INSIGHTS IN NUTRITIONAL EPIDEMIOLOGY

EDITED BY: Mauro Serafini, Francesco Sofi and Megan A. McCrory
PUBLISHED IN: Frontiers in Nutrition

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-83250-460-4
DOI 10.3389/978-2-83250-460-4

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

INSIGHTS IN NUTRITIONAL EPIDEMIOLOGY

Topic Editors:
Mauro Serafini, University of Teramo, Italy
Francesco Sofi, Università degli Studi di Firenze, Italy
Megan A. McCrory, Boston University, United States

Citation: Serafini, M., Sofi, F., McCrory, M. A., eds. (2022). Insights in Nutritional Epidemiology. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83250-460-4

Table of Contents

frontiers | Frontiers in Nutrition

TYPE Editorial
PUBLISHED 27 September 2022
DOI 10.3389/fnut.2022.1032825

OPEN ACCESS

EDITED AND REVIEWED BY
Marwan El Ghoch,
Beirut Arab University, Lebanon

*CORRESPONDENCE
Mauro Serafini
mserafini@unite.it

SPECIALTY SECTION
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

RECEIVED 31 August 2022
ACCEPTED 09 September 2022
PUBLISHED 27 September 2022

CITATION
Serafini M, Sofi F and McCrory MA (2022) Editorial: Insights in nutritional epidemiology. *Front. Nutr.* 9:1032825. doi: 10.3389/fnut.2022.1032825

Editorial: Insights in nutritional epidemiology

Mauro Serafini[1]*, Francesco Sofi[2] and Megan A. McCrory[3]

[1]Faculty of Biosciences and Technologies for Agriculture, Food and Environment, University of Teramo, Teramo, Italy, [2]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy, [3]Department of Health Sciences, Boston University, Boston, MA, United States

KEYWORDS

epidemiology, nutrition, obesity, health, degenerative diseases, dietary assessment, biomarkers

Editorial on the Research Topic
Insights in nutritional epidemiology

We are now entering the third decade of the twenty-first century, and, especially in the last years, the achievements made by scientists have been exceptional, leading to major advancements in the fast-growing field of Nutritional Epidemiology. Frontiers has organized a series of Research Topics to highlight the latest advancements in nutrition in order to be at the forefront of science in different fields of research. This editorial initiative of particular relevance was focused on new insights, novel developments, current challenges, latest discoveries, recent advances and future perspectives in the field of Nutritional Epidemiology. Specifically, the Research Topic solicited brief, forward-looking contributions describing the state of the art, outlining, recent developments and major accomplishments that have been achieved and that need to occur to move the field forward. The goal of this special edition Research Topic was to shed light on the key advancement achieved in the past decade in the field of nutritional epidemiology with a special focus on the role of diet in improving human health, and on its future challenges to provide a thorough overview of the field and to identify novel research avenues.

Regarding cancer, Zhong et al. aimed to understand whether fried food consumption is associated with the risk of pancreatic cancer in a population-based cohort of 101,729 US adults. The review of Fan Y. et al. provides an overview of the current burden, underlying risk factors, and co-existence of malnutrition and other infections including cancer.

In the area of aging, the article by Angelino et al. highlights, for the first time, the importance of meal timing and of a daily caloric restriction lapse, in human longevity.

The paper by Li et al. (A) and corrigendum Li et al. (B) analyzed the trends of obesity and metabolic status among Chinese population in 2012–2020. The work by He et al. focuses on the relationship between serum serine levels and all-cause mortality in general hypertensive patients in a longitudinal cohort. Liu M. et al. evaluate the association between dietary selenium intake and the risk of kidney stones in 30,184 adults.

Fan L. et al. evaluated China's achievements in iodine deficiency disorders (IDDs) prevention. Yang et al. performed a two-sample Mendelian randomization (MR) analysis to evaluate the association between serum vitamin D levels and atrial fibrillation (AF)

risks. The work by Liu H. et al. was aimed to determine the prevalence of malnutrition in tertiary hospitals of China and the associations between malnutrition and adverse clinical outcomes. Zahidi et al. found that food insecurity was associated with common mental health problems (CMHPs) among a sample of reproductive-aged women in Kabul, Afghanistan. Liu Y. et al. explore the relationship between serum 25(OH)D, cadmium, and CRP with all-cause mortality among people in diabetic and non-diabetic individuals in the NHANES. Also the work by Lin et al. utilized data from NHANES study to evaluate the relationship between natural yogurt and dietary probiotic supplements consumption and mortality in US adults. In the epigenetic field, Zhou et al. examine whether the associations between plasma selenium and 3-year lipid changes is modified by rs7579 polymorphism. Song et al. aimed to evaluate the effect of vitamin D on the risk of vitiligo using meta-analysis and Mendelian randomization (MR). Lastly, Orsini et al. investigated the interaction analysis based on Shapley values and extreme gradient boosting in a large epidemiological prospective study.

In conclusion, we hope that this article collection will inspire, inform and provide direction and guidance to researchers in the field of nutritional epidemiology.

Author contributions

MS wrote the initial draft of the manuscript. FS and MM finalized the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

Publisher's note

frontiers in Nutrition

ORIGINAL RESEARCH
published: 07 December 2021
doi: 10.3389/fnut.2021.789577

A Functional Variant in *SEPP1* Interacts With Plasma Selenium Concentrations on 3-Year Lipid Changes: A Prospective Cohort Study

Li Zhou[1,2,3], *Xiaoling Liang*[1,2], *Manling Xie*[4], *Jiawei Yin*[1,2], *Yue Huang*[1,2], *Xiaoqin Li*[1,2], *Zhilei Shan*[1,2], *Liangkai Chen*[1,2], *Yan Zhang*[5], *Cheng Luo*[1,2]* and *Liegang Liu*[1,2]*

[1] Department of Nutrition and Food Hygiene, Hubei Key Laboratory of Food Nutrition and Safety, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [2] Ministry of Education (MOE) Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [3] Academy of Nutrition and Health, Wuhan University of Science and Technology, Wuhan, China, [4] Department of Neurology, Mayo Clinic, Rochester, MN, United States, [5] The Hubei Provincial Key Laboratory of Yeast Function, Yichang, China

OPEN ACCESS

Edited by:
Francesco Sofi, Università degli Studi di Firenze, Italy

Reviewed by:
Holger Steinbrenner, Friedrich Schiller University Jena, Germany
Tommaso Filippini, University of Modena and Reggio Emilia, Italy

***Correspondence:**
Liegang Liu
lgliu@mails.tjmu.edu.cn
Cheng Luo
1343941286@qq.com

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *05 October 2021*
Accepted: *08 November 2021*
Published: *07 December 2021*

Citation:
Zhou L, Liang X, Xie M, Yin J, Huang Y, Li X, Shan Z, Chen L, Zhang Y, Luo C and Liu L (2021) A Functional Variant in SEPP1 Interacts With Plasma Selenium Concentrations on 3-Year Lipid Changes: A Prospective Cohort Study. Front. Nutr. 8:789577. doi: 10.3389/fnut.2021.789577

Background: Excess selenium has been related with adverse lipid levels in previous epidemiological studies. Meanwhile, a functional variant in *SEPP1* (encodes selenoprotein P), namely rs7579, has been suggested to modulate lipid metabolism. However, the interactions between selenium status and rs7579 polymorphism on lipid changes remain unclear.

Objective: To examine whether the associations between plasma selenium and 3-year lipid changes is modified by rs7579 polymorphism.

Methods: A prospective cohort study was conducted among 1,621 individuals to examine the associations between baseline plasma selenium and 3-year lipid changes, as well as the interactions between plasma selenium and rs7579 polymorphism on lipid changes.

Results: The median (interquartile range) concentration of plasma selenium was 91.68 (81.55–104.92) μg/L. Higher plasma selenium was associated with adverse 3-year lipid changes. Comparing the highest to the lowest quartiles of plasma selenium concentrations, 3-year lipid changes were elevated by 8.25% (95% CI: 1.54–14.96%) for triglycerides ($P = 0.016$), 5.88% (3.13–8.63%) for total cholesterol ($P < 0.001$), 7.37% (3.07–11.67%) for low-density lipoprotein cholesterol ($P = 0.0008$), 6.44% (2.66–10.21%) for non-high-density lipoprotein cholesterol ($P = 0.0009$), 4.99% (0.62–9.36%) for total cholesterol/high-density lipoprotein cholesterol ratio ($P = 0.025$), and 7.00% (1.55–12.46%) for low-density lipoprotein cholesterol/high-density lipoprotein cholesterol ratio ($P = 0.012$). In analyses stratified by rs7579 genotypes, positive associations between plasma selenium concentrations and 3-year changes in triglycerides, TC, LDL-C, non-HDL-C, TC/HDL-C ratio, and LDL-C/HDL-C ratio were observed among CC

genotype carriers, but negative associations between plasma selenium and TC/HDL-C ratio, and LDL-C/HDL-C ratio were observed among TT genotype carriers.

Conclusions: Our findings suggested that plasma selenium was associated with 3-year lipid changes differentially by rs7579 genotypes, and higher plasma selenium was associated with adverse lipid changes among rs7579 CC genotype carriers, but not among T allele carriers.

Keywords: selenium, *SEPP1*, gene-environment interactions, lipid changes, cohort study

INTRODUCTION

Selenium is an essential trace element which plays an important role in the antioxidant process by incorporating into selenoproteins (1). Because of this property, selenium supplementation has been suggested to protect against oxidative stress. In recent years, selenium-containing supplements and selenium-rich foods have been widely used due to their potential benefits (2). However, several studies revealed that high selenium was associated with numerous metabolic disorders, including dyslipidemia (3–5). Most epidemiological studies examining the associations between selenium status and lipid levels are cross-sectional, while longitudinal evidence to show prospective associations is limited (3, 6–8).

Genetic factors are believed to play an important role in lipid metabolism (9, 10). A functional variant located in the 3′ untranslated regions of *SEPP1*, namely rs7579 polymorphism, has been suggested to modulate lipid response to Brazil nut supplementation, and Brazil nut is the richest natural source of selenium (11). *SEPP1* encodes selenoprotein P (SELENOP), which mainly functions as a selenium transporter and delivers selenium from the liver to other tissues (12, 13). Moreover, findings from previous studies indicated that SELENOP played a crucial role in the antioxidant-mediated protection against colitis-associated cancer (14, 15). Several studies demonstrated that the rs7579 polymorphism affected the mRNA expression as well as plasma levels of SELENOP, and both the mRNA expression and plasma levels of SELENOP were significantly lower among rs7579 CC genotype than T allele carriers (16, 17). Meanwhile, previous findings showed that the changes in cholesterol levels were greater among rs7579 CC genotype than T allele carriers in response to Brazil nut supplementation (18). Based on the function of rs7579 polymorphism, it can be anticipated that the associations between plasma selenium and changes in lipid levels are more pronounced in rs7579 CC genotype than T allele carriers. However, to our knowledge, no study has examined the interaction between plasma selenium and rs7579 polymorphism on lipid changes.

Hence, the current study aimed to evaluate the associations between plasma selenium concentrations and changes in lipid levels, and then to examine whether the associations differ across rs7579 genotypes.

Abbreviations: BMI, body mass index; FASN, fatty acid synthase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NHANES III, the third National Health and Nutrition Examination Survey; SELENOP, selenoprotein P; SREBP1c, sterol regulatory element binding protein 1c; TC, total cholesterol; TJEZ, Tongji-Ezhou.

METHODS

Study Design and Subjects

The current study was conducted based on the Tongji-Ezhou (TJEZ) cohort study, which is an ongoing prospective cohort study, with the aim of investigating the associations of lifestyle, dietary factors, and genetic factors with chronic diseases. Design of the TJEZ cohort study has been described elsewhere (5). Briefly, 5,533 residents in Ezhou aged above 20 years were recruited between 2013 and 2015. At baseline, all participants underwent comprehensive medical examinations, including semi-structured questionnaires, anthropometric measurements, and biochemical analyses (including lipid measurements). Also, fasting venous blood samples were collected from all participants at baseline enrollment. All blood samples were separated for plasma within 1 h and stored in −80°C freezers until laboratory analysis. Additionally, genotyping of rs7579 was performed on 3,605 subjects at baseline enrollment. The TJEZ cohort will be followed up every 3 years, and a total of 4,695 subjects participated in the first follow-up survey which was conducted between 2016 and 2018, with a follow-up rate of 91.1%. In the first follow-up survey, 3,204 cohort members were invited to undergo lipid remeasurements and genotyping; the 2,691 (84.0%) respondents were broadly representative of the total cohort; 1,957 subjects had lipid measurements, and 1,655 individuals among them had genotyping results at baseline. After excluding subjects on lipid-lowering drugs, a subset of 1,621 subjects who had genotyping results at baseline as well as lipid measurements in the first follow-up survey, were included in the present study.

The study was approved by the Ethics and Human Subject Committee of Tongji Medical College, and written informed consent was obtained from all participants.

Lipid Measurement

Fasting serum lipid levels, including triglycerides, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), were measured by Hitachi automatic analyzer. Both the intra-assay and inter-assay variation coefficients of lipid levels were below 5%. The non-high-density lipoprotein cholesterol (non-HDL-C) level was calculated as TC subtracting HDL-C. Additionally, the TC/HDL-C and LDL-C/HDL-C ratio were calculated. Changes

in lipid levels during 3-year follow-up period were calculated by subtracting the lipids at follow-up from lipids at baseline.

Measurement of Plasma Selenium Concentrations

Plasma selenium concentrations were measured by inductively coupled plasma mass spectrometry (Agilent 7700 Series, Tokyo, Japan) in the Ministry of Education Key Laboratory of Environment and Health at Tongji Medical College of Huazhong University of Science & Technology, as described previously (5). Prior to analysis, thawed samples (40 μL plasma) were diluted with ultrapure water and digestive solution at a ratio of 1:19:20. For quality assurance, standard reference material ClinChek No. 8883 and No. 8884 human plasma controls for trace elements were analyzed in every 20 samples. The detection limit for plasma selenium was 0.024 μg/L, and no samples had a level below this limit. Both intra-assay and inter-assay variation coefficients of plasma selenium were below 5%.

Genotyping

Genomic DNA was isolated from the peripheral blood sample by kit (Tiangen biotech, Beijing, China). The sample DNA was amplified by a multiplex polymerase chain reaction for locus-specific single-base extension reaction. Genotyping of rs7579 was done by the MassArray system (Agena iPLEXassay, San Diego, United States). The sequences for the forward primer are "ACGTTGGATGTGACGCTGAAAGAATCAGGC," and the sequences for the reverse primer are "ACGTTGGAT GTGTGTCTAGACTAAATTGGG." Genotyping was successful in 99.3% (1,609 of 1,621) of the participants. The genotypes distribution of rs7579 was in Hardy-Weinberg equilibrium.

Assessment of Covariates

Basic information including sex, age, smoking status, alcohol drinking status, physical activity, educational level, and medical history (including use of lipid-lowering medication) was obtained *via* semi-structured questionnaires at baseline and was included as adjustment variables. Smoking status was grouped into two categories: current smoking (≥1 cigarette/day over the past 6 months) and non-smoking. Alcohol drinking status was classified as current drinking (≥1 time/week over the past 6 months) and non-drinking. Physical activity was defined as regular exercise for at least 60 min per week over the past 6 months. We classified educational level into three categories: none or elementary school, middle school, and high school or college. Anthropometric measurements were performed by trained staff. Body weight and standing height were measured in light indoor clothing and without shoes. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters.

Statistical Analysis

Descriptive statistics were shown as means ± standard deviations for continuous variables if normally distributed, medians (interquartile ranges) for continuous variables if not normally distributed, and frequencies (percentages) for categorical variables. Basic characteristics were compared across quartiles of plasma selenium by analysis of variance (normal distribution) or Kruskal-Wallis test (skewed distribution) for continuous variables, and χ^2-test for categorical variables. Multivariable linear regression models were used and regression coefficients (95% CIs) were calculated to examine the associations of plasma selenium concentrations, as well as rs7579 polymorphisms, with 3-year changes in lipid levels. In multivariate models, we adjusted for respective baseline lipid (continuous) in model 1. In model 2, we further adjusted for sex (men/women), age (continuous), and BMI (continuous). In model 3, we further adjusted for current smoking status (yes/no), current drinking status (yes/no), physical activity (yes/no), and educational level (none or elementary school/middle school/high school or beyond). Due to right-skewed distribution, all lipids were ln-transformed and multiplied by 100 before analyses. Changes in lipid levels during 3-year follow-up period were calculated by subtracting the 100*ln-transformed lipids at follow-up from 100*ln-transformed lipids at baseline. Hence, regression coefficients in models can be interpreted as percentage difference in means (19).

To examine whether the associations between plasma selenium and 3-year changes in lipid levels were different across rs7579 genotypes, analyses of plasma selenium with 3-year lipid changes were stratified by rs7579 genotypes, and interactions between plasma selenium and rs7579 on 3-year lipid changes were identified through 1df multiplicative terms tests. Statistical power for the selenium×rs7579 interactions with lipid changes was calculated using QUANTO 1.2.4 (http://biostats.usc.edu/Quanto.html). Assuming a marginal R^2_G of 0.008, a marginal R^2_E of 0.01, and a marginal R^2_{GE} of 0.006, our study had 88% power to detect an interaction for a lipid change.

All analyses were conducted using SAS 9.4 (SAS Instituted Inc, Cary, NC), and two-side $P < 0.05$ were considered statistically significant.

RESULTS

General characteristics of participants are shown in **Table 1**. The 1,621 participants had a mean age of 57.86 years, and 65.3% were male. The median (interquartile range) concentration of plasma selenium was 91.68 (81.55–104.92) μg/L. At baseline, participants with higher plasma selenium concentrations were more likely to be men, current drinkers, and had higher levels of TC, LDL-C, and non-HDL-C.

After adjustment for sex, age, BMI, educational level, lifestyle factors, and respective baseline lipid, plasma selenium concentrations were positively associated with 3-year changes in triglycerides, TC, LDL-C, non-HDL-C, TC/HDL-C ratio, and LDL-C/HDL-C ratio (**Table 2**). Comparing the highest to the lowest quartiles of plasma selenium concentrations, 3-year changes in lipid levels were elevated by 8.25% (95% CI: 1.54–14.96%) for triglycerides ($P = 0.016$), 5.88% (3.13–8.63%) for TC ($P < 0.001$), 7.37% (3.07–11.67%) for LDL-C ($P = 0.0008$), 6.44% (2.66–10.21%) for non-HDL-C ($P = 0.0009$), 4.99% (0.62–9.36%) for TC/HDL-C ratio ($P = 0.025$), and 7.00% (1.55–12.46%) for LDL-C/HDL-C ratio ($P = 0.012$). The associations between

TABLE 1 | Baseline characteristics of participants[a].

Characteristics	Total	Quartiles of plasma selenium concentrations (μg/L)				*P*-value
		Q1, <81.56	Q2, 81.57–91.68	Q3, 91.69–104.90	Q4, ≥104.91	
No of participants	1,621	406	405	405	405	
Men, *n* (%)	1,058 (65.3)	253 (62.3)	256 (63.2)	257 (63.5)	292 (72.1)	**0.010**
Age, years	57.86 (10.25)	56.97 (11.20)	58.16 (10.72)	58.13 (9.54)	58.19 (9.40)	0.247
BMI, kg/m^2	23.79 (3.13)	23.80 (3.27)	23.72 (3.02)	23.61 (3.15)	24.03 (3.06)	0.273
Triglycerides, mmol/L	1.24 (0.88, 1.78)	1.19 (0.84, 1.74)	1.28 (0.92, 1.84)	1.23 (0.89, 1.70)	1.28 (0.89, 1.96)	0.105
TC, mmol/L	4.75 (4.18, 5.36)	4.58 (3.91, 5.12)	4.62 (4.10, 5.31)	4.87 (4.28, 5.43)	4.95 (4.32, 5.56)	**<0.001**
HDL-C, mmol/L	1.32 (1.10, 1.58)	1.27 (1.05, 1.54)	1.33 (1.09, 1.57)	1.35 (1.14, 1.59)	1.34 (1.11, 1.61)	**0.008**
LDL-C, mmol/L	2.82 (2.28, 3.33)	2.72 (2.15, 3.17)	2.79 (2.25, 3.31)	2.87 (2.41, 3.36)	2.89 (2.29, 3.56)	**<0.001**
Non-HDL-C, mmol/L	3.37 (2.80, 4.01)	3.20 (2.65, 3.78)	3.29 (2.80, 3.99)	3.48 (2.86, 4.06)	3.58 (2.98, 4.20)	**<0.001**
TC/HDL-C ratio	3.56 (2.96, 4.36)	3.51 (2.87, 4.37)	3.57 (2.94, 4.30)	3.51 (2.94, 4.29)	3.66 (3.05, 4.49)	0.154
LDL-C/HDL-C ratio	2.12 (1.63, 2.72)	2.10 (1.63, 2.72)	2.11 (1.64, 2.72)	2.08 (1.59, 2.71)	2.21 (1.71, 2.75)	0.549
Education level, *n* (%)						0.091
None or elementary school	424 (26.2)	99 (24.4)	95 (23.5)	105 (25.9)	125 (30.9)	
Middle school	734 (45.3)	174 (42.8)	196 (48.4)	191 (47.2)	173 (42.7)	
High school or beyond	463 (28.5)	133 (32.8)	114 (28.1)	109 (26.9)	107 (26.4)	
Current smoker, *n* (%)	479 (29.5)	120 (29.6)	122 (30.1)	116 (28.6)	121 (29.9)	0.970
Current drinker, *n* (%)	480 (29.6)	99 (24.4)	109 (26.9)	120 (29.6)	152 (37.5)	**<0.001**
Vigorous activity, *n* (%)	738 (45.5)	180 (44.3)	198 (48.9)	177 (43.7)	183 (45.2)	0.450
rs7579 genotypes, *n* (%)[b]						
CC	882 (54.7)	209 (51.7)	235 (58.1)	225 (56.1)	213 (53.0)	0.234
CT	614 (38.1)	159 (39.4)	140 (34.7)	156 (38.9)	159 (39.5)	
TT	115 (7.1)	36 (8.9)	29 (7.2)	20 (5.0)	30 (7.5)	
Plasma selenium, μg/L	91.68 (81.55, 104.92)	75.25 (68.92, 78.50)	87.09 (84.16, 89.11)	97.86 (94.50, 101.08)	114.67 (108.91, 126.16)	**<0.001**

[a]*Data are presented as mean (SD) for parametrically distributed data, median (interquartile range) for non-parametrically distributed data, or n (%) for categorical data. BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; TC, total cholesterol.*
[b]*Genotyping of rs7579 polymorphism was failed in 12 participants. Bold values indicates P < 0.05.*

rs7579 polymorphism and 3-year changes in lipids were showed in **Table 3**. After adjustment for sex, age, BMI, educational level, lifestyle factors, and respective baseline lipid, 3-year changes in lipid levels were elevated by 0.90% (95% CI: −8.41–10.21%) for triglycerides ($P = 0.849$), −0.47% (−4.32–3.39%) for TC ($P = 0.813$), 0.67% (−4.85–6.20%) for HDL-C ($P = 0.811$), −2.67% (−8.72–3.39%) for LDL-C ($P = 0.388$), 0.47% (−4.85–5.79%) for non-HDL-C ($P = 0.861$), −1.53% (−7.70–4.63%) for TC/HDL-C ratio ($P = 0.626$), and −3.20% (−10.89–4.49%) for LDL-C/HDL-C ratio ($P = 0.415$), when comparing the TT genotype with the TT genotype carriers.

Analyses of plasma selenium with 3-year changes in lipid levels were stratified by rs7579 genotypes (**Figure 1**). In stratified analyses, positive associations between plasma selenium concentrations and 3-year changes in triglycerides, TC, LDL-C, non-HDL-C, TC/HDL-C ratio, and LDL-C/HDL-C ratio were observed among CC genotype carriers, but negative associations between plasma selenium and TC/HDL-C ratio, and LDL-C/HDL-C ratio were observed among TT genotype carriers. Among rs7579 CC genotype carriers, comparing the highest to the lowest quartiles of plasma selenium concentrations, 3-year changes in lipid levels were elevated by 11.54% (2.29–20.79%) for triglycerides ($P = 0.015$), 9.39% (5.53–13.24%) for TC ($P < 0.001$), −0.41% (−5.94–5.11%) for HDL-C ($P = 0.884$), 11.66% (5.72–17.61%) for LDL-C ($P = 0.0001$), 10.91% (5.64–16.18%) for non-HDL-C ($P < 0.001$), 9.44% (3.36–15.51%) for TC/HDL-C ratio ($P = 0.002$), and 12.35% (5.19–19.51%) for LDL-C/HDL-C ratio ($P = 0.0007$). Among TT genotype carriers, comparing the highest to the lowest quartiles of plasma selenium concentrations, 3-year changes in lipid levels were elevated by 26.45% (−3.43–56.33%) for triglycerides ($P = 0.082$), 6.23% (−4.65–17.10%) for TC ($P = 0.259$), 15.41% (−6.15–36.97%) for HDL-C ($P = 0.159$), 8.56% (−14.93–32.06%) for LDL-C ($P = 0.471$), 4.06% (−11.20–19.31%) for non-HDL-C ($P = 0.599$), −5.45% (−32.65–21.76%) for TC/HDL-C ratio ($P = 0.692$), and −3.88% (−41.99–34.24%) for LDL-C/HDL-C ratio ($P = 0.840$). The interactions between plasma selenium and rs7579 polymorphism on 3-year changes in TC (P for interaction = 0.007), LDL-C (P for interaction = 0.034), non-HDL-C (P for interaction = 0.005), TC/HDL-C ratio (P for interaction = 0.034), and LDL-C/HDL-C ratio (P for interaction = 0.048) were statistically significant.

DISCUSSION

In this study, plasma selenium was positively associated with 3-year changes in triglycerides, TC, LDL-C, non-HDL-C, TC/HDL-C ratio, and LDL-C/HDL-C ratio. Moreover, the positive associations between plasma selenium and 3-year changes in lipid levels were only significant in rs7579

TABLE 2 | Mean % difference (95% CI) in 3-year changes in lipid levels according to quartiles of plasma selenium concentrations[a].

Mean % (95% CI) difference in 3-year changes in lipid levels	Quartiles of plasma selenium concentrations (μg/L)				*P*-trend
	Q1, <81.56	Q2, 81.57–91.68	Q3, 91.69–104.90	Q4, ≥104.91	
Triglycerides					
Model 1	0.00 (referent)	1.57 (−5.25, 8.39)	1.78 (−5.00, 8.55)	6.87 (0.10, 13.64)*	0.056
Model 2	0.00 (referent)	2.85 (−3.87, 9.57)	2.73 (−3.93, 9.40)	7.42 (0.75, 14.09)*	**0.040**
Model 3	0.00 (referent)	2.90 (−3.82, 9.62)	3.13 (−3.55, 9.81)	8.25 (1.54, 14.96)*	**0.021**
TC					
Model 1	0.00 (referent)	2.70 (−0.14, 5.53)	4.08 (1.26, 6.91)*	5.40 (2.58, 8.22)*	**<0.001**
Model 2	0.00 (referent)	3.31 (0.56, 6.05)*	4.28 (1.55, 7.02)*	5.87 (3.14, 8.61)*	**<0.001**
Model 3	0.00 (referent)	3.23 (0.48, 5.98)*	4.26 (1.52, 7.00)*	5.88 (3.13, 8.63)*	**<0.001**
HDL-C					
Model 1	0.00 (referent)	1.09 (−3.10, 5.28)	−1.18 (−5.36, 3.00)	0.17 (−4.00, 4.33)	0.797
Model 2	0.00 (referent)	2.21 (−1.74, 6.16)	−0.95 (−4.88, 2.98)	0.97 (−2.96, 4.89)	0.970
Model 3	0.00 (referent)	2.14 (−1.80, 6.08)	−1.30 (−5.22, 2.62)	0.40 (−3.52, 4.32)	0.729
LDL-C					
Model 1	0.00 (referent)	3.58 (−0.76, 7.92)	4.89 (0.56, 9.21)*	6.65 (2.34, 10.96)*	**0.002**
Model 2	0.00 (referent)	4.27 (−0.03, 8.57)	5.30 (1.02, 9.59)*	7.27 (2.99, 11.55)*	**0.001**
Model 3	0.00 (referent)	4.27 (−0.04, 8.58)	5.39 (1.10, 9.69)*	7.37 (3.07, 11.67)*	**<0.001**
Non-HDL-C					
Model 1	0.00 (referent)	3.20 (−0.65, 7.04)	4.77 (0.94, 8.61)*	5.77 (1.94, 9.59)*	**0.002**
Model 2	0.00 (referent)	3.99 (0.22, 7.76)*	5.21 (1.45, 8.97)*	6.27 (2.51, 10.02)*	**0.001**
Model 3	0.00 (referent)	3.96 (0.18, 7.74)*	5.30 (1.53, 9.07)*	6.44 (2.66, 10.21)*	**<0.001**
TC/HDL-C ratio					
Model 1	0.00 (referent)	1.68 (−2.76, 6.12)	5.00 (0.59, 9.41)*	4.60 (0.20, 9.00)*	**0.016**
Model 2	0.00 (referent)	1.29 (−3.11, 5.69)	4.90 (0.53, 9.27)*	4.31 (−0.05, 8.68)	**0.019**
Model 3	0.00 (referent)	1.33 (−3.07, 5.72)	5.32 (0.95, 9.69)*	4.99 (0.62, 9.36)*	**0.007**
LDL-C/HDL-C ratio					
Model 1	0.00 (referent)	2.73 (−2.81, 8.28)	6.62 (1.11, 12.13)*	6.71 (1.22, 12.21)*	**0.007**
Model 2	0.00 (referent)	2.05 (−3.43, 7.53)	6.38 (0.93, 11.82)	6.35 (0.91, 11.79)*	**0.008**
Model 3	0.00 (referent)	2.16 (−3.32, 7.65)	6.85 (1.41, 12.30)*	7.00 (1.55, 12.46)*	**0.004**

[a]*Model 1, adjusted for respective baseline lipid (continuous); Model 2, further adjusted for sex (men/women), age (continuous), and BMI (continuous); Model 3, further adjusted for current smoking status (yes/no), current drinking status (yes/no), physical activity (yes/no), and educational level (none or elementary school/middle school/high school or beyond). BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; TC, total cholesterol. *P < 0.05. Bold values indicates P < 0.05.*

CC genotype carriers, while non-significant associations were observed in T allele carriers.

The selenium status among people varies by regions and mainly depends on its dietary intake (20, 21). The intakes are high in America, Japan, and Canada, and much lower in the majority of Eastern Europe. China has both high-selenium and low-selenium areas (20). Although the optimal selenium status remains controversial, findings from some studies suggested the plasma selenium concentration around 90 μg/L to be the optimal value, which maximizes the plasma SELENOP level (20). The median concentration of plasma selenium was 91.68 μg/L in our participants, which was considered as selenium-sufficient.

Our findings of positive associations between plasma selenium and changes in triglycerides, TC, LDL-C, non-HDL-C, TC/HDL-C ratio, and LDL-C/HDL-C ratio are consistent with those from cross-sectional studies conducted among subjects with a wide-range of selenium status, which also demonstrated strong, positive associations between selenium status and lipid levels (3, 4, 21–24). For example, in the third National Health and Nutrition Examination Survey (NHANES III), which included a representative American population (median blood selenium 199.88 μg/L), increased serum selenium was significantly associated with higher lipid levels (21). Also, another cross-sectional study conducted among a selenium-excess Chinese population (mean serum selenium 120 μg/L) revealed significant positive associations of plasma selenium with triglycerides, TC, and LDL-C (3). In addition, several cross-sectional studies conducted among selenium-deficient individuals (mean serum selenium 79.5 μg/L) also revealed that higher plasma selenium was associated with adverse lipid levels (7, 25). Limited longitudinal studies have explored relationships between baseline blood selenium and changes in lipid levels, and findings from them indicated non-significant or negative associations between selenium status and changes in lipid levels (6, 8). It seems likely

TABLE 3 | Mean % difference (95% CI) in 3-year changes in lipid levels according to rs7579 genotypes[a].

Mean % (95% CI) difference in 3-year changes in lipid levels	rs7579 genotypes			*P*-trend
	CC	CT	TT	
Triglycerides				
Model 1	0.00 (referent)	−2.58 (−7.61, 2.45)	2.90 (−6.55, 12.35)	0.835
Model 2	0.00 (referent)	−2.92 (−7.87, 2.03)	0.99 (−8.32, 10.30)	0.573
Model 3	0.00 (referent)	−3.13 (−8.10, 1.83)	0.90 (−8.41, 10.21)	0.532
TC				
Model 1	0.00 (referent)	−0.99 (−3.11, 1.12)	0.46 (−3.52, 4.43)	0.683
Model 2	0.00 (referent)	−1.33 (−3.38, 0.72)	−0.41 (−4.26, 3.45)	0.360
Model 3	0.00 (referent)	−1.23 (−3.28, 0.83)	−0.47 (−4.32, 3.39)	0.383
HDL-C				
Model 1	0.00 (referent)	1.86 (−1.25, 4.97)	2.25 (−3.63, 8.12)	0.223
Model 2	0.00 (referent)	1.02 (−1.91, 3.95)	0.78 (−4.76, 6.33)	0.547
Model 3	0.00 (referent)	1.36 (−1.57, 4.29)	0.67 (−4.85, 6.20)	0.473
LDL-C				
Model 1	0.00 (referent)	0.81 (−2.42, 4.04)	−1.71 (−7.81, 4.39)	0.948
Model 2	0.00 (referent)	0.49 (−2.70, 3.69)	−2.68 (−8.72, 3.36)	0.691
Model 3	0.00 (referent)	0.53 (−2.68, 3.74)	−2.67 (−8.72, 3.39)	0.704
Non-HDL-C				
Model 1	0.00 (referent)	−1.60 (−4.45, 1.26)	1.62 (−3.80, 7.03)	0.779
Model 2	0.00 (referent)	−1.97 (−4.77, 0.83)	0.51 (−4.80, 5.82)	0.470
Model 3	0.00 (referent)	−1.95 (−4.76, 0.86)	0.47 (−4.85, 5.79)	0.473
TC/HDL-C ratio				
Model 1	0.00 (referent)	−3.03 (−6.33, 0.26)	−1.88 (−8.10, 4.35)	0.134
Model 2	0.00 (referent)	−2.61 (−5.88, 0.65)	−1.56 (−7.74, 4.61)	0.196
Model 3	0.00 (referent)	−2.89 (−6.16, 0.38)	−1.53 (−7.70, 4.63)	0.165
LDL-C/HDL-C ratio				
Model 1	0.00 (referent)	−1.12 (−5.23, 3.00)	−3.99 (−11.77, 3.79)	0.318
Model 2	0.00 (referent)	−0.54 (−4.61, 3.52)	−3.29 (−10.99, 4.40)	0.471
Model 3	0.00 (referent)	−0.84 (−4.92, 3.24)	−3.20 (−10.89, 4.49)	0.427

[a]*Model 1, adjusted for respective baseline lipid (continuous); Model 2, further adjusted for sex (men/women), age (continuous), and BMI (continuous); Model 3, further adjusted for current smoking status (yes/no), current drinking status (yes/no), physical activity (yes/no), and educational level (none or elementary school/middle school/high school or beyond). BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; TC, total cholesterol.*

that there are some other unmeasured factors might confound the longitudinal association between selenium status and lipid levels. Additionally, in view of genetic factors account for nearly 30% of lipids, such contradictory findings might be partly due to difference in genetic background of participants between studies. More longitudinal studies examining associations of selenium status with changes in lipid levels are warranted to further verify our findings.

Although exact mechanisms underlying the adverse effects of increased selenium on lipid changes among selenium-sufficient or selenium-excess subjects are not clearly understood, several potential mechanisms might be involved, including endoplasmic reticulum stress, mitochondrial dysfunction, and changed miRNA expression. Firstly, excess selenium has been suggested to induce endoplasmic reticulum stress (26). Under endoplasmic reticulum stress, the expression of several key factors in the pathway of *de novo* lipogenesis was upregulated in the liver, including the sterol regulatory element binding protein 1c (SREBP1c) and the fatty acid synthase (FASN) (27). Secondly, high selenium might be involved in abnormal lipid metabolism through disturbing mitochondrial function. Romero et al. revealed that high selenium exposures could induce mitochondrial dysfunction by increasing the generation of reactive oxygen species (28). Mitochondrial dysfunction could disturb lipid metabolism through several mechanisms, including leading to endoplasmic reticulum stress, impairing autophagy, and then increasing lipogenesis (29, 30). And lastly, Guo et al. found blood selenium was positively associated with the expression of miR-122-5p (31), which has been implicated as a key regulator of cholesterol and fatty-acid metabolism in the liver (32). Nevertheless, more mechanistic studies are needed to further illustrate potential mechanisms through which high selenium plays a role in adverse changes in lipid levels.

Our study also revealed significant interactions between plasma selenium and rs7579 polymorphism on changes in lipid levels, which helped to explain the inconsistent findings between

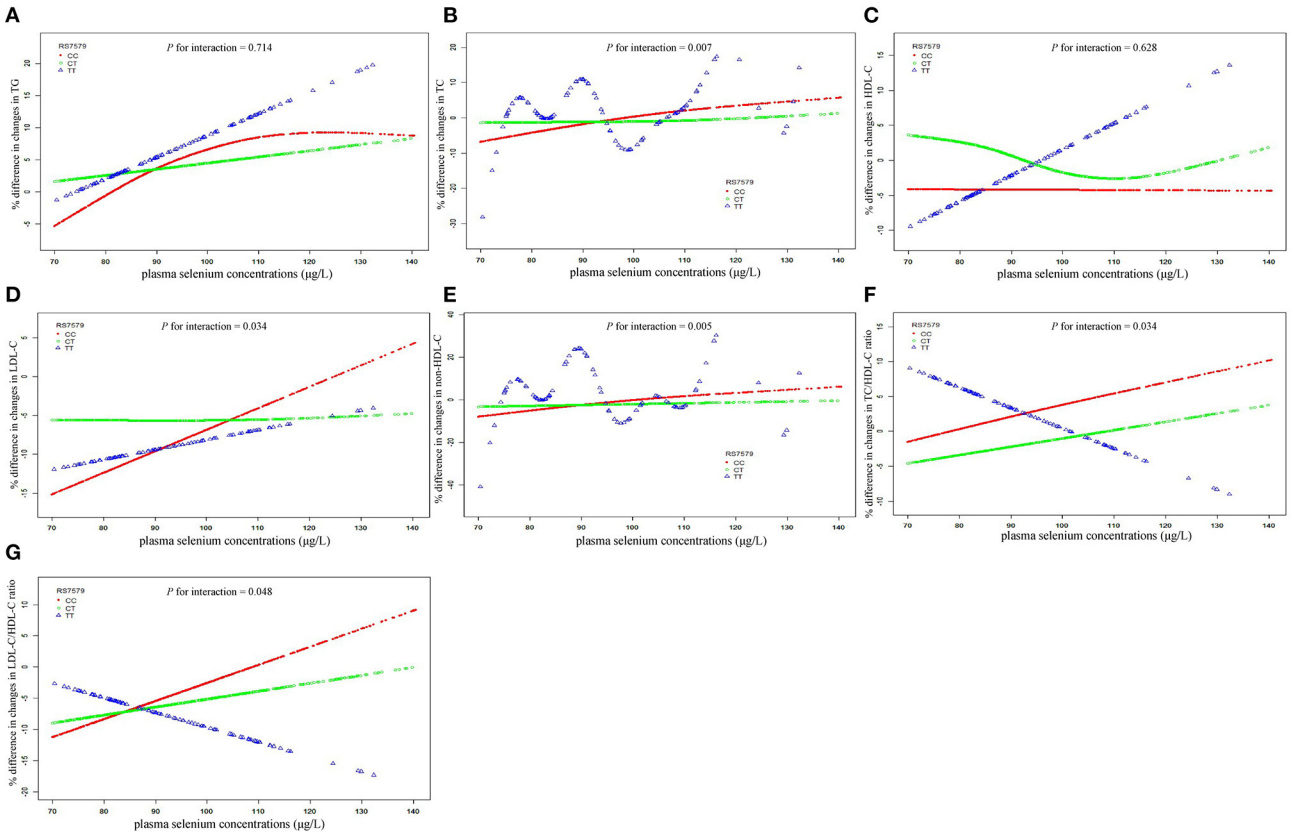

FIGURE 1 | Associations of plasma selenium concentrations with 3-year changes in lipids stratified by rs7579 genotypes. X bars represent plasma selenium concentrations. Y bars represent mean % difference in 3-year changes in triglycerides for **(A)**, TC for **(B)**, HDL-C for **(C)**, LDL-C for **(D)**, non-HDL-C for **(E)**, TC/HDL-C ratio for **(F)**, and LDL-C/HDL-C ratio for **(G)**. Multivariable analyses were adjusted for sex (men/women), age (continuous), BMI (continuous), current smoking status (yes/no), current drinking status (yes/no), physical activity (yes/no), educational level (none or elementary school/middle school/high school or beyond), and respective baseline lipid (continuous). BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; TC, total cholesterol.

studies and suggested that such inconsistency might be partly due to the selenium × rs7579 polymorphism interactions. We showed that the impact of baseline selenium on changes in lipid levels depended on genotype at rs7579. Notably, the adverse effect of high selenium on changes in lipid levels was only significant among rs7579 CC genotype carriers, but non-significant among rs7579 T allele carriers. Although such an interaction has not been reported before, there are studies that corroborate our findings. Findings from an intervention trial suggested that the changes in cholesterol levels in response to Brazil nut supplementation were greater among rs7579 CC genotype than T allele carriers (18), which is in line with the currently observed differential associations of plasma selenium with lipid changes across rs7579 genotypes. Furthermore, it has been suggested that both the mRNA expression and plasma levels of SELENOP were significantly lower among rs7579 CC than T allele carriers (16, 17), and decreased plasma SELENOP was related with increased risks of several dyslipidemia-related diseases, including metabolic syndrome, cerebrovascular events, and cardiovascular disease (33–35). Our study also revealed non-significant associations between rs7579 polymorphism and changes in lipid levels, which is consistent with those from previous studies.

Our study has several strengths. First, to our knowledge, this is the first population-study systematically exploring the interactions between plasma selenium and rs7579 polymorphism with changes in lipid levels. Second, the prospective design allowed us to clarify the temporal sequence of the relationships between selenium and lipid levels. Third, we assess selenium status using plasma selenium concentration instead of dietary intake of selenium to avoid possible bias through dietary survey.

Several limitations should also be acknowledged. First, plasma selenium concentrations were measured only once, and measurement errors and exposure misclassification were inevitable. However, such errors were likely to be non-differential and thus would be more likely to induce the attenuation of the observed associations. Second, we only measured total plasma selenium levels rather than different chemical forms of selenium. Given that different chemical forms of selenium might have different properties and effects on human health, more detailed analyses of different chemical forms of selenium are

needed to better understand the relationships between selenium and lipid changes. Also, we did not have measurement of plasma SELENOP levels, which precluded us from exploring the associations of rs7579 polymorphism as well as lipid changes with plasma SELENOP levels. Third, our study was conducted among a selenium-appropriate population. Thus, the generalizability of our findings to people with different selenium statuses remains unclear, and further studies are needed to determine the generalizability of our findings. Fourth, the study population was limited to a Chinese population, which also limits the generalizability of our findings. Finally, no information on health status (e.g., obese, diabetic) was collected in our population, which should be taken into account in future study. In addition, although multiple potential confounders have been adjusted, we could not rule out the possibility of residual confounding by other unknown or unmeasured factors.

In conclusion, our findings demonstrated that, in a selenium-appropriate population, plasma selenium was associated with lipid changes differentially across rs7579 genotypes, and higher selenium seemed to be associated with adverse changes in lipid levels among rs7579 CC genotype carriers, but not among T allele carriers. These findings warn the widespread use of selenium-fortified foods or selenium supplements, especially among selected study populations, such as rs7579 CC genotype carriers.

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 and Human Subject Committee of Tongji Medical College. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LZ contributed to the statistical analysis, interpretation of the results, and wrote the manuscript. LZ, XLia, MX, JY, and YH contributed to the acquisition of data and researched the data. XLi, ZS, and LC contributed to the study design. YZ contributed to the discussion of the project. LZ, XLia, MX, JY, YH, XLi, ZS, LC, YZ, CL, and LL reviewed and edited the manuscript. LL and CL are the guarantors of this work and as such, have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Major International (Regional) Joint Research Project (NSFC 81820108027), the National Key Research and Development Program of China (2020YFC2006300), the National Natural Science Foundation of China (81803226), and the Angel Nutrition Research Fund (AF2019003). The funding sources had no role in the design and conduct of the study, collection, management, analysis, and interpretation of the data, preparation, review, or approval of the manuscript, and decision to submit the manuscript for publication.

ACKNOWLEDGMENTS

The authors thank all those who volunteered to take part in this research.

