.59, 2.23)****	**1.24 (0.42, 2.07)****	**1.11 (0.28, 1.93)****
Self-employed individual/sales	—	**−1.44 (−2.04, −0.84)*****	**−1.67 (−2.28, −1.05)*****	**−1.70 (−2.32, −1.08)*****
Freelance	—	**−0.71 (−1.35, −0.07)***	**−0.90 (−1.54, −0.25)****	**0.91 (−1.55, −0.26)****
Employee	—	−0.08 (−0.79, 0.63)	−0.29 (−1.01, 0.42)	−0.33 (−1.05, 0.39)
Education				
Low	0	0	0	0
Medium	0.10 (−0.45, 0.64)	0.36 (−0.21, 0.92)	0.23 (−0.34, 0.80)	0.22 (−0.35, 0.79)
High	**1.83 (1.38, 2.29)*****	**1.61 (1.01, 2.20)*****	**1.39 (0.79, 1.99)*****	**1.36 (0.76, 1.96)*****
Flavor preferences				
Light	0	—	0	0
Sour	**−1.16 (−2.07, −0.25)***	—	**−1.15 (−2.06, −0.24)***	**−1.06 (−1.97, −0.15)***
Sweet	−0.64 (−1.53, 0.25)	—	−0.61 (−1.50, 0.28)	−0.58 (−1.46, 0.31)
Spicy	0.07 (−0.34, 0.49)	—	0.06 (−0.36, 0.49)	0.13 (−0.29, 0.56)
Salty	**1.33 (0.61, 2.05)*****	—	**1.14 (0.42, 1.85)****	**1.24 (0.52, 1.96)****
Hypertension				
No	—	0	—	—
Yes	—	−0.59 (−1.37, 0.19)	—	—
Hypertension				
No	—	0	—	—
Yes	—	−0.72 (−1.72, 0.28)	—	—

(Continued)

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TABLE 5 (Continued)

Factor	Standardized KAP total score			
	Yunnan	Guizhou	Sichuan	Chongqing
Diabetes				
No	—		—	0
Yes	—		—	**−1.28 (−2.47, −0.08)***
Gout				
No	—		—	0
Yes	—		—	**−1.12 (−2.22, −0.03)***
Constant	66.66 (65.95, 67.36)	66.61 (65.88, 67.34)	66.44 (65.66, 67.22)	66.50 (65.55, 67.45)

β, regression coefficient; CI, confidence interval; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; —, Variables that were not significant in the univariate analysis of each group were not included in the multivariate analysis. Bolded values denote statistically significant data at the $p < 0.05$ level.

be promoted to help maintain healthy dietary habits. In subsequent studies, quantitative means can be utilized to conduct a further assessment of the specific situation of oil and salt intake in Southwestern China. At the same time, intervention measures can be employed to control oil and salt intake and combine it with the incidence status of cardiovascular diseases in Southwestern China to carry out more systematic and in-depth research.

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 humans were approved by The Ethics Committee of Chongqing Medical University (approval number: 2021041). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LZ: Conceptualization, Data curation, Methodology, Writing – original draft. QX: Conceptualization, Methodology, Writing – original draft. KJ: Writing – review & editing. ZL: Writing – review & editing. YW: Writing – review & editing. ZH: Writing – review & editing. CX: Writing – review & editing. ZS: Writing – review & editing. MS: Writing – review & editing. YZ: Supervision, Writing – review & editing.

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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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