REFERENCES

1. Rayman MP. Selenium and human health. *Lancet*. (2012) 379:1256–68. doi: 10.1016/S0140-6736(11)61452-9
2. Bodnar M, Szczyglowska M, Konieczka P, Namiesnik J. Methods of selenium supplementation: bioavailability and determination of selenium compounds. *Crit Rev Food Sci Nutr*. (2016) 56:36–55. doi: 10.1080/10408398.2012.709550
3. Ju W, Ji M, Li X, Li Z, Wu G, Fu X, et al. Relationship between higher serum selenium level and adverse blood lipid profile. *Clin Nutr*. (2018) 37:1512–17. doi: 10.1016/j.clnu.2017.08.025
4. Liu A, Xu P, Gong C, Zhu Y, Zhang H, Nie W, et al. High serum concentration of selenium, but not calcium, cobalt, copper, iron, and magnesium, increased the risk of both hyperglycemia and dyslipidemia in adults: a health examination center based cross-sectional study. *J Trace Elem Med Biol.* (2020) 59:126470. doi: 10.1016/j.jtemb.2020.126470
5. Zhou L, Luo C, Yin J, Zhu Y, Li P, Chen S, et al. Diverse associations of plasma selenium concentrations and SELENOP gene polymorphism with metabolic syndrome and its components. *Oxid Med Cell Longev*. (2020) 2020:5343014. doi: 10.1155/2020/5343014
6. Stranges S, Galletti F, Farinaro E, D'Elia L, Russo O, Iacone R, et al. Associations of selenium status with cardiometabolic risk factors: an 8-year follow-up analysis of the Olivetti Heart study. *Atherosclerosis*. (2011) 217:274–8. doi: 10.1016/j.atherosclerosis.2011.03.027
7. Stranges S, Laclaustra M, Ji C, Cappuccio FP, Navas-Acien A, Ordovas JM, et al. Higher selenium status is associated with adverse blood lipid profile in British adults. *J Nutr*. (2010) 140:81–7. doi: 10.3945/jn.109.111252
8. Stranges S, Tabak AG, Guallar E, Rayman MP, Akbaraly TN, Laclaustra M, et al. Selenium status and blood lipids: the cardiovascular risk in Young Finns study. *J Intern Med*. (2011) 270:469–77. doi: 10.1111/j.1365-2796.2011.02398.x
9. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature*. (2010) 466:707–13. doi: 10.1038/nature09270
10. Krauss RM. What can the genome tell us about LDL cholesterol? *Lancet*. (2008) 371:450–2. doi: 10.1016/S0140-6736(08)60213-5
11. Thomson CD, Chisholm A, McLachlan SK, Campbell JM. Brazil nuts: an effective way to improve selenium status. *Am J Clin Nutr*. (2008) 87:379–84. doi: 10.1093/ajcn/87.2.379
12. Hill KE, Zhou J, McMahan WJ, Motley AK, Atkins JF, Gesteland RF, et al. Deletion of selenoprotein P alters distribution of selenium in the mouse. *J Biol Chem*. (2003) 278:13640–6. doi: 10.1074/jbc.M300755200
13. Schomburg L, Schweizer U, Holtmann B, Flohe L, Sendtner M, Kohrle J. Gene disruption discloses role of selenoprotein P in selenium delivery to target tissues. *Biochem J*. (2003) 370 (Pt 2):397–402. doi: 10.1042/bj20021853
14. Short SP, Pilat JM, Williams CS. Roles for selenium and selenoprotein P in the development, progression, and prevention of intestinal disease. *Free Radic Biol Med.* (2018) 127:26–35. doi: 10.1016/j.freeradbiomed.2018.05.066

15. Short SP, Pilat JM, Barrett CW, Reddy VK, Haberman Y, Hendren JR, et al. Colonic epithelial-derived selenoprotein P is the source for antioxidant-mediated protection in colitis-associated cancer. *Gastroenterology*. (2021) 160:5:1694–708.e3. doi: 10.1053/j.gastro.2020.12.059
16. Donadio JLS, Rogero MM, Cockell S, Hesketh J, Cozzolino SMF. Influence of genetic cariations in selenoprotein genes on the pattern of gene expression after supplementation with Brazil nuts. *Nutrients*. (2017) 9:7. doi: 10.3390/nu9070739
17. Donadio JLS, Rogero MM, Guerra-Shinohara EM, Barbosa F Jr, Desmarchelier C, Borel P, et al. Genetic variants in selenoprotein genes modulate biomarkers of selenium status in response to Brazil nut supplementation (the SU.BRA.NUT study). *Clin Nutr*. (2019) 38:539–48. doi: 10.1016/j.clnu.2018.03.011
18. Donadio JLS, Rogero MM, Guerra-Shinohara EM, Desmarchelier C, Borel P, Cozzolino SMF. SEPP1 polymorphisms modulate serum glucose and lipid response to Brazil nut supplementation. *Eur J Nutr*. (2018) 57:1873–82. doi: 10.1007/s00394-017-1470-7
19. Pinto Pereira SM, Power C. Life course body mass index, birthweight and lipid levels in mid-adulthood: a nationwide birth cohort study. *Eur Heart J*. (2013) 34:1215–24. doi: 10.1093/eurheartj/ehs333
20. Xia Y, Hill KE, Li P, Xu J, Zhou D, Motley AK, et al. Optimization of selenoprotein P and other plasma selenium biomarkers for the assessment of the selenium nutritional requirement: a placebo-controlled, double-blind study of selenomethionine supplementation in selenium-deficient Chinese subjects. *Am J Clin Nutr*. (2010) 92:525–31. doi: 10.3945/ajcn.2010.29642
21. Christensen K, Werner M, Malecki K. Serum selenium and lipid levels: associations observed in the National Health and Nutrition Examination Survey (NHANES) 2011-2012. *Environ Res.* (2015) 140:76–84. doi: 10.1016/j.envres.2015.03.020
22. Fang H, He X, Wu Y, Chen S, Zhang M, Pan F, et al. Association between selenium level in blood and glycolipid metabolism in residents of Enshi prefecture, China. *Biol Trace Elem Res*. (2021) 199:7:2456–66. doi: 10.1007/s12011-020-02372-9
23. Huang YQ, Shen G, Lo K, Huang JY, Liu L, Chen CL, et al. Association of circulating selenium concentration with dyslipidemia: results from the NHANES. *J Trace Elem Med Biol.* (2020) 58:126438. doi: 10.1016/j.jtemb.2019.126438
24. Loomba R, Filippini T, Chawla R, Chaudhary R, Cilloni S, Datt C, et al. Exposure to a high selenium environment in Punjab, India: effects on blood chemistry. *Sci Total Environ.* (2020) 716:135347. doi: 10.1016/j.scitotenv.2019.135347
25. Yang KC, Lee LT, Lee YS, Huang HY, Chen CY, Huang KC. Serum selenium concentration is associated with metabolic factors in the elderly: a cross-sectional study. *Nutr Metab.* (2010) 7:38. doi: 10.1186/1743-7075-7-38
26. Zachariah M, Maamoun H, Milano L, Rayman MP, Meira LB, Agouni A. Endoplasmic reticulum stress and oxidative stress drive endothelial dysfunction induced by high selenium. *J Cell Physiol*. (2021) 236:4348–59. doi: 10.1002/jcp.30175
27. Fu S, Watkins SM, Hotamisligil GS. The role of endoplasmic reticulum in hepatic lipid homeostasis and stress signaling. *Cell Metab*. (2012) 15:623–34. doi: 10.1016/j.cmet.2012.03.007
28. Zhuang Y, Li L, Feng L, Wang S, Su H, Liu H, et al. Mitochondrion-targeted selenium nanoparticles enhance reactive oxygen species-mediated cell death. *Nanoscale*. (2020) 12:3:1389–96. doi: 10.1039/C9NR09039H
29. Biczo G, Vegh ET, Shalbueva N, Mareninova OA, Elperin J, Lotshaw E, et al. Mitochondrial dysfunction, through impaired autophagy, leads to endoplasmic reticulum stress, deregulated lipid metabolism, and pancreatitis in animal models. *Gastroenterology*. (2018) 154:3:689–703. doi: 10.1053/j.gastro.2017.10.012
30. Peng KY, Watt MJ, Rensen S, Greve JW, Huynh K, Jayawardana KS, et al. Mitochondrial dysfunction-related lipid changes occur in nonalcoholic fatty liver disease progression. *J Lipid Res*. (2018) 59:10:1977–86. doi: 10.1194/jlr.M085613
31. Guo X, Yang Q, Zhang W, Chen Y, Ren J, Gao A. Associations of blood levels of trace elements and heavy metals with metabolic syndrome in Chinese male adults with microRNA as mediators involved. *Environ Pollut.* (2019) 248:66–73. doi: 10.1016/j.envpol.2019.02.015
32. Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, et al. miR-122 regulation of lipid metabolism revealed by *in vivo* antisense targeting. *Cell Metab*. (2006) 3:2:87–98. doi: 10.1016/j.cmet.2006.01.005
33. Gharipour M, Sadeghi M, Salehi M, Behmanesh M, Khosravi E, Dianatkhah M, et al. Association of expression of selenoprotein P in mRNA and protein levels with metabolic syndrome in subjects with cardiovascular disease: results of the selenegene study. *J Gene Med*. (2017) 19:3. doi: 10.1002/jgm.2945
34. Koyama H, Abdulah R, Ohkubo T, Imai Y, Satoh H, Nagai K. Depressed serum selenoprotein P: possible new predicator of increased risk for cerebrovascular events. *Nut Res*. (2009) 29:2:94–9. doi: 10.1016/j.nutres.2009.01.002
35. Schomburg L, Orho-Melander M, Struck J, Bergmann A, Melander O. Selenoprotein-P deficiency predicts cardiovascular disease and death. *Nutrients*. (2019) 11:8. doi: 10.3390/nu11081852

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 22 December 2021
doi: 10.3389/fnut.2021.782270

Circulating Vitamin D Levels and Risk of Vitiligo: Evidence From Meta-Analysis and Two-Sample Mendelian Randomization

Jie Song, Ke Liu, Weiwei Chen, Bin Liu, Hong Yang, Linshuoshuo Lv, Xiaohui Sun, Yingying Mao and Ding Ye**

Department of Epidemiology, School of Public Health, Zhejiang Chinese Medical University, Hangzhou, China

Background: The association between circulating vitamin D levels and risk of vitiligo was inconsistent among observational studies, and whether these observed associations were causal remained unclear. Therefore, we aimed to evaluate the effect of vitamin D on the risk of vitiigo using meta-analysis and Mendelian randomization (MR).

Methods: At the meta-analysis stage, literature search was performed in PubMed and Web of Science to identify eligible observational studies examining the association of circulating 25-hydroxyvitamin D [25(OH)D] or 25-hydroxyvitamin D_3 [25(OH)D_3] levels with risk of vitiligo up to April 30, 2021. Standardized mean differences (SMDs) with 95% confidence intervals (CIs) of 25(OH)D and 25(OH)D_3 in patients with vitiligo relative to controls were pooled. Then at the MR stage, genetic instruments for circulating 25(OH)D ($N = 120,618$) and 25(OH)D_3 ($N = 40,562$) levels were selected from a meta-analysis of genome-wide association studies (GWAS) of European descent, and summary statistics of vitiligo were obtained from a meta-analysis of three GWASs including 4,680 cases and 39,586 controls. We used inverse-variance weighted (IVW) as main method, followed by weighted-median and likelihood-based methods. Pleiotropic and outlier variants were assessed by MR-Egger regression and MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test.

Results: In the meta-analysis, patients with vitiligo had a lower level of circulating 25(OH)D compared with controls [SMD = −1.40; 95% confidence interval (CI): −1.91, −0.89; $P < 0.001$], while no statistically significant difference of 25(OH)D_3 between vitiligo cases and controls was found (SMD = −0.63; 95% CI: −1.29, 0.04; $P = 0.064$). However, in the MR analyses, genetically predicted 25(OH)D [odds ratio (OR) = 0.93, 95% CI = 0.66–1.31, $P = 0.66$] and 25(OH)D_3 levels (OR = 0.95, 95% CI = 0.80–1.14, $P = 0.60$) had null associations with risk of vitiligo using the IVW method. Sensitivity analyses using alternative MR methods and instrumental variables (IV) sets obtained consistent results, and no evidence of pleiotropy or outliers was observed.

Conclusion: Our study provided no convincing evidence for a causal effect of 25(OH)D or 25(OH)D_3 levels on the risk of vitiligo. Further longitudinal and experimental studies, as well as functional studies are warranted to elucidate the role of vitamin D in the development of vitiligo.

Keywords: 25-hydroxyvitamin D, 25-hydroxyvitamin D_3, Mendelian randomization, meta-analysis, vitiligo

OPEN ACCESS

Edited by:
Mauro Serafini,
University of Teramo, Italy

Reviewed by:
Hui Lu,
Capital Medical University, China
Hai-Feng Pan,
Anhui Medical University, China

****Correspondence:***
Ding Ye
yeding@zcmu.edu.cn
Yingying Mao
myy@zcmu.edu.cn

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *24 September 2021*
Accepted: *29 November 2021*
Published: *22 December 2021*

Citation:
Song J, Liu K, Chen W, Liu B, Yang H, Lv L, Sun X, Mao Y and Ye D (2021) Circulating Vitamin D Levels and Risk of Vitiligo: Evidence From Meta-Analysis and Two-Sample Mendelian Randomization. Front. Nutr. 8:782270. doi: 10.3389/fnut.2021.782270

INTRODUCTION

Vitiligo is an acquired pigmented skin disease caused by the loss of melanocyte function, which is characterized by localized or generalized complete depigmentation of skin mucosa. It can occur in all parts of the body, commonly in finger back, wrist, forearm, face, neck and around genitalia, affecting both men and women (1). Globally, the prevalence of vitiligo was estimated to be ranging from a low of 0.5% to a high of 2.0% in adults, and the peak onset period is between 10 and 30 years of age (2). Although vitiligo does not bring fatal risk, it is often accompanied by a variety of autoimmune system-related diseases, such as Hashimoto's thyroiditis, diabetes, Addison's disease and so on (3–6). Therefore, it is necessary to further explore the influence of modifiable risk factors on the incidence of vitiligo, which may provide new ideas for its prevention and treatment.

Previous studies have identified a series of nutritional factors associated with vitiligo, such as selenium (7), copper, and zinc (8). Recently, vitamin D deficiency has been reported to be a potential risk factor for vitiligo (9). With usual physiological intake, the majority of vitamin D is converted to 25-hydroxyvitamin D (25(OH)D) and released into the blood, thus circulating 25(OH)D concentrations can reflect vitamin D status in the human body. As the main metabolite of 25(OH)D, the levels of circulating 25-hydroxy vitamin D_3 [25(OH)D_3] is also widely accepted as an indicator of overall vitamin D status (10). Though no randomized controlled trials have been conducted to investigate the preventive role of vitamin D on the risk of vitiligo to date (11), numerous observational epidemiological studies have evaluated circulating 25(OH)D and 25(OH)D_3 levels in patients with vitiligo relative to controls. For instance, Atazadeh et al. (12) conducted a case-control study with 90 vitiligo patients and 90 healthy controls in Iran, and found that the median (interquartile range) of circulating 25(OH)D level was lower in patients with vitiligo than in matched healthy controls [28.50 (22.52–33.20) ng/mL vs. 29.10 (27.40–35.70) ng/mL, $P = 0.01$). Another case-control study (13) conducted in India with 100 vitiligo patients and 100 healthy controls suggested that circulating 25(OH)D_3 levels were lower in vitiligo patients than controls (16.17 ± 8.63 vs. 25.49 ± 1.02 ng/ml, $P < 0.001$). However, Saniee (14) recruited 98 patients with vitiligo and 98 matched healthy controls and measured 25(OH)D levels, but did not detect statistically significant differences between patients with vitiligo and normal subjects (22.37 ± 10.78 vs. 26.16 ± 17.11 ng/ml, $P = 0.19$). Similarly, Ustun et al. also found no statistically significant association (15). These inconsistent results may be due to differences in the study population, sample size, measurement methods and so on. Besides, findings from traditional observational studies are susceptible to bias such as confounding and reverse causation, therefore, it remains unclear whether the observed association was causal or not.

Mendelian Randomization (MR) study utilizes genetic variants such as single nucleotide polymorphisms (SNPs), which act as instrumental variables (IVs) to estimate the potential causal relationship between exposure and outcome (16). Since genotypes are randomly distributed in the process of gamete formation, the relationship between exposure and outcome of interest will not be biased by common confounding factors, such as postnatal environment, socio-economic status and behavioral factors in conventional observational studies. Moreover, MR study has unique advantages in causal inference because genetic variation is inherited from parents and remains unchanged after birth, so the association between genetic variation and outcome is reasonable in time sequence.

Therefore, in the present study, we first performed a systematic review and meta-analysis of existing observational evidence on the association of circulating 25(OH)D or 25(OH)D_3 levels with risk of vitiligo, and then performed MR analysis to further investigate whether there was evidence for a causal relationship of circulating 25(OH)D and 25(OH)D_3 levels with risk of vitiligo.

METHOD

Meta-Analysis

Data Source

The overall design of the present study is shown in **Figure 1**. We first systematically searched PubMed and Web of Science up to April 30, 2021 to identify observational studies examining the association of circulating 25(OH)D or 25(OH)D_3 levels with risk of vitiligo. The keywords included vitiligo AND (vitamin D OR 25-hydroxyvitamin D OR 25-dihydroxyvitamin D_3 OR 25(OH)D OR 25(OH)D_3 OR calcidiol).

Literature Inclusion and Exclusion Criteria

The inclusion criteria were: (i) The study was cross-sectional, case-control or cohort design; (ii) The original study reported standardized mean difference (SMD) and 95% confidence interval (95% CI) of circulating 25-hydroxyvitamin D or 25-hydroxyvitamin D_3 between patients with vitiligo and controls, or provided sufficient data to calculate SMD and 95% CI; (iii) When two or more literatures recruited overlapped study population, the literature with the largest sample size was included. The exclusion criteria were: (i) Review or meta-analysis; (ii) Full text is unavailable; (iii) Data provided in the literature were insufficient.

Data Extraction and Quality Assessment

Relevant information was extracted and crosschecked by two researchers independently (Liu K and Chen W), and discussed with the third researcher (Ye D) in case of disagreement. The extracted information included the first author, year of publication, region, type of study design, study period, sample size, gender and age of the study participants. Two researchers (Liu K and Chen W) independently scored the quality of the

Abbreviations: CI, confidence interval; GWAS, genome-wide association study; IV, instrumental variable; IVW, inverse-variance weighted; IL-6, interleukin-6; IL-8, interleukin-8; MR, mendelian randomization; MAF, minor allele frequency; NOS, newcastle ottawa scale; OR, odds ratio; PRESSO, Pleiotropy RESidual Sum and Outlier; SD, standard deviation; SMD, standardized mean difference; SNP, single nucleotide polymorphism; TNF-α, tumor necrosis factor α; TNF-γ, tumor necrosis factor γ; 25(OH)D, 25-hydroxyvitamin D; 25(OH)D_3, 25-hydroxyvitamin D_3.

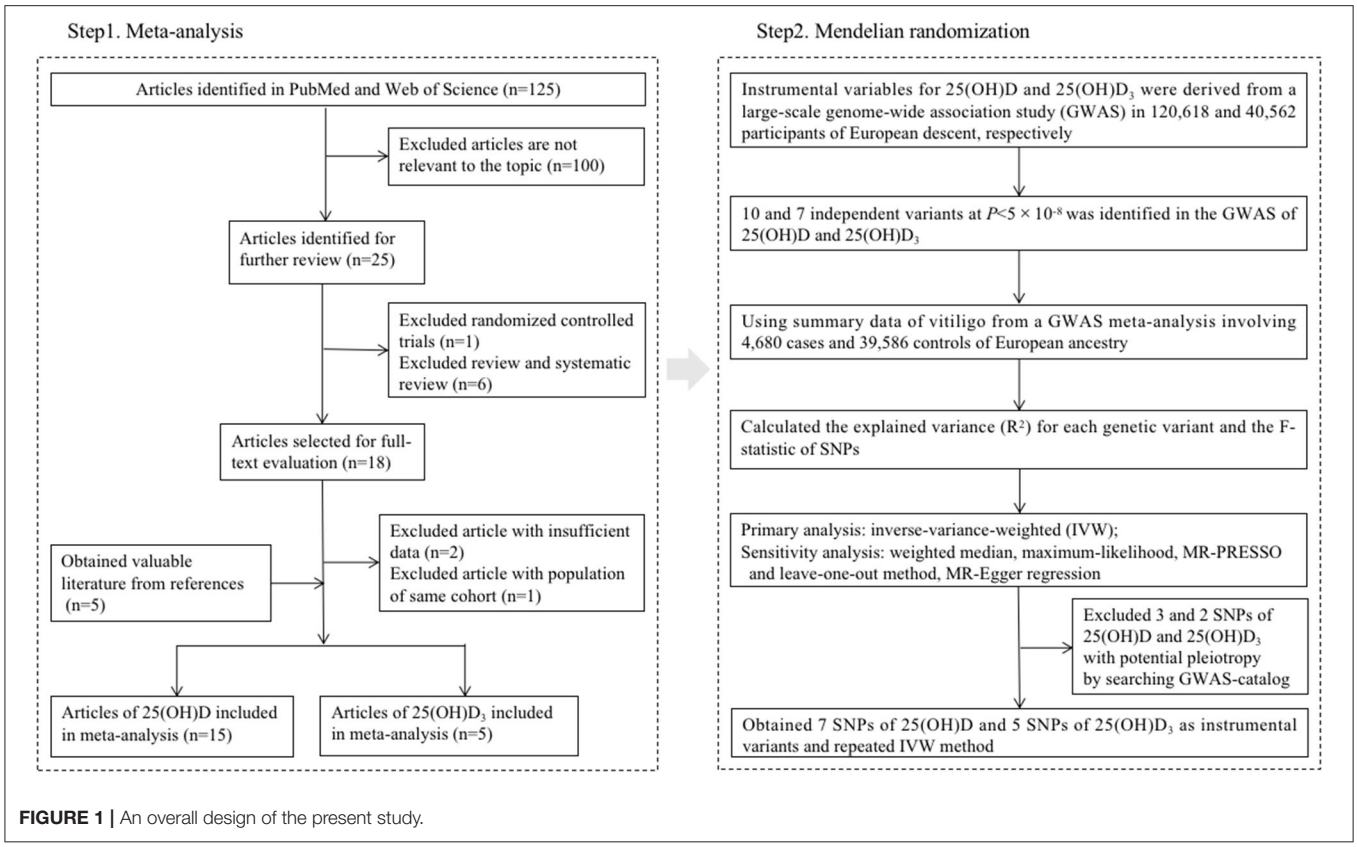

FIGURE 1 | An overall design of the present study.

included articles using Newcastle Ottawa scale (NOS) (17). The scores ranging from 7 to 9 were regarded as high quality, 5–6 as moderate quality, and ≤4 as low quality.

Statistical Analysis

The mean and standard deviations (SD) of circulating levels of 25 (OH) D and 25 (OH) D_3 between patients with vitiligo and controls were used to calculate SMDs and 95% CIs. If the study provided with median and range, we computed mean and SD using previously described formulas (18, 19). If odds ratio (OR) was provided, we computed SMD using the formulae of $SMD = \left(\frac{\sqrt{3}}{\pi}\right)^{*} lnOR$ (20). The heterogeneity among studies was tested by Cochrane's Q test and I^2 statistics. If $I^2 < 50\%$ and $P > 0.10$, a fixed-effects model was used; otherwise, a random-effects model was applied. Sensitivity analysis was performed to evaluate the stability of the association by sequential removal of each study from the analysis. Subgroup analysis was performed in terms of region, publication year, quality score and study design, and meta-regression analysis was conducted for the above factors, respectively. Funnel plot was generated, and Begg's test (21) and Egger's test (22) were used to evaluate potential publication bias.

All statistical analyses were performed using STATA (version 14.0). Two-sided *P*-values < 0.05 were considered statistically significant.

Mendelian Randomization

Outcome Dataset

Genetic association data of vitiligo were obtained from a meta-analysis of three genome-wide association studies (GWAS) involving 4,680 vitiligo cases and 39,586 controls of European ancestry (23). A total of 48 genetic variants associated with risk of vitiligo were identified, accounting for 17.4% of heritability. Detailed information of the above studies has been described in the previous article (23). All study participants provided written informed consent and the study was carried out under the jurisdiction of each local institutional review board.

Selection of Genetic Variants

IVs for 25(OH)D and 25(OH)D_3 were derived from a large-scale GWAS including 120,618 and 40,562 participants of European descent, respectively (24). A total of ten and seven independent SNPs (r^2 threshold < 0.1) associated with circulating 25(OH)D and 25(OH)D_3 levels at genome-wide significance level ($P < 5 \times 10^{-8}$) were identified, respectively. None of the SNPs overlapped or were in linkage disequilibrium ($r^2 < 0.1$) with known risk loci of vitiligo. The variance explained for total 25(OH)D by the ten lead SNPs was 3.95%, and the variance explained for 25(OH)D_3 by the seven SNPs was 4.58%. The details of GWAS studies and datasets used in the present study are listed in **Supplementary Table 1**.

Statistical Analysis

We calculated F-statistics to quantify the strength of the IVs, with the equation of $F = R^2 \times (n - 2)/(1 - R^2)$, in which R^2 represents the variance explained by the IVs and n indicates the sample size (25). We used inverse-variance weighted (IVW) method to evaluate the potential causal relationship of circulating 25(OH)D and 25(OH)D_3 levels with risk of vitiligo as the main analysis.

IVW method combines the causal effect estimates of multiple single genetic IVs by Wald ratio method (26). The estimated effect size by IVW method is essentially the regression coefficient of weighted regression without considering the intercept term (27). Moreover, weighted-median and likelihood-based methods were used to evaluate the robustness of the IVW method. For the weighted median method, the consistent estimation of the causal effect can be obtained as long as the weight of the causal effect calculated by the effective IV reaches 50% (25). Moreover, the likelihood-based method was used to evaluate the casual relationship under the assumption of a linear association between the risk factor and the outcome variables (28).

In addition, we conducted MR-Egger regression analysis to analyze the effect of potential pleiotropy on causal estimation. The intercept term of MR-Egger represents the average pleiotropic effect of genetic variation. If the intercept is different from zero, it indicates that there is evidence of directed pleiotropy (29). Also, we performed Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) method (30) to detect and correct for horizontal pleiotropic outliers. It conducts a global test of heterogeneity by regressing the SNP-outcome association on the SNP-exposure association and comparing the observed distance of each SNP from the regression with that expected under the null hypothesis of no pleiotropy.

We further performed "leave-one-out" method as sensitivity analyses to test the reliability of the association of genetically predicted circulating 25(OH)D and $25(OH)D_3$ with risk of vitiligo. Specifically, we removed each SNP and combined the effect estimates of the remaining SNPs using the IVW method. The fluctuation of the results before and after removing the SNP reflects the stability of the association. Finally, considering the influence of pleiotropy on the results, associated phenotypes of the SNPs used as IVs were manually scanned through the GWAS Catalog (https://www.ebi.ac.uk/gwas, accessed on May 15, 2021). SNPs associated with other traits at genome-wide significance were excluded from the IVs and MR analyses were subsequently performed using the remaining SNPs.

R (version 4.0.5) with packages "MendelianRandomization" and "MR-PRESSO" was used for MR analysis. *P*-values < 0.05 were considered statistically significant.

RESULTS

Patients With Vitiligo Had a Lower Level of 25(OH)D Relative to Controls

The flowchart of literature inclusion and exclusion is shown in **Figure 1**. Briefly, a total of 125 articles were retrieved in the initial stage, with 15 articles and 5 articles investigating the association of 25(OH)D (12, 14, 31–43) and $25(OH)D_3$ (13, 15, 44–46) with risk of vitiligo included in the final analysis, respectively. In total, we collected the data of 987 patients and 770 controls for 25(OH)D, and 305 patients and 321 controls for $25(OH)D_3$ between 2012 and 2020 in this meta-analysis. Overall, there were 14 case-control studies and one cross-sectional study for 25(OH)D, and 4 case-control studies and one cross-sectional study for $25(OH)D_3$. As assessed by NOS, the quality of the included studies was high in general. The basic information of the literature included is shown in **Table 1**.

Statistically significant heterogeneity was observed among studies for the association of 25(OH)D ($I^2 = 95.5\%$, $P < 0.001$) and $25(OH)D_3$ ($I^2 = 93.3\%$, $P < 0.001$) with risk of vitiligo, respectively. Therefore, we used random-effects models to combine the effect sizes. As shown in **Figure 2** Patients with vitiligo had a lower level of 25(OH)D compared with controls (SMD $= -1.40$; 95% CI: -1.91, -0.89; $P < 0.001$). However, the differences of circulating $25(OH)D_3$ levels in cases and controls did not reach statistical significance (SMD $= -0.63$; 95% CI: -1.29, 0.04; $P = 0.064$).

Consistently, subgroup analysis revealed associations between circulating levels of 25(OH)D and risk of vitiligo in different strata. The levels of $25(OH)D_3$ in patients with vitiligo was significantly lower than that of the control group in the subgroups of studies with a high-quality score (SMD $= -0.81$; 95% CI: -1.56, -0.07; $P = 0.032$), published in 2017 and before (SMD $= -0.81$; 95% CI: -1.56, -0.07; $P = 0.032$), and in Asian populations (SMD $= -1.40$; 95% CI: -1.96, -0.83; $P < 0.001$). However, meta-regression analysis did not find potential sources of heterogeneity from region, publication year, quality score, study type and sample size for 25(OH)D and $25(OH)D_3$ (**Supplementary Table 2**). The Begg's test ($P = 0.010$) and Egger's test ($P = 0.003$) suggest evidence of potential publication bias in 25(OH)D, and the funnel plot was not symmetrical (**Supplementary Figure 1A**). For $25(OH)D_3$, the Begg's test ($P = 0.806$) and Egger's test ($P = 0.966$) did not suggest evidence of publication bias (**Supplementary Figure 1B**). Sensitivity analysis omitting one study at a time suggested none of these studies had a strong effect on the combined effect estimates (**Supplementary Figure 2**).

Genetically Determined Circulating 25(OH)D and $25(OH)D_3$ Were Not Associated With Risk of Vitiligo

Supplementary Table 3 presents detailed information of IVs and their effect estimates with 25(OH)D, $25(OH)D_3$ and vitiligo, respectively. The F-statistic was 495.99 for 25(OH)D and 278.07 for $25(OH)D_3$, suggesting the IVs were unlikely to suffer from weak instrument bias.

As shown in **Table 2**, genetically predicted circulating 25(OH)D (OR = 0.93, 95% CI = 0.66–1.31, $P = 0.663$) and $25(OH)D_3$ levels (OR = 0.95, 95% CI = 0.80–1.14, $P = 0.595$) were not associated with altered risk of vitiligo using the IVW method. Similar results were obtained using the weighted-median approach and maximum-likelihood method. The intercept from the MR-Egger regression analysis did not reach statistical significance [25(OH)D: $P = 0.614$, $25(OH)D_3$: $P = 0.061$], suggesting no apparent evidence of directional pleiotropy. Additionally, no outlier SNPs were detected using MR-PRESSO test, and the causal effect estimates of 25(OH)D ($P = 0.529$) and $25(OH)D_3$ ($P = 0.215$) with risk of vitiligo were not statistically significant.

TABLE 1 | Characteristics of individual studies included in the meta-analysis.

References	Region	Study type	Research time	Sample size	Sex, male (%)		Age (years)		NOS
					Cases	Controls	Cases	Controls	
25(OH)D									
Xu et al. (39)	China	Case-control	Mar–May 2011	221	46.7	42	18–60	18–60	7
			Sep–Oct 2010	50			18–60	18–60	
Saleh et al. (35)	Egypt	Case-control	Apr–Jun 2011	80	45	45	34.1 ± 11.4	34.2 ± 11.5	8
Aksu Cerman et al. (31)	Turkey	Cross-sectional	Nov 2012–Mar 2013	102	23.9	29.1	33.64 ± 11.51	32.55 ± 9.78	7
Sehrawat et al. (36)	India	Case-control		60	33.3	33.3	31.33 ± 7.33	31.33 ± 7.33	8
Takci et al. (38)	Turkey	Case-control	Nov 2011–Feb 2012	87	54.5	23.2	34.5 ± 16.1	33.0 ± 12.6	7
Doss et al. (43)	Egypt	Case-control	Jun–Sep 2013	60	66.7	53.3	32.5 ± 14.6	28 ± 5.7	7
Sobeih et al. (37)	Egypt	Case-control	July–December 2013	150			31.5 ± 13.5	31.5 ± 13.5	7
Shalaby et al. (41)	Egypt	Case-control	Dec 2014–Jun 2016	80	75	62.5	28.70 ± 13.44	32.95 ± 9.24	7
Farag et al. (32)	Egypt	Case-control	Mar–Jun 2016	75	36	40	28.76 ± 10.64	28.16 ± 9.84	8
Ibrahim et al. (33)	Egypt	Case-control	Dec 2015–Dec 2016	100	50	50	34.27 ± 11.741	35.20 ± 10.65	7
Omidian and Asadian (34)	Iran	Case-control	Apr 2015–Mar 2016	60	40	36.6	36.93 ± 14.5	32.03 ± 15.08	7
Zhang et al. (40)	China	Case-control	Nov 2016–Mar 2017	214	43.9	47	7.37 ± 3.78	6.95 ± 3.63	7
Amer et al. (42)	Egypt	Case-control	Mar–Oct 2018	42	47.6	47.6	30.8 ± 19.1	30.6 ± 13.2	8
Saniee (14)	Iran	Case-control	Spring–summer 2017	196	51	54.1	30.06 ± 16.18	29.45 ± 13.16	7
Atazadeh et al. (12)	Iran	Case-control	2018–2019	180	62.2	62.2	18–65	19–60	7
25(OH)D_3									
Ustun et al. (15)	Turkey	Cross-sectional	2010–2011	66	52	48.7	33.9 ± 19.4	34.7 ± 15.9	5
Abou Khodair et al. (46)	Egypt	Case-control	Mar–Jun 2018	60	20	33.3	30.7 ± 9.3	30.3 ± 9.7	7
Dinachandran and Pillai (45)	India	Case-control	Jan–Apr 2018	100	54	60	30.96 ± 10.57	31.45 ± 8.33	8
Alshiyab et al. (44)	Jordan	Case-control	May–Dec 2018	200	42	35	2–82	2–74	8
Hassan et al. (13)	India	Case-control	Mar–Apr 2019	200	39	40	28.66 ± 11.98	28.66 ± 11.98	7

25(OH)D, 25-hydroxyvitamin D; 25(OH)D_3, 25-hydroxyvitamin D_3; SNP, single nucleotide polymorphism; NOS, Newcastle Ottawa scale.

In the sensitivity analyses, we performed "leave-one-out" analyses to identify potential influencing SNPs. We found that the risk estimates of genetically predicted 25(OH)D and 25(OH)D_3 with risk of vitiligo did not change substantially after excluding one single SNP at a time (**Supplementary Figure 3**). Then we excluded potential pleiotropic SNPs and used the remaining seven and five SNPs as IVs for 25(OH)D and 25(OH)D_3. Details of the excluded SNPs and their associated phenotypes according to the GWAS Catalog are shown in **Supplementary Table 4**. Consistently, we did not observe statistically significant association of genetically predicted circulating levels of 25(OH)D (OR = 0.98, 95% CI = 0.63–1.53, $P = 0.920$) or 25(OH)D_3 (OR = 1.16, 95% CI = 0.85–1.58, $P = 0.360$) with risk of vitiligo (**Supplementary Table 5**).

DISCUSSION

To date, vitiligo is a disease that cannot be completely cured. Patients often encounter psychological difficulties due to discrimination, such as shame, depression and anxiety, which usually lead to inferiority and social isolation (47, 48), and have an adverse impact on the quality of life (49). At present, the pathogenic mechanism of vitiligo is not completely clear. There are studies suggesting that vitamin D may increase the melanogenesis and tyrosinase content of human melanocytes through its anti-apoptotic effect, thus preventing the loss of skin pigment (50). In addition, vitamin D can also inhibit the autoimmune pathway in the pathogenesis of vitiligo by inhibiting the expression of interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor α (TNF-α) and tumor necrosis factor γ (TNF-γ),

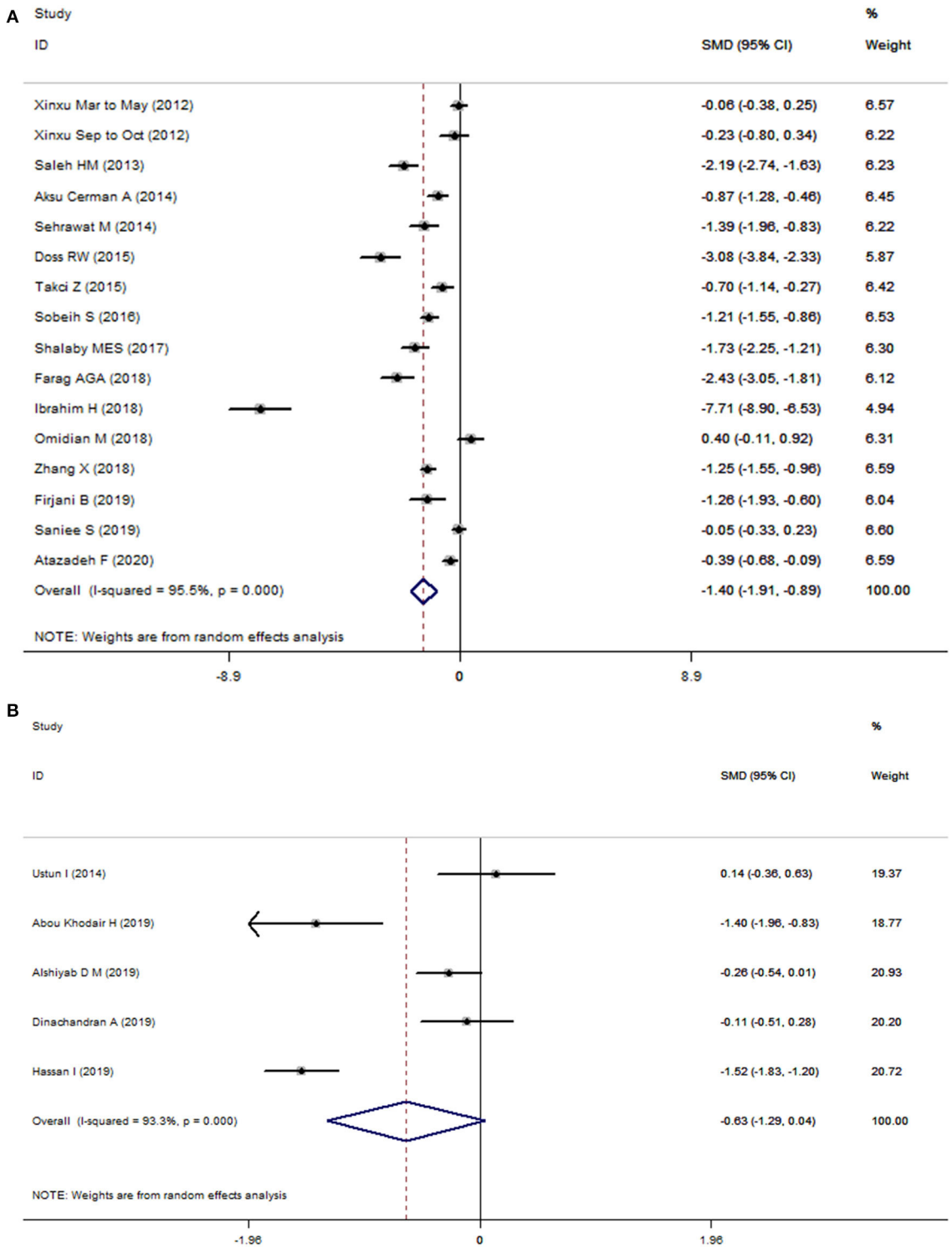

FIGURE 2 | Forest plots of 25(OH)D **(A)** and 25(OH)D3 **(B)** in vitiligo patients relative to controls. The horizontal lines correspond to the study-specific standardized mean differences (SMD) and 95% confidence intervals (CI), respectively. The area of the squares reflects the study-specific weight.

regulating the mature differentiation and activation of dendritic cells and inhibiting antigen presentation (51). For prevention and early diagnosis of vitiligo, the present study investigated the role of circulating 25(OH)D and 25(OH)D_3 on the risk of vitiligo by using meta-analysis and Mendelian randomization, however, we did not find convincing evidence to support

TABLE 2 | Association of genetically predicted circulating levels of 25(OH)D and 25(OH)D_3 levels with risk of Vitiligo.

	No. of SNPs	OR (95% CI)	*P* for association	*P* for heterogeneity	*P* intercept from MR-Egger regression	*P* for MR-PRESSO global test
25(OH)D						
Inverse-variance weighted	10	0.93 (0.66–1.31)	0.667	0.419		
Weighted-median	10	0.90 (0.60–1.36)	0.622			
Maximum-likelihood	10	0.93 (0.66–1.31)	0.667			
MR-PRESSO test	10	0.93 (0.66–1.31)	0.677			0.529
MR-Egger	10	0.85 (0.53–1.39)	0.520		0.614	
25(OH)D_3						
Inverse-variance weighted	7	0.95(0.80–1.14)	0.595	0.326		
Weighted-median	7	0.91(0.75–1.11)	0.337			
Maximum-likelihood	7	0.95 (0.79–1.16)	0.620			
MR-PRESSO test	7	0.95 (0.79–1.15)	0.639			0.215
MR-Egger	7	0.75 (0.55–1.02)	0.066		0.061	

25(OH)D, 25-hydroxyvitamin D; 25(OH)D_3, 25-hydroxyvitamin D_3; CI, confidence interval; MR, Mendelian randomization; MR-PRESSO test, MR-Pleiotropy RESidual Sum and Outlier test; OR, odds ratio; SNP, single nucleotide polymorphism.

a protective role of vitamin D supplementation on the risk of vitiligo.

The results from the meta-analysis indicated that patients with vitiligo had a lower level of 25(OH)D compared with controls. Consistently, a meta-analysis conducted by Zhang et al. (52) in 2018 including 679 patients and 537 controls reported similar results (SMD = −0.94, 95% CI: −1.39, −0.48, $P = 0.0001$). Our updated meta-analysis included five additional recent published studies with a sample size of 987 patients and 770 controls for 25(OH)D, 305 patients and 321 controls for 25(OH)D_3, and therefore had increased statistical power. Similarly, we also found heterogeneity in the studies included in the meta-analysis. However, meta-regression did not suggest potential sources of heterogeneity, and subgroup analysis consistently revealed associations between circulating levels of 25(OH)D and risk of vitiligo in different strata. Publication bias was detected for the association between 25(OH)D and risk of vitiligo, suggesting that our results may be more based on positive results, resulting in overestimation of the summary effect. Previously, no meta-analysis has focused on the association between 25(OH)D_3 and risk of vitiligo. In the present study, we found no statistical differences in circulating levels of 25(OH)D_3 between cases and controls. However, the number of included studies was relatively small, more studies are warranted for obtaining stable results. Additionally, owing to the limitations inherent in observational studies, high heterogeneity among studies may be due to differences in the study design, characteristics of the study population such as age and ethnicity, use of drugs and so on. Finally, all of the included studies were cross-sectional studies and case-control studies with relatively small study sample sizes, which is weak in demonstrating causality, since it is difficult to judge the time sequence of exposure and disease (53). Therefore, low vitamin D levels could also be a consequence of already developed vitiligo.

In order to overcome the limitations inherent in conventional observational studies, we adopted a two-sample MR method which used genetic variants as IVs of exposures to further investigate the relationship of 25(OH)D and 25(OH)D_3 levels with risk of vitiligo. MR approach offers great opportunities to the etiological research of diseases, provided that the following three assumptions are satisfied (54). The first assumption is that the IVs must be associated with the exposure of interest. To ensure this, we used independent loci associated with circulating 25(OH)D and 25(OH)D_3 levels achieving genome-wide significance level ($P < 5 \times 10^{-8}$) as IVs, which was identified from the largest GWAS to date. The second assumption is that the IVs must not be associated with potential confounders of the exposure-outcome association. Since genotypes are randomly allocated during gamete formation, MR analyses using genetic variants as IVs to a large extent solve the problem of confounding in conventional observational studies. The third assumption is that the IVs influence the outcome only through the risk factor. To ensure this, we excluded potential pleiotropic SNPs and retained those solely associated with 25(OH)D and 25(OH)D_3 levels in the MR sensitivity analyses to evaluate the robustness of our results. We also performed different MR methods to test for potential pleiotropy. We did not observe evidence of directional pleiotropy for the causal association between 25(OH)D, 25(OH)D_3 and risk of vitiligo in any of the above MR approaches. However, the estimate of MR studies reflect the effects of lifelong interference, whereas that of observational studies reflects more acute effects.

The limitations of the present study should be noted. First, the data for the meta-analysis were from observational studies, in which there could be potential confounding factors in the baseline characteristics of the selected population. The statistical ability of the small sample is limited, thus the well-designed and large sample studies are necessary to validate the findings.

Second, heterogeneity in meta-analysis is a potential problem that affects the results of statistical analysis. In the present study, subgroup analysis and meta-regression did not indicate sources account for the heterogeneity observed in the study. In addition, publication bias was found in the results of 25(OH)D, which indicated that the results of meta-analysis were affected by publication bias. Third, the variance explained by IVs is limited, with 3.95% total 25(OH)D and 4.58% 25(OH)D_3, respectively. Besides, the number of reported cases in the outcome dataset is relatively small, which could affect the statistical power in MR study. However, it is the largest sample size in the outcome database available at present, which needs more vitiligo GWASs with larger sample size to explore the association. Finally, because our MR analyses were restricted to participants of European ancestry, it is unclear whether our findings can be extrapolated to other study populations. Therefore, further prospective studies and functional studies *in vivo* and *in vitro* are needed to elucidate the exact role of 25(OH)D and 25(OH)D_3 in the occurrence of vitiligo.

CONCLUSIONS

Our study showed that although there seemed to be an inverse association between vitiligo and 25(OH)D level based on meta-analysis of observational studies, MR analysis pointed to a lack of causal association. In addition, meta-analysis and MR study did not provide evidence of an associations between 25(OH)D_3 level and risk of vitiligo. The findings suggested there is no convincing evidence that vitamin D may help to prevent vitiligo.

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/s.

AUTHOR CONTRIBUTIONS

JS performed the literature review, conducted data analysis, interpreted findings, and drafted the manuscript. KL and WC carried on data analysis and interpreted findings of meta-analysis. BL and HY mainly conducted on the data collation of the Mendelian randomization study. JS, LL, and XS can take responsibility for statistical reports, tables, and figures of the data analysis. YM and DY directed analytic strategy, supervised the study from conception to completion and revised drafts of the manuscript. All authors have read and approved the final manuscript.

FUNDING

This work was supported by grants from the National Natural Science Foundation of China (81973663), the Natural Science Foundation of Zhejiang Province (LQ21H260001 and LQ20H260008), the Talent Project of Zhejiang Association for Science and Technology (2018YCGC003), the Foundation of Zhejiang Chinese Medical University (2020ZG01 and 2020ZG16), the Research Project of Zhejiang Chinese Medical University (2021JKZKTS004A).

ACKNOWLEDGMENTS

The authors sincerely thank the researchers and participants of the original GWAS for their collection and management of large-scale data resources, as well as those who actively participated in this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.782270/full#supplementary-material

REFERENCES

1. Ezzedine K, Eleftheriadou V, Whitton M, van Geel N. Vitiligo. *Lancet.* (2015) 386:74–84. doi: 10.1016/S0140-6736(14)60763-7
2. Bergqvist C, Ezzedine K. Vitiligo: a review. *Dermatology.* (2020) 236:571–92. doi: 10.1159/000506103
3. Taieb A, Picardo M. Clinical practice. *Vitiligo N Engl J Med.* (2009) 360:160–9. doi: 10.1056/NEJMcp0804388
4. Elbuluk N, Ezzedine K. Quality of life, burden of disease, co-morbidities, and systemic effects in vitiligo patients. *Dermatol Clin.* (2017) 35:117–28. doi: 10.1016/j.det.2016.11.002
5. Jin Y, Mailloux CM, Gowan K, Riccardi SL, LaBerge G, Bennett DC, et al. NALP1 in vitiligo-associated multiple autoimmune disease. *N Engl J Med.* (2007) 356:1216–25. doi: 10.1056/NEJMoa061592
6. Sandru F, Carsote M, Albu SE, Dumitrascu MC, Valea A. Vitiligo and chronic autoimmune thyroiditis. *J Med Life.* (2021) 14:127–30. doi: 10.25122/jml-2019-0134
7. Huo J, Liu T, Huan Y, Li F, Wang R. Serum level of antioxidant vitamins and minerals in patients with vitiligo, a systematic review and meta-analysis. *J Trace Elem Med Biol.* (2020) 62:126570. doi: 10.1016/j.jtemb.2020.126570
8. Zeng Q, Yin J, Fan F, Chen J, Zuo C, Xiang Y, et al. Decreased copper and zinc in sera of Chinese vitiligo patients: a meta-analysis. *J Dermatol.* (2014) 41:245–51. doi: 10.1111/1346-8138.12392
9. Piotrowska A, Wierzbicka J, Zmijewski MA. Vitamin D in the skin physiology and pathology. *Acta Biochim Pol.* (2016) 63:17–29. doi: 10.18388/abp.2015_1104
10. Wilson LR, Tripkovic L, Hart KH, Lanham-New SA. Vitamin D deficiency as a public health issue: using vitamin D2 or vitamin D3 in future fortification strategies. *Proc Nutr Soc.* (2017) 76:392–9. doi: 10.1017/S0029665117000349
11. Watabe A, Yamasaki K, Asano M, Kanbayashi Y, Nasu-Tamabuchi M, Terui H, et al. Efficacy of oral cholecalciferol on rhododendrol-induced vitiligo: a blinded randomized clinical trial. *J Dermatol.* (2018) 45:456–62. doi: 10.1111/1346-8138.14244
12. Atazadeh F, Fazeli Z, Vahidnezhad H, Namazi N, Younespour S, Youssefian L, et al. Increased level of cathelicidin (LL-37) in vitiligo: possible pathway independent from vitamin D receptor gene polymorphism. *Exp Dermatol.* (2020) 29:1176–85. doi: 10.1111/exd.14200
13. Hassan I, Bhat YJ, Majid S, Sajad P, Rasool F, Malik RA, et al. Association of vitamin D receptor gene polymorphisms and serum 25-hydroxy vitamin D levels in vitiligo - a case-control study. *Indian Dermatol Online J.* (2019) 10:131–8. doi: 10.4103/idoj.IDOJ_97_18

14. Saniee S. Zinc, vitamin D and TSH levels in vitiligo patients. *Erciyes Med J.* (2019) 41:148–52. doi: 10.14744/etd.2019.40316
15. Ustun I, Seraslan G, Gokce C, Motor S, Can Y, Ugur Inan M, et al. Investigation of vitamin D levels in patients with vitiligo vulgaris. *Acta Dermatovenerol Croat.* (2014) 22:110–3.
16. Katan MB. Commentary: Mendelian randomization, 18 years on. *Int J Epidemiol.* (2004) 33:10–11. doi: 10.1093/ije/dyh023
17. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol.* (2010) 25:603–5. doi: 10.1007/s10654-010-9491-z
18. Yehuda S. Nutrients, brain biochemistry, and behavior: a possible role for the neuronal membrane. *Int J Neurosci.* (1987) 35:21–36. doi: 10.3109/00207458708987106
19. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. *BMC Med Res Methodol.* (2005) 5:13. doi: 10.1186/1471-2288-5-13
20. Chinn S. A simple method for converting an odds ratio to effect size for use in meta-analysis. *Stat Med.* (2000) 19:3127–31. doi: 10.1002/1097-0258(20001130)19:22<3127::AID-SIM784>3.0.CO;2-M
21. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics.* (1994) 50:1088–101 doi: 10.2307/2533446
22. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* (1997) 315:629–34. doi: 10.1136/bmj.315.7109.629
23. Jin Y, Andersen G, Yorgov D, Ferrara TM, Ben S, Brownson KM, et al. Genome-wide association studies of autoimmune vitiligo identify 23 new risk loci and highlight key pathways and regulatory variants. *Nat Genet.* (2016) 48:1418–24. doi: 10.1038/ng.3680
24. Zheng JS, Luan J, Sofianopoulou E, Sharp SJ, Day FR, Imamura F, et al. The association between circulating 25-hydroxyvitamin D metabolites and type 2 diabetes in European populations: a meta-analysis and Mendelian randomisation analysis. *PLoS Med.* (2020) 17:e1003394. doi: 10.1371/journal.pmed.1003394
25. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol.* (2016) 40:304–14. doi: 10.1002/gepi.21965
26. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med.* (2008) 27:1133–63. doi: 10.1002/sim.3034
27. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol.* (2013) 37:658–65. doi: 10.1002/gepi.21758
28. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG, Consortium E-I. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. *Eur J Epidemiol.* (2015) 30:543–52. doi: 10.1007/s10654-015-0011-z
29. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol.* (2015) 44:512–25. doi: 10.1093/ije/dyv080
30. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet.* (2018) 50:693–8. doi: 10.1038/s41588-018-0099-7
31. Aksu Cerman A, Sarikaya Solak S, Kivanc Altunay I. Vitamin D deficiency in alopecia areata. *Br J Dermatol.* (2014) 170:1299–304. doi: 10.1111/bjd.12980
32. Farag AGA, Haggag MM, Muharram NM, Mahfouz R, Elnaidany NF, Abd El Ghany HM. Is vitamin D a participant in narrow-band ultraviolet B-induced pigmentation in patients with vitiligo? *J Egypt Womens Dermatol Soc.* (2018) 15:30–4. doi: 10.1097/01.EWX.0000525983.54753.6
33. Ibrahim H, El Taieb M, El Gamel Z, El Saied AR. Effect of narrow-band ultraviolet B on the serum of 25-hydroxyvitamin D in vitiligo patients. *J Cosmet Dermatol.* (2018) 17:911–6. doi: 10.1111/jocd.12515
34. Omidian M, Asadian T. Evaluation of serum vitamin D levels in patients with vitiligo undergoing NBUVB (narrowband UV-B) therapy. *Jundishapur J Nat Pharm Prod.* (2017) 13:e65045. doi: 10.5812/jjnpp.65045
35. Saleh HM, Abdel Fattah NS, Hamza HT. Evaluation of serum 25-hydroxyvitamin D levels in vitiligo patients with and without autoimmune diseases. *Photodermatol Photoimmunol Photomed.* (2013) 29:34–40. doi: 10.1111/phpp.12016
36. Sehrawat M, Arora TC, Chauhan A, Kar HK, Poonia A, Jairath V. Correlation of vitamin D levels with pigmentation in vitiligo patients treated with NBUVB therapy. *ISRN Dermatol.* (2014) 2014:493213. doi: 10.1155/2014/493213
37. Sobeih S, Mashaly HM, Gawdat H, Amr K, Hamid MF, Shaalan E. Evaluation of the correlation between serum levels of vitamin D and vitamin D receptor gene polymorphisms in an Egyptian population. *Int J Dermatol.* (2016) 55:1329–35. doi: 10.1111/ijd.13363
38. Takci Z, TekIN Ö, ErtuGRul DT, KaradaG AS, Akin KO. A case-control study: evaluation of vitamin D metabolism in patients with vitiligo. *Turkish J Med Sci.* (2015) 45:837–41. doi: 10.3906/sag-1405-17
39. Xu X, Fu WW, Wu WY. Serum 25-hydroxyvitamin D deficiency in Chinese patients with vitiligo: a case-control study. *PLoS ONE.* (2012) 7:e52778. doi: 10.1371/journal.pone.0052778
40. Zhang X, Wang W, Li Y, Wang H, Liu R, Zhu L. Serum 25-hydroxyvitamin D status in chinese children with vitiligo: a case-control study. *Clin Pediatr.* (2018) 57:802–5. doi: 10.1177/0009922817734362
41. Shalaby MES, Ibrahim S, Shorbagy M, Ahmed R. Evaluation of serum level of 25-hydroxy vitamin D in vitiligo patients. *Gulf J Dermatol Venereol.* (2017) 24:45–9.
42. Amer AM, Galal Khater EM, Marei AM, A Firjani BA. Evaluation of serum level of 25-hydroxy vitamin D in vitiligo patients. *Zagazig Univ Med J.* (2019) 25:935–40. doi: 10.21608/zumj.2019.10995.11390
43. Doss RW, El-Rifaie AA, Gohary YM, Rashed LA. Vitamin D receptor expression in vitiligo. *Indian J Dermatol.* (2015) 60:544–8. doi: 10.4103/0019-5154.169123
44. Alshiyab DM, Al-Qarqaz FA, Heis LH, Muhaidat JM, Eddin WS, Atwan AA. Assessment of serum vitamin D levels in patients with vitiligo in Jordan: a case-control study. *Dermatol Res Pract.* (2019) 2019:2048409. doi: 10.1155/2019/2048409
45. Dinachandran A, Pillai R. A Study to assess the association of vitamin D (serum 25 hydroxy vitamin D3) and vitiligo. *IP Indian J Clin Exp Dermatol.* (2019) 5:6–8. doi: 10.18231/2581-4729.2019.0002
46. Abou Khodair H, Amer AW-A, Abd Al Samee HS, Gad NFA. Assessment of serum vitamin D level before and after narrowband therapy in vitiligo. *Egypt J Hosp Med.* (2019) 74:310–7. doi: 10.21608/ejhm.2019.23052
47. Kostopoulou P, Jouary T, Quintard B, Ezzedine K, Marques S, Boutchnei S, et al. Objective vs. subjective factors in the psychological impact of vitiligo: the experience from a French referral centre. *Br J Dermatol.* (2009) 161:128–33. doi: 10.1111/j.1365-2133.2009.09077.x
48. Porter J, Beuf AH, Nordlund JJ, Lerner AB. Psychological reaction to chronic skin disorders: a study of patients with vitiligo. *Gen Hosp Psychiatry.* (1979) 1:73–7. doi: 10.1016/0163-8343(79)90081-1
49. Parsad D, Dogra S, Kanwar AJ. Quality of life in patients with vitiligo. *Health Qual Life Outcomes.* (2003) 1:58. doi: 10.1186/1477-7525-1-58
50. AlGhamdi K, Kumar A, Moussa N. The role of vitamin D in melanogenesis with an emphasis on vitiligo. *Indian J Dermatol Venereol Leprol.* (2013) 79:750–8. doi: 10.4103/0378-6323.120720
51. Penna G, Adorini L. 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. *J Immunol.* (2000) 164:2405–11. doi: 10.4049/jimmunol.164.5.2405
52. Zhang JZ, Wang M, Ding Y, Gao F, Feng YY, Yakeya B, et al. Vitamin D receptor gene polymorphism, serum 25-hydroxyvitamin D levels, and risk of vitiligo: a meta-analysis. *Medicine.* (2018) 97:e11506. doi: 10.1097/MD.0000000000011506
53. Schulz KF, Grimes DA. Case-control studies: research in reverse. *Lancet.* (2002) 359:431–4. doi: 10.1016/S0140-6736(02)07605-5

54. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ*. (2018) 362:k601. doi: 10.1136/bmj.k601

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 03 January 2022
doi: 10.3389/fnut.2021.794607

Associations Between Food Insecurity and Common Mental Health Problems Among Reproductive-Aged Women in Kabul-Afghanistan

Fawzia Zahidi[1], *Madiha Khalid*[2], *Pamela J. Surkan*[3] *and Leila Azadbakht*[1,4]*

[1] *Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Science (TUMS), Tehran, Iran,* [2] *Toxicology and Disease Group, Pharmaceutical Sciences Research Center (PSRC), The Institute of Pharmaceutical Sciences (TIPS), Tehran University of Medical Sciences (TUMS), Tehran, Iran,* [3] *Department of International Health, Bloomberg School of Public Health, Johns Hopkins University (JHU), Baltimore, MD, United States,* [4] *Diabetes Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences (TUMS), Tehran, Iran*

OPEN ACCESS

Edited by:
Mauro Serafini,
University of Teramo, Italy

Reviewed by:
Maryam Amini,
National Nutrition and Food Technology Research Institute, Iran
Krishnamachari Srinivasan,
St. John's Research Institute, India

***Correspondence:**
Leila Azadbakht
azadbakhtleila@gmail.com

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *13 October 2021*
Accepted: *19 November 2021*
Published: *03 January 2022*

Citation:
Zahidi F, Khalid M, Surkan PJ and Azadbakht L (2022) Associations Between Food Insecurity and Common Mental Health Problems Among Reproductive-Aged Women in Kabul-Afghanistan. Front. Nutr. 8:794607. doi: 10.3389/fnut.2021.794607

Background: Food insecurity has been linked to poor health outcomes, however this relationship is poorly understood among women of reproductive age. Therefore, we investigated the relationship between food insecurity and common mental health problems (CMHPs) in this population of women in Kabul, Afghanistan.

Method: A cross-sectional study was conducted with 421 women of reproductive age from four health centers located in four randomly selected zones in the city of Kabul. We used the United State Department of Agriculture (USDA) food-insecurity questionnaire, multiple 24-h recall for dietary intake, the Depression, the Anxiety and Stress Scale (DASS-21) to assess major mental health problems, and the International Physical Activity Questionnaire (IPAQ) to assess physical activity.

Result: Food insecurity affected 69.6% of reproductive-aged women. In total, 44.9, 10.9, and 13.9% of food-insecure participants had food insecurity without hunger, food insecurity with hunger, and food insecurity with severe hunger, respectively. Depression, anxiety, and stress were prevalent among food-insecure participants at 89.4, 90.8, and 85.7%, respectively. Food insecurity was associated with depression (OR = 4.9, 95% CI: 2.7–8.9), anxiety (OR = 4.7, 95% CI: 2.5–8.8), and stress (OR = 3.8, 95% CI: 2.2–6.7). Women's household ownership, family size, and hypertension, on the other hand, were not associated with food insecurity.

Conclusion: This study found food insecurity was associated with CMHPs among a sample of reproductive-aged women in Kabul, Afghanistan. Further longitudinal studies are needed to confirm these findings.

Keywords: food insecurity, mental health problems, depression, anxiety, stress

INTRODUCTION

The Food and Agriculture Organization (FAO) describes food insecurity (FI) as a condition in which people have limited access to sufficient nutritious and safe food to live a healthy and productive life (1). Food security, on the other hand, is defined as having enough, safe, and nutritious food to live a healthy and active life (1). Food and nutrition insecurity are important public health issues that affect millions of people globally (2). In 2018, 9.2% of people globally had severe food insecurity, with Sub-Sahara Africa and Asia having the highest rates of severe food-insecurity (3). Household food insecurity (HFI) has a negative impact on the nutritional status and health of vulnerable people, particularly women of reproductive age (4).

Recent studies have found that women who live in food insecure homes are more likely to have inadequate dietary intake (4), depression, and poor mental health (5). Common mental health problems (CMHPs) linked to FI include depression, anxiety, stress, and sleeping disorders (6–8). According to Laraia et al., food shortages and FI are associated with poor mental and physical health among women in the United States (6). A systematic review of FI and mental health among females in high-income countries found a relationship between depression and FI, as well as a relationship between the severity of HFI and chronic stress (9).

As a conflict afflicted country, Afghanistan's health statistics are among the poorest in the world (10, 11). According to research by the Global Network Against Food Crises, approximately two quarters of Afghans suffered from FI in 2018 (12). In a population-based study related to mental health and disability among participants over age 15 years in Afghanistan, depression, anxiety, and posttraumatic stress disorder (PTSD) were prevalent in 68, 72, and 42% of participants, respectively. These mental health problems were more common in women than in men (13). In Afghanistan, there is still a scarcity of data on the relationship between FI and mental health. Therefore, the aim of this study was to determine the association of FI with CMHPs among reproductive-aged women in Kabul, Afghanistan.

METHODS

Kabul has 22 municipalities with more than 160 health centers (14). This cross-sectional study was conducted in four municipalities, at four different health centers that were chosen using multistage random sampling. The aim that we include this four directions, was to include these four major ethnicities of Afghanistan in this present study; In the West mostly Hazara people are living, in the North mostly Tajik People, and in the East and South mostly Pashtun, Tajik and Uzbak people are living (15). Three comprehensive health centers (CHCs) were selected from the 15th (in the North), 13th (in the West), and 9th municipalities (in the East) (16). In the South, one district hospital (DH) was selected from the 16th municipality (16). From those health centers, a convenience sample of 421 reproductive-aged women were sampled by the following formula (17):

$$\alpha = 0.05$$
$$Z = 95\% (1.96)$$

$P = 47$ ($P = 47\%$, the prevalence of overweight among wealthy reproductive-aged women) (18).

$$q = (1 - 0.47) = (0.53)$$
$$n = \frac{(Z_{1-\frac{\alpha}{2}})^2 pq}{(r)^2} = \frac{(1.96)^2\ 0.47\ x\ 0.53}{(0.05)^2} = 382.7 \approx 383$$

For the study drop out or non-response coverage we add the 10% on our sample size which is calculated as **10**% *of participant* $= \frac{10^* 383}{100} = \mathbf{38}$ person. Then we added 38 on to our total sample size, $\mathbf{38 + 383 = 421}$ participants. An equal number of routine visitor women (n = 105) were sampled from each of the four health centers.

Assessment of Food Insecurity

In this study, we used the United States Department of Agriculture (USDA) 18-item questionnaire to assess FI (19, 20). This questionnaire reflects HFI in the last 12 months. In 1995, it was introduced as a valid questionnaire for epidemiologic studies and calculated based on the method of Bickel et al. (20).

Assessment of Dietary Intake

All participants' dietary intakes were obtained using a 24-recall questionnaire on 3 days of the week (two during the week and one on the weekend) (21). Interviewers used a variety of tools to enhance participant reporting of serving sizes, including can sizes, a chunk of bread that fits in the palm of a hand, tablespoons, teaspoons, ladles, plates, bowls, glasses, and photographs of common household meals. We used the Automatic Multiple Pass Method (AMPM) to reduce bias in the 24-h recall questionnaire (22), which asks about (i) foods listed by participants that were consumed in the previous day, (ii) any forgotten foods, (iii) the time of day each food was consumed, (iv) specific details about foods (e.g., quantities consumed, and foods eaten between meals); and (v) whether anything was forgotten. Following that, portions were estimated based on household eating/cooking equipment and converted into grams from reported quantities, before being entered into Nutritionist 4 (NUT4) software for nutrient adequacy analysis.

Assessment of Anthropometric Indices

Anthropometric indices, such as weight, height, and body mass index (BMI) were measured and computed for all participants. BMI was calculated by dividing weight (kg) by height^2 (cm). A calibrated digital scale (SECA 831, Germany) was used for weight measurements. Adult BMI classifications according to the World Health Organization (WHO) are as follows: BMI<18.5 is considered low weight, BMI >18.5<24.9 is considered normal weight, BMI >25<29.9 is considered overweight, and BMI $\geq$30 is considered obese (23).

Assessment of Common Mental Health Problems

The Depression, Anxiety and Stress Scale-21 Items (DASS-21) is a set of self-reported scales used to assess the CMHPs such as depression, anxiety, and stress. The DASS-21 scale has three sections, each of which comprises seven items (24). Dysphoria (a feeling of overall unhappiness with life), hopelessness, devaluation of one's life, self-deprecation, lack of interest, anhedonia (inability to experience pleasure), and inertia (a tendency to do nothing) are assessed on the depression component. Autonomic arousal, skeletal muscular responses, and subjective sensations of anxious affect are measured on the anxiety component. The stress component is sensitive to non-specific stimulant levels that have been present for a long time. CMHPs were categorized into three categories: normal, moderate, and severe.

Assessment of Physical Activity

The International Physical Activity Questionnaire (IPAQ) was developed in the late 1990s to collect international comparable data on health-related physical activity. We used the long version of the IPAQ instrument (IPAQ-27 items) in this study (25).

Statistical Analysis

The quantity of nutrients consumed by each participant was calculated using Nutritionist IV software. The data were analyzed using the Statistical Package for Social Science (SPSS Version 26) software. Histograms and the Kolmogorov-Smirnov tests were used to assess the normality of distributions of the variables. For general characteristics of individuals, the Chi-square test was performed, and one-way ANOVA was used to compare the means of categorical variables among FI categories. To assess the risk of CMHPs based on FI status, we fitted logistic regression models.

RESULTS

The data were collected from February to May 2021 and included an equal number of participants from each of the four health centers ($N = 105$ at each). Hazara, Tajik, Pashtun, and Uzbak ethnicities made up the majority of participants, accounting for 33.5, 32.8, 27.8, and 5.9%, respectively. The mean age and BMI of all participants were 31 ± 9 years and 23.3 ± 5.06 kg/m^2, respectively. We also found the mean age 29.8 ± 9.1 years and mean BMI 24.2 ± 6.3 kg/m^2 amongst FS participants, while among FI without hunger, FI with hunger and FI with severe hunger participants the mean age was 30.8 ± 8.9, 29.8 ± 9.3, and 35.3 ± 9.0 years and mean BMI was 23.0 ± 4.5, 23.1 ± 4.4, and 22.7 ± 3.5 kg/m^2, respectively.

The average home size was 6 ± 3 people, with more than 91% of women having a family size of <10. The mean monthly income was US $241.4 ± 204.3, with 79.1% having a monthly income of <$300 and 14.3% having a monthly income of $500–1,200. In addition, the mean monthly income among FS participants $374 ± 275 were higher than food insecure participant's $183 ± 126. Meanwhile, the mean of monthly income was $194 ± 109, $168 ± 130, and $167 ± 160 among FI without hunger, FI with hunger, and FI with severe hunger.

Half of the women were illiterate, with just 14.8% having a high level of education (bachelor's or master's degree). We found that 30.4% ($n = 129$) of reproductive-aged women were food secure, whereas a large number of women ($n = 293$; 69.6%) were food insecure, in addition, more than three quarters of reproductive-aged women in West and South municipalities were suffering from FI. According to the food-insecurity categories, 44.9% ($n = 189$), 10.9% ($n = 46$), and 13.8% ($n = 53$) of participants, respectively, had food insecurity with hunger, food insecurity with mild hunger, and food insecurity with severe hunger. About four fifths of the women (81%) had low levels of physical activity, whereas 29% had at least a moderate level of physical activity.

In this study, food insecurity categories were significantly related to age, marital status, household size, and income ($P < 0.05$). Furthermore, ethnicity, education, and women's education level, as well as the occupation and education level of their husbands, were found to be associated with food insecurity ($P = 0.001$) (**Table 1**). BMI, hypertension, and physical activity, on the other hand, were not associated with food-insecurity categories. We found that the overall prevalence of depression, anxiety, and stress were 79, 81, and 74.6%, respectively, while the prevalence of these conditions among food insecure people were 89, 91, and 87%, respectively (**Table 2**). We found that common mental health problems (depression, anxiety, and stress) were significantly associated with food insecurity levels ($P = 0.001$). Severe depression, anxiety, and stress affected nearly 85, 88, and 84% of the food insecure population, respectively (**Table 3**). We found that FI was associated an increased risk of symptoms of depression (OR = 4.9; CI: 2.7–8.9), anxiety (OR = 4.7; CI: 2.5–8.8), and stress (OR = 3.8; CI: 2.2–6.7). Women's BMI >25 (OR = 1.3, CI: 0.7–2.4), household ownership (OR = 0.7, CI: 0.2–1.7), family size (OR = 1; CI: 0.6–1.9), and hypertension (OR = 0.6, CI: 0.2–1.9) were not associated with FI status (**Table 4**).

DISCUSSION

In this study, we found that about 70% of reproductive-aged women were food insecure, nearly half were food insecure, and 11, 14, and 11%, respectively, were affected by FI without hunger, FI with hunger, and FI with severe hunger. Findings show that the prevalence of FI in our study is nearly 40% higher than the National Risk and Vulnerability Assessment (NRVA) in 2011/2012, reporting food and nutrition insecurity to be about 30% (7.6 millions) among Afghans generally (26). The high level of FI status among reproductive-aged women in this study may have been elevated due to the civil war and the COVID-19 pandemic. Depressive symptoms were present in 79% of reproductive-aged women, whereas symptoms of anxiety and stress were present in 81 and 75%, respectively. In contrast to our findings, a national survey of anxiety disorders and major depressive episodes

TABLE 1 | Socio-demographic characteristic by different food-insecurity categories among reproductive-aged women in Kabul-Afghanistan.

Background	Food-security status		*p*-value	Food-insecurity categories			*p*-value*
	Food-secure *N* (%)	Food insecure *N* (%)		FI without hunger *N* (%)	FI with moderate hunger *N* (%)	FI with severe hunger *N* (%)	
Marital status							
Single	51 (47.7)	56 (52.3)	0.001	39 (36.4)	11 (10.3)	6 (5.6)	0.001
Married	73 (24.9)	220 (75.1)		114 (49.1)	34 (11.6)	42 (14.3)	
Widow	4 (19)	17 (81)		6 (28.6)	1 (4.8)	10 (47.6)	
Income (US $)							
Less than 300$	68 (20.4)	265 (79.6)	0.001	167 (50.2)	42 (12.6)	56 (16.8)	0.001
300–500$	31 (58.5)	22 (41.5)		19 (35.8)	2 (3.8)	1 (1.9)	
500–1,200$	29 (82.9)	6 (17.1)		3 (8.6)	2 (5.7)	1 (2.9)	
Household-size (number)							
Less than 10	121 (30.9)	270 (69.1)	0.04	179 (45.8)	43 (11.0)	48 (12.8)	0.015
More than 10	7 (23.3)	23 (76.7)		10 (33.3)	3 (10.0)	10 (33.3)	
Ethnicity							
Pashtun	32 (27.4)	85 (72.8)	0.001	71 (60.7)	8 (6.8)	6 (5.1)	0.001
Tajik	58 (42)	80 (58)		58 (62)	11 (80)	11 (8)	
Hazara	25 (17.7)	116 (82.3)		54 (38.3)	24 (17)	38 (19.4)	
Uzbak	13 (52)	12 (48.0)		6 (24)	3 (12)	3 (3.4)	
Municipalities							
North	52 (49.1)	54 (50.9)	0.0001	30 (28.0)	14 (13.2)	10 (9.4)	0.0001
West	12 (11.4)	93 (88.6)		41 (39.0)	21 (20.0)	31 (29.5)	
East	38 (36.2)	67 (63.8)		53 (50.5	5 (4.8)	9 (8.6)	
South	26 (24.8)	79 (75.2)		65 (61.9)	6 (5.7)	8 (7.6)	
Education level							
Illiterate	39 (17.6)	182 (82.4)	0.001	110 (49.8)	31 (14)	41 (18.6)	0.001
Primary	10 (25.6)	29 (74.4)		16 (41)	7 (17.9)	6 (15.4)	
Secondary	8 (24.2)	25 (75.8)		18 (54.4)	3 (9.1)	4 (12.1)	
High school	25 (37.9)	41 (62.1)		34 (51.5)	3 (4.5)	4 (6.1)	
Bachelor	38 (73.1)	14 (26.9)		9 (17.3)	2 (3.8)	3 (5.8)	
Master	8 (80)			2 (20)	0 (0)	0 (0)	
Husband's education level							
Illiterate	14 (10.6)	121 (89.6)	0.001	71 (52.6)	22 (16.3)	28 (20.7)	0.001
Primary	6 (18.2)	27 (81.8)		18 (54.5)	5 (15.2)	4 (12.1)	
Secondary	4 (16)	21 (84)		16 (64)	2 (8)	3 (12)	
High school	17 (45.9)	20 (54.0)		12 (32.4)	3 (8.1)	5 (13.5)	
Bachelor	30 (49.2)	31 (50.8)		24 (39.3)	4 (6.6)	3 (4.9)	
Master	6 (60.0)	4 (40.0)		4 (40.0)	0 (0)	0 (0)	
Occupation							
Jobless	68 (21.6)	242 (78.1)	0.001	157 (50.6)	36 (11.6)	49 (15.8)	0.001
Worker	18 (30)	42 (70)		25 (41.7)	9 (15)	8 (13.3)	
Employee	37 (90.2)	4 (9.8)		3 (7.7)	1 (2.4)	0 (0)	
Scientific member	4(50)	4 (50)		4 (50)	0 (0)	0 (0)	
Husband's occupation							
Jobless	0 (0)	18 (100)	0.001	11 (61.1)	4 (22.2)	3(16.7)	0.001
Worker	34 (18.3)	152 (81.7)		97 (52.2)	23 (12.4)	32 (17.2)	
Farmer	1 (16.7)	5 (83.3)		1 (16.7)	1 (16.7)	3 (50)	
Employee	30 (42.9)	40 (57.1)		30 (42.9)	7 (10)	3 (4.3)	
Scientific member	7 (70)	3 (30.0)		3 (30)	0 (0)	0 (0)	
Hypertension illness	10 (7.8)	23 (7.8)	0.581	14 (7.4)	4 (8.7)	5 (8.6)	0.986
Physical activity							
Low	108 (31.7)	233 (68.3)	0.001	152 (44.6)	36 (10.6)	45 (13.2)	0.719
Moderate	20 (26.0)	57 (74.0)		35 (45.5)	10 (13.0)	12 (15.6)	
Intensive	0 (0)	3 (100)		2 (66.7)	0 (0)	1 (33.3)	

**P-values are based on the chi-square test.*

FI, food-insecurity.

TABLE 2 | Mental health status among reproductive-aged women in Kabul, Afghanistan.

Mental health	Food secure *N* (%)	Food insecure *N* (%)	*P*-value*	FI without hunger *N* (%)	FI with hunger *N* (%)	FI with severe hunger *N* (%)	*P*-value
Depression	70 (54.7)	262 (89.4)	0.001	161 (85.2)	43 (93.5)	58 (100)	0.001
Anxiety	76 (59.4)	266 (90.8)	0.001	164 (86.8)	44 (95.7)	58 (100)	0.001
Stress	63 (49.2)	251 (85.7)	0.001	153 (81.0)	40 (87.0)	58 (100)	0.001

**P-values are based on Chi-square tests.*

TABLE 3 | Mental health status in different food-insecurity categories among reproductive-aged women in Kabul, Afghanistan.

Major mental health problems	Level	Food-insecurity status		*P*-value	Food-insecurity categories			*P*-value*
		Food-Security *N* (%)	Food-insecurity *N* (%)		FI without hunger *N* (%)	FI with mild hunger *N* (%)	FI with severe hunger *N* (%)	
Depression	Normal	58 (45.3)	31 (10.6)	0.001	28 (14.8)	3 (6.5)	0 (0)	0.001
	Moderate	8 (6.3)	13 (4.4)		10 (5.3)	3 (6.5)	0 (0)	
	Severe	62 (48.4)	249 (85.0)		151 (79.9)	40 (87.7)	58 (100)	
Anxiety	Normal	52 (40.6)	27 (9.2)	0.001	25 (13.2)	2 (4.3)	0 (0)	0.001
	Moderate	8 (6.3)	9 (3.1)		7 (3.7)	2 (4.3)	0 (0)	
	Severe	68 (53.1)	257 (87.7)		157 (83.1)	42 (91.3)	58 (100)	
Stress	Normal	65 (50.8)	42 (14.3)	0.001	36 (19.0)	6 (13.0)	0 (0)	0.001
	Moderate	3 (2.3)	4 (1.4)		3 (1.6)	0 (0)	1 (1.7)	
	Severe	60 (46.9)	247 (84.3)		150 (79.4)	40 (87.0)	57 (98.3)	

*P-values are based on Chi-square tests.
FI, Food-insecurity.

TABLE 4 | Odds ratios (ORs) of common mental health problems according to food-insecurity status among reproductive-aged women in Kabul-Afghanistan.

Variables**	Food-insecure vs. food-secure unadjusted OR (95% CI)			Food-insecure vs. food-secure adjusted OR (95% CI)		
	OR	CI	*P*-value	OR	CI	*P*-value*
Depression	7.0	4.2–11.6	0.001	4.9	2.7–8.9	0.001
Anxiety	6.7	3.9–11.4	0.001	4.7	2.5–8.8	0.001
Stress	6.1	3.8–9.9	0.001	3.8	2.2–6.7	0.001
BMI >25	1.4	0.8–2.2	0.138	1.3	0.7–2.4	0.255
Hypertension	1.0	0.4–2.1	0.990	0.7	0.2–1.7	0.471
Household ownership	1.5	0.9–2.2	0.056	1.0	0.6–1.7	0.753
Family size >10 members	1.4	0.5–3.3	0.449	0.6	0.2–1.9	0.455

*P-values are based on binary logistic regression.
**All the variables were adjusted for BMI, income, marital status, education level, occupation, ethnicity, family size, financial level, and house ownership.

conducted in 2021 found that the prevalence of general anxiety disorder and major depressive episode were significantly lower, at 11.7 and 2.7%, respectively (27). However, such higher prevalence may be expected since our study used symptom measures (interview) rather than evaluation of clinical diagnoses (observation, psychological tests, neurological tests, and interviews) (28). Based on our study, 89, 90, and 85% of FI participants had symptoms of depression, anxiety, and stress, respectively. Further, FI was associated with having these symptoms.

Consistent with our results, a growing body of evidence suggests that FI is linked to common mental health problems (29–31). A systematic review of females in developed countries found a strong relationship between FI and depression and stress (9). Lachance et al. found a positive relationship between FI and mental health problems in a quantitative community-based

study of Canadians. They also concluded that FI and mental health problems, particularly depression, had a bidirectional relationship, with poor mental health often causing people to make poor food choices (32). In contrast to our study, Chung et al. investigated the relationship between household FI and adverse mental health problems in Korean adults and found that FI was associated with stress, anxiety and depression (29). Similarly, Scanlon et al. investigated depression and social vulnerability in African-American men and found that FI did not enhance the likelihood of depression (33). Moreover, we found a high prevalence of depression, anxiety and stress among FS participants, it may be because of gender-based violence, civil war and insecure situation in Afghanistan especially in Kabul (34).

We also found that sociodemographic factors such as household income and education level were associated with FI status. In comparison to their husbands, most participating women were uneducated. This may be due to a variety of factors, including civil wars, poverty, minority status, early marriage and pregnancy, and gender-based violence, which impede women and girls from fully exercising their education (35). Omidvar et al. observed that household income, socioeconomic status, and education level among Afghan refugees in Iran were associated with FI (36). Similarly, in a study on food insecurity and its determinants in Nigeria, Amaza et al. found that household income, education level, and gender were the most important indicators of FI status (37).

We also found that marital status and household size were associated with food insecurity. Married women with children are more prone to utilize risky coping techniques like restricting food intake to ensure that their children and other household members are well-fed (38). A study from a South African Township indicated that marital status, household income, and household size were significantly associated with FI (39). However, in another study of South African households, marital status and household size were negatively associated with FI (40). Among socio-demographic factors we found age, ethnicity and house ownership to also be associated with FI. These findings were similar to those of Fernandes et al.'s cohort study, which indicated that 23% of older adults lived in a food-insecure households. The odds of FI was higher for participants in the 70–74 year old age category (41). In a study of FI, depression, and race among university students, Reeder et al. reported that African-American students had 3.5 times higher odds of FI than Caucasian students (42). Similarly, a US study of diet quality and FI among people of various races found that FI was most prevalent among non-Hispanic white individuals and Asians.

We found that FI is associated with depression, anxiety and stress. More research is needed to confirm these findings and to examine if the same patterns hold true for different populations (e.g. in different regions in Afghanistan, age groups, etc.). Furthermore, additional development activities, such as economic and education programs, could be beneficial for Afghan women to improve their home food security. Limitations of this study include that it was a cross-sectional study that only included reproductive-aged women, with no other age groups or males. Our study also had several strengths. We used the USDA long version scale for FI measurements, the DASS-21 for CMHPs measurements, and it was the first study to examine the relationship between FI and CMHPs in reproductive-aged women in Kabul, Afghanistan.

CONCLUSION

A considerable number of women of reproductive age in our study, over two-thirds, were food insecure. Notably, almost 25% reported food insecurity with hunger or food insecurity with severe hunger. Common mental health problems were extremely prevalent among food-insecure individuals, with 90% of this subgroup displaying symptoms for depression and anxiety and 86% reporting stress. FI was associated with about a five-fold risk of depression and anxiety and about a four-fold risk of stress. Women's household ownership, family size, and hypertension were not associated with food insecurity status. Given the strong association between FI and CMHPs among reproductive-aged women and high prevalence of FI in Kabul policies should prioritize access to food among these women. Furthermore, due to the high prevalence of CMHPs a psychoanalytic intervention should be done for reproductive-aged women.

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 Tehran University of Mediccal Sciences Ethical approval ID (IR.TUMS.MEDICINE.REC.1399.656). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

LA conceptualized this study. FZ and MK designed the study. FZ performed statistical analysis. The study was finalized by LA and PS who contributed to the writing and interpretation of the results. All authors read and approved the final manuscript.

FUNDING

This research was a MSc thesis project, which was funded by Tehran University of Medical Sciences (TUMS Grant No. 99-2-163-49080).

ACKNOWLEDGMENTS

The author would like to thank all authors that made contribution in this research.

REFERENCES

1. FAO. *World Food Security: A Reappraisal of the Concepts and Approaches. Director General's Report*. Roma: FAO (1983).
2. Ghattas H. *Food Security and Nutrition in the Context of the Global Nutrition Transition.* Rome: Food and Agriculture Organization (2014).
3. Ritchie MRaH. *Hunger and Undernutrition.* Our World in Data. (2019).
4. Hamelin AM, Beaudry M, Habicht JP. Characterization of household food insecurity in Québec: food and feelings. *Soc Sci Med.* (2002) 54:119–32. doi: 10.1016/S0277-9536(01)00013-2
5. Effect of famine on later mental performance. *Nutr Rev*. (1973) 31:140–2. doi: 10.1111/j.1753-4887.1973.tb05154.x
6. Laraia BA, Siega-Riz AM, Gundersen C, Dole N. Psychosocial factors and socioeconomic indicators are associated with household food insecurity among pregnant women. *J Nutr*. (2006) 136:177–82. doi: 10.1093/jn/136.1.177
7. Brostow DP, Gunzburger E, Thomas KS. Food insecurity among veterans: findings from the health and retirement study. *J Nutr Health Aging*. (2017) 21:1358–64. doi: 10.1007/s12603-017-0910-7
8. Brostow DP, Gunzburger E, Abbate LM, Brenner LA, Thomas KS. Mental illness, not obesity status, is associated with food insecurity among the elderly in the health and retirement study. *J Nutr Gerontol Geriatr*. (2019) 38:149–72. doi: 10.1080/21551197.2019.1565901
9. Maynard M, Andrade L, Packull-McCormick S, Perlman CM, Leos-Toro C, Kirkpatrick SI. Food insecurity and mental health among females in high-income countries. *Int J Environ Res Public Health*. (2018) 15:1424. doi: 10.3390/ijerph15071424
10. Frost A, Wilkinson M, Boyle P, Patel P, Sullivan R. An assessment of the barriers to accessing the Basic Package of Health Services (BPHS) in Afghanistan: was the BPHS a success? *Globalization Health*. (2016) 12:71. doi: 10.1186/s12992-016-0212-6
11. Loevinsohn B, Sayed GD. Lessons from the health sector in Afghanistan: how progress can be made in challenging circumstances. *JAMA*. (2008) 300:724–6. doi: 10.1001/jama.300.6.724
12. Network Fsi. *Global Network Agains Food Crises Relief Web* (2021).
13. Cardozo BL, Bilukha OO, Crawford CA, Shaikh I, Wolfe MI, Gerber ML, et al. Mental health, social functioning, and disability in postwar Afghanistan. *JAMA*. (2004) 292:575–84. doi: 10.1001/jama.292.5.575
14. Governmet of Islamic Republic of Afghanistan. *2015 GK, Kabul Municipality and Ministry of Urban Development Affairs; State of Afghanistan Cities*. (2015). p. 1.
15. Lamer W, Foster E. *Afghan Ethnic Groups: A Brief Investigation.* Afghanistan Thematic Report (2011).
16. Ministry of Public Health. *A Basic Package of Health Services (BPHS) in Afghanistan* (2009).
17. Charan J, Biswas T. How to calculate sample size for different study designs in medical research? *Indian J Psychol Med*. (2013) 35:121. doi: 10.4103/0253-7176.116232
18. UNICEF. *National Nutrition Survey: Afghanistan.* Kabul: United Nations ChildrenLs Fund (2013). Available online at: https://reliefweb.int/sites/reliefweb.int/files/resources/Report%20NNS%20Afghanistan
19. Mancino L, Gregory CA. Food-Insecure households score lower on diet quality compared to food-secure households. Amber Waves: The Economics of Food, Farming, Natural Resources, and Rural America, (2020). doi: 10.22004/ag.econ.302915
20. Bickel G, Nord M, Price C, Hamilton W, Cook J. *Guide to Measuring Household Food Security*. Alexandria, VI; Washington DC: Department of Agriculture Food and Nutrition Service (2000).
21. Castell GS, Serra-Majem L, Ribas-Barba L. What and how much do we eat? 24-hour dietary recall method. *Nutricion Hospitalaria*. (2015) 31:46–8. doi: 10.3305/nh.2015.31.sup3.8750
22. Conway JM, Ingwersen LA, Moshfegh AJ. Accuracy of dietary recall using the USDA five-step multiple-pass method in men: an observational validation study. *J Am Dietetic Assoc.* (2004) 104:595–603. doi: 10.1016/j.jada.2004.01.007
23. WHO. *BMI Classification.*
24. Lovibond SHL PF. *Manual for the Depression Anxiety & Stress Scales*. 2nd ed. Sydney, NSW: Psychology Foundation (1995).
25. Biernat E, Stupnicki R, Gajewski A. International physical activity questionnaire (IPAQ)–Polish version. *Phys Educ Sport*. (2007) 51:47-54. doi: 10.2478/v10030-007-0004-0
26. Organization Faa. *Afghanistan Food Security and Nutirion Agenda* (2013).
27. Kovess-Masfety V, Keyes K, Karam E, Sabawoon A, Sarwari BA. A national survey on depressive and anxiety disorders in Afghanistan: a highly traumatized population. *BMC Psychiatry*. (2021) 21:1–12. doi: 10.1186/s12888-021-03273-4
28. Carlson J. Clinical assessment. *Encyclopedia Mental Health.* (2015) 2015:293. doi: 10.1016/B978-0-12-397045-9.00017-3
29. Chung HK, Kim OY, Kwak SY, Cho Y, Lee KW, Shin MJ. Household food insecurity is associated with adverse mental health indicators and lower quality of life among Koreans: results from the Korea National Health and Nutrition Examination Survey 2012-2013. *Nutrients*. (2016) 8:819. doi: 10.3390/nu8120819
30. Kolovos S, Zavala GA, Leijen AS, Melgar-Quiñonez H, van Tulder M. Household food insecurity is associated with depressive symptoms: results from a Mexican population-based survey. *Food Security*. (2020) 12:407–16. doi: 10.1007/s12571-020-01014-1
31. Pinard CA, Calloway EE, Fricke HE, Yaroch AL. A cross-sectional exploration of food security, depression, and CHAOS in low-income households with children. *J Appl Res Child*. (2015) 6:6.
32. Lachance L, Martin MS, Kaduri P, Godoy-Paiz P, Ginieniewicz J, Tarasuk V, et al. Food insecurity, diet quality, and mental health in culturally diverse adolescents. *Ethnicity Inequalities Health Social Care*. (2014) 7:14–22. doi: 10.1108/EIHSC-02-2013-0002
33. Scanlon FA, Scheidell JD, Cuddeback GS, Samuelsohn D, Wohl DA, Lejuez CW, et al. Depression, executive dysfunction, and prior economic and social vulnerability associations in incarcerated African American men. *J Correctional Health Care*. (2018) 24:295–308. doi: 10.1177/1078345818782440
34. Mannell J, Grewal G, Ahmad L, Ahmad A. A qualitative study of women's lived experiences of conflict and domestic violence in Afghanistan. *Violence Against Women*. (2021) 27:1862–78. doi: 10.1177/1077801220935191
35. Parvazian S, Gill J, Chiera B. Higher education, women, and sociocultural change: a closer look at the statistics. *Sage Open*. (2017) 7:2158244017700230. doi: 10.1177/2158244017700230
36. Omidvar N, Ghazi-Tabatabie M, Sadeghi R, Mohammadi F, Abbasi-Shavazi MJ. Food insecurity and its sociodemographic correlates among Afghan immigrants in Iran. *J Health Popul Nutr*. (2013) 31:356. doi: 10.3329/jhpn.v31i3.16828
37. Amaza P, Umeh JC, Helsen J, Adejobi AO. *Determinants and Measurements of Food Insecurity in Nigeria: Some Empirical Policy Guide*. Borno (2006).
38. Ihab A, Rohana AJ, Manan WW, Suriati WW, Zalilah MS, Rusli AM. Nutritional outcomes related to household food insecurity among mothers in rural Malaysia. *J Health Popul Nutr*. (2013) 31:480. doi: 10.3329/jhpn.v31i4.20031
39. Sekhampu TJ. Association of food security and household demographics in a South African township. *Int J Soc Sci Humanity Stud*. (2017) 9:157–70.
40. Sekhampu TJ. *Determinants of the Food Security Status of households Receiving Government Grants in Kwakwatsi, South Africa*. Portugal (2013).
41. Fernandes SG, Rodrigues AM, Nunes C, Santos O, Gregório MJ, de Sousa RD, et al. Food insecurity in older adults: results from the epidemiology of chronic diseases cohort study 3. *Front Med*. (2018) 5:203. doi: 10.3389/fmed.2018.00203
42. Reeder N, Tapanee P, Persell A, Tolar-Peterson T. Food insecurity, depression, and race: correlations observed among college students at a University in the

Southeastern United States. *Int J Environ Res Public Health*. (2020) 17:8268. doi: 10.3390/ijerph17218268

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 11 January 2022
doi: 10.3389/fnut.2021.811244

Trends in Obesity and Metabolic Status in Northern and Southern China Between 2012 and 2020

Ying Li[1], Lin Yang[2,3], Lu Yin[4], Qingqi Liu[5], Yaqin Wang[1], Pingting Yang[1], Jiangang Wang[1], Zhiheng Chen[1], Xiaohui Li[6], Qinyu Yang[6], Yongmei He[7†] and Xin Huang[8†]*

[1] Department of Health Management, The Third Xiangya Hospital, Central South University, Changsha, China, [2] Department of Cancer Epidemiology and Prevention Research, Cancer Care Alberta, Alberta Health Services, Calgary, AB, Canada, [3] Departments of Oncology and Community Health Sciences, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada, [4] Medical Research & Biometrics Center, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [5] Department of Biostatistics, Bioinformatics & Biomathematics, Georgetown University, Washington, DC, United States, [6] Department of Pharmacology, Xiangya School of Pharamceutical Science, Central South University, Changsha, China, [7] Department of Health Management, Aerospace Center Hospital, Beijing, China, [8] Department of Epidemiology, School of Medicine, Hunan Normal University, Changsha, China

OPEN ACCESS

Edited by:
Megan A. McCrory,
Boston University, United States

Reviewed by:
Norbert Stefan,
University of Tübingen, Germany
Rachael Mira McLean,
University of Otago, New Zealand

****Correspondence:***
Xin Huang
xin.huang@hunnu.edu.cn

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *08 November 2021*
Accepted: *17 December 2021*
Published: *11 January 2022*

Citation:
Li Y, Yang L, Yin L, Liu Q, Wang Y, Yang P, Wang J, Chen Z, Li X, Yang Q, He Y and Huang X (2022) Trends in Obesity and Metabolic Status in Northern and Southern China Between 2012 and 2020. Front. Nutr. 8:811244. doi: 10.3389/fnut.2021.811244

Background: The trends of obesity-associated metabolic status in Chinese are lacking, especially those from different regions.

Objectives: To examine the trends of obesity and metabolic status among Chinese population in 2012–2020.

Methods: In a series cross-sectional study, data on 256,782 participants surveyed between 2014 and 2020 in Beijing, northern China, and 697,170 participants surveyed between 2012 and 2020 in Hunan, southern China were analyzed. Anthropometrics, blood pressure measurements, and blood tests were performed according to standard protocols. Trends in obesity and metabolic status were evaluated using the Joinpoint software.

Results: Based on age- and sex-standardized values, the mean BMI values in northern and southern participants were 23.94 (95% CI: 23.93, 23.95) and 23.68 (95% CI: 23.67, 23.69) kg/m^2, respectively. Between 2014 and 2020, the overall obesity prevalence among northern participants increased from 12.70% (95% CI: 12.17, 13.23%) to 14.33% (95% CI: 13.97, 14.70%) ($P = 0.009$), mainly derived by the 20–39 and 40–59 age groups. Moreover, the prevalence of metabolically healthy obese significantly increased from 2.07% (95% CI: 1.84, 2.30%) to 4.33% (95% CI: 4.13, 4.53%) in Northerners. Between 2012 and 2020, no significant trend in obesity was found among overall southern participants, but the prevalence of metabolically unhealthy obese significantly increased from 5.36% (95% CI: 5.18, 5.54%) to 7.35% (95% CI: 7.11, 7.58%), mainly derived by the 20–39 and 40–59 age groups.

Conclusions: The trends in obesity and metabolic status were different between southern and northern Chinese. A national weight control plan is needed in China, focusing on young and middle-aged population.

Keywords: obesity, metabolic status, trend, series cross-sectional study, China

INTRODUCTION

Obesity is a major risk factor for hypertension, diabetes, coronary heart disease, certain types of cancer, and poor mental health (1–5). Approximately 4 million global deaths were due to high body mass index (BMI) in 2015 (6). According to the Global Burden of Disease Study, the worldwide prevalence of overweight and obesity has doubled from 1980 to 2015 (7). With the increasing spread of the global obesity pandemic, China also saw a dramatic increase in overweight and obese adults (8). For example, during 1993–2015, the prevalence of overweight, obesity, and abdominal obesity increased by 14.7, 11.5, and 26.7%, respectively (9). The most recent national nutrition survey during 2015–2019 indicates that obesity was 16.4% in Chinese adults (10). However, the annual trend of change in obesity, especially in different regions, is not available.

According to the metabolic status and the BMI level, the population could be further classified into the following four phenotypes: metabolically healthy non-obese (MHNO), metabolically unhealthy non-obese (MUNO), metabolically healthy obese (MHO), and metabolically unhealthy obese (MUO) (11). Different phenotypes present different cardiovascular and metabolic complications risks (11–14). However, the data on trends in obesity-related metabolic status in the Chinese population are lacking.

Moreover, the coronavirus disease 2019 (COVID-19) pandemic has caused significant disruption in everyday lifestyle since 2020. The previous study indicated that the COVID-19 pandemic has serious consequences for the obesity epidemic. And in turn, obesity and impaired metabolic health also emerged as important determinants of severe COVID-19 (15). The changes in body weight and metabolic status that occurred during the COVID-19 pandemic in China are unknown.

The current study provides new estimates of the prevalence of obesity and its metabolic status in large populations from two southern and northern areas in China.

PATIENTS AND METHODS

Study Population

The serial cross-sectional study population comprised more than 900,000 individuals from a mixed urban and rural area who visited health management centers in Beijing and Hunan, two northern and southern regions in China, between 2012 and 2020. The overall population of Hunan is over 60 million, and that of Beijing is around 20 million. In the present study, ∼80% of the participants in Hunan were from urban areas, while 95% of the Beijing area was urban. Participants with diverse socioeconomic background (public services employees, workers, self-employed persons, farmers, and others) came to health management centers to check their health status was enrolled in the current study. All participants signed informed consent forms, and the Ethics Committee of the Third Xiangya Hospital approved the study (2020-S498).

All enrolled participants underwent a routine clinical examination. Participants recorded age, sex, current medication use, and previous medical diagnoses by themself in Beijing, northern China. Beginning in 2018, physicians reconfirmed the questionnaires during the physical examination. More detailed questionnaires, including exercise, smoking history, alcohol consumption, and food consumption, were obtained and checked by physicians in Hunan, southern China (16). Individuals with missing data or unreasonable values on age (<20 or >80 years), height (<140 or >210 cm), or weight (<26 or >175 kg) were excluded. Participants with missing or unreasonable blood pressure [Systolic Blood Pressure (SBP) <60 or >270 mmHg; Diastolic Blood Pressure (DBP) <30 or >220 mmHg; Pulse Pressure (PP) <10 mmHg], Fasting Serum Glucose (FSG) (<2.00 or >42 mmol/l) or lipids [Triglyceride (TG) >35 mmol/l; Total Cholesterol (TC) >20 mmol/l; Low-Density Lipoprotein cholesterol (LDL-c) >15 mmol/l; High-Density Lipoprotein cholesterol (HDL-c) >13 mmol/l] were further excluded from metabolically status classification. The enrollment process was listed in **Figure 1**. Assessment methods are detailed in the **Supplementary Material**.

Measurement and Definition

Physical examinations were conducted with the same methods described in our previous study (17). Briefly, blood pressure, height, and weight were measured by trained physicians. Participants were categorized into non-obese (<28 kg/m^2) and obese (≥28 kg/m^2) groups (18). Moreover, participants who met two or more of the following four criteria were considered metabolically unhealthy: high TG (≥1.7 mmol/L) or using lipid-lowering drugs, elevated SBP (≥130 mmHg) or DBP (≥85 mmHg) or using anti-hypertensive drugs, high FSG (≥5.6 mmol/L) or using medications for diabetes (insulin and oral anti-diabetic), and low HDL-c (<1.04 mmol/L for men and <1.29 mmol/L for women) (11). Then the participants were furtherly classified into the MHNO MUNO, MHO, and MUO phenotypes. Definition of chronic diseases are detailed in the **Supplementary Material**.

Laboratory Measurements

Fasting venous blood samples were collected and were immediately processed and analyzed at the clinical laboratory of Third Xiangya Hospital (in Hunan) or Aerospace Center Hospital (in Beijing), as detailed in the **Supplementary Material**.

Statistical Analysis

Continuous variables were expressed as means with 95% confidence intervals (95% CIs), categorical variables were expressed as percentages with 95% CIs, and differences among different BMI groups were tested by analysis of variance and the Chi-square test. Restricted cubic spline regression models were used to test the overall and non-linear association between survey years and BMI, HDL-c, TG, SBP, DBP, and FSG. Then, the linear association would be evaluated when the test for non-linearity is not significant. Means of BMI and metabolic status were further stratified by location and sex because of significant 3-way interactions. Trends in the prevalence of obesity and metabolic subtypes were evaluated using the Joinpoint Regression Program (Version 4.9.0.0) (19), and annual percentage changes (APCs) in slopes were reported. Moreover, the prevalence of obesity and

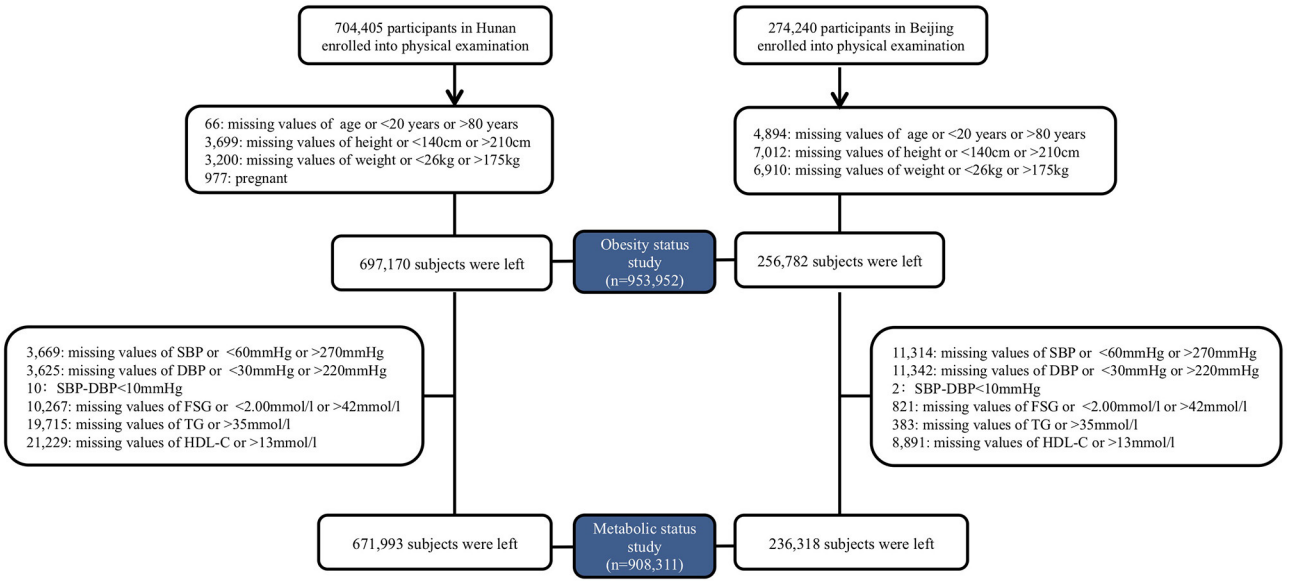

FIGURE 1 | Enrollment flowchart.

metabolic subtypes were stratified by location, age group (20–39, 40–59, 60, and older years), and sex because of significant 4-way interactions. The prevalence of obesity, MHNO, MUNO, MHO, and MUO and the mean levels of BMI, TC, HDL-c, and TG, SBP, DBP, and FSG were all estimated for females and males after age standardization according to the population distribution in China in 2010 (20). The weights were 0.4385, 0.3860, and 0.1755 for the 20–39, 40–59, and over 60 years old groups, respectively. When calculating estimates for each survey year, sex was additionally adjusted, and weights for females and males were the same as 0.5. A two-sided $P < 0.05$ was considered to be statistically significant. SAS version 9.4 (SAS Institute Inc) was used for analyses.

RESULTS

Characteristics of Selected Study Participants

A total of 256,782 participants were surveyed from January 1, 2014, to December 31, 2020, in northern China, and 697,170 participants were surveyed from January 1, 2012, to December 31, 2020, in southern China (**Figure 1**). Overall, the mean BMI was 23.91 (95% CI: 23.90, 23.92) kg/m^2 and 108,914 participants [11.41% (95% CI: 11.34, 11.47%)] were obese. **Table 1** shows the demographic and clinical characteristics of the study participants by obesity status. Compared to the non-obese, participants in the obese group were more likely to be male, from northerners, older, with a higher level of TC, TC, LDL-c, and FSG, having elevated blood pressure, with hypertension, diabetes, or hyperlipidemia ($P < 0.001$, **Table 1**).

Trends in BMI and Obesity

The age- and sex-standardized mean BMI levels between northerners and southerners were 23.94 (95% CI: 23.93, 23.95) and 23.68 (95% CI: 23.67, 23.69) kg/m^2, respectively (**Table 2**). During 2014–2020, the BMI levels in northerners showed significant non-linear changes by surveyed year ($P < 0.001$) and grew rapidly after 2018 (**Figure 2**). A non-linear trend in BMI level among female from south was also observed, which increased from 2012 to 2016 and declined after 2016. However, a significant and linear increasing trend in BMI trend was observed among the southern male population ($P < 0.001$; **Figure 2**). Due to the interaction between BMI and age, we further stratified the population by age. As a result, the BMI level was found to significantly increase among 20–39 aged groups in both areas, and the trends between southern male and northern female groups were linear. Among the 40–59 years old, non-linear upward trends were observed in both sexes from southern and northern China. On the other hand, a significant and downward trend in BMI was found among female participants over 60 from southern China (**Supplementary Table 1**, **Supplementary Figure 1**).

Between 2014 and 2020, the prevalence of obesity among northerners increased from 12.70% (95% CI: 12.17, 13.23%) to 14.33% (95% CI: 13.97, 14.70%) ($P = 0.009$; **Figure 3A**). The obesity prevalence among northern females and males showed similar annual percentage changes. Among the 20–39 and 40–59 age groups, the APCs were 6.07 (95% CI: 3.97, 8.21) and 3.17 (95% CI: 1.02, 5.37), respectively. However, the trends of obesity in the over 60 years age group were non-significant ($P = 0.668$; **Figures 3B–D**).

The trends of obesity among southerners were different from those among northerners. Between 2012 and 2020, no significant trend in obesity was found among the total sample of southerners ($P = 0.240$; **Figure 3A**). After stratification by age and sex, the prevalence of obesity among females and males aged 20–39 years was found to increase from 1.87% (95% CI, 1.63, 2.11%) to 2.99% (95% CI, 2.69, 3.29%) and from

TABLE 1 | Characteristics of selected study participants by obesity status.

Characteristics		Obesity *N* (%) /mean (SD)	Non-obesity *N* (%) /mean (SD)
Location**	Northern China	35,953 (33.01)	220,829 (26.13)
	Southern China	72,961 (66.99)	624,209 (73.87)
Sex**	Female	23,584 (21.65)	386,537 (45.74)
	Male	85,330 (78.35)	458,501 (54.26)
Age**, year (*N* = 953,952)		46.13 (14.20)	44.56 (14.79)
BMI, kg/m^2 (*N* = 953,952)		30.10 (2.10)	23.11 (2.65)
TG**, mmol/L (*N* = 933,854)		2.46 (2.15)	1.53 (1.39)
TC**, mmol/L (*N* = 933,872)		5.14 (1.01)	4.90 (0.95)
HDL-c**, mmol/L (*N* = 923,832)		1.15 (0.29)	1.39 (0.39)
LDL-c**, mmol/L (*N* = 923,715)		2.84 (0.85)	2.72 (0.80)
SBP**, mmHg (*N* = 938,969)		132.81 (16.08)	121.36 (16.31)
DBP**, mmHg (*N* = 938,985)		83.27 (11.57)	74.79 (10.92)
FSG**, mmol/L (*N* = 942,864)		5.84 (1.61)	5.35 (1.18)
Hypertension**	No	62,506 (58.53)	681,807 (81.93)
	Yes	44,278 (41.47)	150,342 (18.07)
Diabetes**	No	94,512 (87.50)	791,011 (94.75)
	Yes	13,500 (12.50)	43,841 (5.25)
Dyslipidemia**	No	40,345 (38.01)	546,177 (66.82)
	Yes	65,785 (61.99)	271,260 (33.18)

***p < 0.01.*

SD, standard deviation; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FSG, fasting serum glucose; TG, triglyceride; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol.

Obesity was defined as BMI ≥ 28 kg/m^2.

Hypertension was defined as self-reported hypertension diagnosed by a physician, self-reported regular use of antihypertensive medications, or systolic/diastolic blood pressure at recruitment ≥ 140/90 mmHg.

Dyslipidemia was defined as meeting any of the following criteria: (1) TC ≥ 6.22 mmol/L; (2) LDL-C ≥ 4.14 mmol/L; (3) HDL-C < 1.04 mmol/L; (4) TG ≥ 2.26 mmol/L; (5) self-reported dyslipidemia or use of lipid-lowering medications; Diabetes mellitus was defined as self-reported diabetes diagnosed by a physician, self-reported regular use of antidiabetic medications, or fasting glucose at recruitment ≥ 7.0 mmol/L.

TABLE 2 | Age-Standardized mean and 95% confidence interval of BMI levels among adults aged 20 years and older in northern and southern China, 2012–2020*.

Year	Northern China BMI, kg/m^2 [mean (95% CI)]			Southern China BMI, kg/m^2 [mean (95% CI)]		
	All$	Female	Male	All$	Female	Male
2012				23.46 (23.44, 23.49)	22.33 (22.30, 22.37)	24.59 (24.56, 24.62)
2013				23.63 (23.61, 23.66)	22.46 (22.43, 22.49)	24.80 (24.78, 24.83)
2014	23.85 (23.80, 23.90)	22.76 (22.69, 22.84)	24.94 (24.87, 25.00)	23.74 (23.72, 23.76)	22.60 (22.57, 22.63)	24.88 (24.85, 24.91)
2015	23.67 (23.63, 23.71)	22.59 (22.54, 22.65)	24.75 (24.70, 24.80)	23.75 (23.73, 23.77)	22.59 (22.56, 22.62)	24.90 (24.87, 24.93)
2016	23.78 (23.74, 23.82)	22.71 (22.66, 22.76)	24.85 (24.80, 24.90)	23.61 (23.58, 23.63)	22.46 (22.43, 22.49)	24.75 (24.72, 24.78)
2017	23.78 (23.75, 23.82)	22.68 (22.63, 22.73)	24.89 (24.85, 24.93)	23.68 (23.66, 23.70)	22.54 (22.51, 22.57)	24.82 (24.79, 24.85)
2018	23.97 (23.94, 24.00)	22.92 (22.87, 22.96)	25.02 (24.98, 25.06)	23.67 (23.65, 23.70)	22.46 (22.43, 22.49)	24.89 (24.86, 24.92)
2019	24.04 (24.02, 24.07)	22.99 (22.95, 23.03)	25.10 (25.06, 25.13)	23.79 (23.77, 23.81)	22.60 (22.57, 22.63)	24.98 (24.95, 25.01)
2020	24.16 (24.13, 24.19)	22.98 (22.94, 23.03)	25.34 (25.29, 25.38)	23.73 (23.70, 23.75)	22.49 (22.45, 22.53)	24.96 (24.93, 25.00)
p for overall trend	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001#
p for non-linear trend	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.207
Subtotal	23.94 (23.93, 23.95)	22.86 (22.84, 22.88)	25.02 (25.01, 25.04)	23.68 (23.67, 23.69)	22.51 (22.50, 22.53)	24.84 (24.83, 24.85)

**Estimates are age-standardized to the 2010 Chinese Census population using age groups 20–39, 40–59, and 60 or older; $ additional adjusted by sex; #P for linear trends<0.001.*

13.10% (95% CI, 12.53, 13.67%) to 16.04% (95% CI, 15.34, 16.74%), respectively (**Figure 3B**). However, obesity among over 60 years old southern females showed significant decreasing trends ($P < 0.006$), and among 40–59 years aged southern females, the prevalence of obesity increased between 2012 and 2014 [slope = 0.12 (95% CI: −0.07,0.30), $P = 0.17$], and decreased after 2014 [slope = −0.03 (95% CI: −0.06, 0.00), $P = 0.04$; **Figures 3C,D**].

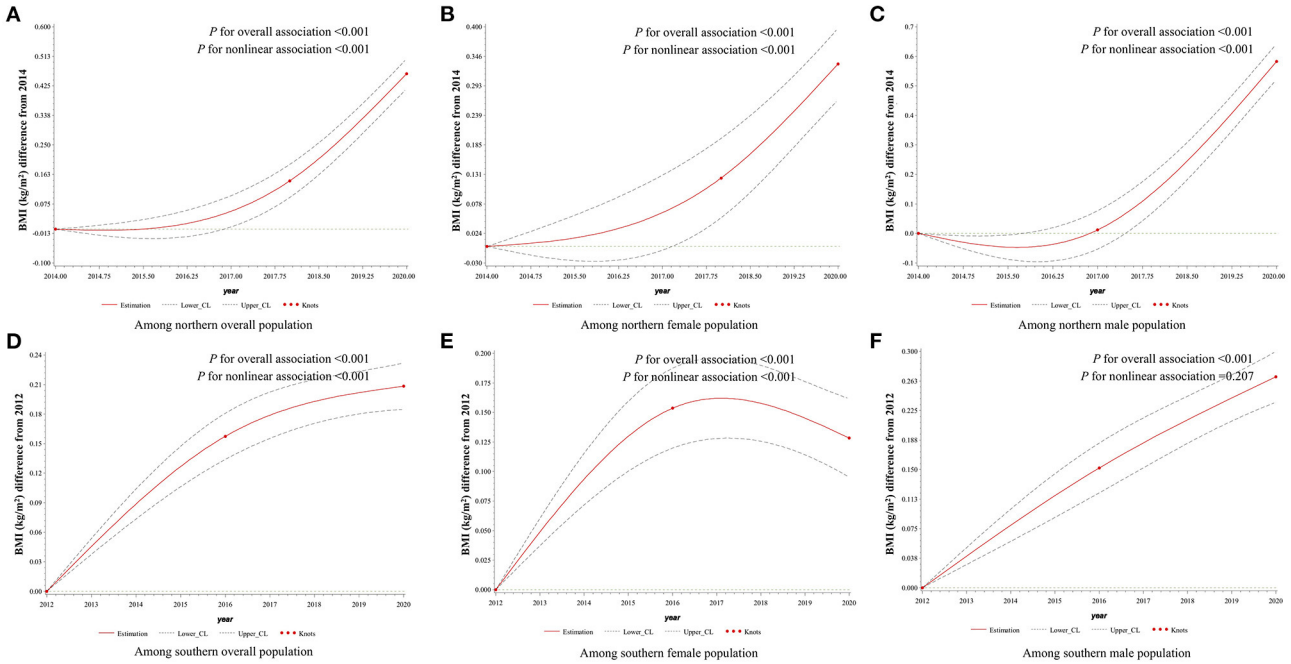

FIGURE 2 | Association between survey years and BMI among adults aged 20 years and older in northern and southern China, 2012–2020. Surveyed year was coded using the RCS function with three knots located at the 5th, 50th, and 95th percentiles of the distribution of survey years. Y-axis represents the BMI difference from referenced year. Referenced year in **(A–C)** was 2014, and in **(D–F)** was 2012. Dashed lines are 95 percent confidence intervals. Knots are represented by dots. In **(A)** and **(D)**, age and sex were included as adjustment variables. In **(B,C,E,F)**, age was included as an adjustment variable. BMI, body mass index.

Trends in Metabolic Factors

Overall, a total of 908,311 persons were included for metabolic analysis. The age-adjusted means of FSG, TG, HDL-c, SBP, and DBP are listed in **Supplementary Tables 2–6**. Overall, the changing trend in metabolic factors was similar between different sexes but showed regional differences in FSG, which were both rising in the two regions (**Supplementary Figure 2**). The TG levels showed a downward trend in 2014-2018 and an upward trend in 2018–2020 among the northern population, and in the south, it showed a non-linear monotonous rising trend (**Supplementary Figure 3**). The HDL-c levels had opposite trends in the two places, with northern rising and southern declining (**Supplementary Figure 4**). The SBP levels showed an upward trend in the north, and in the south, it showed a downward trend in 2012–2016 and an upward trend in 2016–2020 (**Supplementary Figure 5**). The DBP levels rose first and then fell in the north, and fell first and then rose in the south (**Supplementary Figure 6**).

Trends of Obesity Phenotypes

The trends of obesity phenotypes have been listed in **Figures 4A–D**. Between 2014 and 2020, among the northern participants, the prevalence of the MUNO subtype significantly decreased from 36.03% (95% CI: 35.16, 36.90%) to 27.85% (95% CI: 27.31, 28.39%) with an annual percentage change of −6.53 (95% CI: −12.33, −0.34) (**Figure 4B**), but the trends in MUO were non-significant ($P = 0.384$; **Figure 4D**). However, the trends in MHNO were non-significant (**Figure 4A**), and the prevalence of MHO increased significantly from 2.07% (95% CI: 1.84, 2.30%) to 4.33% (95% CI: 4.13, 4.53%) (**Figure 4C**). All obesity phenotypes in different sex and age groups among northerners showed similar annual percentage changes (**Supplementary Figure 7**).

Unlike the trends in obesity phenotypes among northerners, between 2012 and 2020, the prevalence of the metabolically unhealthy subtypes MUNO and MUO among southerners increased significantly from 21.92% (95% CI: 21.55, 22.30%) to 29.58% (95% CI: 29.10, 30.07%) and from 5.36% (95% CI: 5.18, 5.54%) to 7.35% (95% CI: 7.11, 7.58%), respectively (**Figures 4B,D**). Meanwhile, the prevalence of the metabolically healthy subtypes MHNO and MHO decreased significantly from 69.24% (95% CI: 68.57, 69.92%) to 60.21% (95% CI: 59.55, 60.88%) and from 3.47% (95% CI: 3.32, 3.61%) to 2.86% (95% CI: 2.71, 3.00%), respectively (**Figures 4A,C**). Among southerners aged 40–59 and over 60 years old, females and males showed a similar annual percentage change in all obesity phenotypes (**Supplementary Figure 7**), but among the 20–39 year age group, the prevalence of MUNO and MUO in females showed a greater annual percentage increase than that in males [14.84 (95% CI: 9.40, 20.56) vs. 5.18 (95% CI: 2.62, 7.81) and 15.02 (95% CI: 13.55, 16.51) vs. 5.32 (95% CI: 3.74, 6.92)] (**Supplementary Figure 7**).

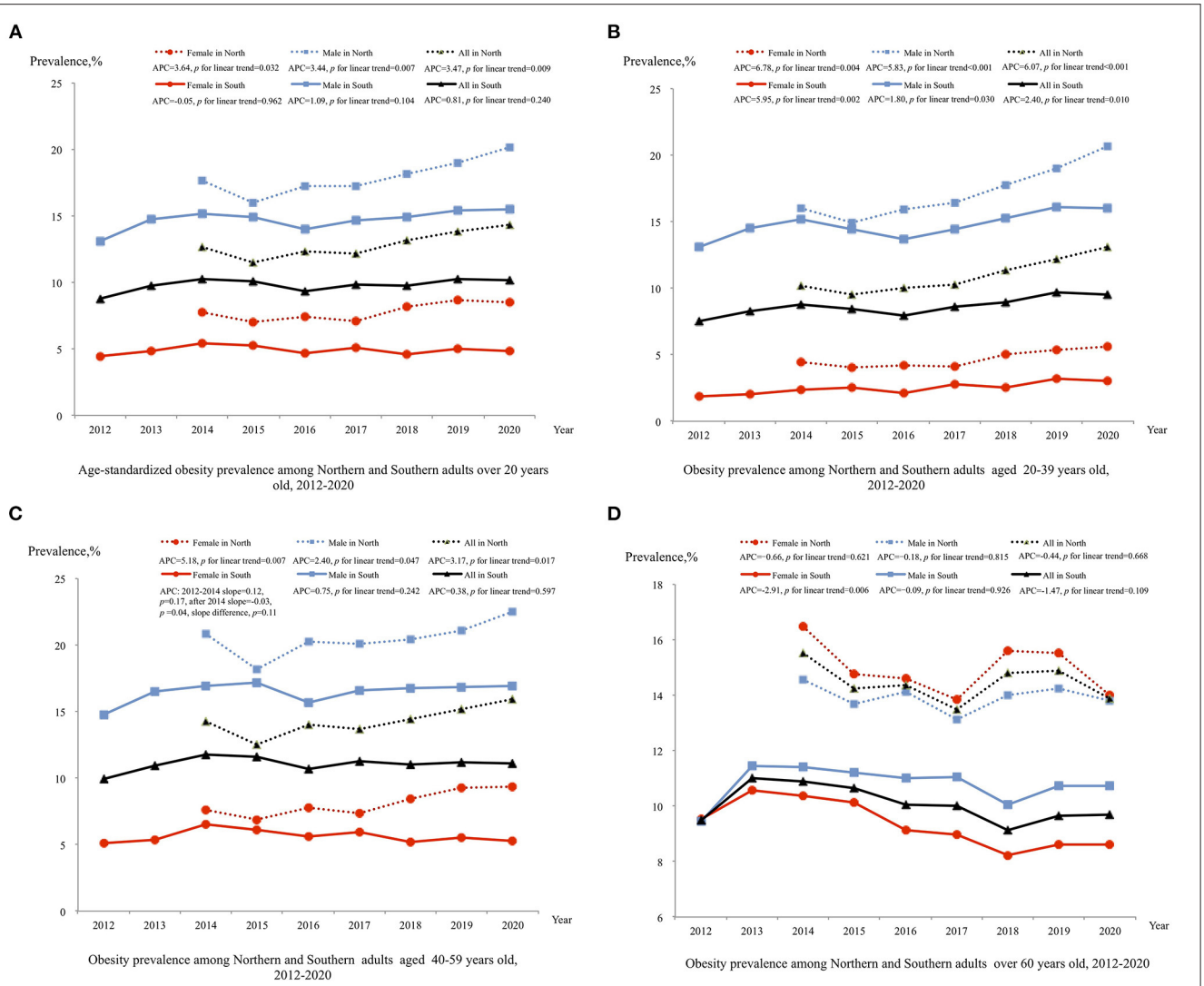

FIGURE 3 | Trend in prevalence of obesity adults in northern and southern China, 2012–2020. Estimates by different sex are age-standardized to the 2010 Chinese Census population using age groups 20–39, 40–59, and 60 or older and estimates for overall population are additional adjusted by sex; Significant linear trends ($P < 0.05$) for the following groups:(1) increased age-standardized obesity among all sex groups from northern China; (2) increased obesity among all 20–39 aged groups from northern and southern China; (3) increased obesity among all 40–59 aged groups from northern ($P < 0.05$); (4) among 40–59 aged female from southern China, obesity during 2012–2014 showed increased level [slope = 0.12 (95% CI: −0.07,0.30), $P = 0.17$], and level decreased after 2014 [slope = −0.03 (95% CI: −0.06, 0.00, $P = 0.04$], slope difference, $P = 0.11$; (5) decreased obesity among 60 and over aged female from southern. BMI, body mass index; APC, annual percentage change.

DISCUSSION

The present analyses show that the mean BMI and the prevalence of obesity in northern China were significantly higher than in southern China. However, compared to the north, marked changes in metabolic abnormalities occurred in the south from 2012 to 2020. The mean BMI and the prevalence of obesity increased significantly in the north, driven primarily by 20–39- and 40–59-years adult males and females from 2014 to 2020. In the south, the mean BMI increased significantly, but the overall obesity prevalence was stable. Moreover, the proportion of MUO increased, especially in 20–39 years females, and 20–39- and 40–59-years male population.

In a previous study, the age-standardized mean BMI increased by 2.0 kg/m^2 from 1993 to 2015 in China (9). Our study further confirms that the mean BMI continued to grow in both areas from 2012 to 2020. But the trend in BMI differed between northern and southern China. The mean BMI level in northern China increased rapidly after 2018, whereas in southern China, it increased obviously from 2012 to 2016. Moreover, unlike the other study (9), the increased mean BMI level was mainly derived by young and middle-aged population, especially in the north.

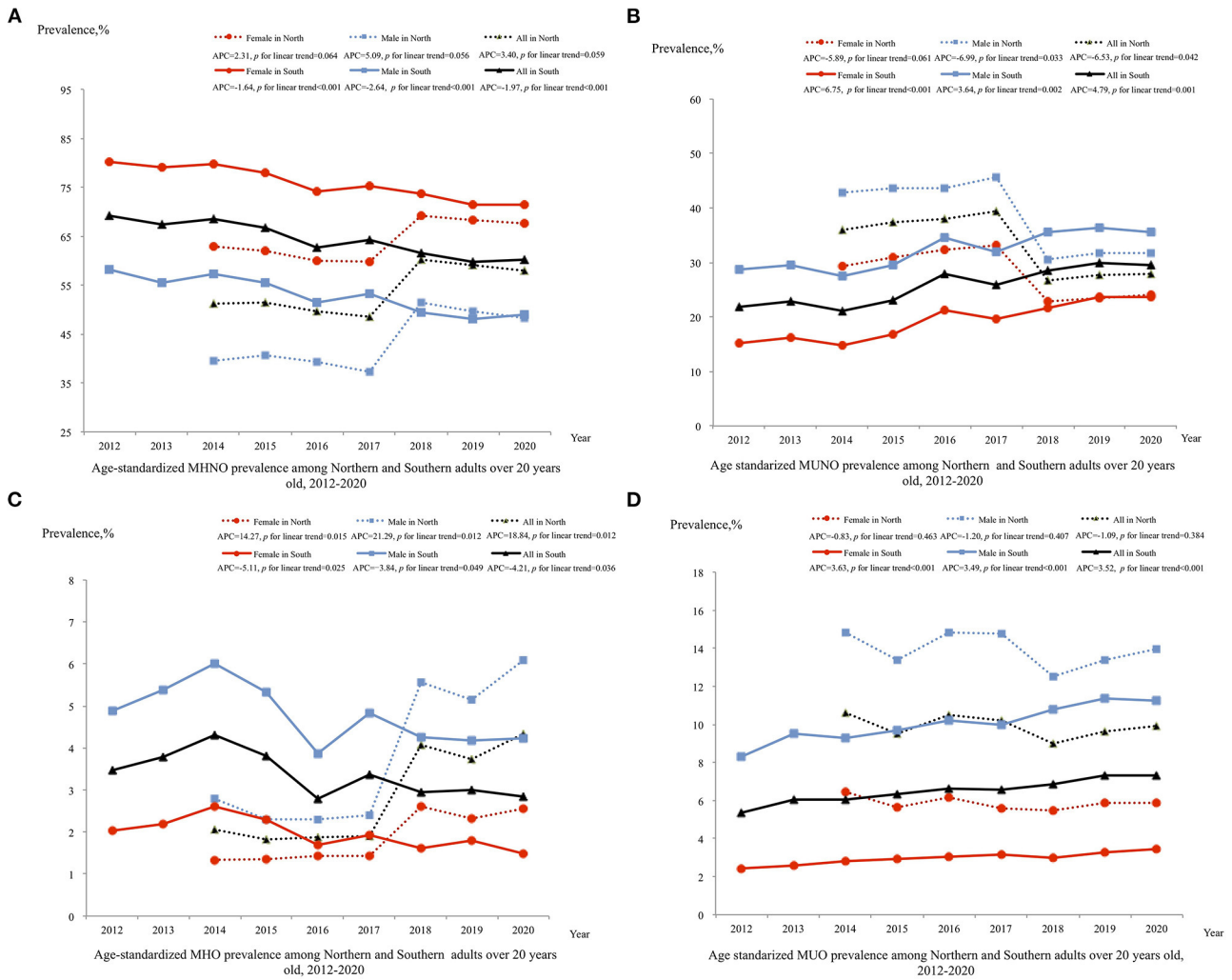

FIGURE 4 | Trend in prevalence of obesity by metabolic status phenotype among adults in northern and southern China, 2012–2020. Estimates by different sex are age-standardized to the 2010 Chinese Census population using age groups 20–39, 40–59, and 60 or older and estimates for overall population are additionally adjusted by sex; Significant linear trends ($P < 0.05$) for the following groups: (1) decreased MHNO among all sex groups from southern; (2) decreased MUNO among male and overall groups from northern ($P < 0.05$); increased MUNO among all sex groups from southern ($P < 0.05$); (3) increased MHO among all sex groups from northern and decreased MHO among all sex groups from southern; (4) increased MUO among all sex groups from southern ($P < 0.05$). MHNO, metabolic healthy non-obese; MUNO, metabolic unhealthy non-obese; MHO, metabolic healthy obese; MUO, metabolic unhealthy obese; APC, annual percentage change.

The trend in obesity prevalence in our study was inconsistent with other studies. Globally, the prevalence of obesity increased from 5% in 1980 to 10.1% in males and from 8.9 to 14.8% in females in 2015 (21). Based on cross-sectional surveys, the prevalence of obesity increased from 4.2 to 15.7% in the Chinese population between 1993 and 2015 (9). In the other regional studies, the prevalence of obesity in Jilin and Nanjing, two cities located in eastern China increased by 3.6 and 4.0% for males, and by 5.6 and 1.5% for females from 2007 to 2013 and 2008 to 2016 respectively (22). However, the increase in obesity in northern was 3.47% per year, while the current study found no significant increasing trend in Hunan, southern China. In Wang's study, the change in the prevalence of obesity in Liangshan Yi Autonomous Prefecture migrants was −0.6% in Sichuan, western China, from 2007 to 2015 (23). The geographical position of Hunan Province borders Sichuan Province; thus, the demographic characteristics might be similar between those two areas.

In addition, aged 20–39 and 40–59 populations drove the increasing trend of the prevalence of obesity in Beijing. The turning point of the prevalence of obesity in females aged 40–59 and the declining trend in females over 60 years old have evened out the rising rate in both females and males aged 20–39. Thus, the overall obesity rate did not show an increasing trend in southern China. In previous studies, the increasing trend in obesity was driven by all age subgroups, especially by the 40–80 aged population surveyed before 2015 (24). Our study indicated that more attention should be given to the young and middle-aged populations in China.

It is demonstrated that the COVID-19 pandemic could have serious consequences for the obesity epidemic (15). A study conducted in Italy has shown that home isolation and adverse mental health burden linked to the COVID-19 pandemic were associated with significant weight gain in 2020 (25). In the current study, neither the mean BMI nor the prevalence of the obesity rate showed a significant change in 2020 that deviated from the previous trend. In our view, the rapidly controlled COVID-19 pandemic might not have had significantly impact on the weight in China. Some previous reports of weight gain during the pandemic might have obtained significant results because they did not compare to previous trends.

In our study, the prevalence of MHO was 4.33 and 2.95% in northern and southern China in 2020, respectively. In the China Kadoorie Biobank study, the MHO phenotype accounted for 3.3% of the total population from 2004 to 2008 in China (26). According to a previous study, the prevalence of MHO has been shown to range between 4.2 and 13.6% in a random sample from a Chinese adult population, depending on the definition used for MHO (27). The trends in the prevalence of obesity metabolic status differed between northern and southern China. Overall, the increasing trend in the prevalence of obesity was predominantly driven by MHO in all age groups in the north. During the period of 1973–1980, Keyes and Reuben Andres suggested that MHO could be benign and not contribute to cardiovascular risk (28). However, an increasing number of studies have demonstrated MHO is indeed associated with an increased risk of cardiovascular disease, chronic kidney disease, non-alcoholic fatty liver disease, and death (29–32). Therefore, MHO could be a risk factor for chronic disease (33) and finally transit to MUO (26) if timely intervention is not performed in northern China. In support, although the overall prevalence of obesity was stable, the prevalence of MUO phenotype has increased, while the prevalence of MHO has decreased, especially in young and middle-aged groups in southern China. A previous study done in Shanghai (eastern China) adults indicated the prevalence of metabolism problem was doubled with an increase in metabolically unhealthy overweight from 2002 to 2017 as well (34). There is no doubt that MUO has the most significant impact on health. Therefore, residents in southern China should pay more attention to metabolic status.

High energy intake, especially sugary drinks, and other energy-dense foods, and low levels of physical activity contributed to the increasing trend of obesity in China (35, 36). In the current study, the relatively slow growth trend in the north and the stable trend in the south may be due to the following reasons: First, recognizing the immediacy of chronic disease challenges, the "China Healthy Lifestyle for All" initiative launched in 2007 was developed to raise awareness of a range of preventive health issues, such as knowledge of dietary guidelines, and the adoption of health-promoting behaviors. Currently, much evidence suggests a positive role in healthy lifestyle action after 2015 (37). Thus, good knowledge of healthy lifestyles may help control weight in Chinese adults. Second, the current study participants were from annual health check-up population who received health education and guidance on weight intervention from the Health Management Center. Therefore, the result may not be representative of the data of the national epidemiologic survey.

Our study has several strengths. First, it included a large sample size (>900,000 participants) from Beijing and Hunan, two regions of northern and southern China. Second, the surveys were performed every year between 2012 and 2020 to facilitate the analyses for annual trend of change. Third, we also used physical examination data to analyze the metabolic status and further understand the types of obesity. However, several limitations should be considered. First, only health check-up subjects were investigated in two institutions of health management centers, who were community-derived but not represent random samples. Hence, our results may not be generalizable to overall Chinese population due to selection bias. Second, BMI could not differentiate fat from lean mass or consider the distribution of adipose tissue, while waist circumference (WC), hip circumstance (HC), and waist-to-hip ratio (WHR), which focus on abdominal adiposity, have been identified as useful weight-related anthropometric measures to predict the risk of chronic disease (38). But unfortunately, waist and hip circumferences were not regular measurements in our northern participants. Thus, WC and HC were not analyzed in the current study. Third, the questionnaires were obtained from participants under voluntary principle before 2018 in Beijing. Therefore, some information such as medication use history may be lower than the real-world data. Fourth, lifestyle was associated with obesity and metabolism disorder (39). But we were unable to examine the roles of nutrition and lifestyle factors (e.g., physical activity and sleep duration) on obesity trends because these data were not continuously collected in northerners.

CONCLUSIONS

The trends in the prevalence of obesity and metabolic status were different between northern and southern Chinese. The northerners were dominated by the growth of young and middle-aged obesity and MHO phenotype, while although the overall obesity rate was stable in the south, the proportion of MUO increased, especially in the young and middle-aged population. The weight control plan should generalize to the young and middle-aged Chinese.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

YL, LYa, LYi, QL, and XH produced data for analysis. YL, LYa, XH, and YH wrote the manuscript. YL, XH, and YH designed the study and handled funding and supervision. PY, YW, JW, ZC, XL, QY, and YH included patients for the study. All authors reviewed and edited the manuscript, read, and approved the final manuscript.

FUNDING

This work was supported by funding from the National Natural Science Foundation of China (81973324 to YL, 81872685 to XH, and 82003537 to XH), Hunan Young Talent grant (2020RC3063 to YL), Natural Science Foundation of Hunan Province (2020JJ5858 to YL, 2020JJ4439 to XH), and the Wisdom Accumulation and Talent Cultivation Project of the Third XiangYa hospital of Central South University (YX202002 to YL). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the voluntary participation of all study subjects.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.811244/full#supplementary-material

REFERENCES

1. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. *JAMA*. (1999) 282:1523–9. doi: 10.1001/jama.282.16.1523
2. Lauby-Secretan B, Scoccianti C, Loomis D, Grosse Y, Bianchini F, Straif K, et al. Body fatness and cancer–viewpoint of the IARC working group. *N Engl J Med*. (2016) 375:794–8. doi: 10.1056/NEJMsr1606602
3. Ayton A, Ibrahim A. Obesity is a public health emergency. *BMJ*. (2019) 366:l5463. doi: 10.1136/bmj.l5463
4. Anandacoomarasamy A, Caterson I, Sambrook P, Fransen M, March L. The impact of obesity on the musculoskeletal system. *Int J Obes*. (2008) 32:211–22. doi: 10.1038/sj.ijo.0803715
5. Anstey KJ, Cherbuin N, Budge M, Young J. Body mass index in midlife and late-life as a risk factor for dementia: a meta-analysis of prospective studies. *Obes Rev*. (2011) 12:e426–37. doi: 10.1111/j.1467-789X.2010.00825.x
6. GBD 2015 Obesity Collaborators, Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, et al. Health effects of overweight and obesity in 195 countries over 25 years. *N Engl J Med*. (2017) 377:13–27. doi: 10.1056/NEJMoa1614362
7. Global Burden of Disease Study 2015. *Global Burden of Disease Study 2015 (GBD 2015) Obesity and Overweight Prevalence 1980–2015*. Seattle, WA: Institute for Health Metrics and Evaluation (IHME) (2017).
8. Pan X-F, Wang L, Pan A. Epidemiology and determinants of obesity in China. *Lancet Diabetes Endocrinol*. (2021) 9:373–92. doi: 10.1016/S2213-8587(21)00045-0
9. Ma S, Xi B, Yang L, Sun J, Zhao M, Bovet P. Trends in the prevalence of overweight, obesity, and abdominal obesity among Chinese adults between 1993 and 2015. *Int J Obes*. (2021) 45:427–37. doi: 10.1038/s41366-020-00698-x
10. The State Council Information Office of the People's Republic of China. *Press Briefing for the Report on Chinese Residents' Chronic Diseases and Nutrition 2020*. Beijing: The State Council Information Office of the People's Republic of China (2020).
11. Hinnouho G-M, Czernichow S, Dugravot A, Nabi H, Brunner EJ, Kivimaki M, et al. Metabolically healthy obesity and the risk of cardiovascular disease and type 2 diabetes: the whitehall II cohort study. *Eur Heart J*. (2015) 36:551–9. doi: 10.1093/eurheartj/ehu123
12. Eckel N, Mühlenbruch K, Meidtner K, Boeing H, Stefan N, Schulze MB. Characterization of metabolically unhealthy normal-weight individuals: risk factors and their associations with type 2 diabetes. *Metabolism*. (2015) 64:862–71. doi: 10.1016/j.metabol.2015.03.009
13. Hinnouho G-M, Czernichow S, Dugravot A, Batty GD, Kivimaki M, Singh-Manoux A. Metabolically healthy obesity and risk of mortality: does the definition of metabolic health matter? *Diabetes Care*. (2013) 36:2294–300. doi: 10.2337/dc12-1654
14. Ko S-H, Baeg MK, Ko SY, Jung HS, Kim P, Choi M-G. Obesity and metabolic unhealthiness have different effects on colorectal neoplasms. *J Clin Endocrinol Metab*. (2017) 102:2762–9. doi: 10.1210/jc.2017-00152
15. Stefan N, Birkenfeld AL, Schulze MB. Global pandemics interconnected-obesity, impaired metabolic health and COVID-19. *Nat Rev Endocrinol*. (2021) 17:135–49. doi: 10.1038/s41574-020-00462-1
16. Yang P, Chen Z, Yin L, Peng Y, Li X, Cao X, et al. Salt intake assessed by spot urine on physical examination in Hunan, China. *Asia Pac J Clin Nutr*. (2019) 28:845–56. doi: 10.6133/apjcn.201912_28(4).0022
17. Lu Y, Pechlaner R, Cai J, Yuan H, Huang Z, Yang G, et al. Trajectories of age-related arterial stiffness in chinese men and women. *J Am Coll Cardiol*. (2020) 75:870–80. doi: 10.1016/j.jacc.2019.12.039
18. National Health and Family Planning Commission of the People's Republic of China. *WST 428-2013 Criteria of Weight for Adults*. Beijing: Standards Press of China.
19. Kim HJ, Fay MP, Feuer EJ, Midthune DN. Permutation tests for joinpoint regression with applications to cancer rates. *Stat Med*. (2000) 19:335–51. doi: 10.1002/(SICI)1097-0258(20000215)19:3<335::AID-SIM336>3.0.CO;2-Z
20. Population Census Office under the State Council Department of Population and Employment Statistics National Bureau of Statistics. *Tabulation on the 2010 Population Census of the People's Republic of China*. Beijing: China Statistics Press. Available online at: http://www.stats.gov.cn/tjsj/pcsj/rkpc/6rp/left.htm
21. NCD Risk Factor Collaboration (NCD-RisC). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. *Lancet*. (2016) 387:1377–96. doi: 10.1016/S0140-6736(16)30054-X
22. Sun J, Zhou W, Gu T, Zhu D, Bi Y. A retrospective study on association between obesity and cardiovascular risk diseases with aging in Chinese adults. *Sci Rep*. (2018) 8:5806. doi: 10.1038/s41598-018-24161-0
23. Zhang J, Wan S, Zhang B, Dong F, Pan L, Yihuo W, et al. Twenty-year time trends in hypertension prevalence in Yi people of China: three successive cross-sectional studies, 1996-2015. *BMJ Open*. (2018) 8:e022714. doi: 10.1136/bmjopen-2018-022714
24. Mi Y-J, Zhang B, Wang H-J, Yan J, Han W, Zhao J, et al. Prevalence and secular trends in obesity among Chinese adults, 1991-2011. *Am J Prev Med*. (2015) 49:661–9. doi: 10.1016/j.amepre.2015.05.005
25. Pellegrini M, Ponzo V, Rosato R, Scumaci E, Goitre I, Benso A, et al. Changes in weight and nutritional habits in adults with obesity during the "lockdown" period caused by the COVID-19 virus emergency. *Nutrients*. (2020) 12:E2016. doi: 10.3390/nu12072016
26. Gao M, Lv J, Yu C, Guo Y, Bian Z, Yang R, et al. Metabolically healthy obesity, transition to unhealthy metabolic status, and vascular disease in Chinese adults: a cohort study. *PLoS Med*. (2020) 17:e1003351. doi: 10.1371/journal.pmed.1003351

27. Liu C, Wang C, Guan S, Liu H, Wu X, Zhang Z, et al. The prevalence of metabolically healthy and unhealthy obesity according to different criteria. *Obes Facts.* (2019) 12:78–90. doi: 10.1159/000495852
28. Sims EA. Are there persons who are obese, but metabolically healthy? *Metabolism.* (2001) 50:1499–504. doi: 10.1053/meta.2001.27213
29. Opio J, Croker E, Odongo GS, Attia J, Wynne K, McEvoy M. Metabolically healthy overweight/obesity are associated with increased risk of cardiovascular disease in adults, even in the absence of metabolic risk factors: a systematic review and meta-analysis of prospective cohort studies. *Obes Rev.* (2020) 21:e13127. doi: 10.1111/obr.13127
30. Chang Y, Jung H-S, Cho J, Zhang Y, Yun KE, Lazo M, et al. Metabolically healthy obesity and the development of nonalcoholic fatty liver disease. *Am J Gastroenterol.* (2016) 111:1133–40. doi: 10.1038/ajg.2016.178
31. Stanford FC, Butsch WS. Metabolically healthy obesity and development of chronic kidney disease. *Ann Intern Med.* (2016) 165:742–3. doi: 10.7326/L16-0408
32. Stefan N, Häring HU, Schulze MB. Metabolically healthy obesity: the low-hanging fruit in obesity treatment? *Lancet Diabetes Endocrinol.* (2018) 6:249–58. doi: 10.1016/S2213-8587(17)30292-9
33. Kim HS, Lee J, Cho YK, Park J-Y, Lee WJ, Kim Y-J, et al. Differential effect of metabolic health and obesity on incident heart failure: a nationwide population-based cohort study. *Front Endocrinol.* (2021) 12:625083. doi: 10.3389/fendo.2021.625083
34. Yang Y, Li Y, Pei J, Cheng M, Xu W, Shi Y. Dynamic changes in metabolic health status in Chinese adults: multiple population-based surveys in Shanghai, China. *J Diabetes Investig.* (2021) 12:1784–96. doi: 10.1111/jdi.13556
35. Popkin BM. Synthesis and implications: China's nutrition transition in the context of changes across other low- and middle-income countries. *Obes Rev.* (2014) 15 (Suppl. 1):60–7. doi: 10.1111/obr.12120
36. Li Y, Wang DD, Ley SH, Howard AG, He Y, Lu Y, et al. Potential impact of time trend of life-style factors on cardiovascular disease burden in China. *J Am Coll Cardiol.* (2016) 68:818–33. doi: 10.1016/j.jacc.2016.06.011
37. Zhang J, Astell-Burt T, Seo D-C, Feng X, Kong L, Zhao W, et al. Multilevel evaluation of "China healthy lifestyles for all", a nationwide initiative to promote lower intakes of salt and edible oil. *Prev Med.* (2014) 67:210–5. doi: 10.1016/j.ypmed.2014.07.019
38. Stefan N. Causes, consequences, and treatment of metabolically unhealthy fat distribution. *Lancet Diabetes Endocrinol.* (2020) 8:616–27. doi: 10.1016/S2213-8587(20)30110-8
39. Sotos-Prieto M, Ortolá R, Ruiz-Canela M, Garcia-Esquinas E, Martínez-Gómez D, Lopez-Garcia E, et al. Association between the Mediterranean lifestyle, metabolic syndrome and mortality: a whole-country cohort in Spain. *Cardiovasc Diabetol.* (2021) 20:5. doi: 10.1186/s12933-020-01195-1

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Nutrition

CORRECTION
published: 08 March 2022
doi: 10.3389/fnut.2022.872237

Corrigendum: Trends in Obesity and Metabolic Status in Northern and Southern China Between 2012 and 2020

Ying Li[1], Lin Yang[2,3], Lu Yin[4], Qingqi Liu[5], Yaqin Wang[1], Pingting Yang[1], Jiangang Wang[1], Zhiheng Chen[1], Xiaohui Li[6], Qinyu Yang[6], Yongmei He[7†] and Xin Huang[8†]*

[1] Department of Health Management, The Third Xiangya Hospital, Central South University, Changsha, China, [2] Department of Cancer Epidemiology and Prevention Research, Cancer Care Alberta, Alberta Health Services, Calgary, AB, Canada, [3] Departments of Oncology and Community Health Sciences, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada, [4] Medical Research & Biometrics Center, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [5] Department of Biostatistics, Bioinformatics & Biomathematics, Georgetown University, Washington, DC, United States, [6] Department of Pharmacology, Xiangya School of Pharamceutical Science, Central South University, Changsha, China, [7] Department of Health Management, Aerospace Center Hospital, Beijing, China, [8] Department of Epidemiology, School of Medicine, Hunan Normal University, Changsha, China

Keywords: obesity, metabolic status, trend, series cross-sectional study, China

OPEN ACCESS

Edited and reviewed by:
Megan A. McCrory,
Boston University, United States

***Correspondence:**
Xin Huang
xin.huang@hunnu.edu.cn

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *09 February 2022*
Accepted: *14 February 2022*
Published: *08 March 2022*

Citation:
Li Y, Yang L, Yin L, Liu Q, Wang Y, Yang P, Wang J, Chen Z, Li X, Yang Q, He Y and Huang X (2022) Corrigendum: Trends in Obesity and Metabolic Status in Northern and Southern China Between 2012 and 2020. Front. Nutr. 9:872237. doi: 10.3389/fnut.2022.872237

A Corrigendum on

Trends in Obesity and Metabolic Status in Northern and Southern China Between 2012 and 2020

by Li, Y., Yang, L., Yin, L., Liu, Q., Wang, Y., Yang, P., Wang, J., Chen, Z., Li, X., Yang, Q., He, Y., and Huang, X. (2022). Front. Nutr. 8:811244. doi: 10.3389/fnut.2021.811244

In the original article, there were some mistakes in **Table 1** as published. The means and SD of TG, TC, HDL-c, LDL-c, SBP, DBP and FSG in the non-obesity group were incorrect. The corrected **Table 1** appears below.

The authors apologize for these errors and state that they do not change the scientific conclusions of the article in any way. The original article has been updated.

TABLE 1 | Characteristics of selected study participants by obesity status.

Characteristics		Obesity *N* (%) /mean (SD)	Non-obesity *N* (%) /mean (SD)
Location**	Northern China	35,953 (33.01)	220,829 (26.13)
	Southern China	72,961 (66.99)	624,209 (73.87)
Sex**	Female	23,584 (21.65)	386,537 (45.74)
	Male	85,330 (78.35)	458,501 (54.26)
Age**, year (*N* = 953,952)		46.13 (14.20)	44.56 (14.79)
BMI, kg/m^2 (*N* = 953,952)		30.10 (2.10)	23.11 (2.65)
TG**, mmol/L (*N* = 933,854)		2.46 (2.15)	1.53 (1.39)
TC**, mmol/L (*N* = 933,872)		5.14 (1.01)	4.90 (0.95)
HDL-c**, mmol/L (*N* = 923,832)		1.15 (0.29)	1.39 (0.39)
LDL-c**, mmol/L (*N* = 923,715)		2.84 (0.85)	2.72 (0.80)
SBP**, mmHg (*N* = 938,969)		132.81 (16.08)	121.36 (16.31)
DBP**, mmHg (*N* = 938,985)		83.27 (11.57)	74.79 (10.92)
FSG**, mmol/L (*N* = 942,864)		5.84 (1.61)	5.35 (1.18)
Hypertension**	No	62,506 (58.53)	681,807 (81.93)
	Yes	44,278 (41.47)	150,342 (18.07)
Diabetes**	No	94,512 (87.50)	791,011 (94.75)
	Yes	13,500 (12.50)	43,841 (5.25)
Dyslipidemia**	No	40,345 (38.01)	546,177 (66.82)
	Yes	65,785 (61.99)	271,260 (33.18)

$^{**}p < 0.01$.

SD, standard deviation; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FSG, fasting serum glucose; TG, triglyceride; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol.

Obesity was defined as BMI ≥ 28 kg/m^2.

Hypertension was defined as self-reported hypertension diagnosed by a physician, self-reported regular use of antihypertensive medications, or systolic/diastolic blood pressure at recruitment ≥ 140/90 mmHg.

Dyslipidemia was defined as meeting any of the following criteria: (1) TC ≥ 6.22 mmol/L; (2) LDL-C ≥ 4.14 mmol/L; (3) HDL-C < 1.04 mmol/L; (4) TG ≥ 2.26 mmol/L; (5) self-reported dyslipidemia or use of lipid-lowering medications; Diabetes mellitus was defined as self-reported diabetes diagnosed by a physician, self-reported regular use of antidiabetic medications, or fasting glucose at recruitment ≥ 7.0 mmol/L.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 25 January 2022
doi: 10.3389/fnut.2022.815578

Nutritional Status According to the Short-Form Mini Nutritional Assessment (MNA-SF) and Clinical Characteristics as Predictors of Length of Stay, Mortality, and Readmissions Among Older Inpatients in China: A National Study

Hongpeng Liu[1], Jing Jiao[1†], Minglei Zhu[2], Xianxiu Wen[3], Jingfen Jin[4], Hui Wang[5], Dongmei Lv[6], Shengxiu Zhao[7], Xiang Sun[1], Xinjuan Wu[1*†] and Tao Xu[8*†]*

[1] Department of Nursing, Chinese Academy of Medical Sciences—Peking Union Medical College, Peking Union Medical College Hospital (Dongdan Campus), Beijing, China, [2] Department of Geriatrics, Chinese Academy of Medical Sciences—Peking Union Medical College, Peking Union Medical College Hospital (Dongdan Campus), Beijing, China, [3] Department of Nursing, Sichuan Provincial People's Hospital, Chengdu, China, [4] Department of Nursing, The Second Affiliated Hospital Zhejiang University School of Medicine, Hangzhou, China, [5] Department of Nursing, Tongji Medical College, Tongji Hospital, Huazhong University of Science and Technology, Wuhan, China, [6] Department of Nursing, The Second Affiliated Hospital of Haerbin Medical University, Haerbin, China, [7] Department of Nursing, Qinghai Provincial People's Hospital, Xining, China, [8] Department of Epidemiology and Statistics, Institute of Basic Medical Sciences, Peking Union Medical College, Chinese Academy of Medical Sciences and School of Basic Medicine, Beijing, China

Background: Studies are scarce in China that explore the association of nutritional status, measured using the Short-Form Mini Nutritional Assessment (MNA-SF) and biochemical data, on adverse clinical outcomes among older inpatients. In this study, we aimed to determine the prevalence of malnutrition in tertiary hospitals of China and the associations between malnutrition and adverse clinical outcomes.

Methods: This prospective study involved 5,516 older inpatients (mean age 72.47 ± 5.77 years) hospitalized in tertiary hospitals between October 2018 and February 2019. The tertiary hospitals refer to the hospital with more than 500 beds and can provide complex medical care services. The MNA-SF was used to assess nutritional status. Multiple logistic regression and negative binomial regression were used to analyze the relationship between nutritional parameters and risk of hospital length of stay (LoS), mortality, and rehospitalization.

Results: We found that 46.19% of hospitalized patients had malnutrition or malnutrition risk, according to the MNA-SF. Death occurred in 3.45% of patients. MNA-SF scores 0–7 (odds ratio [OR] 5.738, 95% confidence interval [CI] 3.473 to 9.48) were associated with a six-fold higher likelihood of death, and scores 8–11 (OR 3.283, 95% CI 2.126–5.069) with a three-fold higher likelihood of death, compared with MNA-SF scores 12–14 in the logistic regression model, after adjusting for potential confounders. A low MNA-SF score of 0–7 (regression coefficient 0.2807, 95% CI 0.0294–0.5320; $P < 0.05$) and a score of

OPEN ACCESS

Edited by:
Mauro Serafini,
University of Teramo, Italy

Reviewed by:
Yuan Hu,
Chinese Center for Disease Control and Prevention, China
Lili Ding,
Beijing Hospital, China
Jon Irazusta,
University of the Basque Country, Spain
Siti Setiati,
University of Indonesia, Indonesia

***Correspondence:**
Jing Jiao
jiaojing.2006@aliyun.com
Xinjuan Wu
wuxinjuan@sina.com
Tao Xu
xutaosd@126.com

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *15 November 2021*
Accepted: *03 January 2022*
Published: *25 January 2022*

Citation:
Liu H, Jiao J, Zhu M, Wen X, Jin J, Wang H, Lv D, Zhao S, Sun X, Wu X and Xu T (2022) Nutritional Status According to the Short-Form Mini Nutritional Assessment (MNA-SF) and Clinical Characteristics as Predictors of Length of Stay, Mortality, and Readmissions Among Older Inpatients in China: A National Study. Front. Nutr. 9:815578. doi: 10.3389/fnut.2022.815578

8–11 (0.2574, 95% CI 0.0863–0.4285; $P < 0.01$) was associated with a significantly higher (28.07 and 25.74%, respectively) likelihood of increased LoS, compared with MNA-SF score 12–14. MNA-SF scores 0–7 (OR 1.393, 95% CI 1.052–1.843) and 8–11 (OR 1.356, 95% CI 1.124–1.636) were associated with a nearly 1.5-fold higher likelihood of 90-day readmission compared with MNA-SF scores 12–14 in the logistic regression model. Moreover, hemoglobin level, female sex, education level, former smoking, BMI 24–27.9 kg/m^2, age 75 years and above, and current alcohol consumption were the main factors influencing clinical outcomes in this population.

Conclusions: Malnutrition increases the risk of hospital LoS, mortality, and 90-day readmission. The use of nutritional assessment tools in all hospitalized patients in China is needed. The MNA-SF combined with hemoglobin level may be used to identify older inpatients with a high risk of adverse clinical outcomes. These findings may have important implications for the planning of hospital services.

Keywords: malnutrition parameters, mortality, length of stay, readmission, older inpatients, nutritional epidemiology

INTRODUCTION

With the rapid development of health science and the global economy, the life span of the world's population is increasing (1–3). By 2050, it is estimated that 16% of the world's population would be aged 65 years and older (4), with 80% of those living in low- and middle-income countries (5). China, as the world's second-largest economy, has the world's largest population of 1.44 billion (19% of the world's population) and is rapidly becoming an aging nation (6–8).

Older adults are likely to have poor nutritional status and decreased quality of life (8–10). Nutritional deficits in the elderly can be caused by insufficient nutrient and energy intake (11). A few age-related pathophysiological, psychosocial, and pharmacological factors determine changes in dietary habits, as well as intake and use of nutrients, leading to specific deficits (12). The adverse effects of malnutrition or malnutrition risk on health substantially affect the quality of life by increasing the risk of physical frailty, disability, mortality, and deterioration during hospitalization (15, 19).

The prevalence of hospital malnutrition or the risk of malnutrition in elderly patients is considerable (30–50%) (11, 13, 14). This is primarily due to challenges in identifying and appropriately managing at-risk patients (15). As the European Society for Parenteral and Enteral Nutrition (ESPEN) recommends (16), Nutrition Risk Screening (NRS 2002) should be used to screen undernutrition in all inpatients. Several nutritional measures have been developed to determine nutritional status among inpatients in China, including the NRS 2002 and Short-Form Mini Nutritional Assessment (MNA-SF) (14, 17). However, appropriate nutritional risk screening is not performed in many Chinese hospitals, only in some large-scale nationwide, provincial, and municipal tertiary hospitals with more than 500 beds (18), where nutritional risk screening is mandatory.

Previous reports have suggested that malnutrition is associated with increased mortality among older residents of nursing homes (19). Other studies have used various nutritional screening tools, including the NRS 2002 and Malnutrition Universal Screening Tool, to estimate nutritional status and adverse clinical outcomes in the general hospitalized population (17, 20, 21) and in surgical patients (10, 22, 23), or these have used hospital data only or a smaller sample size (10, 24–27).

The European Society for Parenteral and Enteral Nutrition (ESPEN) suggests the use of NRS 2002 and Malnutrition Universal Screening Tool, whereas for older populations ESPEN recommends the use of the Mini Nutritional Assessment (MNA) either in its full or short form (MNA-SF) (28). Previous studies also indicated that the MNA-SF is a valid instrument with good specificity and sensitivity for the diagnosis of malnutrition, and this tool is specific for the older population (28–30). Nevertheless, few studies have reported on the prevalence of malnutrition in Asia hospitals (31), and studies are scarce in China that explore the associations between nutritional status, measured using the MNA-SF and biochemical data, on adverse clinical outcomes among older inpatients. To address this issue, we used data from a cohort study to examine the implementation of nutritional risk screening. We also assessed the prevalence, determinants, and associations between nutritional status and hospital length of stay (LoS), mortality, and readmission.

METHODS

Participants and Data Collection

Participants were inpatients aged 65 years and older from an ongoing, prospective, large-scale cohort study of elder patients hospitalized in tertiary hospitals in China (Eastern: Zhejiang Province; South-Central: Hubei Province; southwest: Sichuan

Abbreviations: MNA-SF, Mini Nutritional Assessment Short-Form; BMI, body mass index; SD, standard deviation; CI, confidence interval; ICU, intensive care unit; CRF, Case Report Form.

Province; Northeast: Heilongjiang Province; Northern: Beijing municipality/city; Northwest: Qinghai Province). Details can be found elsewhere (14, 32). Eligible study subjects are recruited from neurology, surgical, orthopedics departments, intensive care unit (ICU), as well as internal medicine of selected hospitals, are consecutively enrolled. The surveys are managed by trained nurses using a structured Case Report Form (CRF). Our research team developed manuals for the project survey and operation process. To ensure data quality, the nurses received training and test before they apply the assessment to the patients. All CRF results were reviewed by the head nurse. The research team also developed a quality control team, and a communication platform based on the WeChat App to guarantee timely feedback. If the participant is unable to answer questions on his or her own, proxy interviewees (usually spouses or other legal guardians) are interviewed.

The current study was based on baseline survey data collected from October 2018 to February 2019. During this period, there were 9,996 hospitalizations; of these, 8,326 patients had complete data at 90-day follow-up, and 5,516 had an LoS of at least 2 days or had initial biochemical data during hospitalization. Thus, the study subjects included a total of 5,516 patients aged 65 years and older.

Measurements

Nutritional status was assessed using the MNA-SF, a six-item instrument with scores ranging from 0 to 14 points (19). The MNA-SF has been validated and shows good specificity and sensitivity for the diagnosis of malnutrition, mainly in older adults (10, 29). For the purpose of our study, participants were categorized into a group with normal nutritional status (12–14 points), the patient at risk of malnutrition (8–11 points), or malnourished patients (0–7 points) (33). The MNA-SF has been verified in the Chinese elderly and has extraordinary test characteristics (14, 34).

Participants' height (cm) was measured to the nearest 1 mm using a stadiometer, while weight (kg) was measured to the nearest 0.1 kg using a digital electronic chair scale. Patients were weighed while wearing light clothing and without shoes. Considering most of the ICU patients were immobility, the body weight and height were recorded when they were first admitted to the general ward, and this data can be synchronized to the ICU nursing information system. Therefore, our research team record their body weight and height according to the information system. Body mass index (BMI) was calculated as body weight divided by height (in meters) squared (kg/m^2) (35) and was used to classify patients of underweight ($<18.5\ kg/m^2$), normal (18.5–23.9 kg/m^2), overweight (24–27.9) kg/m^2), and obese greater or equal to 28 kg/m^2) (36, 37).

The following parameters were also analyzed: age, alcohol consumption, smoking (former smokers refer to at least 6 months without smoking), sex, education level, ethnicity, and marital status. All biochemical parameters included in the analysis (such as hemoglobin, serum albumin, blood urea nitrogen, and creatinine) were the first determinations during patients' respective hospitalizations (21).

Measured Outcomes

The following outcomes were measured: death (record all-cause mortality within 90 days, which consists of in-hospital deaths), LoS (duration of hospitalization) (38), and nonelective readmission (second and subsequent hospitalizations during the period analyzed) during the 90 days following discharge.

Bioethics

This study was ethically approved by the review board of Peking Union Medical College Hospital (S-K540). All patients participating in this study provided written informed consent. If patients had cognitive decline, the investigator interviewed their proxy respondents and obtained their consent for the patient to participate in this study. Patients were excluded if they were persistently unconscious, or if their proxy respondents were unable to provide effective information.

Statistical Analyses

Statistical analysis using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). Continuous variables are summarized as mean and standard deviation (SD). Categorical variables were summarized as counts and percentages. Bivariate analyses were performed using the χ^2 test or Fisher's exact test for qualitative variables and the Student *t*-test, analysis of variance, or Kruskal–Wallis test for quantitative variables. We ran separate models for each parameter of nutritional status in all analyses, owing to the collinearity between them. Both logistic and negative binomial regression analyses were conducted. The strength of the associations was estimated as odds ratios (ORs) for the logistic models, and as the regression coefficient for negative binomial regression, with 95% confidence intervals (95% CIs). A *P*-value of <0.05 was considered statistically significant.

RESULTS

Participant Characteristics

In **Table 1**, the mean age of the included patients was 72.47 ± 5.77 years at baseline, and 59.77% of participants (3,297/5,516) were men. Approximately 54% of patients had a middle school education and above, and 90.1% were married. A major proportion of respondents were of Han nationality (94.65%). More than half of the participants were non-smokers (65.07%) and non-drinkers (74.85%). The average BMI was 23.56 ± 3.52 kg/m^2, and approximately half (49.02%) had a BMI between 18.5 and 23.9 kg/m^2. 40.08% of patients were from medicine departments and only 5.73% of patients were from ICU. According to the MNA-SF, 46.19% (2,548/5,516) of hospitalized individuals were at risk of malnutrition or malnourishment (**Table 2**). Patients who were malnourished were with higher mortality and 90-day readmission, and longer LoS. 11.89% (656/5,516) of malnourished patients had low serum albumin [cut-off 35 g/L for serum albumin levels (19)] compared with the well-nourished patients or patients at risk of malnutrition. The remaining data for hospitalizations analyzed according to MNA-SF values for malnutrition, malnutrition risk, or normal, are presented in **Tables 1**, **2**.

TABLE 1 | Demographic and clinical characteristics of patients in relation to MNA-SF score on admission.

Parameter	Cases (*N* = 5,516)
Age (years)	
Age ranges	72.47 ± 5.77
<75	3,799 (68.87)
≥75	1,717 (31.13)
Sex (*n*, %)	
Male gender	3,297 (59.77)
Female gender	2,219 (40.23)
Education (*n*, %) (*n* = 5,514)	
University	754 (13.67)
Middle school	2,203 (39.95)
Primary school	1,644 (29.82)
Illiterate	913 (16.56)
Marital status (*n*, %) (*n* = 5,510)	
Married	4,964 (90.1)
Divorced or widowed	546 (9.91)
Ethnicity (*n*, %)	
Han	5221 (94.65)
Others	295 (5.35)
Smoking (*n*, %)	
Current smoker	641 (11.62)
Former smoker	1,286 (23.31)
Non-smoker	3,589 (65.07)
Alcohol consumption (*n*, %)	
Current drinker	686 (12.44)
Former drinker	701 (12.71)
Non-drinker	4,129 (74.85)
BMI (kg/m^2)	23.56 ± 3.52
BMI ranges (*n*, %) (*n* = 5,447)	
<18.5	418 (7.67)
18.5–23.9	2,670 (49.02)
24–27.9	1,835 (33.69)
≥28	524 (9.62)
Death (*n*, %) (*n* = 5215)	180 (3.45)
Length of hospital stay (days)	9.79 ± 7.56
Number of non-scheduled rehospitalizations 90 days after discharge (*n*, %) (*n* = 5139)	742 (14.44)
Initial biochemical data	
Hemoglobin (g/l)	122.68 ± 23.86
Serum albumin (g/l)	36.48 ± 7.48
Blood urea nitrogen (BUN, mmol/l)	7.37 ± 18.19
Creatinine (μmol/l)	87.38 ± 94.60
Department (*n*, 100%)	
Surgical	1,978 (35.86)
Medicine	2,211 (40.08)
Neurology	511 (9.26)
Orthopedics	500 (9.06)
ICU	316 (5.73)
Province or municipality/city (*n*, 100%)	
Sichuan province	1,584 (28.72)
Heilongjiang province	287 (5.20)
Hubei province	836 (15.16)
Beijing municipality/city	742 (13.45)
Qinghai province	758 (13.74)
Zhejiang province	1,309 (23.73)

Data presented as mean ± standard deviation or frequency (%), as appropriate.
Some data were missing in the calculations.
MNA-SF, Mini Nutritional Assessment Short-Form; BMI, body mass index.

TABLE 2 | Data for hospitalizations, analyzed according to MNA-SF score.

Parameter	Malnourished (*N* = 656)	Malnutrition risk (*N* = 1892)	Normal (*N* = 2968)	*P*-Value
Age (years)	73.34 ± 6.02	73.16 ± 6.01	72.18 ± 5.66	<0.001
Age ranges				<0.001
<75	413 (62.96)	1,248 (65.96)	2,138 (72.04)	
≥75	243 (37.04)	644 (34.04)	830 (27.96)	
Sex (*n*, %)				<0.001
Male gender	358 (54.57)	1,101 (58.19)	1,838 (61.93)	
Female gender	298 (45.43)	791 (41.81)	1,130 (38.07)	
Education (*n*, %) (*n* = 5,514)				<0.001
University	76 (11.62)	219 (11.58)	459 (15.46)	
Middle school	223 (34.10)	708 (37.42)	1,272 (42.86)	
Primary school	186 (28.44)	615 (32.51)	843 (28.40)	
Illiterate	169 (25.84)	350 (18.50)	394 (13.27)	
Marital status (*n*, %) (*n* = 5510)				<0.001
Married	568 (86.98)	1,652 (87.41)	2,744 (92.48)	
Divorced or widowed	85 (13.02)	238 (12.59)	223 (7.52)	
Ethnicity (*n*, %)				<0.001
Han	598 (91.16)	1,776 (93.87)	2,847 (95.92)	
Others	58 (8.84)	116 (6.13)	121 (4.08)	
Smoking (*n*, %)				<0.05
Current smoker	53 (8.08)	239 (12.63)	349 (11.76)	
Former smoker	144 (21.95)	425 (22.46)	717 (24.16)	
Non-smoker	459 (69.97)	1,228 (64.90)	1,902 (64.08)	
Alcohol consumption (*n*, %)				0.06
Current drinker	51 (7.77)	209 (11.05)	426 (14.35)	
Former drinker	85 (12.96)	265 (14.01)	351 (11.83)	
Non-drinker	520 (79.27)	1,418 (74.95)	2,191 (73.82)	
BMI (kg/m^2)	20.47 ± 3.28	22.05 ± 3.25	24.97 ± 2.94	<0.001
BMI ranges (*n*, %) (*n* = 5447)				0.001
<18.5	187 (29.92)	231 (12.42)	0	
18.5–23.9	345 (55.20)	1,179 (63.39)	1,146 (38.69)	
24–27.9	78 (12.48)	361 (19.41)	1,396 (47.13)	
≥28	15 (2.40)	89 (4.78)	420 (14.18)	
Death (*n*, %) (*n* = 5215)	61 (10.29)	84 (4.72)	35 (1.23)	<0.001
Length of hospital stay (days)	12.58 ± 9.25	11.52 ± 8.54	9.87 ± 7.49	<0.001
Number of non-scheduled rehospitalizations 90 days after discharge (*n*, %) (*n* = 5139)	106 (18.76)	295 (16.92)	341 (12.05)	<0.001
Initial biochemical data				
Hemoglobin (g/l)	112.36 ± 25.89	120.27 ± 24.44	126.51 ± 22.08	<0.001
Serum albumin (g/l)	34.37 ± 7.26	35.96 ± 7.07	37.29 ± 7.65	<0.001
Blood urea nitrogen (BUN, mmol/l)	8.40 ± 18.76	7.54 ± 21.20	7.04 ± 15.83	0.41
Creatinine (μmol/l)	91.74 ± 100.79	91.66 ± 105.22	83.69 ± 85.46	<0.001

Data presented as mean ± standard deviation or frequency (%), as appropriate.
Some data were missing in the calculations.
MNA-SF, Mini Nutritional Assessment Short-Form; BMI, body mass index.

Mortality

Table 1 displays mortality among the study participants. Overall, death occurred in 3.45% of study participants. In **Table 2**, the number of patients who died who were malnourished, at risk of malnutrition, and normal was 61 (10.29%), 84 (4.72%),

and 35 (1.23%), respectively ($P < 0.001$). MNA-SF scores 0–7 (OR 5.738, 95% CI 3.473 to 9.48; $P < 0.001$) were associated with a six-fold higher likelihood of death and scores 8–11 (OR 3.283, 95% CI 2.126–5.069) with a three-fold higher likelihood of death, compared with MNA-SF scores 12–14 in the logistic regression model, after adjusting for potential confounders (**Table 3**). However, hemoglobin (OR 0.992, 95% CI 0.985–0.998; $P < 0.05$) and serum albumin (OR 0.927, 95% CI 0.902–0.951; $P < 0.001$) had a lower likelihood of increased mortality. Additionally, female patients had a lower likelihood of death than male patients (OR 0.646, 95% CI 0.433–0.962; $P < 0.05$). A higher level of education (OR 0.484, 95% CI 0.247–0.947; $P < 0.05$) was statistically associated with a decline in the risk of death, in comparison with illiterate patients.

Length of Stay (LoS)

Table 2 also indicates the LoS among the patients, with an average LoS in the group with MNA-SF scores 0–7 of 12.58 ± 9.25 days, 11.52 ± 8.54 days in the group with MNA-SF scores 8–11, and 9.87 ± 7.49 in the group with MNA-SF scores 12–14. In **Table 3**, after adjusting for potential confounders in the negative binomial regression model, a low MNA-SF score of 0–7 (regression coefficient 0.2807, 95% CI 0.0294–0.5320; $P < 0.05$) and a score of 8–11 (0.2574, 95% CI 0.0863–0.4285; $P < 0.01$) was associated with a significantly (28.07 and 25.74%, respectively) higher likelihood of increased LoS compared with an MNA-SF score 12–14. Additionally, compared with nonsmokers, former smokers had a 40.41% (0.4041, 95% CI 0.1994–0.6088; $P < 0.001$) higher likelihood of increased LoS, whereas there was no significance among current smokers. However, BMI 24–27.9 kg/m^2 (−0.2737, 95% CI −0.4558 to −0.0916; $P < 0.01$) and hemoglobin (−0.0081, 95% CI −0.0114 to −0.0047; $P < 0.001$) had a lower likelihood of increased LoS. Age 75 years and above (−0.2592, 95% CI −0.4294 to −0.0891; $P < 0.01$) and current alcohol consumption (−0.5579, 95% CI −0.8452 to −0.2706; $P < 0.001$) were also protective factors.

Ninety-Day Readmission

Table 1 also shows that 742 participants (14.44%) had unscheduled rehospitalization 90 days after discharge. In **Table 2**, the number of patients with malnutrition, malnutrition risk, and normal nutritional status who had 90-day readmission was 106 (18.76%), 295 (16.92%), and 341 (12.05%), respectively ($P<0.001$). In **Table 3**, MNA-SF scores 0–7 (OR 1.393, 95% CI 1.052–1.843) and 8–11 (OR 1.356, 95% CI 1.124–1.636) were associated with a nearly 1.5-fold higher likelihood of 90-day readmission, compared with MNA-SF scores 12–14 scores in the logistic regression model, after adjusting for potential confounders. Additionally, former smokers (OR 1.632, 95% CI 1.301–2.047; $P < 0.001$) had a higher risk of 90-day readmission than nonsmokers. However, BMI 24–27.9 kg/m^2 (OR 0.723, 95% CI 0.594–0.881; $P < 0.05$), hemoglobin (OR 0.99, 95% CI 0.986–0.993; $P < 0.001$), age 75 years and above (OR 0.733, 95% CI 0.609–0.882; $P < 0.01$), and current drinking (OR 0.52, 95% CI 0.383–0.707; $P < 0.001$) were significantly associated with a lower risk of 90-day readmission in the multivariate model.

DISCUSSION

This study is among the first to examine the association between nutritional status and LoS, mortality, and readmission in a nationally representative sample of Chinese elder inpatients of tertiary hospitals. We found malnutrition (MNA-SF score 0–7) in 11.89% of all older hospitalized patients, and low MNA-SF scores were associated with increased average LoS, a greater likelihood of death, and 90-day readmission.

Studies are scarce in China that explore the associations between nutritional status and health outcomes among older inpatients. Adigüzel et al. (39) indicated that nutritional parameters and sociodemographic features could affect health-related quality of life and functional ability among home care patients in Turkey. A study conducted in Germany also suggested that malnutrition could increase the risk of morbidity, mortality, hospital LoS, and costs among surgical patients (22). Additionally, Valmorbida et al. (19) suggested that malnutrition is associated with an increased risk of hospital admission and death among the 144 Italian older adults. Therefore, these studies are in accordance with our results and emphasize the urgent need for physicians, nurses, and clinical institutions to be aware of the high prevalence of malnutrition in the elderly, periodic nutritional screening of geriatric inpatients is critical.

Malnutrition is a crucial condition among older inpatients, both as a cause and consequence of disease (14, 40). The prevalence of malnutrition is 46.19% in tertiary hospitals of China, which is higher than the results of a cross-sectional study conducted in nursing homes of Spain reporting that 40.1% of older residents had malnutrition or risk of malnutrition, as measured using the Mini Nutritional Assessment (MNA®) (41). Previous research has indicated that 38.2% of nursing home residents in Italy are malnourished or at risk of malnutrition according to the MNA-SF (19), and the prevalence of malnutrition and malnutrition risk among older patients with hip fracture in Israel ranges from 55.81% using the MNA-SF (10) to 56.59% (medium risk and nutritionally at risk, measured using the NRS-2002) in Hyogo, Japan (42). These differences are likely owing to a variety of factors, including differences in the nutritional assessment tools used, study participants, study design, study periods, and local factors, such as the health systems in different countries and the standards of medical treatment received (10, 15, 19, 21, 43).

In comparison with well-nourished patients, malnourished older patients in our study had higher mortality (OR 5.738, 95% CI 3.473–9.48; 10.29% vs. 1.23%), longer LoS (0.2807, 95% CI 0.0294–0.5320; $P < 0.05$; 12.58 ± 9.25 vs. 9.87 ± 7.49) and were more likely to be readmitted within 90 days (OR 1.393, 95% CI 1.052–1.843; 18.76% vs. 12.05%). In line with our results, Lim et al. (31) suggested that approximately 29% of malnourished Singaporean patients had a longer hospital stay (6.9 ± 7.3 days vs. 4.6 ± 5.6 days, $P < 0.001$) and were more likely to be readmitted within 15 days (adjusted relative risk = 1.9, 95% CI 1.1–3.2, P=0.025) than well-nourished patients (31). Agarwal et al. (44) suggested that the 90-day in-hospital mortality in malnourished patients was around 1.09–3.34 times that of well-nourished patients, readmission

TABLE 3 | Prevalence of measured outcomes in relation to nutritional status and clinical parameters analyzed.

Parameter which acted as a risk factor for measured outcome	Death[a] Odds ratio, (95% CI)	Length of hospital stay[b] Coefficients (95% CI)	90-day readmission[a] Odds ratio, (95% CI)
Malnourished (0–7)	5.738 (3.473, 9.48)***	0.2807 (0.0294, 0.5320)*	1.393 (1.052, 1.843)
Malnutrition risk (8–11)	3.283 (2.126, 5.069)	0.2574 (0.0863, 0.4285)**	1.356 (1.124, 1.636)
Normal (12-14)	1.0 (Ref.)	0 (Ref.)	1.0 (Ref.)
BMI			
<18.5	1.448 (0.939, 2.233)	−0.2317 (−0.5197, 0.0562)	0.747 (0.543, 1.027)
18.5–23.9	1.0 (Ref.)	0 (Ref.)	1.0 (Ref.)
24–27.9	1.254 (0.837, 1.878)	−0.2737 (−0.4558, −0.0916)**	0.723 (0.594, 0.881)*
≥28	0.877 (0.392, 1.96)	−0.0185 (−0.2926, 0.2556)	0.974 (0.723, 1.313)
Hemoglobin (mg/dl)	0.992 (0.985, 0.998)*	−0.0081 (−0.0114, −0.0047)***	0.99 (0.986, 0.993)***
Serum albumin (g/l)	0.927 (0.902, 0.951)***	−0.0051 (−0.0164, 0.0061)	0.994 (0.982, 1.007)
Blood urea nitrogen (BUN, mg/dl)	1.004 (0.998, 1.01)	−0.0003 (−0.0021, 0.0015)	1.001 (0.997, 1.005)
Creatinine (μmol/l)	1.001 (1.000, 1.002)	0.0002 (−0.0005, 0.0009)	1.000 (0.999, 1.001)
Age (years)			
<75	1.0 (Ref.)	0 (Ref.)	1.0 (Ref.)
≥75	1.336 (0.962, 1.854)	−0.2592 (−0.4294, −0.0891)**	0.733 (0.609, 0.882)**
Sex			
Male gender	1.0 (Ref.)	0 (Ref.)	1.0 (Ref.)
Female gender	0.646 (0.433, 0.962)*	−0.1262 (−0.3243, 0.0718)	0.848 (0.683, 1.053)
Education			
University	0.484 (0.247, 0.947)*	−0.2416 (−0.5324, 0.0491)	0.746 (0.544, 1.024)
Middle school	0.846 (0.538, 1.328)	−0.0275 (−0.2463, 0.1914)	0.968 (0.76, 1.232)
Primary school	0.953 (0.606, 1.5)	−0.1392 (−0.3686, 0.0901)	0.841 (0.654, 1.082)
Illiterate	1.0 (Ref.)	0 (Ref.)	1.0 (Ref.)
Marital status			
Married	1.0 (Ref.)	0 (Ref.)	1.0 (Ref.)
Divorced or widowed	0.844 (0.496, 1.437)	−0.2380 (−0.5286, 0.0525)	0.765 (0.56, 1.044)
Ethnicity			
Han	1.0 (Ref.)	0 (Ref.)	1.0 (Ref.)
Others	1.041 (0.544, 1.991)	0.1661 (−0.1600, 0.4922)	1.246 (0.868, 1.788)
Smoking			
Current smoker	0.748 (0.411, 1.361)	0.0606 (−0.2119, 0.3331)	1.079 (0.802, 1.452)
Former smoker	0.993 (0.645, 1.529)	0.4041 (0.1994, 0.6088)***	1.632 (1.301, 2.047)***
Non-smoker	1.0 (Ref.)	0 (Ref.)	1.0 (Ref.)
Alcohol drinking			
Current drinker	0.54 (0.287, 1.018)	−0.5579 (−0.8452, −0.2706)***	0.52 (0.383, 0.707)***
Former drinker	0.89 (0.541, 1.463)	0.0534 (−0.1656, 0.2724)	1.072 (0.838, 1.371)
Non-drinker	1.0 (Ref.)	0 (Ref.)	1.0 (Ref.)

$^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

[a]Logistic regression models.

[b]Negative binomial regression model.

BMI, body mass index; CI, confidence interval.

rates and mean LoS among the malnourished patients were 36% and 15 days, respectively. These differences might result from the demographic characteristics of study participants and different follow-up periods after discharge. These findings confirm that early and compulsory nutritional assessment to identify malnutrition or malnutrition risk in elder inpatients is critical and may help in targeting interventions to tackle malnutrition (16).

Despite the association between malnutrition assessed by the MNA-SF and adverse clinical outcomes, hemoglobin level was associated with the hospital LoS, mortality, and 90-day readmission, after adjusting for confounders in our sample. These results are in line with the current literature (45–47) that low hemoglobin is associated with adverse clinical outcomes among older adults. Moreover, previous research indicates that the Geriatric Nutrition Risk Index (GNRI) (42) combined with

biochemical objective indicators, such as albumin, can be used to identify and diagnose malnutrition and can better predict 30-day mortality rate in older patients (48). In addition, Hong et al. (49) and Pérez-Ros et al. (50) indicated that compared with the robust, levels of hemoglobin showed lower values among the prefrail and frail group, and nutritional markers may be used for the evaluation of adverse clinical outcomes in older patients. Therefore, our study suggests that the MNA-SF may be used in combination with hemoglobin levels to predict clinical outcomes among older inpatients. Although further evaluations are needed to clarify this, we believe that such findings may have concrete, helpful implications in the clinical practice.

Serum albumin is a protective factor of death among this study population. Only 11.89% of older inpatients had low serum albumin [cut-off of 35 g/L (19)] in our sample. Besides, Bouillanne et al. suggested that serum albumin lower than 35 g/L was associated with a higher risk of death (51). Therefore, during nutritional assessment in older inpatients, we might take albumin values into consideration, which may inform prognosis and hospital services planning.

Concerning BMI <18.5 kg/m^2 as an indicator of undernutrition, we did not find significant associations with LoS, mortality or readmission, which differs from reports that individuals with BMI <25 kg/m^2 had a higher risk of death than those who were overweight or obese (19). Interestingly, among BMI categories, only BMI 24–27.9 kg/m^2 was significantly associated with a lower LoS and risk of 90-day readmission than BMI 18.5–23.9 kg/m^2. This can hypothetically indicate that older overweight inpatients have significantly higher muscle mass than their normal counterparts. Thus, characteristics related to overweight and higher muscle mass could introduce a positive association for LoS and readmission (52). However, other relevant clinical data were lacking, including muscle mass in older inpatients; therefore, prospective studies with more sophisticated assessments are needed to confirm our findings.

In addition to the nutritionally related parameters assessed, former smokers were associated with a 40.41% higher likelihood of increased LoS and 90-day readmission than nonsmokers, which further supports previous research findings (14, 53, 54). However, current alcohol consumption and age $\geq$75 years were protective factors for LoS and readmission. This can possibly be explained by evidence that older people who consume small to moderate amounts of alcohol [1–25 g/day (55)] are more likely to maintain functional status and mobility than nondrinkers (32, 56); limited alcohol consumption has been associated with a decreased risk of adverse clinical outcomes (57). Another possible explanation is that compared with inpatients aged 75 years and above, those younger than 75 years may be able to withstand surgical stresses, and underwent more surgical treatment, which increases the risk of medical complications and longer LoS (58, 59).

Older female inpatients had a lower likelihood of death than male inpatients, and higher education level was significantly associated with a lower mortality risk than low education levels. The clinical phenomenon that female individuals survive longer than male adults is well described (57, 60), and well-educated older people may better comprehend the importance of nutrient intake, daily exercise, and disease prevention (61, 62).

The main limitations of our study include the limited follow-up; investigations with a longer duration are needed to better clarify the present findings. A further limitation of this paper is not having assessed all nutritional parameters over time; we only used the MNA-SF with a few anthropometric measures and limited biochemical data. Moreover, the older inpatients enrolled in our study were all from tertiary hospitals and only one hospital in each province or municipality/city, patients may have more complex clinical features, which may limit the generalizability of our results to different settings. Additionally, patients are taken from multiple hospitals or wards with differing baselines, the severity of the comorbidity along with different characteristics of the patients may influence the mortality rate and length of stay. The patients enrolled in this study were relatively young, 68.87% of the patients were $<$75 years old, which also limited the generalizability of this study. ICU patients have a higher mortality rate compared to general wards, it is meaningful to explore the nutritional status and adverse clinical outcomes among the ICU older inpatients. We are going to conduct this study in the future. Besides, our use of a limited number of nutritional screening tools restricted the comparison of our results with those of other studies, and the nutritional status was analyzed using the original MNA-SF which was derived based on Western populations, whereas the recent studies conducted in Taiwan of China have resulted in a modified MNA-SF, we will use this modified MNA-SF for further study (63, 64). Finally, the study participants were hospitalized in multiple wards or departments; we did not analyze the medical and nursing care received by patients, nor did we analyze the reason for the hospital admission. The patients enrolled in the present study were older adults from surgical, neurology, orthopedics departments, internal medicine, and ICU of selected hospitals, thus there would be great differences in nutritional status among these participants who have these health conditions, have chronic diseases, or undergoing surgery could be associated with poor nutritional status among hospitalized older people. To be meaningful, we will learn from previous research and results will be analyzed according to the like-kind of patients only (65, 66). Chronic diseases including cancer, diabetes, and cardiovascular diseases could be associated with poor nutritional status among elder patients, whereas we did not analyze the impact of chronic diseases in this study. There is a lack of other relevant clinical data, including albumin data, comorbidities, clinical severity of disease, comprehensive geriatric assessment, sarcopenia, the fat fold, upper arm circumference, and thigh circumference, because these features were not recorded in the first place, which is an inherent drawback of retrospective analysis of a prospectively collected database. Prospective studies with more sophisticated assessments are needed in the future.

CONCLUSIONS

The findings of this study suggested that malnutrition increases the risk of hospital LoS, mortality, and 90-day

readmission among inpatients, in comparison with well-nourished inpatients. Use of nutritional assessment tools in all hospitalized Chinese patients is needed. The MNA-SF in combination with hemoglobin level may be used to identify a high risk of adverse clinical outcomes among older inpatients and may be a good predictor of patient outcomes. These findings could have major importance for the planning of hospital services, discharge planning, and post-discharge care.

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 Peking Union Medical College Hospital (S-K540). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XWu: study concept and design. HL, JJia, and TX: analysis and interpretation of data. HL: editing of the manuscript and drafting of tables. XWu and HL: critical review of the manuscript for important intellectual content. MZ, XWe, JJin, HW, DL, XS, and SZ: patient recruitment, data collection, and manuscript editing. All authors critically reviewed and approved the manuscript before it was submitted.

FUNDING

The Special Research Fund for Central Universities, Peking Union Medical College [Grant Number 2018PT33001] supported this study but had no role in study design or data collection, analysis, and interpretation, or manuscript conception and writing.

ACKNOWLEDGMENTS

The authors are grateful to all the persons who participated in the collection of the data. We thank Analisa Avila, ELS, of Liwen Bianji, for editing the English text of a draft of this paper.

REFERENCES

1. The L: global elderly care in crisis. *Lancet.* (2014) 383:927. doi: 10.1016/S0140-6736(14)60463-3
2. Bloom DE, Luca DL: Chapter 1—the global demography of aging: facts, explanations, future. In: Piggott J, Woodland A, editors. *Handbook of the Economics of Population Aging. vol. 1: North-Holland.* (2016). p. 3–56. doi: 10.1016/bs.hespa.2016.06.002
3. Liu H, Jiao J, Zhu M, Wen X, Jin J, Wang H, et al. An early predictive model of frailty for older inpatients according to nutritional risk: protocol for a cohort study in China. *BMC Geriatr.* (2021) 21:465. doi: 10.1186/s12877-021-02396-3
4. Søreide K, Wijnhoven BP. Surgery for an ageing population. *Br J Surg.* (2016) 103:e7–9. doi: 10.1002/bjs.10071
5. The L: how to cope with an ageing population. *Lancet.* (2013) 382. doi: 10.1016/S0140-6736(13)62080-2
6. Lv X, Li W, Ma Y, Chen H, Zeng Y, Yu X, et al. Cognitive decline and mortality among community-dwelling Chinese older people. *BMC Med.* (2019) 17:63. doi: 10.1186/s12916-019-1295-8
7. Fang EF, Scheibye-Knudsen M, Jahn HJ Li J, Ling L, Guo H, Zhu X, et al. A research agenda for aging in China in the 21st century. *Ageing Res Rev.* (2015) 24:197–205. doi: 10.1016/j.arr.2015.08.003
8. The L: ageing and health—an agenda half completed. *Lancet.* (2015) 386. doi: 10.1016/S0140-6736(15)00521-8
9. Kelaiditi E, Demougeot L, Lilamand M, Guyonnet S, Vellas B, Cesari M. Nutritional status and the incidence of pneumonia in nursing home residents: results from the INCUR study. *J Am Med Dir Assoc.* (2014) 15:588–92. doi: 10.1016/j.jamda.2014.04.012
10. Koren-Hakim T, Weiss A, Hershkovitz A, Otzrateni I, Anbar R, Gross Nevo RF, et al. Comparing the adequacy of the MNA-SF, NRS-2002 and MUST nutritional tools in assessing malnutrition in hip fracture operated elderly patients. *Clin Nutr.* (2016) 35:1053–8. doi: 10.1016/j.clnu.2015.07.014
11. Martínez-Reig M, Gómez-Arnedo L, Alfonso-Silguero SA, Juncos-Martínez G, Romero L, Abizanda P. Nutritional risk, nutritional status and incident disability in older adults. *FRADEA study J Nutr Health Aging.* (2014) 18:270–6. doi: 10.1007/s12603-013-0388-x
12. Inzitari M, Doets E, Bartali B, Benetou V, Di Bari M, Visser M, et al. Nutrition in the age-related disablement process. *J Nutr Health Aging.* (2011) 15:599–604. doi: 10.1007/s12603-011-0053-1
13. Vanderwee K, Clays E, Bocquaert I, Gobert M, Folens B, Defloor T. Malnutrition and associated factors in elderly hospital patients: a Belgian cross-sectional, multi-centre study. *Clin Nutr.* (2010) 29:469–76. doi: 10.1016/j.clnu.2009.12.013
14. Liu H, Jiao J, Zhu C, Zhu M, Wen X, Jin J, Wang H, Lv D, Zhao S, Wu X et al. Associations between nutritional status, sociodemographic characteristics, and health-related variables and health-related quality of life among Chinese elderly patients: a multicenter prospective study. *Front Nutr.* (2020) 7:583161. doi: 10.3389/fnut.2020.583161
15. Khalatbari-Soltani S, Marques-Vidal P. Impact of nutritional risk screening in hospitalized patients on management, outcome and costs: a retrospective study. *Clin Nutr.* (2016) 35:1340–6. doi: 10.1016/j.clnu.2016.02.012
16. Kondrup J, Allison SP, Elia M, Vellas B, Plauth M. ESPEN guidelines for nutrition screening 2002. *Clin Nutr.* (2003) 22:415–21. doi: 10.1016/S0261-5614(03)00098-0
17. Jie B, Jiang ZM, Nolan MT, Efron DT, Zhu SN Yu K, Kondrup J. Impact of nutritional support on clinical outcome in patients at nutritional risk: a multicenter, prospective cohort study in Baltimore and Beijing teaching hospitals. *Nutrition.* (2010) 26:1088–93. doi: 10.1016/j.nut.2009.08.027
18. Liu H, Zhu D, Cao J, Jiao J, Song B, Jin J, et al. The effects of a standardized nursing intervention model on immobile patients with stroke: a multicenter study in China. *Eur J Cardiovasc Nurs.* (2019) 18:753–63. doi: 10.1177/1474515119872850
19. Valmorbida E, Trevisan C, Imoscopi A, Mazzochin M, Manzato E, Sergi G. Malnutrition is associated with increased risk of hospital admission and death in the first 18 months of institutionalization. *Clin Nutr.* (2020) 39:3687–94. doi: 10.1016/j.clnu.2020.03.029
20. Marques-Vidal P, Khalatbari-Soltani S, Sahli S, Coti Bertrand P, Pralong F, Waeber G. Undernutrition is associated with increased financial losses in hospitals. *Clin Nutr.* (2018) 37:681–6. doi: 10.1016/j.clnu.2017.02.012
21. Budzynski J, Tojek K, Czerniak B, Banaszkiewicz Z. Scores of nutritional risk and parameters of nutritional status assessment as predictors of in-hospital mortality and readmissions in the general hospital population. *Clin Nutr.* (2016) 35:1464–71. doi: 10.1016/j.clnu.2016.03.025
22. Thomas MN, Kufeldt J, Kisser U, Hornung HM, Hoffmann J, Andraschko M, et al. Effects of malnutrition on complication rates, length of hospital stay, and revenue in elective surgical patients in the G-DRG-system. *Nutrition.* (2016) 32:249–54. doi: 10.1016/j.nut.2015.08.021

23. Schiesser M, Muller S, Kirchhoff P, Breitenstein S, Schafer M, Clavien PA. Assessment of a novel screening score for nutritional risk in predicting complications in gastro-intestinal surgery. *Clin Nutr.* (2008) 27:565–70. doi: 10.1016/j.clnu.2008.01.010
24. Rasheed S, Woods RT. An investigation into the association between nutritional status and quality of life in older people admitted to hospital. *J Human Nutr Diet.* (2014) 27:142–51. doi: 10.1111/jhn.12072
25. Kostka J, Borowiak E, Kostka T. Nutritional status and quality of life in different populations of older people in Poland. *Eur J Clin Nutr.* (2014) 68:1210–5. doi: 10.1038/ejcn.2014.172
26. Jimenez-Redondo S, Beltran de. Miguel B, Gavidia Banegas J, Guzman Mercedes L, Gomez-Pavon J, Cuadrado Vives C: Influence of nutritional status on health-related quality of life of non-institutionalized older people. *J Nutr Health Aging.* (2014) 18:359–64. doi: 10.1007/s12603-013-0416-x
27. Eriksson BG, Dey DK, Hessler RM, Steen G, Steen B. Relationship between MNA and SF-36 in a free-living elderly population aged 70 to 75. *J Nutr Health Aging.* (2005) 9:212–20.
28. Cederholm T, Barazzoni R, Austin P, Ballmer P, Biolo G, Bischoff SC, et al. ESPEN guidelines on definitions and terminology of Clin Nutr. *Clin Nutr.* (2017) 36:49–64. doi: 10.1016/j.clnu.2016.09.004
29. Fernández-Barrés S, García-Barco M, Basora J, Martínez T, Pedret R, Arija V. The efficacy of a nutrition education intervention to prevent risk of malnutrition for dependent elderly patients receiving Home Care: A randomized controlled trial. *Int J Nurs Stud.* (2017) 70:131–41. doi: 10.1016/j.ijnurstu.2017.02.020
30. Guigoz Y, Vellas B, Garry PJ. Assessing the nutritional status of the elderly: The Mini Nutritional Assessment as part of the geriatric evaluation. *Nutr Rev.* (1996) 54:S59–65. doi: 10.1111/j.1753-4887.1996.tb03793.x
31. Lim SL, Ong KCB, Chan YH, Loke WC, Ferguson M, Daniels L. Malnutrition and its impact on cost of hospitalization, length of stay, readmission and 3-year mortality. *Clin Nutr.* (2012) 31:345–50. doi: 10.1016/j.clnu.2011.11.001
32. Liu H, Jiao J, Zhu C, Zhu M, Wen X, Jin J, et al. Potential associated factors of functional disability in Chinese older inpatients: a multicenter cross-sectional study. *BMC Geriatr.* (2020) 20:319. doi: 10.1186/s12877-020-01738-x
33. Verlaan S, Aspray TJ, Bauer JM, Cederholm T, Hemsworth J, Hill TR, et al. Nutritional status, body composition, and quality of life in community-dwelling sarcopenic and non-sarcopenic older adults: A case-control study. *Clin Nutr.* (2017) 36:267–74. doi: 10.1016/j.clnu.2015.11.013
34. Lei Z, Qingyi D, Feng G, Chen W, Hock RS, Changli W. Clinical study of mini-nutritional assessment for older Chinese inpatients. *J Nutr Health Aging.* (2009) 13:871–5. doi: 10.1007/s12603-009-0244-1
35. Weir CB, Jan A. BMI classification percentile and cut off points. In: *StatPearls.* (2019). Treasure Island (FL).
36. Zhou BF. Effect of body mass index on all-cause mortality and incidence of cardiovascular diseases–report for meta-analysis of prospective studies open optimal cut-off points of body mass index in Chinese adults. *Biomed Environ Sci.* (2002) 15:245–52.
37. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser.* (2000) 894:i–xii, 1–253.
38. Liu H, Song B, Jin J, Liu Y, Wen X, Cheng S, Nicholas S, Maitland E, Wu X, Zhu D. Length of stay, hospital costs and mortality associated with comorbidity according to the charlson comorbidity index in immobile patients after ischemic stroke in China: a national study. *Int J Health Policy Manag.* (2021) 1–8. doi: 10.34172/ijhpm.2021.79
39. Adigüzel E, Acar-Tek N. Nutrition-related parameters predict the health-related quality of life in home care patients. *Exp Gerontol.* (2019) 120:15–20. doi: 10.1016/j.exger.2019.02.018
40. Lorenzo-López L, Maseda A, de Labra C, Regueiro-Folgueira L, Rodríguez-Villamil JL, Millán-Calenti JC. Nutritional determinants of frailty in older adults: A systematic review. *BMC Geriatr.* (2017) 17:108. doi: 10.1186/s12877-017-0496-2
41. Serrano-Urrea R, Garcia-Meseguer MJ. Malnutrition in an elderly population without cognitive impairment living in nursing homes in Spain: study of prevalence using the Mini Nutritional Assessment test. *Gerontology.* (2013) 59:490–8. doi: 10.1159/000351763
42. Inoue T, Misu S, Tanaka T, Kakehi T, Ono R. Acute phase nutritional screening tool associated with functional outcomes of hip fracture patients: A longitudinal study to compare MNA-SF, MUST, NRS-2002 and GNRI. *Clin Nutr.* (2019) 38:220–6. doi: 10.1016/j.clnu.2018.01.030
43. Jeejeebhoy KN, Keller H, Gramlich L, Allard JP, Laporte M, Duerksen DR, et al. Nutritional assessment: comparison of clinical assessment and objective variables for the prediction of length of hospital stay and readmission. *Am J Clin Nutr.* (2015) 101:956–65. doi: 10.3945/ajcn.114.098665
44. Agarwal E, Ferguson M, Banks M, Batterham M, Bauer J, Capra S, et al. Malnutrition and poor food intake are associated with prolonged hospital stay, frequent readmissions, and greater in-hospital mortality: results from the Nutrition Care Day Survey 2010. *Clin Nutr.* (2013) 32:737–45. doi: 10.1016/j.clnu.2012.11.021
45. Grossman A, Beloosesky Y, Schlesinger A, Gafter-Gvili A, Koren-Morag N, Meyerovitch J, et al. The association between glycated hemoglobin levels and mortality in non-diabetic elderly subjects. *Eur J Intern Med.* (2016) 27:57–61. doi: 10.1016/j.ejim.2015.10.010
46. Lee G, Choi S, Kim K, Yun JM, Son JS, Jeong SM, Kim SM, Park SM. Association of hemoglobin concentration and its change with cardiovascular and all-cause mortality. *J Am Heart Assoc.* (2018) 7:e007723. doi: 10.1161/JAHA.117.007723
47. Zakai NA, French B, Arnold AM, Newman AB, Fried LF, Robbins J, et al. Hemoglobin decline, function, and mortality in the elderly: the cardiovascular health study. *Am J Hematol.* (2013) 88:5–9. doi: 10.1002/ajh.23336
48. Yenibertiz D, Cirik MO. The comparison of GNRI and other nutritional indexes on short-term survival in geriatric patients treated for respiratory failure. *Aging Clin Exp Res.* (2020) 33:611–7. doi: 10.1007/s40520-020-01740-8
49. Hong X, Yan J, Xu L, Shen S, Zeng X, Chen L. Relationship between nutritional status and frailty in hospitalized older patients. *Clin Interv Aging.* (2019) 14:105–11. doi: 10.2147/CIA.S189040
50. Pérez-Ros P, Vila-Candel R, López-Hernández L, Martínez-Arnau FM. Nutritional status and risk factors for frailty in community-dwelling older people: a cross-sectional study. *Nutrients.* (2020) 12:1041. doi: 10.3390/nu12041041
51. Bouillanne O, Morineau G, Dupont C, Coulombel I, Vincent JP, Nicolis I, et al. Geriatric Nutritional Risk Index: a new index for evaluating at-risk elderly medical patients. *Am J Clin Nutr.* (2005) 82:777–83. doi: 10.1093/ajcn/82.4.777
52. Sousa AS, Guerra RS, Fonseca I, Pichel F, Amaral TF. Sarcopenia and length of hospital stay. *Eur J Clin Nutr.* (2016) 70:595–601. doi: 10.1038/ejcn.2015.207
53. Debbi EM, Rajaee SS, Spitzer AI, Paiement GD. Smoking and Total Hip Arthroplasty: Increased Inpatient Complications, Costs, and Length of Stay. *J Arthroplasty.* (2019) 34:1736–9. doi: 10.1016/j.arth.2019.03.059
54. Sari AA, Rezaei S, Arab M, Majdzadeh R, Matin BK, Zandian H. Effects of Smoking on Cost of Hospitalization and Length of Stay among Patients with Lung Cancer in Iran: a Hospital-Based Study. *Asian Pac J Cancer Prev.* (2016) 17:4421–6.
55. Miyagi S, Takamura T, Nguyen TTT, Tsujiguchi H, Hara A, Nakamura H, et al. Moderate alcohol consumption is associated with impaired insulin secretion and fasting glucose in non-obese non-diabetic men. *J Diabetes Investig.* (2021) 12:869–76. doi: 10.1111/jdi.13402
56. LaCroix AZ, Guralnik JM, Berkman LF, Wallace RB, Satterfield S. Maintaining mobility in late life. II Smoking, alcohol consumption, physical activity, and body mass index. *Am J Epidemiol.* (1993) 137:858–69. doi: 10.1093/oxfordjournals.aje.a116747
57. Stuck AE, Walthert JM, Nikolaus T, Büla CJ, Hohmann C, Beck JC. Risk factors for functional status decline in community-living elderly people: a systematic literature review. *Soc Sci Med.* (1999) 48:445–69. doi: 10.1016/S0277-9536(98)00370-0
58. Dworsky JQ, Russell MM. Surgical Decision Making for Older Adults. *JAMA.* (2019) 321:716. doi: 10.1001/jama.2019.0283
59. Kow AW. Prehabilitation and its role in geriatric surgery. *Ann Acad Med Singap.* (2019) 48:386–92.
60. Gordon EH, Peel NM, Samanta M, Theou O, Howlett SE, Hubbard RE. Sex differences in frailty: A systematic review and meta-analysis. *Exp Gerontol.* (2017) 89:30–40. doi: 10.1016/j.exger.2016.12.021
61. Snowdon DA, Ostwald SK, Kane RL. Education, survival, and independence in elderly Catholic sisters, 1936-1988. *Am J Epidemiol.* (1989) 130:999–1012. doi: 10.1093/oxfordjournals.aje.a115433
62. Luo Y, Zhang Z, Gu D. Education and mortality among older adults in China. *Soc Sci Med.* (2015) 127:134–42. doi: 10.1016/j.socscimed.2014.09.039
63. Tsai AC, Chang TL, Wang YC, Liao CY. Population-specific short-form mini nutritional assessment with body mass index or calf circumference can predict

risk of malnutrition in community-living or institutionalized elderly people in taiwan. *J Am Diet Assoc.* (2010) 110:1328–34. doi: 10.1016/j.jada.2010.06.003

64. Tsai AC, Chang TL, Chen JT, Yang TW. Population-specific modifications of the short-form Mini Nutritional Assessment and Malnutrition Universal Screening Tool for elderly Taiwanese. *Int J Nurs Stud.* (2009) 46:1431–8. doi: 10.1016/j.ijnurstu.2009.05.004
65. Tsai AC, Chang MZ. Long-form but not short-form Mini-Nutritional Assessment is appropriate for grading nutritional risk of patients on hemodialysis–a cross-sectional study. *Int J Nurs Stud.* (2011) 48:1429–35. doi: 10.1016/j.ijnurstu.2011.05.004
66. Tsai AC, Chou YT, Chang TL, Chang-Lee SN, Tsay SF. A modified Mini Nutritional Assessment without BMI can effectively assess the nutritional status of neuropsychiatric patients. *J Clin Nurs.* (2009) 18:1916–22. doi: 10.1111/j.1365-2702.2008.02686.x

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.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 07 February 2022
doi: 10.3389/fnut.2022.803076

Association of Yogurt and Dietary Supplements Containing Probiotic Consumption With All-Cause and Cause-Specific Mortality in US Adults: A Population-Based Cohort Study

Ping Lin[1†], *Xuezhen Gui*[2†], *Zongan Liang*[1*] *and Ting Wang*[1*]

[1] *Department of Respiratory and Critical Care Medicine, West China School of Medicine and West China Hospital, Sichuan University, Chengdu, China,* [2] *Division of Pulmonary Diseases, State Key Laboratory of Biotherapy of China, Sichuan University, Chengdu, China*

OPEN ACCESS

Edited by:
Mauro Serafini,
University of Teramo, Italy

Reviewed by:
Wen Yen Juan,
United States Food and Drug Administration, United States
Donato Angelino,
University of Teramo, Italy

***Correspondence:**
Zongan Liang
2020324025198@stu.scu.edu.cn;
liangza@scu.edu.cn
Ting Wang
w.t33@foxmail.com

[†] *These authors have contributed equally to this work*

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *03 November 2021*
Accepted: *14 January 2022*
Published: *07 February 2022*

Citation:
Lin P, Gui X, Liang Z and Wang T (2022) Association of Yogurt and Dietary Supplements Containing Probiotic Consumption With All-Cause and Cause-Specific Mortality in US Adults: A Population-Based Cohort Study. Front. Nutr. 9:803076. doi: 10.3389/fnut.2022.803076

Background: Although probiotic intake had beneficial effects on several specific disorders, limited evidence was available about the benefits of probiotic intake in the general population. This study aimed to evaluate the relationship between yogurt (as a natural probiotic source) and dietary supplements containing probiotic consumption and mortality in US adults.

Methods: We conducted an observational cohort study comprised of a nationally representative sample of adults who were enrolled in the National Health and Nutrition Examination Survey (NHANES) between 1999 and 2014. Individuals were linked to the US National Death Index.

Results: We included 32,625 adults in our study. Of the study cohort, 3,539 participants had yogurt consumption, 213 had dietary supplements containing probiotic consumption, and the remaining participants (28,873) did not have yogurt and/or dietary supplements containing probiotic consumption. During 266,432 person-years of follow-up, 3,881 deaths from any cause were ascertained, of which 651 were due to cardiovascular disorders and 863 were due to cancer. Weighted Cox proportional hazards models suggested that yogurt consumption was inversely associated with all-cause mortality (adjusted hazard ratio (HR), 0.83 [95% confidence interval (CI), 0.71–0.98]) but not cardiovascular mortality (adjusted HR, 0.68 [95%CI, 0.43–1.08]) and cancer mortality (adjusted HR, 1.00 [95%CI, 0.72–1.38]). However, dietary supplements containing probiotic were not associated with decreased all-cause and cause-specific mortality.

Conclusions: The present study suggested that yogurt consumption was associated with a lower risk of all-cause mortality among U.S. adults. Yogurt consumption in diet might be a sensible strategy for reducing the risk of death.

Keywords: probiotic, yogurt, NHANES, mortality, population

INTRODUCTION

The human gastrointestinal tract harbors a complex community of microbes called the gut microbiota (1). The composition and metabolic activity of gut microbiota are now known to co-develop with the host beginning at birth and are under the influence of numerous factors (2). Gut microbiota and its disturbance have been associated with the pathogenesis of both intestinal and extra-intestinal disorders including gastroenterological diseases, metabolic disorders, cardiovascular diseases, autoimmune diseases, and neuropsychiatric disorders (3–7).

Probiotic intake might restore and maintain normal microbiota composition and function (8). Therefore, probiotic intake might have a positive impact on human health (9). Previous studies had suggested that probiotic might improve cognitive function and metabolic status (10), alleviate symptoms of various gastrointestinal disorders (11), decrease circulating levels of inflammatory biomarkers (12), improve glycemic and blood pressure control (13, 14), assist weight management in patients with type 2 diabetes (15), reduce antibiotic resistance (16), and prevent rhinovirus infections in preterm infants (17). Although published studies showed that probiotic intake had beneficial effects on people with specific disorders, limited evidence was available on the benefits of probiotic intake in the general population. Therefore, the recommendation to implement using of probiotic to provide health benefits in the general population needed further investigation (18).

In this study, we aimed to examine the relationship between yogurt (as a natural probiotic source) and dietary supplements containing probiotic consumption and mortality in a large cohort of participants in the National Health and Nutrition Examination Survey (NHANES), 1999–2014. We hypothesized that yogurt and dietary supplements containing probiotic consumption would be negatively associated with mortality.

METHODS

Study Population

The NHANES survey is a national program of studies aimed to evaluate the health and nutritional status of the non-institutionalized US population using a complex, multistage, probability sampling design (19). The survey was conducted periodically since 1999 and a nationally representative sample of about 5,000 persons was examined each year. Data were collected by standardized in-person interviews and physical examinations.

In the present study, 82,091 participants from the NHANES surveys between 1999 and 2014 were included. All participants were linked to the US National Death Index (NDI), which provided mortality follow-up data through December 31, 2015 (20). We excluded participants aged <18 years at interviews (n = 34,812) and those with missing information on BMI (n = 3,232), laboratory tests (white blood cell count, hemoglobin, platelet count, total bilirubin, creatinine, and blood urea nitrogen) (n = 2,861), medical conditions (hypertension, diabetes, asthma, congestive heart failure, coronary heart disease, stroke, chronic bronchitis, and cancer) (n = 4,353), yogurt and dietary supplements containing probiotic consumption (4,208). Therefore, a total of 32,625 participants remained in our cohort for analysis. **Figure 1** presents a flowchart of participant selection.

Abbreviations: NHANES, the National Health and Nutrition Examination Survey; NDI, the National Death Index; HR, hazard ratio; CI, confidence interval; BMI, body mass index.

Assessments of Probiotic Intake

In this study, probiotic intake was considered when a participant reported consumption of yogurt or dietary supplements containing probiotics (21, 22). We utilized the Dietary Interview—First Day (the 24-h dietary recall interview before the survey) and Dietary Interview—Second Day (the second 24-h dietary recall interview collected by telephone 3 to 10 days after the first interview) to assess yogurt consumption. We utilized the Dietary Supplement Use 30-Day (1999–2014), a questionnaire that collected personal interview data on food supplement use during a 30-day period before the interview date, to assess dietary supplements containing probiotic. The detailed probiotic consumption information is described in **Supplementary Material 1**.

Statistical Analysis

We analyzed the data using appropriate sampling weights (1/4*WTDR4YR for 1999–2002 and 1/8*WTDR2D for 2003–2014) to account for the complex survey design applied by the NHANES survey. We described baseline characteristics using percentages for categorical variables and means and standard deviations for continuous variables. We compared baseline characteristics using the Mantel–Haenszel χ^2 test for categorical variables and the linear regression for continuous variables. We used weighted Cox proportional hazards models to calculate the hazard ratios (HRs) and corresponding 95% confidence intervals (CIs). Survey-weight adjusted multivariable Cox proportional model was adjusted for potential confounders that had been shown to be associated with mortality. Model 1 was not adjusted for any factors. Model 2 was adjusted for age, sex, race, and body mass index (BMI). Model 3 was adjusted for age, sex, race, BMI, white blood cell count, hemoglobin, platelet count, total bilirubin, creatinine, blood urea nitrogen, hypertension, diabetes, asthma congestive heart failure, coronary heart disease, stroke, chronic bronchitis, and cancer. Subgroup analyses were conducted by examining demographic characteristics, including age (<60 y, ≥60 y), sex (man, female), race/ethnicity (Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, Other Race), and BMI (kg/m^2) (<18.5, 18.5 to 24.9, 25 to 29.9, ≥30). We used Stata version 14.0 (Stata Corp) and R version 3.6.3 (R Foundation for Statistical Computing) for statistical analysis and considered a two-tailed p-value less than 0.05 to be statistically significant.

RESULTS

Participant Characteristics

A total of 32,625 adults aged 18 years or older were included in this analysis. Of the study cohort, 3,539 participants had yogurt

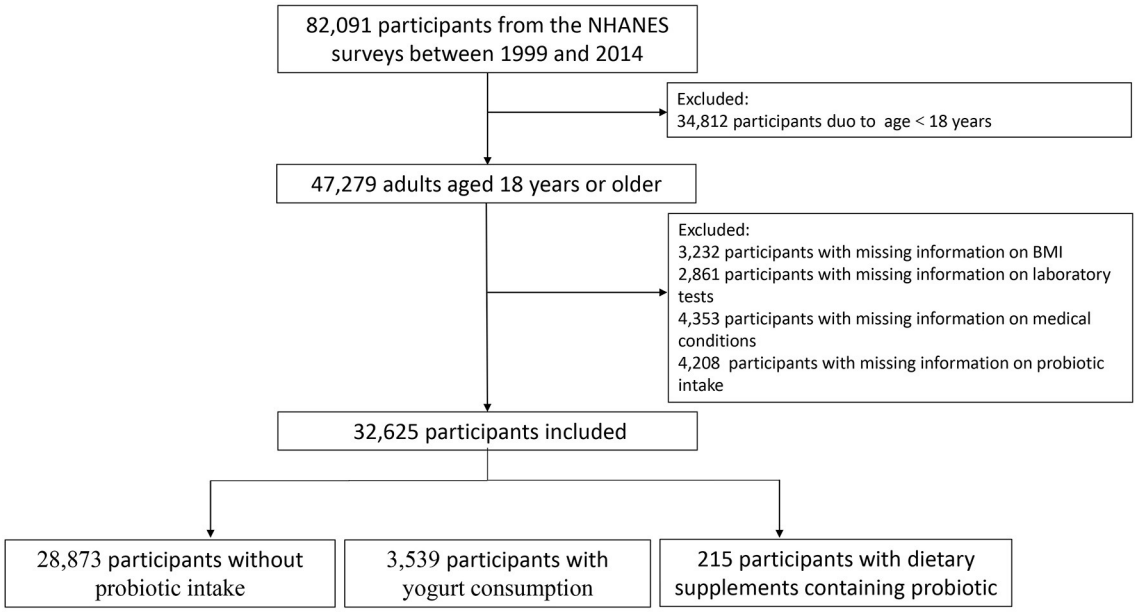

FIGURE 1 | Flowchart of patient selection.

consumption, 213 had dietary supplements containing probiotic consumption, and the remaining participants (28,873) did not have yogurt and/or dietary supplements containing probiotic consumption. Characteristics of participants with different statuses of probiotic consumption were significantly different. Participants with yogurt or dietary supplements containing probiotic consumption were more likely to be older, to be a woman, to have low BMI, to be Non-Hispanic White, to have less hypertension, and to have cancer. The detailed baseline characteristics are shown in **Table 1**.

Associations Between Probiotic Exposure and Mortality

During 266,432 person-years of follow-up, 3,881 deaths from any cause were ascertained, of which 651 were due to cardiovascular disorders and 863 were due to cancer. We evaluated associations of probiotic intake with mortality by logistic regression models with unadjusted and adjusted covariates (**Table 2**).

For unadjusted analyses (**Model 1**), yogurt consumption was not associated with a reduced risk of all-cause mortality (HR, 0.86 [95%CI, 0.73–1.02]), cardiovascular mortality (HR, 0.66[95%CI, 0.41–1.04]), and cancer mortality (HR, 1.03 [95%CI, 0.75–1.41]). After adjusting for potential confounders (**Model 2 and 3**), yogurt consumption was inversely associated with all-cause mortality (HR, 0.83 [95%CI, 0.71–0.98]) but not cardiovascular mortality (HR, 0.68 [95%CI, 0.43–1.08]) and cancer mortality (HR, 1.00 [95%CI, 0.72–1.38]) (**Model 3**).

However, dietary supplements containing probiotic were not associated with decreased all-cause and cause-specific mortality (**Table 2**). The unadjusted RRs of all-cause, cardiovascular, and cancer mortality were 0.64 (95% CI 0.36, 1.14), 0.83 (95% CI 0.25, 2.72), 1.12 (95% CI 0.41, 3.07), respectively. The adjusted RRs of all-cause, cardiovascular, and cancer mortality were 0.74 (95% CI 0.43, 1.29), 1.13 (95% CI 0.34, 3.67), 1.34 (95% CI 0.50, 3.62), respectively.

Subgroup Analysis

We further explored the association of yogurt consumption with all-cause mortality according to gender, age, race/ethnicity, and BMI **(Table 3)**. We found that the association between yogurt consumption and decreased all-cause mortality was statistically significant among female, older participants (aged $\geq$ 60 y), and Non-Hispanic Black, while the association was weak and not statistically significant in other gender, ages, race/ethnicity, and weight subgroups.

DISCUSSION

In this large, nationally representative sample of US adults, we observed that participants with yogurt consumption had a lower risk for all-cause mortality compared with participants without yogurt consumption that persisted after adjustment for demographic characteristics, laboratory tests, and clinic comorbidities. In subsequent subgroup analyses, this yogurt–mortality inverse association was found to be present predominantly among female, older participants, and Non-Hispanic Black.

The association between yogurt consumption and mortality was inconsistent in previous studies. Schmid et al. reported that yogurt consumption was associated with a decreased risk of all-cause mortality (HR, 0.89 [95%CI, 0.86–0.93]) in the Nurses' Health Study and The Health Professionals Follow-Up Study, whereas Soedamah-Muthu et al. and Praagman et al. failed to discover a significant association between yogurt

TABLE 1 | Participants baseline demographic and clinical characteristics.

Characteristic	Without probiotic intake (*N* = 32,965)	Yogurt consumption (*N* = 3,539)	Dietary supplements containing probiotic (*N* = 213)	*p*-value
Baseline characteristics				
Age (y)	46.2 ± 16.8	48.3 ± 16.4	50.1 ± 15.7	<0.001
Man (%)	49.7	35.4	27.3	<0.001
BMI (kg/m^2)	28.6 ± 6.6	27.7 ± 6.4	27.1 ± 6.5	<0.001
Race (%)				<0.001
Mexican American	8.4	6.0	2.6	
Other Hispanic	5.1	4.2	5.2	
Non-Hispanic White	69.3	79.0	81.7	
Non-Hispanic Black	11.6	4.8	6.5	
Others	5.5	6.0	3.9	
Laboratory tests				
Hemoglobin (g/dL)	14.4 ± 1.5	14.1 ± 1.3	14.2 ± 1.1	<0.001
WBC (1,000 cells/uL)	7.3 ± 2.3	7.1 ± 2.4	6.8 ± 1.8	<0.001
Platelets (1,000 cells/uL)	258.5 ± 67.2	252.5 ± 65.4	249.0 ± 63.7	<0.001
Total bilirubin (umol/L)	12.2 ± 5.3	12.5 ± 5.1	12.8 ± 4.8	0.0012
Creatinine (umol/L)	78.1 ± 33.2	75.2 ± 20.4	75.1 ± 16.8	<0.001
BUN (mmol/L)	4.7 ± 1.9	4.8 ± 1.7	4.7 ± 1.6	0.0190
Comorbidities				
Hypertension (%)	29.7	26.8	24.7	0.0002
Diabetes mellitus (%)	8.1	6.4	9.9	0.0002
CHD (%)	3.4	3.1	2.8	0.5734
CHF (%)	2.3	1.8	1.5	0.0707
Asthma (%)	13.4	12.5	18.7	0.0070
Stroke (%)	2.6	1.7	2.3	0.0014
Chronic bronchitis (%)	6.3	4.1	9.4	<0.001
Cancer (%)	8.7	10.8	16.4	<0.001

BMI, body mass index; WBC, white blood cell; BUN, blood urea nitrogen; CAD, coronary heart disease; CHF, congestive heart failure.
Continuous variables were expressed as means and standard deviations and categorical variables were expressed as percentages. Means and percentages are weighted.

consumption and all-cause mortality in the Whitehall II cohort (HR, 0.74 [95%CI, 0.53–1.05]) and the European Prospective Investigation into Cancer and Nutrition-Netherlands cohort (HR, 0.95 [95%CI, 0.85–1.07]) (23, 24). The inconsistent findings might be due to different sociodemographic characteristics and follow-up times. In the present analysis, we observed that individuals with yogurt consumption had a 17% lower risk for all-cause mortality. This study was a national survey with a large sample size and a long follow-up (mean 8.1 years), which could offer reliable evidence and further reinforce earlier results. Moreover, we further conducted subgroup analyses by examining demographic characteristics (age, sex, race/ethnicity, and BMI). We found that the health benefits of yogurt consumption were particular among female, older participants (aged $\geq$ 60 y), and Non-Hispanic Black, indicating that these individuals were more likely to benefit from yogurt consumption. Unfortunately, the exact reasons for these observed demographic characteristics differences in the inverse associations were not clear. Sex-related differences might be due to the metabolism of sex hormones regulated by the gut microbiome (25).

We observed a reduction in mortality in yogurt consumption rather than dietary supplements containing probiotic in this study. Yogurt is a nutrient-dense fermented dairy product and is associated with high nutritional value (26). In addition to probiotic strains, yogurt is also a good source of abundant micronutrients and macronutrients, including calcium, Fe, Mn, zinc, vitamins B-6/B-12, riboflavin, and protein (27, 28). These nutrients are essential for the sustenance of the functioning of the human body. Therefore, these nutrients might have a role in the beneficial effects of yogurt consumption compared to the supplementation of probiotic strains into a carrier liquid only. In addition, only a small number of people (about 0.65%) who had a consumption of dietary supplements containing probiotic in diet might be responsible, at least in part, for these unexpected results.

Our results were supported by the existence of data showing a biological plausibility for the health benefits of probiotic. First, probiotic intake might be helpful to reduce risk factors for all-cause mortality and cardiovascular mortality such as obesity, hypertension, and type 2 diabetes (29–31). Second, probiotic foods had been reported to repress oxidative stress and inflammatory marker profile in both healthy persons and patients with disorders (32–34). Third, data also suggested that the use of probiotic might improve endothelial function and reduce arterial stiffness (35). Fourth, previous investigators found that

TABLE 2 | Association of probiotic exposure with mortality in 32,625 participants of national health and nutrition examination survey, 1999–2014.

Exposure	Model 1[a] HR (95% CI)	*p*-value	Model 2[b] HR (95% CI)	*p*-value	Model 3[c] HR (95% CI)	*p*-value
All-cause mortality						
Yogurt	0.86 (0.73, 1.02)	0.096	0.79 (0.67, 0.93)	0.005	0.83 (0.71, 0.98)	0.035
Probiotic supplements	0.64 (0.36, 1.14)	0.132	0.67 (0.38, 1.19)	0.181	0.74 (0.43, 1.29)	0.300
Cardiovascular mortality						
Yogurt	0.66 (0.41, 1.04)	0.076	0.62 (0.39, 0.99)	0.048	0.68 (0.43, 1.08)	0.109
Probiotic supplements	0.83 (0.25, 2.72)	0.763	0.99 (0.30, 3.24)	0.993	1.13 (0.34, 3.67)	0.837
Cancer mortality						
Yogurt	1.03 (0.75, 1.41)	0.845	0.98 (0.71, 1.35)	0.904	1.00 (0.72, 1.38)	0.972
Probiotic supplements	1.12 (0.41, 3.07)	0.815	1.21 (0.45, 3.27)	0.699	1.34 (0.50, 3.62)	0.555

[a] *Unadjusted.*

[b] *Adjusted for age, sex, race, and body mass index.*

[c] *Adjusted for age, sex, race, body mass index, white blood cell count, hemoglobin, platelet count, total bilirubin, creatinine, blood urea nitrogen, hypertension, diabetes, asthma congestive heart failure, coronary heart disease, stroke, chronic bronchitis, and cancer.*

TABLE 3 | Association of yogurt consumption with mortality according to age, sex, race/ethnicity, and BMI.

Subgroup	All-cause mortality HR (95% CI)*	*p*-value
Gender		
Male	0.89 (0.70, 1.15)	0.403
Female	0.77 (0.62, 0.97)	0.028
Age		
Aged < 60 y	0.96 (0.65, 1.40)	0.851
Aged ≥ 60 y	0.80 (0.68, 0.96)	0.017
Race/Ethnicity		
Mexican American	0.66 (0.40, 1.10)	0.113
Other Hispanic	1.85 (0.94, 3.64)	0.073
Non-Hispanic White	0.87 (0.73, 1.05)	0.170
Non-Hispanic Black	0.54 (0.30, 0.97)	0.042
Other Race	0.40 (0.13, 1.21)	0.107
Weight		
Underweight (BMI < 18.5)	0.44 (0.16, 1.18)	0.104
Normal (BMI, 18.5 to 24.9)	0.78 (0.59, 1.04)	0.096
Overweight (BMI, 25 to 29.9)	0.86 (0.66, 1.14)	0.313
Obese (BMI ≥ 30)	0.83 (0.60, 1.15)	0.275

**Adjusted for age, sex, race, body mass index, white blood cell count, hemoglobin, platelet count, total bilirubin, creatinine, blood urea nitrogen, hypertension, diabetes, asthma congestive heart failure, coronary heart disease, stroke, chronic bronchitis, and cancer.*

probiotic intake might reduce enteric bacterial translocation and suppress the growth of pathogenic microbiota, indicating that probiotic could prevent enteric infections and inhibit the growth of intestinal carcinoma (36, 37). Furthermore, intake of probiotic significantly might improve vitamins and minerals status such as vitamin K and calcium which played an important role in maintaining normal physiological activities (38).

An important implication from this study was that yogurt consumption might be an important public health strategy that had been associated with decreased mortality risk among adults. Our study revealed that only 11.4% of participants had exposure to yogurt and/or dietary supplements containing probiotic among U.S. adults, which were similar to those previously reported (13.1%) (39). It indicated that the consumption of probiotic food remained relatively unpopular and there was still much potential for improvement in US adults. Appropriate strategies were needed to encourage the wider population to accept probiotic food such as health promotion and health education around the benefits of probiotic food.

This study had several strengths. The use of a large size, nationally representative sample would improve the ability to address the impact of possible confounding and increase the generalizability and external validity of our results. In addition, we further conducted subgroup analyses according to age, gender, race/ethnicity, and BMI, and found that female, older participants (aged ≥ 60 y), and Non-Hispanic Black were more likely to benefit from yogurt consumption. At the same time, several limitations of this study should be recognized. First, dietary data was self-reported in the NHANES survey, which might result in measurement error inevitably. Second, due to the lack of detailed information about yogurt consumption, we could not conduct subgroup analyses to explore the dose-response association between yogurt consumption and mortality. Third, this was a retrospective, observational study, which was subject to confounding bias. Although we carefully controlled for possible confounding factors, residual confounding or unmeasured confounding could not be fully ruled out. Moreover, because dietary data was collected based on the Dietary Interview-First Day/Second Day and Dietary Supplement Use 30-Day, dietary changes could not be excluded after the survey. However, dietary changes were likely to lead to an underestimation instead of an overestimation of probiotic benefits.

CONCLUSIONS

In this study, we found that yogurt consumption was associated with a lower risk of all-cause mortality in the general population, especially among female, older participants, and Non-Hispanic Black. Yogurt consumption in diet might be a sensible strategy for reducing the risk of death.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. Data used for this study are available on the NHANES website: https://wwwn.cdc.gov/nchs/nhanes/.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional review board of the National Center for Health Statistics, CDC. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PL and XG contributed to study design, data collection, data analysis, and drafting the article. ZL and TW contributed to study design, critical revision and submitted the report for publication. All authors read and approved the final manuscript.

FUNDING

This study was funded by the National Key Research and Development Program of China (Grant No. 2016YFC1304303) and the National Natural Science Foundation of China (Grant No. 31701011).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.803076/full#supplementary-material

REFERENCES

1. Sommer F, Bäckhed F. The gut microbiota—masters of host development and physiology. *Nat Rev Microbiol.* (2013) 11:227–38. doi: 10.1038/nrmicro2974
2. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, et al. Host-gut microbiota metabolic interactions. *Science.* (2012) 336:1262–7. doi: 10.1126/science.1223813
3. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. *Cell.* (2012) 148:1258–70. doi: 10.1016/j.cell.2012.01.035
4. Kamada N, Seo SU, Chen GY, Núñez G. Role of the gut microbiota in immunity and inflammatory disease. *Nat Rev Immunol.* (2013) 13:321–35. doi: 10.1038/nri3430
5. Tang WHW, Kitai T, Hazen SL. Gut microbiota in cardiovascular health and disease. *Circ Res.* (2017) 120:1183–96. doi: 10.1161/CIRCRESAHA.117.309715
6. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol.* (2021) 19:55–71. doi: 10.1038/s41579-020-0433-9
7. Cryan JF, O'Riordan KJ, Sandhu K, Peterson V, Dinan TG. The gut microbiome in neurological disorders. *Lancet Neurol.* (2020) 19:179–94. doi: 10.1016/S1474-4422(19)30356-4
8. Korpela K, Salonen A, Vepsäläinen O, Suomalainen M, Kolmeder C, Varjosalo M, et al. Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. *Microbiome.* (2018) 6:182. doi: 10.1186/s40168-018-0567-4
9. Quigley EMM. Prebiotics and probiotics in digestive health. *Clin. Gastroenterol Hepatol.* (2019) 17:333–44. doi: 10.1016/j.cgh.2018.09.028
10. Akbari E, Asemi Z, Daneshvar Kakhaki R, Bahmani F, Kouchaki E, Tamtaji OR, et al. Effect of probiotic supplementation on cognitive function and metabolic status in Alzheimer's disease: a randomized, double-blind and controlled trial. *Front Aging Neurosci.* (2016) 8:256. doi: 10.3389/fnagi.2016.00256
11. Ford AC, Quigley EM, Lacy BE, Lembo AJ, Saito YA, Schiller LR, et al. Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. *Am J Gastroenterol.* (2014) 109:1547–61. doi: 10.1038/ajg.2014.202
12. Malik M, Suboc TM, Tyagi S, Salzman N, Wang J, Ying R, et al. *Lactobacillus* plantarum 299v supplementation improves vascular endothelial function and reduces inflammatory biomarkers in men with stable coronary artery disease. *Circ Res.* (2018) 123:1091–102. doi: 10.1161/CIRCRESAHA.118.313565
13. Soleimani A, Zarrati Mojarrad M, Bahmani F, Taghizadeh M, Ramezani M, Tajabadi-Ebrahimi M, et al. Probiotic supplementation in diabetic hemodialysis patients has beneficial metabolic effects. *Kidney Int.* (2017) 91:435–42. doi: 10.1016/j.kint.2016.09.040
14. Khalesi S, Sun J, Buys N, Jayasinghe R. Effect of probiotics on blood pressure: a systematic review and meta-analysis of randomized, controlled trials. *Hypertension.* (2014) 64:897–903. doi: 10.1161/HYPERTENSIONAHA.114.03469
15. Khalili L, Alipour B, Asghari Jafarabadi M, Hassanalilou T, Mesgari Abbasi M, Faraji I. Probiotic assisted weight management as a main factor for glycemic control in patients with type 2 diabetes: a randomized controlled trial. *Diabetol Metab Syndr.* (2019) 11:5. doi: 10.1186/s13098-019-0400-7
16. Montassier E, Valdés-Mas R, Batard E, Zmora N, Dori-Bachash M, Suez J, et al. Probiotics impact the antibiotic resistance gene reservoir along the human GI tract in a person-specific and antibiotic-dependent manner. *Nat Microbiol.* (2021) 6:1043–54. doi: 10.1038/s41564-021-00920-0
17. Luoto R, Ruuskanen O, Waris M, Kalliomäki M, Salminen S, Isolauri E. Prebiotic and probiotic supplementation prevents rhinovirus infections in preterm infants: a randomized, placebo-controlled trial. *J Allergy Clin Immunol.* (2014) 133:405–13. doi: 10.1016/j.jaci.2013.08.020
18. Khalesi S, Bellissimo N, Vandelanotte C, Williams S, Stanley D, Irwin C, et al. review of probiotic supplementation in healthy adults: helpful or hype? *Eur J Clin Nutr.* (2019) 73:24–37. doi: 10.1038/s41430-018-0135-9
19. Centers for Disease Control and Prevention (CDC). *About the National Health and Nutrition Examination Survey.* (2017). Available online at: https://www.cdc.gov/nchs/nhanes/about_nhanes.htm.
20. National Center for Health Statistics. *Office of Analysis and Epidemiology, Public-use Linked Mortality File.* Hyattsville: Maryland (2015). Available online at:https://www.cdc.gov/nchs/data-linkage/mortality-public.htm.
21. Lourens-Hattingh A, Viljoen BC. Yogurt as probiotic carrier food. *Int Dairy J.* (2001) 11:1–17. doi: 10.1016/S0958-6946(01)00036-X
22. Yacoub R, Kaji D, Patel SN, Simoes PK, Busayavalasa D, Nadkarni GN, et al. Association between probiotic and yogurt consumption and kidney disease: insights from NHANES. *Nutr J.* (2016) 15:1–4. 10. doi: 10.1186/s12937-016-0127-3
23. Soedamah-Muthu SS, Masset G, Verberne L, Geleijnse JM, Brunner EJ. Consumption of dairy products and associations with incident diabetes, CHD and mortality in the Whitehall II study. *British J Nutr.* (2013) 109:718–26. doi: 10.1017/S0007114512001845
24. Praagman J, Dalmeijer GW, Van Der Schouw YT, Soedamah-Muthu SS, Verschuren WMM. Bueno-de-Mesquita HB, et al. The relationship between fermented food intake and mortality risk in the European Prospective investigation into cancer and nutrition-Netherlands cohort. *British J Nutr.* (2015) 113:498–506. doi: 10.1017/S0007114514003766
25. Yoon K, Kim N. Roles of Sex Hormones and Gender in the Gut Microbiota. *J Neurogastroenterol Motil.* (2021) 27:314–25. doi: 10.5056/jnm20208
26. Gad AS, Kholif AM, Sayed AF. Evaluation of the nutritional value of functional yogurt resulting from combination of date palm syrup and skim milk. *Am J Food Technol.* (2010) 5:250–9. doi: 10.3923/ajft.2010.250.259

27. Williams EB, Hooper B, Spiro A, Stanner S. The contribution of yogurt to nutrient intakes across the life course. *Nutr Bull.* (2015) 40:9–32. doi: 10.1111/nbu.12130
28. Bakircioglu D, Topraksever N, Yurtsever S, Kizildere M, Kurtulus YB. Investigation of macro, micro and toxic element concentrations of milk and fermented milks products by using an inductively coupled plasma optical emission spectrometer, to improve food safety in Turkey. *Microchemical J.* (2018) 136:133–8. doi: 10.1016/j.microc.2016.10.014
29. Pontes K, Guedes MR, Cunha MRD, Mattos SS, Barreto Silva MI, Neves MF, et al. Effects of probiotics on body adiposity and cardiovascular risk markers in individuals with overweight and obesity: a systematic review and meta-analysis of randomized controlled trials. *Clin Nutr.* (2021) 40:4915–31. doi: 10.1016/j.clnu.2021.06.023
30. Liang T, Wu L, Xi Y, Li Y, Xie X, Fan C, et al. Probiotics supplementation improves hyperglycemia, hypercholesterolemia, and hypertension in type 2 diabetes mellitus: an update of meta-analysis. *Crit Rev Food Sci Nutr.* (2021) 61:1670–88. doi: 10.1080/10408398.2020.1764488
31. Panahi S, Tremblay A. The potential role of yogurt in weight management and prevention of type 2 diabetes. *J Am Coll Nutr.* (2016) 35:717–31. doi: 10.1080/07315724.2015.1102103
32. Asemi Z, Jazayeri S, Najafi M, Samimi M, Mofid V, Shidfar F, et al. Effect of daily consumption of probiotic yogurt on oxidative stress in pregnant women: a randomized controlled clinical trial. *Ann Nutr Metab.* (2012) 60:62–8. doi: 10.1159/000335468
33. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. *Nutr.* (2012) 28:539–43. doi: 10.1016/j.nut.2011.08.013
34. Vasquez EC, Pereira T, Peotta VA, Baldo MP, Campos-Toimil M. Probiotics as beneficial dietary supplements to prevent and treat cardiovascular diseases: uncovering their impact on oxidative stress. *Oxid Med Cell Longev.* (2019) 2019:3086270. doi: 10.1155/2019/3086270
35. Szulińska M, Łoniewski I, Skrypnik K, Sobieska M, Korybalska K, Suliburska J, et al. Multispecies probiotic supplementation favorably affects vascular function and reduces arterial stiffness in obese postmenopausal Women-A 12-week placebo-controlled and randomized clinical study. *Nutr.* (2018) 10:1672. doi: 10.3390/nu10111672
36. Sato J, Kanazawa A, Azuma K, Ikeda F, Goto H, Komiya K, et al. Probiotic reduces bacterial translocation in type 2 diabetes mellitus: a randomised controlled study. *Sci Rep.* (2017) 7:12115. doi: 10.1038/s41598-017-12535-9
37. Perdigon G, Alvarez S, Rachid M, Agüero G, Gobbato N. Immune system stimulation by probiotics. *J Dairy Sci.* (1995) 78:1597–606. doi: 10.3168/jds.S0022-0302(95)76784-4
38. Knapen MHJ, Braam LA, Teunissen KJ, Zwijsen RML, Theuwissen E, Vermeer C. Yogurt drink fortified with menaquinone-7 improves vitamin K status in a healthy population. *J Nutr Sci.* (2015) 4:e35. doi: 10.1017/jns.2015.25
39. Lau E, Neves JS, Ferreira-Magalhães M, Carvalho D, Freitas P. Probiotic ingestion, obesity, and metabolic-related disorders: results from NHANES, 1999–2014. *Nutr.* (2019) 11:1482. doi: 10.3390/nu11071482

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Nutrition

REVIEW
published: 23 February 2022
doi: 10.3389/fnut.2022.814095

Underlying Causes and Co-existence of Malnutrition and Infections: An Exceedingly Common Death Risk in Cancer

Yuanyuan Fan[1†], *Qianqian Yao*[2†], *Yufeng Liu*[2], *Tiantian Jia*[2,3], *Junjuan Zhang*[3*] and *Enshe Jiang*[2,4*]

[1] School of Life Sciences, Henan University, Kaifeng, China, [2] Institute of Nursing and Health, Henan University, Kaifeng, China, [3] Department of Orthopedics, Henan Provincial People's Hospital, Zhengzhou, China, [4] Henan International Joint Laboratory for Nuclear Protein Regulation, Henan University, Kaifeng, China

In nutrition science, malnutrition is a state of imbalance between intake and the needs of the organism, leading to metabolic changes, impaired physiological functions, and weight loss. Regardless of the countless efforts being taken and researched for years, the burden of malnutrition is still alarming and considered a significant agent of mortality across the globe. Around 45% of 12 million children deaths (0–5 years old) annually are due to malnutrition, mostly from developing countries. Malnutrition develops associations with other infections and leads to substantial clinical outcomes, such as mortality, more visits to hospitals, poor quality of life and physical frailty, and socioeconomic issues. Here, in this review, we intend to provide an overview of the current burden, underlying risk factors, and co-existence of malnutrition and other infections, such as cancer. Following the rising concern of the vicious interplay of malnutrition and other medical illnesses, we believed that this narrative review would highlight the need to re-make and re-define the future strategies by giving comprehensive and sustainable programs to alleviate poverty and combat the rampant infectious diseases and those nutrition-related health problems. Furthermore, the study also raises the concern for hospitalized malnourished cancer patients as it is crucially important to knowledge the caregiver healthcare staff for early interventions of providing nutritional support to delay or prevent the onset of malnutrition.

Keywords: malnutrition, infections, children under five, cancer, developing countries, future strategies

OPEN ACCESS

Edited by:
Francesco Sofi,
Università degli Studi di Firenze, Italy

Reviewed by:
Sorush Niknamian,
Liberty University, United States
Dewi Marhaeni Diah Herawati,
Universitas Padjadjaran, Indonesia
Victor Musiime,
Makerere University, Uganda

***Correspondence:**
Enshe Jiang
esjiang@gmail.com
Junjuan Zhang
383583496@qq.com

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *16 November 2021*
Accepted: *24 January 2022*
Published: *23 February 2022*

Citation:
Fan Y, Yao Q, Liu Y, Jia T, Zhang J and Jiang E (2022) Underlying Causes and Co-existence of Malnutrition and Infections: An Exceedingly Common Death Risk in Cancer. Front. Nutr. 9:814095. doi: 10.3389/fnut.2022.814095

INTRODUCTION

For in nutrition sciences, malnutrition is the state of the disproportion of food intake and energy requirement of an organism, which leads to impaired metabolism, body dysfunction, and diminishing of body mass (1, 2). Malnutrition is considered as a multifactorial and diverse ailment of all ages. It has been reported worldwide and has gained substantial significance as a leading health issue of the current time. Statistically, literature revealed that about 12 million children (aged 0–5years) die every year and the major portion of these deaths is malnutrition (3). Findings reveal that malnutrition is the major contributor to disease burden in developing countries where one out of every three preschool-age children were affected by malnutrition (4–6). Sub-Saharan Africa is experiencing the highest burden of malnutrition with the high level of undernutrition and growing

burden of overweight/obesity and diet-related non-communicable diseases (7). It is estimated that around 1.9 billion adults are overweight while more than 462 million adults are underweight (8). According to a study supported by Bill and Melinda Gates Foundation, malnutrition is the predominant risk factor for death in children younger than 5 years of age in every state of India (9). The results of prevalence studies demonstrate the worse look of malnutrition in hospitals where 50% of patients were found malnourished at admission and more patients may be malnourished upon the hospital discharge (10, 11). In health-related outcomes, malnutrition affects individual physical growth, mobility, cognitive development, and extended length of hospital stay (12), institutionalization (11), poor quality of life (13), and physical frailty in hospitalized patients (14). Malnutrition develops a vicious association with other diseases, such as diarrhea, measles, cancer, and HIV, that lead to increased malnutrition-related death that further imposes both manpower and economic loss epically in poor countries (15–17).

Presently, malnutrition is considered as a significant agent of mortality across the globe, and it is urgently needed to design the strategies to cope with the challenges about the prevention of malnutrition: common nomenclature, screening methods, accurately reported data, and defined distinct silos to address the malnutrition by bridging the existing gaps (18–21). The current narrative review aimed to summarize the literature and provide up to date on underlying causes, characteristics, and the prevalence of malnutrition. With more emphasis, the review highlights the complex interactions and co-existence of malnutrition and other diseases, which govern a vicious cycle to end life especially in cancer-related malnutrition.

METHOD AND LITERATURE MINING STRATEGY

Relevant articles were selected against the specific keywords as per outlines of study by using the different search databases, PubMed, Google, Google Scholar, and Research Gate. In addition to the relevancy of the title and abstracts, for strong conceptual and logical understanding, more recent studies were selected for inclusion based on the year of publication, which was between 2016 and 2021. Though, little older publications and some news agencies, government data reports were also cited to strengthen the background of the subject. All selected articles have been cited accordingly.

MALNUTRITION: NOW AND THEN

Nowadays, malnutrition is considered as a complex problem to solve and has been recognized as a world health crisis: a major risk factor for various medical illnesses. Thanks to the vision "Sustainable Development Goals (SDG) 2030, since 1990, there has been a 50% reduction in under-5 years" children deaths by malnutrition (22). To address the challenge of malnutrition, SDG aimed to reduce the under-5 mortality in all countries to at least as low as 25/1,000 live births by 2030 (23). WHO and United Nations International Children's Emergency Fund (UNICEF) integrated measurements are aimed to reduce the number of stunted children by 40% and wasting to <5% by 2025 (24). Although, we have made large-scale efforts on the lives of malnourished children in developing countries around the world, however, due to the exponentially raised population number, however, due to rising population, complexities in screening, and the association of malnutrition with other infections, current estimates predict that these measures are still insufficient to treat, control, and reduce the burden. More effective prevention and treatment of malnutrition are needed urgently.

UNDERLYING CAUSES OF MALNUTRITION

Any disease, whether chronic or acute, is a significant risk factor of malnutrition in third world countries and could cause or exaggerate malnutrition in some respects. As aforementioned, malnutrition is considered as a complex problem to solve due to the contribution of several driving factors in its development, so there is no way to list out the factors that may contribute to malnutrition in the population (21). With this complex manifestation across the life courses in different ways, it has been found that the risk factors and co-occurrence of malnutrition are not only related to nutrient intake but also linked with the levels of water sanitation and hygiene and the influence of infections on nutritional status (25–28).

In elderly persons, malnutrition is a serious threat that leads to disability, institutionalization, and more exposure to illnesses. In these patients, the underlying cause of being malnourished is anorexia of aging: an individual's food intake decreases with age (29). Clinically, metabolism-related malnutrition might be a result of reduced assimilation of nutrients and malabsorption, any infection in the digestive system, trauma, or any type of inflammation (30–32). Different studies have revealed that there are several mediators, such as cytokines (interleukin 1, interleukin 6, and tumor necrosis factors alpha), glucocorticoid, and lack of insulin growth factor 1, whose catabolic effects have altered food intake (33–35). Poor dentistry is also the result of a number of factors, especially in geriatric patients, such as dementia and immobilization (36–38). Other factors, such as loss of appetite, altered taste, and nausea, can reduce food intake and thus malnutrition (39, 40). Socioeconomic factors, such as isolation, poverty, and poor living conditions, are involved in the progression of malnutrition (17, 41). Malnutrition commonly occurs in elderly patients and is highly prevalent in patients with malignant/severe chronic liver disease, heart or kidney disease, HIV/AIDS, chronic obstructive pulmonary disease, impaired intestinal flora and cystic fibrosis, respiratory tuberculosis, and other diseases (42–51). Various studies have shown that patients tend to receive less nutritional care due to the inexperience of hospital staff (52). In addition, malnutrition is also associated with loss of appetite, poor diet, physical disability, depression, and loneliness (53, 54). Diseases are one of the major factors of malnutrition development and the risk increases with the severity of the diseases and it makes it exceedingly difficult to analyze the effect of malnutrition on prognosis alone. This can only be done in well-characterized settings, where analysis

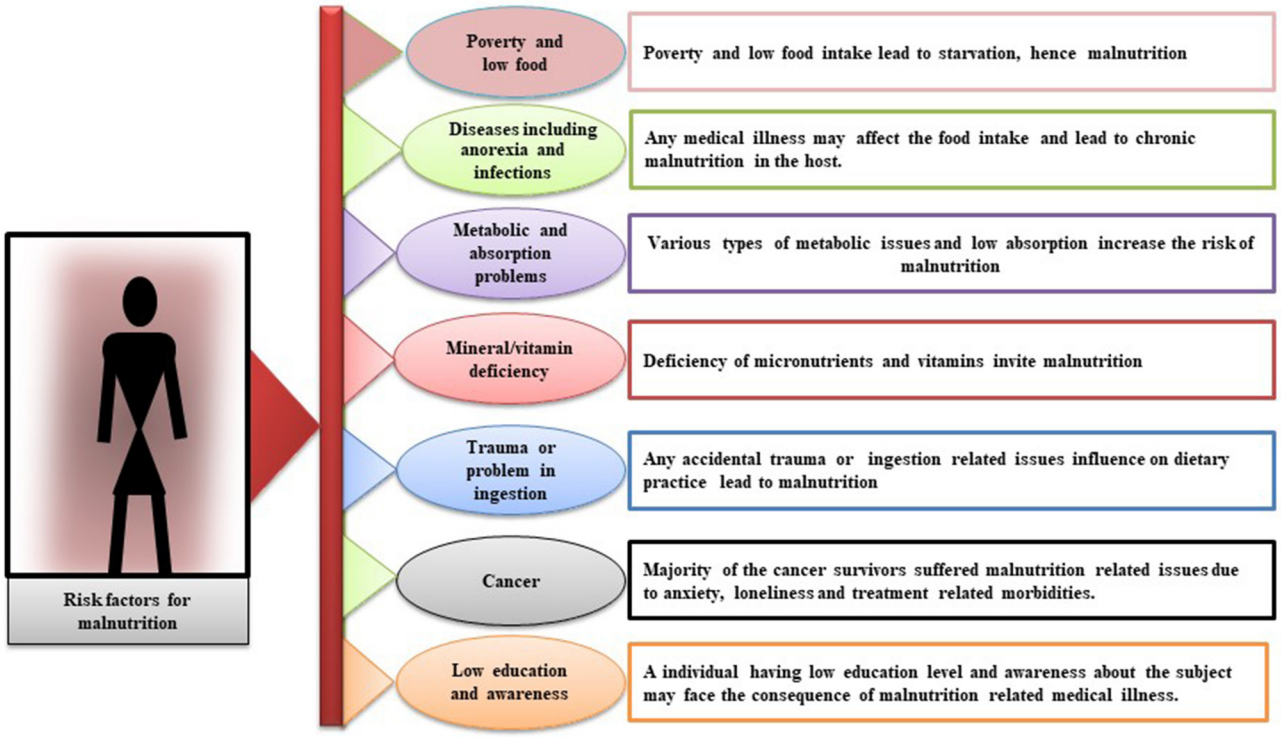

FIGURE 1 | Diagrammatic description of risk factors that contribute directly or indirectly to the development of malnutrition. Leading facts are poverty and low food, diseases, such as anorexia and infections, metabolic and absorption problems, mineral/vitamin deficiency, trauma or problem in ingestion, cancer, and low education and awareness.

can be stratified according to the severity of the disease. A diagrammatic description of all direct or indirect factors that mediate in bringing the malnutrition has been shown in **Figure 1**.

PROTEIN ENERGY MALNUTRITION AND INFECTION

Protein energy malnutrition is a range of pathological conditions arising out of coincident of lack of protein and energy in varying proportions, most frequently seen in infants, and young children and usually associated with infections (55). It is understandable that the body needs extra energy to activate and replenish the body's immune system in response to an infection. In nutrition sciences, this association of energy expenditure during infection can be expressed in the form of PEM, which provides an outlook to understand the relationship between malnutrition and infection (56). WHO has identified PEM as the most lethal form of malnutrition as it affects the most vulnerable segment of the population: children under the age of 5 (57). Through the years of research on the association of malnutrition and infection, it has been well-established that, in malnourished individuals, the immune capacity of energy expenditure is further impaired, and therefore the host becomes susceptible to infection and determines the outcome of infection (58).

Although, deficiencies in non-nutrients, such as vitamins, amino acids, iron, and even micronutrients, can affect the normal functioning of the body, however, PEM is considered to be associated with delayed recovery from disease, poorer quality of life, and increased risk of morbidity and mortality (59). It makes the body vulnerable to major human infectious diseases, especially in children in low-income countries (22). Overviewing the published data, it has been found that around half of the 10.8 million annual deaths of children under the age of 5 by malnutrition in less developed countries are under the deadly co-existence of PEM and infections (60). Hence, PEM determines the susceptibility of infection in the body and energy of the individual, which further leads to a loss of work efficiency at the community level and catalyzes the alarming spiral of infection, disease, and blatant poverty in society. **Figure 2** demonstrates impose-impact of malnutrition on an individuals' life, such as impaired growth, economic loss, hard to recover from other medical illnesses, low immunity, and prone to infections.

MALNUTRITION INCREASE RISK OF INFECTION

A vicious relationship between malnutrition and infectious diseases has been long established. Malnutrition causes an exponential decrease in the chances of survival per exposure

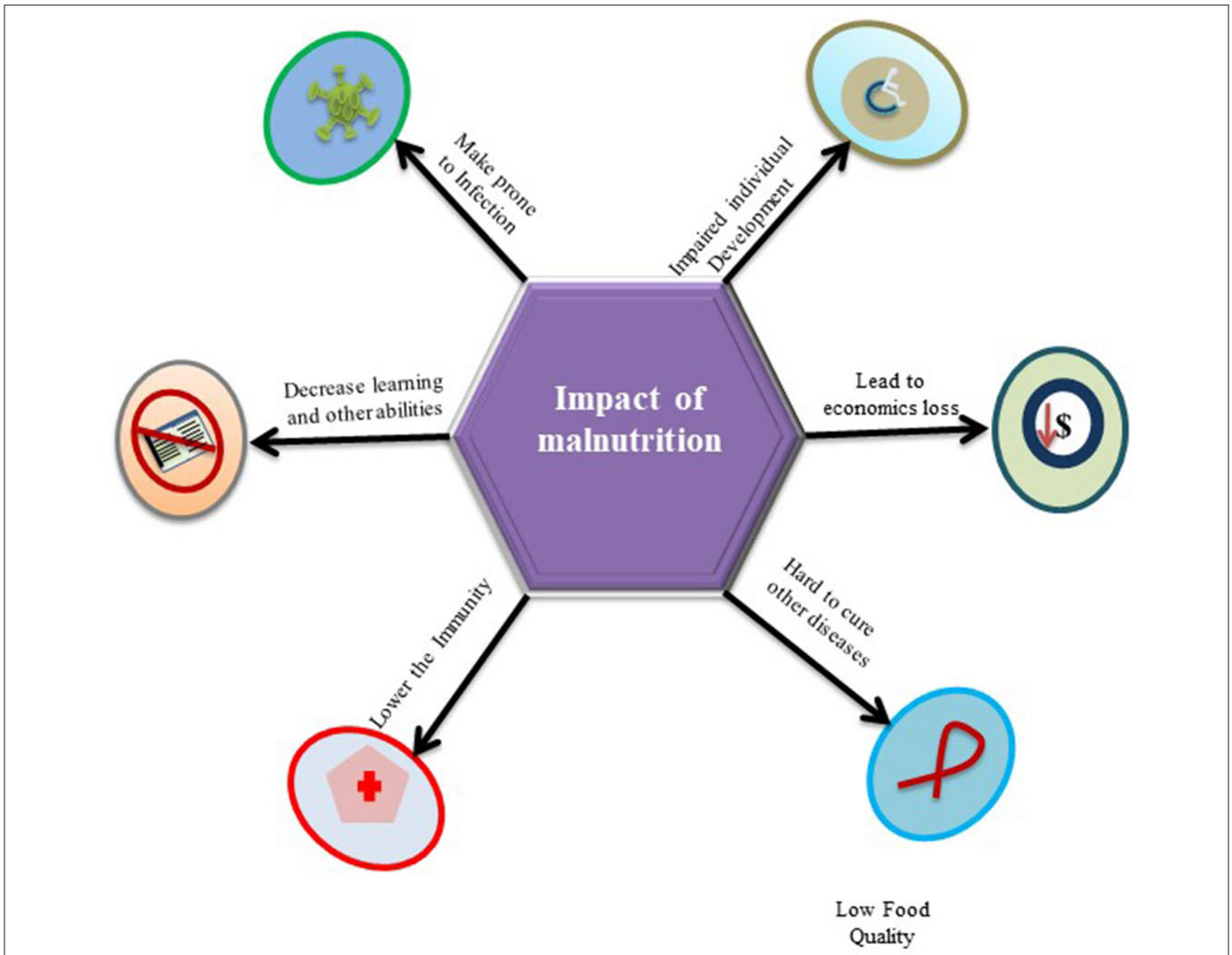

FIGURE 2 | The figurative presentation of impose-impact in the form of low immunity and prone to infections with other related outcomes by malnutrition on an individuals' life.

to any fatal infectious disease while non-fatal and subclinical infections may impair the process of growth (61). Published research revealed that malnourished children have a clear excess risk of infectious morbidity and mortality (62). Concurrently, the latest estimates show that about 50% of 10.6 million under 5 child death per year globally has been ascribed to the five infectious diseases (pneumonia, diarrhea, malaria, measles, and AIDS) (25, 63). Malnutrition impaired the integrity of the gastrointestinal mucosa of patients leading to reduced gastric acid secretion and increased susceptibility to some pathogens (64, 65). Besides the direct organ-specific effects of infection (e.g., intestinal loss of nutrients during diarrhea), there are other illnesses, such as metabolic and immune systems, which are the result of this association (66). It has been well-established that HIV infection and malnutrition are inter-linked by the complex interplay of different etiological factors, which contribute to the progression of the disease and increase the risk of mortality. Hence, addressing the nutrition right from the time of HIV diagnosis is a good strategy. This reduction of immune cells, known as nutritionally acquired immunodeficiency syndrome, plays a key role in the body's susceptibility to any infection (67). Severely malnourished patients have lacked a strong immunological response and show weak acquired immunity and innate host defense mechanisms. These patients are more susceptible to infection factors than the patients with HIV/AIDS and their opportunistic pathogens (68). A remarkable example of malnutrition and infection was seen after the Second World War when thousands of patients with AIDS and malnourished children were repeatedly diagnosed with opportunistic fungal pneumonia with other illnesses (69). Similarly, Noma is another deadly opportunistic infection. Noma is a result of complex interactions of different factors, such as poverty, malnutrition, compromised immune system, and poor oral hygiene, typically occurring in sub-Saharan Africa in children under 4 years of

age (70). Severe malnutrition, PEM, promotes acute and chronic infections, further reduces food intake, increases metabolic requirements, and reduces anabolic nutrient losses by impairing linear growth in affected children. Additionally, the acute phase of PEM disrupts bone remodeling required for longitudinal growth (71). It has been pointed out that the association between malnutrition and growth retardation helps researchers to assess the nutritional status of individuals. Numerous studies have demonstrated a significant correlation between acute malnutrition and acute infections. It has been well-established by the years of research that one cannot separate infection and its risk factors as determinants of the whole malnutrition burden. These determinants of malnutrition and infections are significantly present in the under-5 years of age children in developing countries and adversely impact children's health and development (25, 72, 73). Overviewing the published literature, it has been concluded that the relationship between malnutrition and infection is considered bidirectional (74). Evidence of the inverse relationship between malnutrition and infection is also found where an infection alters the nutrients intake, absorption, secretion, catabolism, and consumption. Such a vicious cycle of infection and malnutrition appears commonly in nature (25). **Figure 3** represents the bidirectional associations between malnutrition and infections.

MALNUTRITION AND CHRONIC DISEASES

It is a need of time to add important nuance to establish facts and understandings about the complex associations of chronic diseases, such as diarrhea, respiratory infection, HIV, malaria, measles, and the most hateful disease cancer, with malnutrition for the specific therapeutic approaches to break these associations and treat the diseases.

The vicious cycle between malnutrition and diarrhea is being explored for decades of years. According to a statistic report, around 1.5 million children die every year of diarrhea (75). It has been understood that, as compared to better nourished children with diarrhea, malnourished children with diarrhea have a far higher risk of death (76, 77). Diarrhea is the most common morbidity in severe acute malnutrition children and needs to be addressed. Effective adherence to the management protocol of dehydration and prompt modification of therapeutic feed can bring satisfactory health outcomes.

Followed by diarrhea, respiratory infections are the leading causes of death in children under the age of 5 years (78). It is plausible to assess the relationship between respiratory infection and malnutrition as poor growth. The low immune response of the host and susceptibility to pathogens lead to mortality and morbidity among the children across the globe (79). Going through the literature, it has been found that the published articles that concluded that malnourished children are very susceptible to several respiratory infections as compared to the others (80–82). Malnutrition especially in children is the risk factor for many bacterial and viral infections. A study on hospitalized children with the severe respiratory syncytial virus (RSV) demonstrates that poor infant growth increases the risk for severe RSV infection and leads to prolonging hospitalized stay (83). Similarly, there are also findings about the frequency of co-morbidities related to measles, pneumonia, tuberculosis, and malaria in the children presenting with severe acute malnutrition (84, 85). In an observational study, it has been known that 22% of 104 severe acute malnourished children were diagnosed with tuberculosis while 3.8% were diagnosed with malaria and measles (86). The research published in the *American Journal of Clinical Nutrition*, with the objective to find whether undernutrition is an underlying cause of children deaths associated with diarrhea, pneumonia, malaria, and measles, reveals that overall 52.5% of all deaths in young children were attributable to undernutrition varying from 44.8% for death by measles and 60.7% for death because of diarrhea (87).

Due to the desperate consequences of malnutrition, more than 1 million children die each year with the co-occurrence of HIV (88). The comorbidity rate of malnutrition and HIV makes HIV-infected children three times more likely to die than non-infected children (88, 89). Sub-Saharan Africa is the most affected region by the deadly association of malnutrition and HIV where 31.2% of stunting, 7.4% of wastage, and 5.2% of overweight children are reported (90). HIV infection in malnourished individuals is the result of several physiological and socioeconomic factors (91, 92). Muenchhoff confirmed strong associations with other comorbidities, such as diversification of the gut microbiota, epithelial malformations, chronic intestinal inflammation, and immune activation in malnourished HIV-infected children, which further lead to advances in disease pathogenesis (88). According to a recent study, it has been observed that micronutrient deficiency, such as selenium and vitamin C, determines the mycobacterial progression in HIV-positive patients, thus an imbalance of these micronutrients can be considered as an important risk factor (93). Furthermore, it has been found that in HIV-positive cases, metabolic problems, such as high lipids, can lead to increased long-term risk of atherosclerosis and cardiovascular abnormalities in malnourished patients (94). **Figure 4** represents the vicious cycle of malnutrition and HIV.

Despite the immense volume of laboratory and field research, there is still an adequate gap in knowledge about the subject and in defining the relationship between malnutrition and chronic diseases. To deal with this worldwide health crisis, diverse minds of researchers are continuously exploring the deadly associations of malnutrition and chronic diseases for the vision of a "world free of malnutrition."

Here, we are illuminating the current understanding of the interactions between malnutrition and cancer. As secondary data, this is an objective study to summarizing the literature and providing an update on the association of malnutrition and said disease.

MALNUTRITION AND CANCER

Malnutrition is exceedingly common in cancer patients. Cancer-associated malnutrition is a multifactorial syndrome characterized by persistent skeletal muscle loss due to different

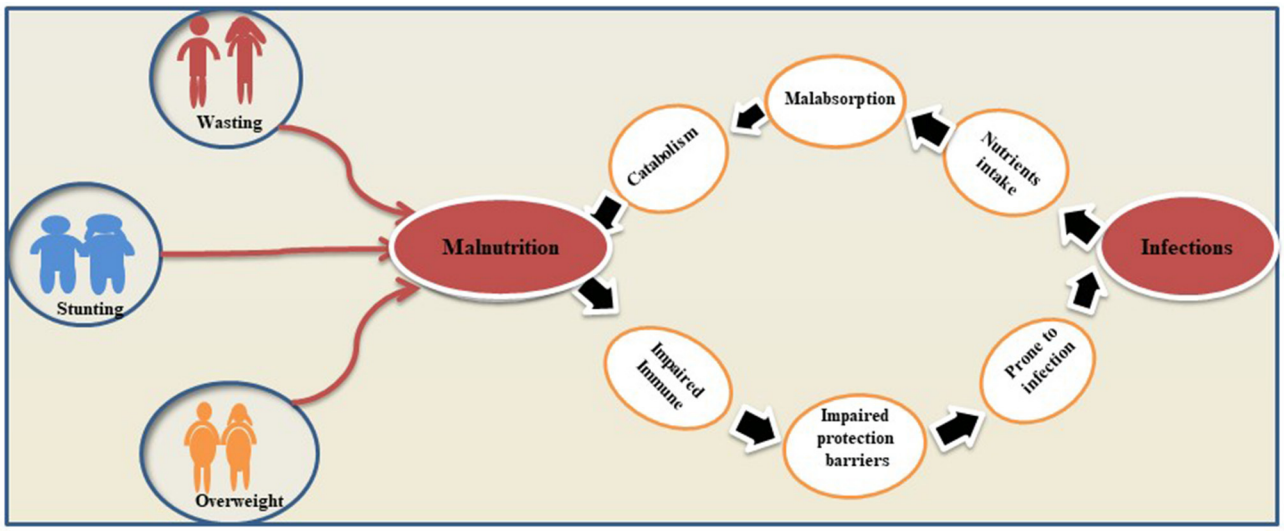

FIGURE 3 | Bidirectional associations between malnutrition and infections. The figure gives an explanation on how malnutrition and infections reinforce each other's and lead to maximum health damage of host.

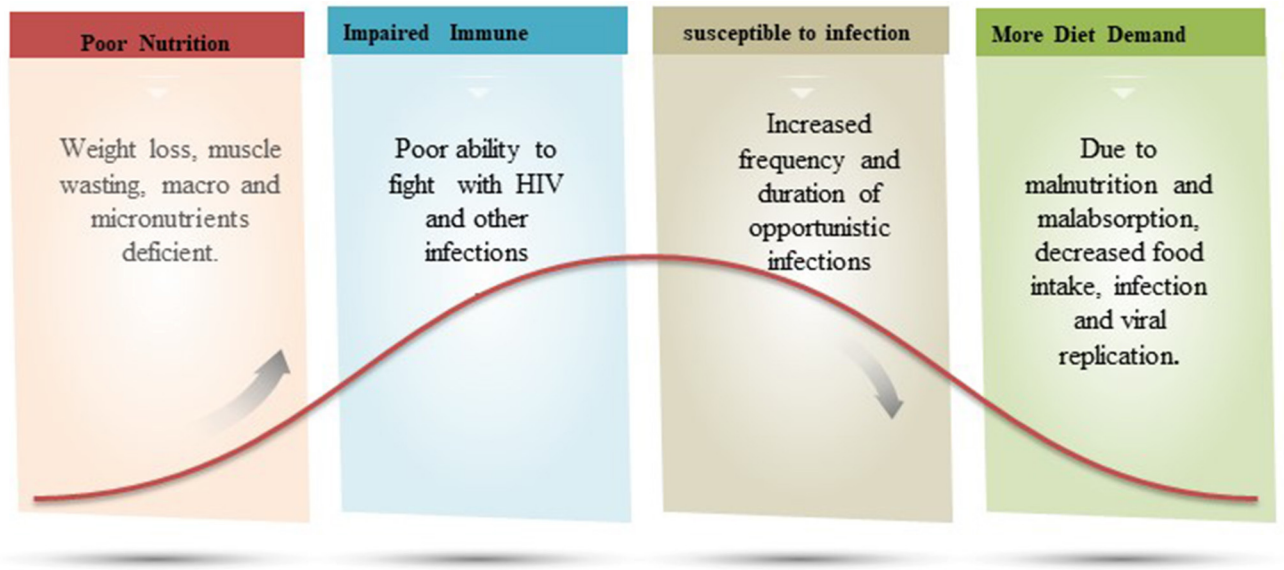

FIGURE 4 | A figurative explanation on the relation of malnutrition and immune fighting ability against HIV infection. In a vicious cycle of malnutrition-HIV, the patient has poor immunity and increased frequency and duration of opportunistic infection.

combinations of reduced dietary intake and metabolic changes (42). This association might express at the time of diagnosis or develop later and as the treatment and disease progressed, the situation became worse. The nutritional status of cancer patients who underwent treatment plays a vital role in promoting multimodality cancer care (95).

Recently, studies demonstrate that many European hospitals have 30–60% of cancer patients at risk of malnutrition who receive nutrition through other means, such as oral supplements, enteral nutrition, or parenteral nutrition (96, 97). Another European study showed that 40% of cancer-related severe malnutrition was misclassified by doctors, resulting in patients losing nutritional interventions (43). It has been also noted that damage of cancer-related malnutrition went uncontrolled when patients and their relatives underestimate it after the doctor's diagnosis (98–100).

The increasing frequency of malnutrition has become an emerging health problem of cancer survivors as it decreases the efficacy of homeopathy treatments and prolongs hospital stay especially in the citizen of the United States of America

(101, 102). According to a follow-up study, it has been obtained that malnourished patients with cancer are at about 2- to 5-fold greater risk of death (103). The development of cancer-related malnutrition is the result of several factors, which are considerably different from simple starvation, such as mental health issues, poor food intake, dysfunction of the gastrointestinal tract, increase in the energy need, low physical activity, and altered metabolism (42, 43, 104). Tumor-derived cytokines also mediate the disruption of metabolism, suppress the appetite, enhance muscle wasting, depression, fatigue, and subsequently, lead to impaired physical activity and anorexia (43, 105). Mentioned conditions may worsen the prognosis and lead to poor quality of life, prolonged hospital stay, tolerance to treatment, and therapeutic efficacy that lead to the end of life (106, 107). Additionally, cancer patients often report poor appetite along with reduced food enjoyment putting them at risk for malnutrition. This reduction in food intake may increase due to the site of the tumor. For example, oral or esophagus cancer may halt the eating and support the malnutrition onset (107, 108). On the other hand, the use of steroid drugs and hormone therapy has been considered as a standard of care for a different advanced type of cancer, however, there are findings that reveal that these steroid or hormone intake may contribute to the development of malnutrition (109, 110). Furthermore, unlikely other disease-related malnutrition, cancer-related malnutrition, are thought to be different from those that lack nutrients in the absence of underlying diseases (i.e., hunger and anorexia nervosa). It has been observed that metabolic changes in cancer patients, such as inflammation, increased catabolic rate, and ineffective cyclic anabolic resistance, achieved through a tumor or cancer treatment may invite malnutrition in the patient (111, 112). Apart from other clinical aspects in cancer-related malnutrition, the lack of adequate knowledge of nutritional intervention for cancer patients in caregivers is also a significant contribution in cancer-related malnutrition loss. A recent study demonstrates that almost half of the hospital oncologists involved in the survey report ascribe the underestimation of nutritional treatment to the insufficient training of healthcare professionals (95). In conclusion, to end up this deadly link of malnutrition and cancer, medical science is an urgent need to introduce advanced, state-of-the-art diagnostics, and care mechanisms and emphasizes the concern institutes to spread and raised the prevention and awareness literature to the public. **Figure 5** gives the multi ways associations and outcomes of the relation between malnutrition and cancer.

PREVENTION AND AWARENESS OF MALNUTRITION: PUBLIC HEALTH NURSING

Exceedingly damage by malnutrition-related co-morbidities demands the development of the specific protocol and practical recommendations rather than official meetings for the prevention and awareness about this global health crisis.

Multidisciplinary approaches are needed for the long-term nutritional support and benefits of geriatric malnutrition prevention, detection, and treatment. These approaches must explain acceptable different screening/assessment models and involve healthcare professionals (113). To examine and prevent the PEM especially in growing children from developing countries, there is a critical need of employing electrical health records and other mobiles systems. Adaptation of health information technology is a single advanced way forward of achieving better health outcomes across the globe (114). Malnutrition manifests its worse outcome in hospitalized malnutrition form and needs effective strategies to promote the awareness and overcome the burden. The nutrition committee of the French pediatric society (ePINUT) has endorsed an awareness strategy to lessen the burden and released its recommendations about the prevention and promotion of malnutrition education awareness in patient relatives, healthcare staff, and other caregivers (115). The vicious association of malnutrition cancer imposes significant health and economic loss. Cancer-related malnutrition is a globally rising health concern and appeals to add novel nuance to break this association. According to a current survey report, there is a lack of collaboration between oncologists and clinical nutritionists, which is the first obstacle to overcome. We are in urgent need to start internationally accepted educational intersociety initiatives, aimed to strengthen oncologist knowledge to improve nutritional support management and identify and remove the barriers to delivering optimal nutrition care in cancer survivors (116, 117). To lessen the burden and to the way of the world free of malnutrition, current understandings of knowledge about the subject deduced the following key steps to start a war against malnutrition. (1) Awareness about all forms of malnutrition must be raised: frontline staff who provide care must understand the importance of nutrition to basic patient care and consequences to patients if they neglected; (2) there is a critical need to develop worldwide accepted state of the art screening and assessments methods; (3) malnourished individual or those at risk must be on the right care pathway and received the right treatments; (4) frontline staff in all care settings must receive appropriate training on the importance of good nutritional care to speed up the recovery; and (5) world organizations must development management structure in place to ensure best practice especially in high prevalence parts of the world: Sub-Saharan Africa.

CONCLUSION

The literature revealed the need for important implications of nutritional intervention drives for elderly and child survival programs for developing countries to ensure the access of children who fight against chronic diseases. In conclusion, to fill the gaps in prioritizing standardized measurements and reporting of malnutrition status worldwide, it is imperative to enhance an in-depth understanding of the development causes and co-existence of malnutrition with other illnesses. To overcome malnutrition, it also demands to re-make and re-define the future by giving comprehensive but achievable and sustainable programs to alleviate poverty and combat the rampant infectious diseases and other nutrition-related health

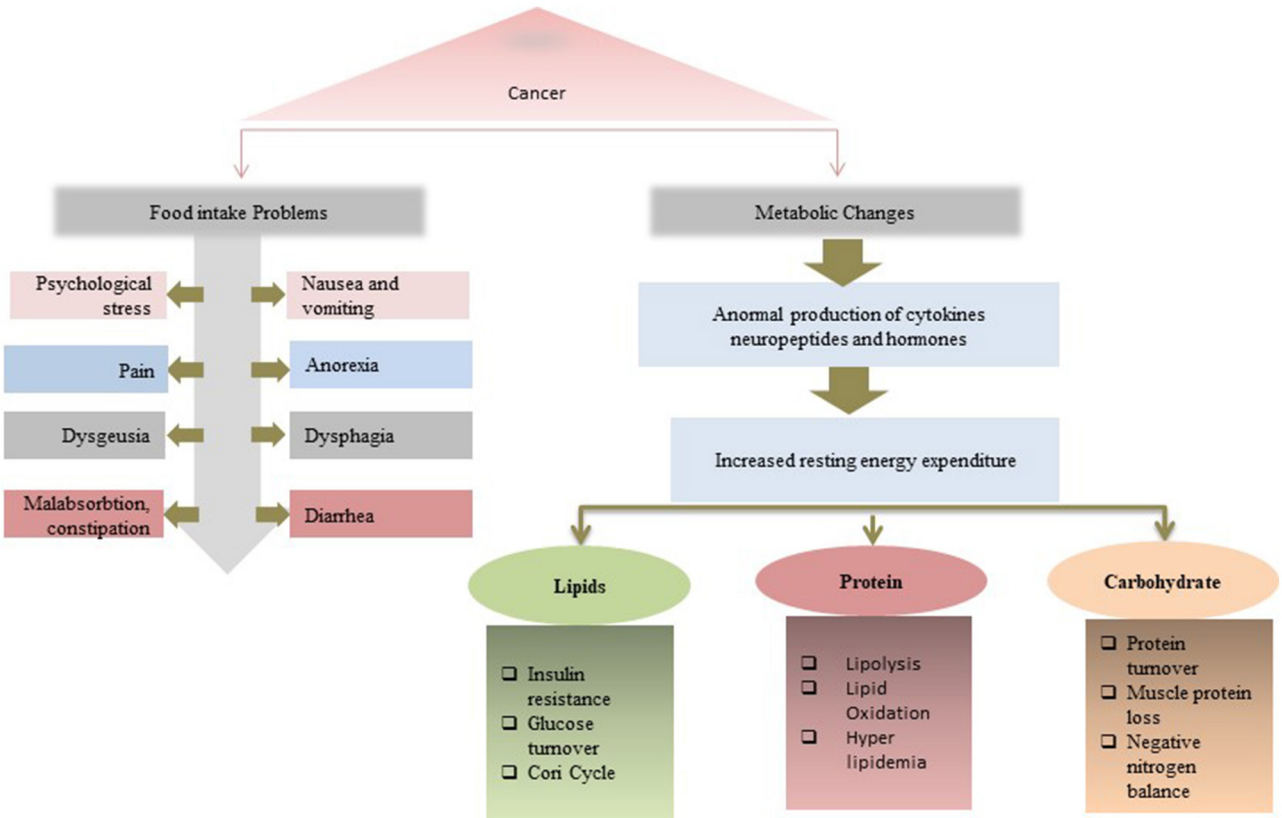

FIGURE 5 | Multi ways associations and outcomes of the relation between malnutrition and cancer. A malnutrition-cancer patient faces issues both in food intake and metabolic changes that lead to the reduction in treatment efficiency.

problems. To improve health outcomes especially in hospitalized cancer patients, it is crucially important to knowledge the caregiver healthcare staff for early interventions of supplying nutritional support to delay or prevent the onset of malnutrition.

AUTHOR CONTRIBUTIONS

YF, YL, TJ, and QY reviewed the literature and prepared the manuscript draft and the figure. EJ and JZ made the final editing and offered his expert suggestions and insights in preparing this work. All authors have read and agreed to the published version of the manuscript.

FUNDING

This review was supported by the National Natural Science Foundation of China (No. 81900375) and the Henan Provincial Science and Technology Research Project (No. 212102310147).

REFERENCES

1. da Silva Fink J, Marcadenti A, Rabito EI, Silva FM. The new european society for clinical nutrition and metabolism definition of malnutrition: application for nutrition assessment and prediction of morbimortality in an emergency service. *JPEN J Parenter Enteral Nutr.* (2018) 42:550–6. doi: 10.1177/0148607117695248
2. Ooi PH, Thompson-Hodgetts S, Pritchard-Wiart L, Gilmour SM, Mager DR. Pediatric sarcopenia: a paradigm in the overall definition of malnutrition in children? *JPEN J Parenter Enteral Nutr.* (2020) 44:407–18. doi: 10.1002/jpen.1681
3. Shrivastava SR, Shrivastava P. Necessity to urgently respond to the challenge of malnutrition: world health organization. *Cukurova Med J.* (2019) 44:303–4. doi: 10.17826/cumj.483943
4. Pelletier DL, Frongillo EA. Changes in child survival are strongly associated with changes in malnutrition in developing countries. *J Nutr.* (2003) 133:107–19. doi: 10.1093/jn/133.1.107
5. Jensen GL, Cederholm T. Global leadership initiative on malnutrition: progress report from aspen clinical nutrition week 2017. *JPEN J Parenter Enteral Nutr.* (2018) 42:266–7. doi: 10.1177/0148607117707761
6. Clark H, Coll-Seck AM, Banerjee A, Peterson S, Dalglish SL, Ameratunga S, et al. A future for the world's children? a WHO-UNICEF-lancet commission. *Lancet.* (2020) 395:605–58. doi: 10.1016/S0140-6736(19)32540-1
7. Onyango AW, Jean-Baptiste J, Samburu B, Mahlangu TLM. Regional overview on the double burden of malnutrition and examples of program and policy responses: african region. *Ann Nutr Metab.* (2019) 75:127–30. doi: 10.1159/000503671
8. World Health Organization. *Malnutrition* (2021). Available online at: https://www.who.int/news-room/fact-sheets/detail/malnutrition (accessed June 9, 2021).
9. Swaminathan S, Hemalatha R, Pandey A, Kassebaum NJ, Laxmaiah A, Longvah T, et al. The burden of child and maternal malnutrition and trends in its indicators in the states of India: the global

burden of disease study 1990–2017. *The Lancet Child.* (2019) 3:855–70. doi: 10.1016/S2352-4642(19)30273-1
10. Pineda JCC, Garcia AG, Velasco N, Graf JIDP, Adames AM, de la Torre AM. Nutritional assessment of hospitalized patients in Latin America: association with prognostic variables. The ENHOLA study. *Nutr Hosp.* (2016) 33:275. doi: 10.20960/nh.275
11. Correia MI, Hegazi RA, Diaz-Pizarro Graf JI, Gomez-Morales G, Fuentes Gutiérrez C, Goldin MF, et al. Addressing disease-related malnutrition in healthcare: a Latin American perspective. *JPEN J Parenter Enteral Nutr.* (2016) 40:319–25. doi: 10.1177/0148607115581373
12. Bwakura-Dangarembizi M, Amadi B, Bourke CD, Robertson RC, Mwapenya B, Chandwe K, et al. Health outcomes, pathogenesis and epidemiology of severe acute malnutrition (HOPE-SAM): rationale and methods of a longitudinal observational study. *BMJ Open.* (2019) 9:e023077. doi: 10.1136/bmjopen-2018-023077
13. Rasheed S, Woods RT. Malnutrition and quality of life in older people: a systematic review and meta-analysis. *Ageing Res Rev.* (2013) 12:561–6. doi: 10.1016/j.arr.2012.11.003
14. Lorenzo-Lopez L, Maseda A, de Labra C, Regueiro-Folgueira L, Rodriguez-Villamil JL, Millan-Calenti JC. Nutritional determinants of frailty in older adults: a systematic review. *BMC Geriatr.* (2017) 17:1–13. doi: 10.1186/s12877-017-0496-2
15. Bagriansky J, Champa N, Pak K, Whitney S, Laillou A. The economic consequences of malnutrition in Cambodia, more than 400 million US dollar lost annually. *Asia Pac J Clin Nutr.* (2014) 23:524–31. doi: 10.6133/apjcn.2014.23.4.08
16. Besora-Moreno M, Llaurado E, Tarro L, Sola R. Social and economic factors and malnutrition or the risk of malnutrition in the elderly: a systematic review and meta-analysis of observational studies. *Nutrients.* (2020) 12:737. doi: 10.3390/nu12030737
17. Adebisi YA, Ibrahim K, Lucero-Prisno DE, Ekpenyong A, Micheal AI, Chinemelum IG, et al. Prevalence and socio-economic impacts of malnutrition among children in Uganda. *Nutr Metab Insights.* (2019) 12. doi: 10.1177/1178638819887398
18. Elia M. Defining, recognizing, and reporting malnutrition. *Int J Low Extrem Wounds.* (2017) 16:230–7. doi: 10.1177/1534734617733902
19. Harris PS, Payne L, Morrison L, Green SM, Ghio D, Hallett C, et al. Barriers and facilitators to screening and treating malnutrition in older adults living in the community: a mixed-methods synthesis. *BMC Fam Pract.* (2019) 20:100. doi: 10.1186/s12875-019-0983-y
20. Craven DL, Lovell GP, Pelly FE, Isenring E. Community-living older adults' perceptions of body weight, signs of malnutrition and sources of information: a descriptive analysis of survey data. *J Nutr Health Aging.* (2018) 22:393–9. doi: 10.1007/s12603-017-0942-z
21. Wells JC, Sawaya AL, Wibaek R, Mwangome M, Poullas MS, Yajnik CS, et al. The double burden of malnutrition: aetiological pathways and consequences for health. *Lancet.* (2020) 395:75–88. doi: 10.1016/S0140-6736(19)32472-9
22. Adepoju AA, Allen S. Malnutrition in developing countries: nutrition disorders, a leading cause of ill health in the world today. *J Paediatr Child Health.* (2019) 29:394–400. doi: 10.1016/j.paed.2019.06.005
23. Sabbahi M, Li J, Davis C, Downs SM. The role of the sustainable development goals to reduce the global burden of malnutrition. Advances in food security and sustainability. *Elsevier.* (2018) 3:277–333. doi: 10.1016/bs.af2s.2018.09.007
24. World Health Organization. *Levels and trends in child malnutrition: UNICEF/WHO/The World Bank Group joint child malnutrition estimates: key findings of the 2020 edition.* (2020). Available online at: https://www.who.int/publications/i/item/jme-2020-edition (accessed March 31, 2020).
25. De Vita MV, Scolfaro C, Santini B, Lezo A, Gobbi F, Buonfrate D, et al. Malnutrition, morbidity and infection in the informal settlements of Nairobi, Kenya: an epidemiological study. *Ital J Pediatr.* (2019) 45:12. doi: 10.1186/s13052-019-0607-0
26. Wang XF, Gao P, Liu YF, Li HF, Lu F. Predicting thermophilic proteins by machine learning. *Curr Bioinform.* (2020) 15:493–502. doi: 10.2174/1574893615666200207094357
27. Niu MT, Lin Y, Zou Q. sgRNACNN: identifying sgRNA on-target activity in four crops using ensembles of convolutional neural networks. *Plant Mol Biol.* (2021) 105:483–95. doi: 10.1007/s11103-020-01102-y
28. Sun SW, Xu L, Zou Q, Wang GH. BP4RNAseq: a babysitter package for retrospective and newly generated RNA-seq data analyses using both alignment-based and alignment-free quantification method. *Bioinformatics.* (2021) 37:1319–21. doi: 10.1093/bioinformatics/btaa832
29. Di Francesco V, Pellizzari L, Corrà L, Fontana G. The anorexia of aging: impact on health and quality of life. *Geriatr Care.* (2018) 4:7324. doi: 10.4081/gc.2018.7324
30. Wu T-T. Malabsorption and malnutrition disorders. In: *Surgical Pathology of Non-Neoplastic Gastrointestinal Diseases,* eds Zhang L, Chandan VS, and Wu T-T (Cham: Springer Nature Switzerland AG) (2019). p. 191–238.
31. Blanar V, Hödl M, Lohrmann C, Amir Y, Eglseer D. Dysphagia and factors associated with malnutrition risk: a 5-year multicentre study. *J Adv Nurs.* (2019) 75:3566–76. doi: 10.1111/jan.14188
32. Dijkink S, Meier K, Krijnen P, Yeh DD, Velmahos GC, Schipper IB. Malnutrition and its effects in severely injured trauma patients. *Eur J Trauma Emerg Surg.* (2020) 46:993–1004. doi: 10.1007/s00068-020-01304-5
33. Roberts BL, Zhu M, Zhao H, Dillon C, Appleyard SM. High glucose increases action potential firing of catecholamine neurons in the nucleus of the solitary tract by increasing spontaneous glutamate inputs. *Am J Physiol Regul Integr Comp Physiol.* (2017) 313:R229–39. doi: 10.1152/ajpregu.00413.2016
34. Rachakonda V, Borhani AA, Dunn MA, Andrzejewski M, Martin K, Behari J. Serum leptin is a biomarker of malnutrition in decompensated cirrhosis. *PLoS ONE.* (2016) 11:e0159142. doi: 10.1371/journal.pone.0159142
35. DeBoer MD, Scharf RJ, Leite AM, Ferrer A, Havt A, Pinkerton R, et al. Systemic inflammation, growth factors, and linear growth in the setting of infection and malnutrition. *Nutrition.* (2017) 33:248–53. doi: 10.1016/j.nut.2016.06.013
36. Jacobsen EL, Brovold T, Bergland A, Bye A. Prevalence of factors associated with malnutrition among acute geriatric patients in Norway: a cross-sectional study. *BMJ Open.* (2016) 6:e011512. doi: 10.1136/bmjopen-2016-011512
37. Soysal P, Dokuzlar O, Erken N, Gunay FSD, Isik AT. The relationship between dementia subtypes and nutritional parameters in older adults. *J Am Med Dir Assoc.* (2020) 21:1430–5. doi: 10.1016/j.jamda.2020.06.051
38. Pourhassan M, Rommersbach N, Lueg G, Klimek C, Schnatmann M, Liermann D, et al. The impact of malnutrition on acute muscle wasting in frail older hospitalized patients. *Nutrients.* (2020) 12:1387. doi: 10.3390/nu12051387
39. Lau S, Pek K, Chew J, Lim JP, Ismail NH, Ding YY, et al. The simplified nutritional appetite questionnaire (SNAQ) as a screening tool for risk of malnutrition: optimal cutoff, factor structure, and validation in healthy community-dwelling older adults. *Nutrients.* (2020) 12:2885. doi: 10.3390/nu12092885
40. Chen CC, Schilling LS, Lyder CH. A concept analysis of malnutrition in the elderly. *J Adv Nurs.* (2001) 36:131–42. doi: 10.1046/j.1365-2648.2001.01950.x
41. Islam MR, Rahman MS, Rahman MM, Nomura S, de Silva A, Lanerolle P, et al. Reducing childhood malnutrition in Bangladesh: the importance of addressing socio-economic inequalities. *Public Health Nutr.* (2020) 23:72–82. doi: 10.1017/S136898001900140X
42. Baracos VE. Cancer-associated malnutrition. *Eur J Clin Nutr.* (2018) 72:1255–9. doi: 10.1038/s41430-018-0245-4
43. Arends J, Baracos V, Bertz H, Bozzetti F, Calder PC, Deutz NEP, et al. ESPEN expert group recommendations for action against cancer-related malnutrition. *Clin Nutr.* (2017) 36:1187–96. doi: 10.1016/j.clnu.2017.06.017
44. Widodo AD, Soelaeman EJ, Dwinanda N, Narendraswari PP, Purnomo B. Chronic liver disease is a risk factor for malnutrition and growth retardation in children. *Asia Pac J Clin Nutr.* (2017) 26:S57–60. doi: 10.6133/apjcn.062017.s10
45. Tabib A, Aryafar M, Ghadrdoost B. Prevalence of malnutrition in children with congenital heart disease. *J Compr Pediatr.* (2019) 10:e84274. doi: 10.5812/compreped.84274
46. Noce A, Vidiri M, Marrone G, Moriconi E, Bocedi A, Capria A, et al. Is low-protein diet a possible risk factor of malnutrition in chronic kidney disease patients? *Cell Death Discov.* (2016) 2:16026. doi: 10.1038/cddiscovery.2016.26
47. Mukhopadhyay DK, Biswas R, Chakraborty M, Sadhukhan SK and Banik KK. Anthropometric failure, a new approach to measure undernutrition: an experience from a rural community of West Bengal, India. *J Indian Med Assoc.* (2009) 107:211–4.

48. Sehgal IS, Dhooria S, Agarwal R. Chronic obstructive pulmonary disease and malnutrition in developing countries. *Curr Opin Pulm Med.* (2017) 23:139–48. doi: 10.1097/MCP.0000000000000356
49. Attia S, Feenstra M, Swain N, Cuesta M, Bandsma RHJ. Starved guts: morphologic and functional intestinal changes in malnutrition. *J Pediatr Gastroenterol Nutr.* (2017) 65:491–5. doi: 10.1097/MPG.0000000000001629
50. Slae M, Wilschanski M. Prevention of malnutrition in cystic fibrosis. *Curr Opin Pulm Med.* (2019) 25:674–9. doi: 10.1097/MCP.0000000000000629
51. Tellez-Navarrete NA, Ramon-Luing LA, Munoz-Torrico M, Osuna-Padilla IA, Chavez-Galan L. Malnutrition and tuberculosis: the gap between basic research and clinical trials. *J Infect Dev Countr.* (2021) 15:310–9. doi: 10.3855/jidc.12821
52. Eglseer D, Halfens RJG, Lohrmann C. Use of an electronic malnutrition screening tool in a hospital setting: effects on knowledge, attitudes and perceived practices of healthcare staff. *Brit J Nutr.* (2018) 120:150–7. doi: 10.1017/S0007114518001447
53. Schorr AV, Yehuda I, Tamir S. Ethnic differences in loneliness, depression, and malnutrition among older adults during COVID-19 quarantine. *J Nutr Health Aging.* (2021) 25:311–7. doi: 10.1007/s12603-020-1540-z
54. Ramic E, Pranjic N, Batic-Mujanovic O, Karic E, Alibasic E, Alic A. The effect of loneliness on malnutrition in elderly population. *Med Arh.* (2011) 65:92–5. Available online at: https://www.ncbi.nlm.nih.gov/pubmed/21585182
55. Jee KO. Protein energy malnutrition: an overview. *International Journal of Homoeopathic Sciences.* (2021) 5:368–73. doi: 10.33545/26164485.2021.v5.i1f.341
56. Batool R, Butt MS, Sultan MT, Saeed F, Naz R. Protein–energy malnutrition: a risk factor for various ailments. *Crit Rev Food Sci Nutr.* (2015) 55:242–53. doi: 10.1080/10408398.2011.651543
57. Satapathy A, Satapathy A, Rout DS, Prusty AK, Rout S. Prevalence of protein energy malnutrition among under-five children in Odisha: a review. *J Phytopharm.* (2021) 10:272–6. doi: 10.31254/phyto.2021.10410
58. Bartelt LA, Bolick DT, Mayneris-Perxachs J, Kolling GL, Medlock GL, Zaenker EI, et al. Cross-modulation of pathogen-specific pathways enhances malnutrition during enteric co-infection with Giardia lamblia and enteroaggregative Escherichia coli. *PLoS Pathog.* (2017) 13:e1006471. doi: 10.1371/journal.ppat.1006471
59. Cederholm T, Barazzoni R, Austin P, Ballmer P, Biolo G, Bischoff SC, et al. ESPEN guidelines on definitions and terminology of clinical nutrition. *Clin Nutr.* (2017) 36:49–64. doi: 10.1016/j.clnu.2016.09.004
60. Echendu ST, Okeke KN, Ebenebe JC, Ugochukwu EF, Onubogu CU, Umeadi EN, et al. Prevalence of protein energy malnutrition in HIV-infected under five children and the effects of highly active antiretroviral therapy on their nutritional status in Nigeria. *MSARR.* (2021) 1:53–61. doi: 10.30574/msarr.2021.1.3.0024
61. Jones KD, Thitiri J, Ngari M, Berkley JA. Childhood malnutrition: toward an understanding of infections, inflammation, and antimicrobials. *Food Nutr Bull.* (2014) 35(2 Suppl):S64–70. doi: 10.1177/15648265140352S110
62. Prendergast AJ. Malnutrition and vaccination in developing countries. *Philos Trans R Soc Lond B Biol Sci.* (2015) 370:20140141. doi: 10.1098/rstb.2014.0141
63. UNICEF. *The State of the World's Children.* (2019). Available online at: https://www.unicef.org/reports/state-of-worlds-children-2019 (accessed October 31, 2019).
64. Martinsen TC, Bergh K, Waldum HL. Gastric juice: a barrier against infectious diseases. *Basic Clin Pharmacol Toxicol.* (2005) 96:94–102. doi: 10.1111/j.1742-7843.2005.pto960202.x
65. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nat Rev Immunol.* (2014) 14:141–53. doi: 10.1038/nri3608
66. Benson MJ, Pino-Lagos K, Rosemblatt M, Noelle RJ. All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. *J Exp Med.* (2007) 204:1765–74. doi: 10.1084/jem.20070719
67. Bourke CD, Berkley JA, Prendergast AJ. Immune dysfunction as a cause and consequence of malnutrition. *Trends Immunol.* (2016) 37:386–98. doi: 10.1016/j.it.2016.04.003
68. Weger-Lucarelli J, Auerswald H, Vignuzzi M, Dussart P, Karlsson EA. Taking a bite out of nutrition and arbovirus infection. *PLoS Negl Trop Dis.* (2018) 12:e0006247. doi: 10.1371/journal.pntd.0006247
69. Evans HM, Bryant GL, Garvya BA. The life cycle stages of pneumocystis murina have opposing effects on the immune response to this opportunistic fungal pathogen. *Infect Immun.* (2016) 84:3195–205. doi: 10.1128/IAI.00519-16
70. Brattström-Stolt L, Funk T, Sié A, Ndiaye C, Alfvén T. Noma—knowledge and practice competence among primary healthcare workers: a cross-sectional study in Burkina Faso. *Int Health.* (2019) 11:290–6. doi: 10.1093/inthealth/ihy088
71. Dolan E, Varley I, Ackerman KE, Pereira RMR, Elliott-Sale KJ, Sale C. The bone metabolic response to exercise and nutrition. *Exerc Sport Sci Rev.* (2020) 48:49–58. doi: 10.1249/JES.0000000000000215
72. Ibrahim MK, Zambruni M, Melby CL, Melby PC. Impact of childhood malnutrition on host defense and infection. *Clin Microbiol Rev.* (2017) 30:919–71. doi: 10.1128/CMR.00119-16
73. Walson JL, Berkley JA. The impact of malnutrition on childhood infections. *Curr Opin Infect Dis.* (2018) 31:231–6. doi: 10.1097/QCO.0000000000000448
74. Schlaudecker EP, Steinhoff MC, Moore SR. Interactions of diarrhea, pneumonia, and malnutrition in childhood: recent evidence from developing countries. *Curr Opin Infect Dis.* (2011) 24:496–502. doi: 10.1097/QCO.0b013e328349287d
75. Black RE, Morris SS, Bryce J. Where and why are 10 million children dying every year? *Lancet.* (2003) 361:2226–34. doi: 10.1016/S0140-6736(03)13779-8
76. Troeger C, Blacker BF, Khalil IA, Rao PC, Cao SJ, Zimsen SRM, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the global burden of disease study 2016. *Lancet Infect Dis.* (2018) 18:1211–28. doi: 10.1016/S1473-3099(18)30362-1
77. Olofin I, McDonald CM, Ezzati M, Flaxman S, Black RE, Fawzi WW, et al. Associations of suboptimal growth with all-cause and cause-specific mortality in children under five years: a pooled analysis of ten prospective studies. *PLoS ONE.* (2013) 8:e64636. doi: 10.1371/journal.pone.0064636
78. Cunha AL. Relationship between acute respiratory infection and malnutrition in children under 5 years of age. *Acta Paediatrica.* (2000) 89:608–9. doi: 10.1111/j.1651-2227.2000.tb00347.x
79. Farhadi S, Ovchinnikov RS. The relationship between nutrition and infectious diseases: a review. *Biomed Biotech Res J.* (2018) 2:168–72. doi: 10.4103/bbrj.bbrj_69_18
80. Bhat RY, Manjunath N. Correlates of acute lower respiratory tract infections in children under 5 years of age in India. *Int J Tuberc Lung Dis.* (2013) 17:418–22. doi: 10.5588/ijtld.12.0117
81. Mukhopadhyay DK, Biswas R, Chakraborty M, Sadhukhan SK, Banik KK. Anthropometric failure, a new approach to measure undernutrition: an experience from a rural community of West Bengal, India. *J Indian Med Assoc.* (2009) 107:211–4, 236. Available online at: https://www.ncbi.nlm.nih.gov/pubmed/19810363
82. Brennhofer S, Reifsnider E, Bruening M. Malnutrition coupled with diarrheal and respiratory infections among children in Asia: a systematic review. *Public Health Nursing.* (2017) 34:401–9. doi: 10.1111/phn.12273
83. Paynter S, Ware RS, Lucero MG, Tallo V, Nohynek H, Weinstein P, et al. Malnutrition: a risk factor for severe respiratory syncytial virus infection and hospitalization. *Pediatr Infect Dis J.* (2014) 33:267–71. doi: 10.1097/INF.0000000000000096
84. Page AL, de Rekeneire N, Sayadi S, Aberrane S, Janssens AC, Rieux C, et al. Infections in children admitted with complicated severe acute malnutrition in Niger. *PLoS ONE.* (2013) 8:e68699. doi: 10.1371/journal.pone.0068699
85. Fatima S, Haider M, Hameed A, Saleem SG, Karim S. Comorbidities and their outcomes in children with severe acute malnutrition visiting pediatric emergency department at a tertiary care hospital in urban slums of Karachi, Pakistan. *Ann Pediatr.* (2021) 4:1068.
86. Kumar R, Singh J, Joshi K, Singh HP, Bijesh S. Co-morbidities in hospitalized children with severe acute malnutrition. *Indian Pediatr.* (2014) 51:125–7. doi: 10.1007/s13312-014-0343-x
87. Caulfield LE, de Onis M, Blossner M, Black RE. Undernutrition as an underlying cause of child deaths associated with diarrhea, pneumonia, malaria, and measles. *Am J Clin Nutr.* (2004) 80:193–8. doi: 10.1093/ajcn/80.1.193
88. Muenchhoff M, Healy M, Singh R, Roider J, Groll A, Kindra C, et al. Malnutrition in HIV-Infected children is an indicator of severe disease with

an impaired response to antiretroviral therapy. *AIDS Res Hum Retroviruses.* (2018) 34:46–55. doi: 10.1089/aid.2016.0261
89. Lazzari TK, Forte GC, Silva DR. Nutrition status among HIV-positive and HIV-negative inpatients with pulmonary tuberculosis. *Nutr Clin Pract.* (2018) 33:858–64. doi: 10.1002/ncp.10006
90. Penda CI, Moukoko ECE, Nolla NP, Evindi NOA, Ndombo PK. Malnutrition among HIV infected children under 5 years of age at the Laquintinie hospital Douala, Cameroon. *Pan Afr Med J.* (2018) 30:91. doi: 10.11604/pamj.2018.30.91.15832
91. Takarinda KC, Mutasa-Apollo T, Madzima B, Nkomo B, Chigumira A, Banda M, et al. Malnutrition status and associated factors among HIV-positive patients enrolled in ART clinics in Zimbabwe. *BMC Nutrition.* (2017) 3:1–11. doi: 10.1186/s40795-017-0132-8
92. Jesson J, Leroy V. Challenges of malnutrition care among HIV-infected children on antiretroviral treatment in Africa. *Med Mal Infect.* (2015) 45:149–56. doi: 10.1016/j.medmal.2015.03.002
93. Rautiainen S, Manson JE, Lichtenstein AH, Sesso HD. Dietary supplements and disease prevention—a global overview. *Nat Rev Endocrinol.* (2016) 12:407–20. doi: 10.1038/nrendo.2016.54
94. Jesson J, Dahourou DL, Folquet MA, Malateste K, Yonaba C, N'Gbeche MS, et al. Malnutrition, growth response and metabolic changes within the first 24 months after ART Initiation in HIV-infected children treated before the age of 2 Years in West Africa. *Pediatr Infect Dis J.* (2018) 37:781–7. doi: 10.1097/INF.0000000000001932
95. Muscaritoli M, Corsaro E, Molfino A. Awareness of cancer-related malnutrition and its management: analysis of the results from a survey conducted among medical oncologists. *Front Oncol.* (2021) 11:682999. doi: 10.3389/fonc.2021.682999
96. Planas M, Alvarez-Hernandez J, Leon-Sanz M, Celaya-Perez S, Araujo K, Garcia de Lorenzo A, et al. Prevalence of hospital malnutrition in cancer patients: a sub-analysis of the PREDyCES(R) study. *Support Care Cancer.* (2016) 24:429–35. doi: 10.1007/s00520-015-2813-7
97. Hebuterne X, Lemarie E, Michallet M, de Montreuil CB, Schneider SM, Goldwasser F. Prevalence of malnutrition and current use of nutrition support in patients with cancer. *JPEN J Parenter Enteral Nutr.* (2014) 38:196–204. doi: 10.1177/0148607113502674
98. Caccialanza R, Lobascio F, Cereda E, Aprile G, Farina G, Traclò F, et al. Cancer-related malnutrition management: a survey among italian oncology units and patients' associations. *Curr Probl Cancer.* (2020) 44:100554. doi: 10.1016/j.currproblcancer.2020.100554
99. Wang S, Zhao Y, Li J, Lai H, Qiu C, Pan N, et al. Neurostructural correlates of hope: dispositional hope mediates the impact of the SMA gray matter volume on subjective well-being in late adolescence. *Soc Cogn Affect Neurosci.* (2020) 15:395–404. doi: 10.1093/scan/nsaa046
100. Zou Q, Xing P, Wei L, Liu B. Gene2vec: gene subsequence embedding for prediction of mammalian N6-methyladenosine sites from mRNA. *RNA.* (2019) 25:205–18. doi: 10.1261/rna.069112.118
101. Mays LC, Drummonds JW, Powers S, Buys DR, Watts PI. Identifying Geriatric Patients at Risk for Malnutrition: A Quality Improvement Project. *J Nutr Gerontol Geriatr.* (2019) 38:115–29. doi: 10.1080/21551197.2019.1604464
102. Zhang XT, Tang TL, Pang LD, Sharma SV, Li RS, Nyitray AG, et al. Malnutrition and overall survival in older adults with cancer: a systematic review and meta-analysis. *J Geriatr Oncol.* (2019) 10:874–83. doi: 10.1016/j.jgo.2019.03.002
103. Karami K, Pourmahmoudi A, Toori MA, Imani H, Hosseinikia M, Jonghani MN, et al. Malnutrition risk and related factors in cancer patients undergoing chemotherapy: a cross-sectional study. *World Cancer Res J.* (2021) 8:e1925. doi: 10.32113/wcrj_20213_1925
104. Muscaritoli M, Lucia S, Farcomeni A, Lorusso V, Saracino V, Barone C, et al. Prevalence of malnutrition in patients at first medical oncology visit: the PreMiO study. *Oncotarget.* (2017) 8:79884–96. doi: 10.18632/oncotarget.20168
105. Zhu CJ, Wang BQ, Gao Y, Ma XL. Prevalence and relationship of malnutrition and distress in patients with Cancer using questionnaires. *BMC Cancer.* (2018) 18:1–6. doi: 10.1186/s12885-018-5176-x
106. Sanz EA, Siles MG, Fernandez LR, Roldan RV, Dominguez AR, Abiles J. Nutritional risk and malnutrition rates at diagnosis of cancer in patients treated in outpatient settings: early intervention protocol. *Nutrition.* (2019) 57:148–53. doi: 10.1016/j.nut.2018.05.021
107. de Pinho NB, Martucci RB, Rodrigues VD, D'Almeida CA, Thuler LCS, Saunders C, et al. Malnutrition associated with nutrition impact symptoms and localization of the disease: results of a multicentric research on oncological nutrition. *Clin Nutr.* (2019) 38:1274–9. doi: 10.1016/j.clnu.2018.05.010
108. Galaniha LT, McClements DJ, Nolden A. Opportunities to improve oral nutritional supplements for managing malnutrition in cancer patients: a food design approach. *Trends Food Sci Technol.* (2020) 102:254–60. doi: 10.1016/j.tifs.2020.03.020
109. Sydes MR, Spears MR, Mason MD, Clarke NW, Dearnaley DP, de Bono JS, et al. Adding abiraterone or docetaxel to long-term hormone therapy for prostate cancer: directly randomised data from the STAMPEDE multi-arm, multi-stage platform protocol. *Ann Oncol.* (2018) 29:1235–48. doi: 10.1093/annonc/mdy072
110. Beirer A. Malnutrition and cancer, diagnosis and treatment. *Memo-Mag Eur Med Oncol.* (2021) 14:168–73. doi: 10.1007/s12254-020-00672-3
111. Verlande A, Chun SK, Goodson MO, Fortin BM, Bae H, Jang C, et al. Glucagon regulates the stability of REV-ERBα to modulate hepatic glucose production in a model of lung cancer–associated cachexia. *Sci Adv.* (2021) 7:eabf3885. doi: 10.1126/sciadv.abf3885
112. de van der Schueren MAE, Laviano A, Blanchard H, Jourdan M, Arends J, Baracos VE. Systematic review and meta-analysis of the evidence for oral nutritional intervention on nutritional and clinical outcomes during chemo(radio)therapy: current evidence and guidance for design of future trials. *Ann Oncol.* (2018) 29:1141–53. doi: 10.1093/annonc/mdy114
113. Keller H, Slaughter S, Gramlich L, Namasivayam-MacDonald A, Bell JJ. Multidisciplinary nutrition care: Benefitting patients with malnutrition across healthcare sectors. In: *Interdisciplinary Nutritional Management and Care for Older Adults*, eds Ólöf GG, Jack JB (Cham: Springer) (2021). p. 177–88.
114. Onaleye F. Protein energy malnutrition in children: Prevention system. In: *Optimizing Health Monitoring Systems With Wireless Technology*, ed Wickramasinghe N (Hershey: IGI Global) (2021). p. 248–57. doi: 10.4018/978-1-5225-6067-8.ch017
115. De Luca A, Patel M, Mantha O, Peretti N, Hankard R. Promoting the awareness of hospital malnutrition in children: ePINUT 10th anniversary in 2020. *Nutrition Clinique et Métabolisme.* (2021) 35:85–92. doi: 10.1016/j.nupar.2021.01.001
116. Kiss N, Bauer J, Boltong A, Brown T, Isenring L, Loeliger J, et al. Awareness, perceptions and practices regarding cancer-related malnutrition and sarcopenia: a survey of cancer clinicians. *Support Care Cancer.* (2020) 28:5263–70. doi: 10.1007/s00520-020-05371-7
117. Caccialanza R, Cereda E, Pinto C, Cotogni P, Farina G, Gavazzi C, et al. Awareness and consideration of malnutrition among oncologists: insights from an exploratory survey. *Nutrition.* (2016) 32:1028–32. doi: 10.1016/j.nut.2016.02.005

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.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 22 March 2022
doi: 10.3389/fnut.2022.863106

Early Dinner Time and Caloric Restriction Lapse Contribute to the Longevity of Nonagenarians and Centenarians of the Italian Abruzzo Region: A Cross-Sectional Study

*Donato Angelino, Francesca Pietrangeli and Mauro Serafini**

Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy

Recent findings showed the role of late-night eating in metabolic disorders, highlighting the importance of meal timing for health. No evidence is available on the role of meal timing for longevity. The aim of this study was to survey, in a cross-sectional study, meal timing and dietary habits of 68 nonagenarians and centenarians of the Abruzzo region, Italy. Results showed an early dinner (7:13 p.m.) and a calorie restriction lapse of 17.5 h between dinner and the following lunch. The frequency of consumption was high for cereals, vegetables, fruits, and legumes; low for meat, processed meat, and eggs; and negligible for sweets. Subjects were physically active throughout life. Our results support the importance of a daily caloric restriction lapse, hampering nocturnal postprandial stress and optimizing metabolic response, associated with high consumption of plant-based foods and physical activity for the longevity of centenarians from Abruzzo.

Keywords: centenarians, longevity, chrono-nutrition, caloric restriction lapse, Abruzzo, dietary habits, lifestyle

OPEN ACCESS

Edited by:
Thea Magrone,
University of Bari Aldo Moro, Italy

Reviewed by:
Aoife Caffrey,
Ulster University, United Kingdom
Giovanni Mario Pes,
University of Sassari, Italy

***Correspondence:**
Mauro Serafini
mserafini@unite.it

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *26 January 2022*
Accepted: *22 February 2022*
Published: *22 March 2022*

Citation:
Angelino D, Pietrangeli F and Serafini M (2022) Early Dinner Time and Caloric Restriction Lapse Contribute to the Longevity of Nonagenarians and Centenarians of the Italian Abruzzo Region: A Cross-Sectional Study. Front. Nutr. 9:863106. doi: 10.3389/fnut.2022.863106

INTRODUCTION

The life expectancy of human individuals is consistently increasing across the decades, almost doubling in the last two centuries (1). The World Health Organization (WHO) forecasts that the rate of 900 million people aged over 60 years will increase up to 2 billion in 2050 (2), with the number of centenarians rising from 573,000 in 2020 to 19,000,000 by 2100 (3). Aging involves a general decay of organ function, in terms of muscle and bone frailties, impairment of renal and hepatic function, as well as loss of cardiovascular and immunological functionality (4). Among lifestyle factors, the diet has been found to significantly impact the health status of individuals and, in turn, to be a key variable impacting the physiological decay of aging processes (5, 6).

Italy is the first country in Europe and the second one in the world, in terms of percentage of citizens older than 65 years of age (22.8% of the total population, following a high of 28% in Japan) and over 85 years (3.6% of the total population, following a high of 4.5% in Japan) (7). The Greek island Ikaria (8) and the Italian Sardinian "*Ogliastra*" subregion are the two locations in the Mediterranean area identified as the "Longevity Blue Zone," for the extremely high rate of centenarians among its citizens (9). Concerning the "*Ogliastra*" region, the first hypothesis proposed to explain these demographic data concerning the genetic isolation of the Sardinian population, due to limited immigration into the island as well as to many conserved anthropological traditions (9). However, more recently, the food habits of these citizens have been taken into consideration to determine if they might have a role in longevity, both today and in the past (10,

11). In fact, their dietary habits seem to resemble the building blocks of the Mediterranean diet, known to be characterized by a high intake of plant-based foods, a moderate intake of fish and dairy products, and a low intake of meat and processed foods.

Based on the data from the Italian National Institute of Statistics (ISTAT), the region of Abruzzo, placed in center Italy, is among the top areas of Italy for the number of nonagenarians (N = 19,673, the 2.43% of the Italian nonagenarians) and centenarians (N = 485, the 2.82% of the Italian centenarians). In fact, with respect to the total population, Italian nonagenarians are the 1.37% and Italian centenarians the 0.03% (12). Particularly, the province of L'Aquila, which shows a high rate of nonagenarians and an extremely higher number of centenarians, compared with the Italian average: 0.043% vs. 0.028%, with the highest numbers occurring in the most rural, internal areas of the region (12).

The inhabitants of the Abruzzo region have typically followed a peculiarly unique dietary habit called "*sdijuno,*" representing the salty breakfast of the morning that was "breaking" the fasting of the night after an early dinner. The putative advantages of this practice are as follows: (i) negligible postprandial stress during the evening, in agreement with the circadian rhythm and lower metabolic efficiency in the night (13, 14); (ii) a period of caloric restriction from dinner to lunch. The "*sdijuno*" practice, consistently followed for decades, might have tuned the endogenous response to daily meals, optimizing both immune and metabolic responses to dietary stressors, playing a role in Abruzzo's longevity rates. However, despite recent findings on the importance of meal timing for health (15, 16), no evidence is available on the meal timing of nonagenarians and centenarians. The aim of this study was to survey, in a cross-sectional study, mealtime, adherence to comply with "*sdijuno*" practice, as well as general dietary habits and the level of physical activity throughout life, of nonagenarians and centenarians of the Abruzzo region.

MATERIALS AND METHODS

Study Design and Participants

A cross-sectional study was conducted in the time frame September 2019–June 2020 in the province of L'Aquila, Abruzzo Region, Italy (L'Aquila capital coordinates: 42°21′14″N; 13°23′31″E). The study protocol was approved by the local Ethics Committee for Human Research for the Provinces of L'Aquila and Teramo of the Local Health Authority "A.S.L. 1 – Avezzano – Sulmona – L'Aquila" (protocol no. 151856/20). The primary and secondary endpoints were those declared when the study was registered at clinicaltrials.gov (as NCT04840381). Inclusion criteria were as follows: (i) being 90 years or older at the moment of the interview; (ii) being born and living for most of one's life in the Abruzzo region; and (iii) mentally active.

Data Collection

Volunteers were recruited by public announcements in local newspapers, through social networks, and by personal acquaintances among the personnel of the University of Teramo. A trained nutritionist first contacted a relative of each subject to inquire about their general cognitive conditions. In positive cases, a trained nutritionist contacted the subjects – in the presence of a relative – for the interview. The interviewers fluently understood the local dialect, in order to make participants feel comfortable.

Gender, age, body weight, and height were retrieved from general questions posed to the volunteer, together with information about their health status, e.g., presence of pathologies, hypercholesterolemia, hypertriglyceridemia, hypertension, or hypotension. Other questions were related to the following: (i) the daily mealtime during the subjects' youth and old age; (ii) the source of the food supply – own production, exchange, and local vendor; and (iii) the amount of moderate or vigorous physical activity, in terms of minutes per week, throughout life.

Nutritional Assessment

Information on dietary intake of some foods and food groups during the subject's youth was retrieved by means of a food frequency questionnaire (FFQ). The questions were related to the frequency of consumption, in terms of the number of times per week, of the following items: cereals, legumes, vegetables, fruit, milk and dairy products, meat, processed meat, eggs, and fish. Furthermore, questions on the use of fat during meal preparation were asked, e.g., lard, *strutto* (fat from pork), bacon, and extra-virgin olive oil. Finally, participants were asked to indicate whether they followed the *sdijuno* practice.

RESULTS

Demographics, anthropometric measures, and health status of the study participants are shown in **Table 1**. For a total of 68 volunteers, i.e., 46 women and 22 men, 46 were aged in the range of 90–99 years, while 22 were centenarians, up to 107 years of age. Subjects were all normal weight, and, except for hypertension which affected 72% of the individuals, they were characterized by an extremely low frequency of hypertriglyceridemia (4%) and hypercholesterolemia (19%).

TABLE 1 | Demographic, anthropometric, and health status of the study participants.

Total (*N*)	**68**
Female	46
Male	22
Age (*N*)	
Nonagenarian (*90–99 y*)	46
Centenarian (*100–107 y*)	22
BMI (kg/m^2)	24.8 ± 0.4
Main pathologies (%)	
Diabetes	13
Hypercholesterolemia	19
Hypertriglyceridemia	4
Hypertension	49
Hypotension	3

BMI, body mass index, expressed as mean ± SEM.

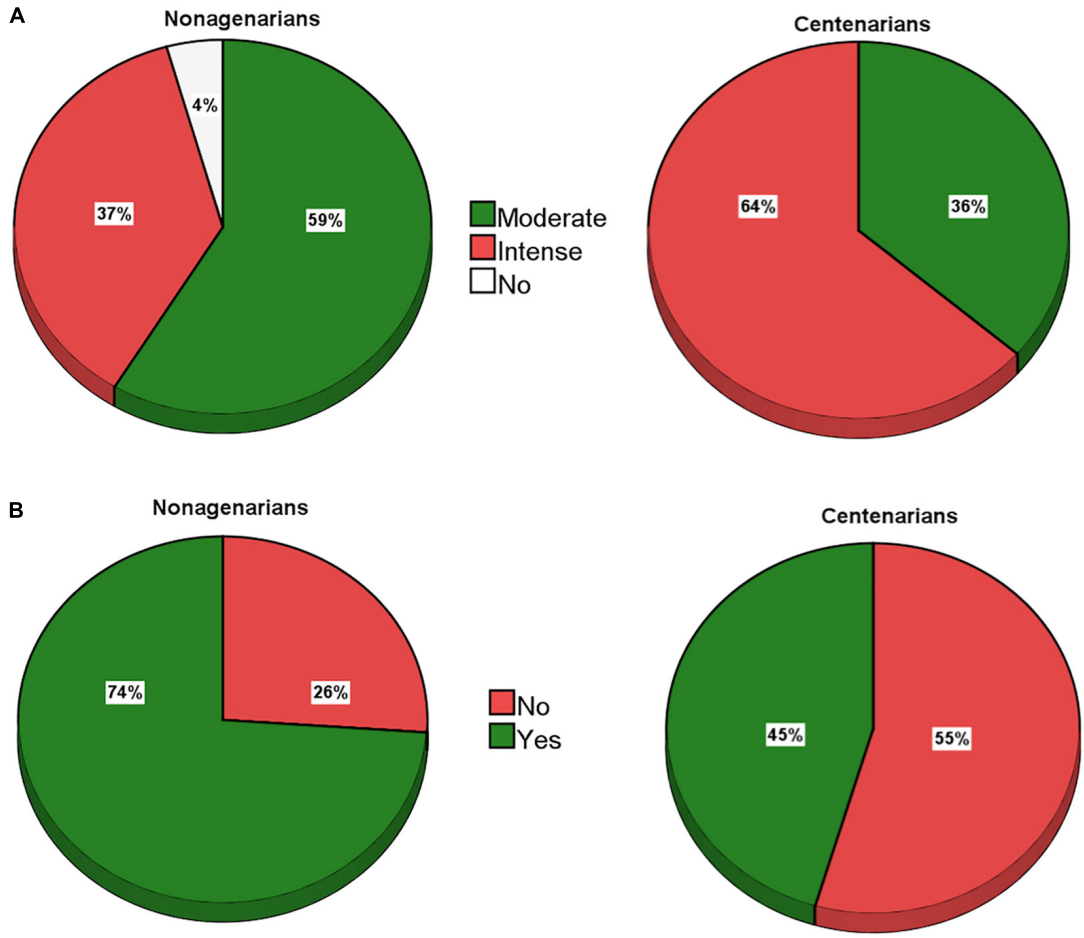

FIGURE 1 | Physical activity status of the study participants in their youth **(A)** and elderly **(B)** age.

Almost all the subjects had been physically active throughout life, and most of them were still active at the time of the interview, refer to **Figure 1**.

Notably, 59 and 36% of the nonagenarians and centenarians, respectively, stated that they had been moderately physically active in their youth, i.e., walking, biking, and horse riding (data not shown). Again, 37 and 64% of the nonagenarians and centenarians, respectively, reported intense physical activity earlier in life, due to the long hours spent on their work activity, i.e., work in the fields of animal husbandry in mountainous areas (data not shown). In fact, only 3% of the nonagenarians and none of the centenarians reported less or no significant physical activity earlier in life (**Figure 1A**). Concerning the present level of physical activity, reported in **Figure 1B**, 74% of the nonagenarians and 45% of the centenarians still practiced moderate physical activity, mainly walking or taking care of the garden or homeworking (data not shown). The remaining 26% and 55% of nonagenarians and centenarians, respectively, did not report their physical activity, mainly due to their deteriorating physical conditions or due to permanent wheelchair use (data not shown).

Data retrieved from the subjects' dietary habits in their young age, in terms of frequency and serving size, split for their decade of age, are summarized in **Figure 2**. Results were very similar among nonagenarians and centenarians, with a median intake of five or more servings of each of several plant-based foods per week, such as cereals, legumes, vegetables, and fruit, with the lowest consumptions accounting for 1–2 servings per week. Among the animal-based products, milk and dairy products were consumed, in moderation, four and five times per week by nonagenarians and centenarians, respectively. Concerning meat and fish products, consumption was only one to two servings per week, while processed meats and eggs had a median consumption of two to three servings per week in the whole population. Lastly, consumption of sweets was negligible, with a median intake of one serving per week and mostly on Sundays or festive days (data not shown).

Subjects were also asked to report the main seasonings used during the preparation of meals. **Figure 3** shows that a higher number of nonagenarians and centenarians mainly used animal-derived fats compared with extra-virgin olive oil, used by 34 and 36% of nonagenarians and centenarians, respectively.

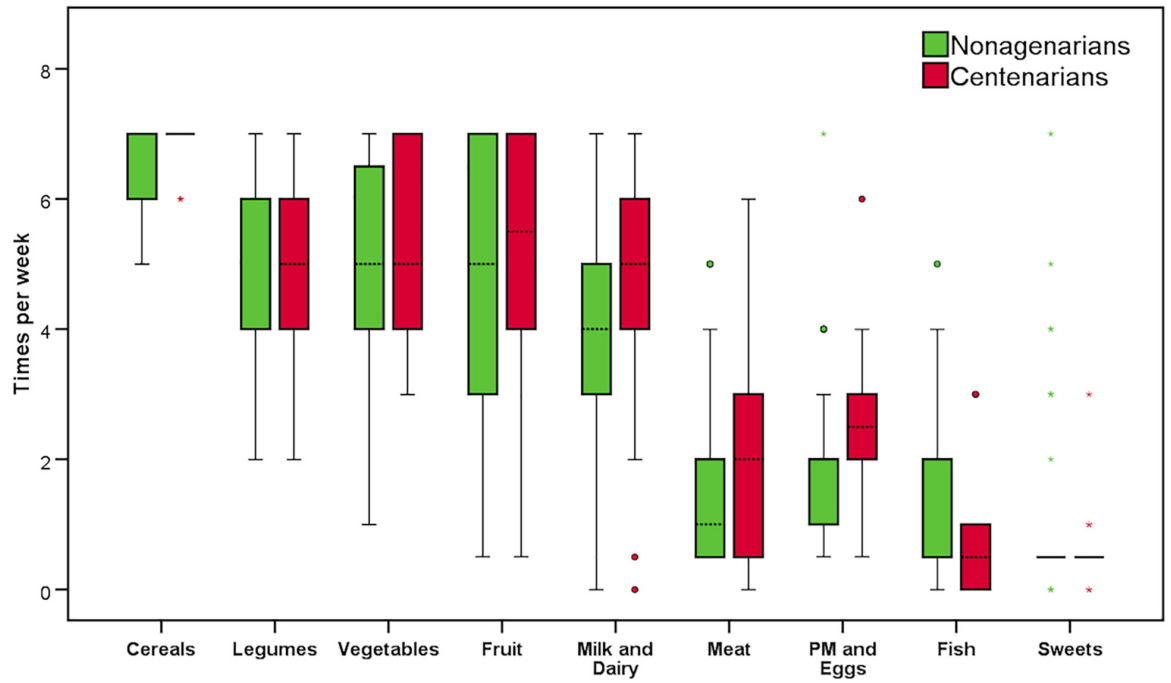

FIGURE 2 | Frequency of consumption of food groups in the nonagenarian and centenarian populations. PM, processed meat. Circles and asterisks represents outlier and extreme outlier values, respectively.

In fact, more than 84% of the nonagenarians and 68% of the centenarians, respectively, used lard and *strutto* for the preparation of recipes, while bacon (also including the Italian *pancetta*) was used by 16 and 14% of nonagenarians and centenarians, respectively.

The survey of the condiments also included questions about the main spices used by participants during food preparation. On the whole, both nonagenarians and centenarians most often used rosemary, garlic, onion, chili pepper, parsley, and basil (data not shown).

Since the subjects resided in rural and relatively inaccessible areas, we also investigated the way of the food supply. **Figure 4** shows that almost 95% of the nonagenarians and centenarians made their own meals from scratch, while 30 and 36% of the nonagenarians and centenarians, respectively, used foods exchanged with other people in the neighborhood or the surroundings. Finally, 20 and 46% of the nonagenarians and centenarians, respectively, bought foods only from local vendors.

A very high percentage of the subjects – 90% of the total – stated that they had adhered to the *sdijuno* practice (data not shown). Regarding meal timing, dinner, consisting mainly of vegetable soups, polenta, vegetables, eggs, or cheese, was consumed on average at 7.13 p.m. The breakfast meal was on average at 6:18 a.m. and included dishes leftover from dinner, milk and bread, or a slice of bread with ham. The main meal of the day was lunch, composed of meat, pasta or polenta, and beans, and was on average at 12.38 p.m., highlighting a caloric restriction period of approximately 17.5 h between dinner and the following lunch (**Figure 5**).

DISCUSSION

In this study, we provided novel studies about the importance of meal timing and of a caloric restriction period of approximately 17–18 h, together with physical activity and a dietary pattern based on high consumption of plant-based foods, moderate consumption of the animal product, and negligible consumption of sweets, for the longevity of Abruzzo's centenarians.

Epidemiological studies have pointed out to a late-night dinner being associated with an increased incidence of cardiovascular diseases, i.e., stroke, heart failure, etc. (17). These findings have been confirmed in two intervention studies, where Japanese volunteers, affected by type-2 diabetes, showed lower postprandial glycemia and insulinemia when they ate their main meal at 6 p.m., compared with 9 p.m. (18, 19). The authors discussed these important findings, emphasizing that just a 3-h shift of the mealtime resulted in a significant health benefit was quite remarkable. This implicates the importance of the circadian rhythm, as "*the diurnal variation in insulin resistance is higher at night than in the daytime,*" and that the long fasting period from lunch to late dinner disrupted this (19). These latter aspects induce an increase of the plasma-free fatty acid values and a decrease of the blood insulin concentrations, leading to a higher postprandial glycemia and metabolic stress (19). Thus, the metabolic impact due to non-optimal eating behaviors likely increases the risk for cardiovascular events, playing a role in life expectancy.

As drawn in the biological clock in **Figure 5**, "*sdijuno*" implies a ∼17.5 h period of low caloric intake, which might be considered

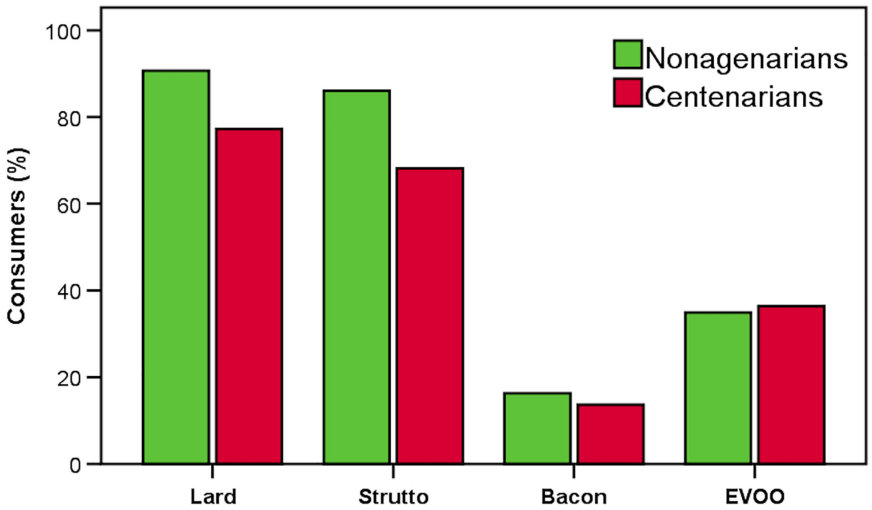

FIGURE 3 | Percentage of consumer use of cooking fat during meal preparation. EVOO, extra-virgin olive oil.

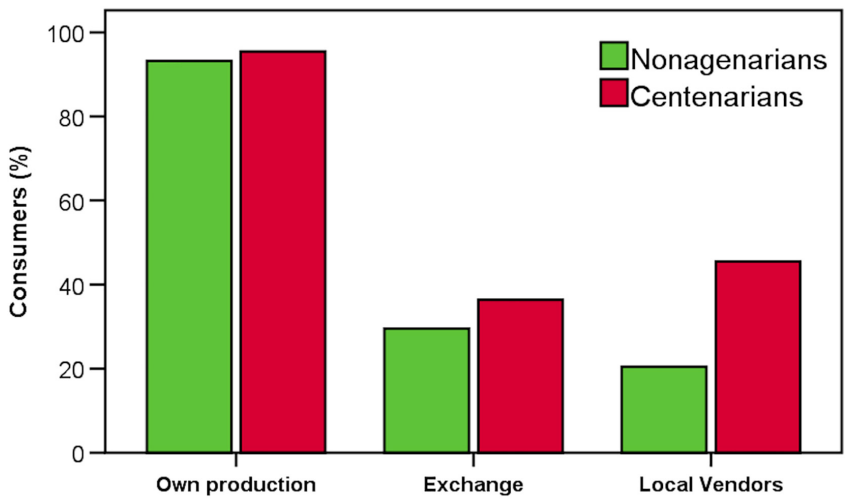

FIGURE 4 | Percentage of subjects consuming foods produced by themselves, exchanged with other locals, or bought from local vendors.

a "time-restricted feeding" regimen, under the frame of the intermittent fasting and calorie restriction (20, 21). If we analyze the proposed metabolic postprandial stress periods after each of the three meals of the day (**Figure 5**, detailed in red), an early dinner causes minimal nocturnal stress, as does the low-calorie intake at breakfast (200–300 kcal). The daily caloric restriction lapse might allow both metabolism and the immune system to efficiently minimize the stress induced by the main meal of the day. Despite very few available human intervention studies, recent findings show that time-restricted feeding has a positive role in the control of insulin sensitivity and β-cell responsiveness, through modulation of the gut microbiota profile and metabolic products and through the alignment of activity of the microbiome with the circadian rhythm (22), confirming previous suggestions of the importance of biological rhythms in health. This metabolic virtual cycle, recurring each day in the life of the centenarians, may have positively affected both glycemic control and lipid metabolism, playing an important role in their longevity.

We showed that subjects had an almost daily consumption of plant-based foods, above all cereals, legumes, fruits, and vegetables, and moderate weekly consumption of milk and dairy in agreement with the consumption pattern of the Mediterranean diet (23) with the exception of the frequent use of animal fats instead of olive oil. Our findings are in agreement with the results of a recent study conducted in the Italian Blue Zone of Sardinia, showing that individuals in the range of 90–101 years old had a higher intake of lard over olive oil (3.8 times/week vs. 2.51 times/week, respectively) (11). As L'Aquila rural area is particularly mountainous, olive trees are not a local culture, and the inhabitants had their own production of foods from the ground and with animal breeding, and it is reliable to think that the consumption of animal fats is higher than the olive ones. In

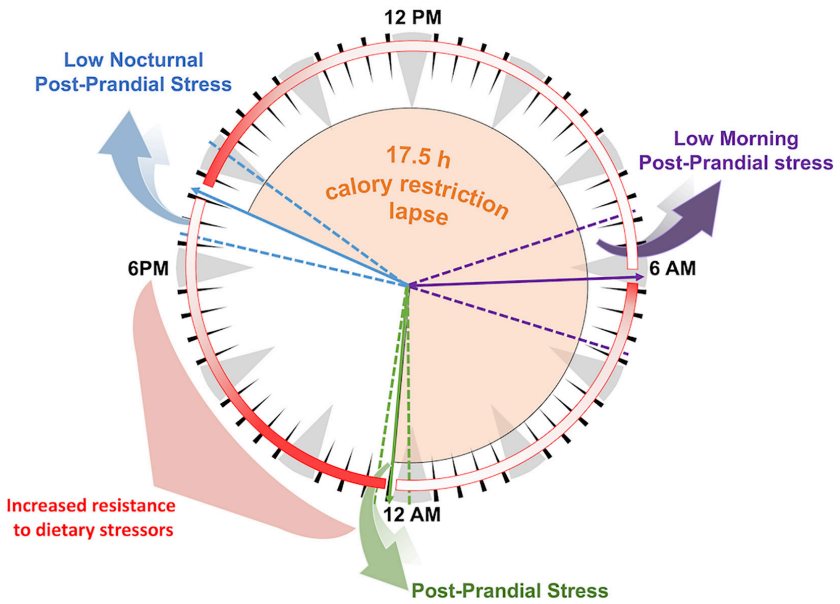

FIGURE 5 | Graphical representation of the day's mealtimes and of the fasting/calorie restriction lapse for nonagenarians and centenarians from Abruzzo. The arrows within the clockface indicate the median time of meal consumption: purple: breakfast; green: lunch; and blue: dinner. Dotted lines represent the earliest and latest time of each meal consumption. Light-orange areas represent an average time of restricted calorie intake. Red shaded circle areas represent hypothesized postprandial stress periods after each meal, the bolder the red, the greater the postprandial stress.

fact, the 20%–40% of the subjects who chose to use vendors for their food supply were also those consuming olive oil. Last, but not least, being olive oil imported from surroundings, it could have resulted more expensive than pork-based ones, letting the product not affordable.

Median intakes of other food groups consumed by Abruzzo elderlies are almost overlapping with the ones found for Sardinian Blue Zone inhabitants (11). Among these, cereals and cereal-based foods are confirmed to be almost daily consumed by all the Sardinians elderlies, as in the Abruzzo ones; regarding other plant-based products, legumes and vegetables are also consumed on average ∼4 times per week. The only difference has been pointed out for fruits, which in the Abruzzo elderlies was found to be consumed with a double frequency compared with Sardinian ones. Also, animal-based food categories mostly agreed in terms of frequency of consumption of the following: milk and dairy products ∼4 times per week, meat between 1 and 2 times per week, and fish less than 2 times per week (11). In addition, we found an abundant and frequent use of herbs and spices during meal preparation – data missing in Sardinian Blue Zone related papers – which may have had a role in the counteraction of inflammatory-related disorders due to their content in bioactive compounds (24).

During the interview, elderlies shared their main recipes prepared and consumed during the three main meals of the day. Among these, breakfast was usually what in Italy is known as "*salted breakfast,*" with the only sweet-tasting item being milk – although it was not consumed daily– and with most of the dishes done with the dinner leftovers, i.e., *polenta*, potatoes, stuffed leafy vegetables and bread spread with animal fats, cheese, and eggs. The lunch, which was the main meal of the day consumed during the working activities, consisted of home-made egg-based recipes or whole wheat pasta with vegetables or *polenta* and soups made with local cultivars of lentils, beans, chickpeas (among which the local spread *cicerchia*, alias *Lathyrus sativus)* or meat, such as lamb or beef. Dinner was mainly based on vegetables or soups or *polenta* and some other animal-based products, i.e., own pecorino cheese, sheep ricotta. Fish was mainly present with local species from the surrounding river or (salt) cod, consumed with lower frequency than meat. Last, but not least, the sugar consumption was close to zero, with sweets or cakes eaten only on special occasions.

A further aspect related to their longevity is the high level of physical activity for most of the nonagenarians and centenarians. This is mainly linked to their work on the land for most of the day, as also found in a Polish survey about the dietary and lifestyle habits of Warsaw centenarians (25). More than 50% of the nonagenarians and the centenarians remained physically active, even at the time of the interview, walking around their community and even still doing some light work on the land, maybe supporting the advantages of regular physical activity throughout life for healthy longevity (26, 27).

It is worth highlighting the limitations of the present survey. First, being a small study and limited to a single province of the Abruzzo region, data retrieved from the few interviewed individuals may not be applicable for all the Abruzzo centenarians and nonagenarians as well as for individuals out of region and country. Then, our study is based on a retrospective survey of nonagenarians and centenarians who gave information on their food habits, dating back sometimes only 60 years, which

barely covered their youth. So, there is a bias relative to their physiological loss of clarity in their memories and another in "summarizing" the food habits in a unique answer.

Concerning food habits, the main limitation to this study is the physiological loss of precision in the memories of dietary and lifestyle habits. Moreover, FFQ does not allow any quantitative assessment, and, as previously found (11), this may result in only a rough grouping of foods consumed by the subjects in their youth, yet their memories are supported by the availability of local ingredients and food preparation methods passed down within families.

CONCLUSION

Our findings support the importance of a daily caloric restriction lapse, associated with high consumption of plant-based foods and physical activity for the longevity of centenarians from Abruzzo. Despite more studies needed, our results support the importance of considering meal timing as a feature involved in longevity processes.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee for Human Research for the Provinces of L'Aquila and Teramo of the Local Health Authority "A.S.L. 1 – Avezzano – Sulmona – L'Aquila." The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DA was involved in the data analyses, interpretation of the results, and drafting of the manuscript. FP was involved in the protocol design and data collection. MS conceived the study and involved in the protocol design, interpretation of the results, drafting of the manuscript, and had primary responsibility for the final content. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We thank Elizabeth H. Jeffery, Department of Food Science and Nutrition, the University of Illinois at Urbana-Champaign, IL, the United States for the critical revision of the manuscript and English language.

REFERENCES

1. Partridge L, Deelen J, Slagboom PE. Facing up to the global challenges of ageing. *Nature.* (2018) 561:45–56. doi: 10.1038/s41586-018-0457-8
2. WHO. *Ageing and Health*. (2021). Available online at: https://www.who.int/news-room/fact-sheets/detail/ageing-and-health (accessed December 20, 2021).
3. Statista. *Number of Centenarians Worldwide 2100*. (2019). Available online at: https://www.statista.com/statistics/996597/number-centenarians-worldwide/ (accessed December 20, 2021).
4. Chrysohoou C, Stefanadis C. Longevity and diet. Myth or pragmatism? *Maturitas.* (2013) 76:303–7. doi: 10.1016/j.maturitas.2013.09.014
5. Rimm EB, Stampfer MJ. Diet, lifestyle, and longevity - The next steps? *J Am Med Assoc.* (2004) 292:1490–2. doi: 10.1001/jama.292.12.1490
6. Dinu M, Pagliai G, Angelino D, Rosi A, Dall'Asta M, Bresciani L, et al. Effects of popular diets on anthropometric and cardiometabolic parameters: an umbrella review of meta-analyses of randomized controlled trials. *Adv Nutr.* (2020) 11:815–33. doi: 10.1093/advances/nmaa006
7. Eurostat. *Ageing Europe - Statistics on Population Developments*. (2020). Available online at: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Ageing_Europe_-_statistics_on_population_developments#Older_people_.E2.80.94_where_do_they_come_from.3F (accessed December 20, 2021).
8. Panagiotakos DB, Chrysohoou C, Siasos G, Zisimos K, Skoumas J, Pitsavos C, et al. Sociodemographic and lifestyle statistics of oldest old people (>80 years) living in Ikaria Island: the Ikaria study. *Cardiol Res Pract.* (2011) 2011:679187. doi: 10.4061/2011/679187
9. Poulain M, Pes GM, Grasland C, Carru C, Ferrucci L, Baggio G, et al. Identification of a geographic area characterized by extreme longevity in the Sardinia island: the AKEA study. *Exp Gerontol.* (2004) 39:1423–9. doi: 10.1016/j.exger.2004.06.016
10. Nieddu A, Vindas L, Errigo A, Vindas J, Pes GM, Dore MP. Dietary habits, anthropometric features and daily performance in two independent long-lived populations from nicoya peninsula (Costa rica) and ogliastra (sardinia). *Nutrients.* (2020) 12:1621. doi: 10.3390/nu12061621
11. Pes GM, Poulain M, Errigo A, Dore MP. Evolution of the dietary patterns across nutrition transition in the sardinian longevity blue zone and association with health indicators in the oldest old. *Nutrients.* (2021) 13:1495. doi: 10.3390/nu13051495
12. ISTAT. *Popolazione Residente al 1° Gennaio per Fascia di età*. (2021). Available online at: http://dati.istat.it/Index.aspx?QueryId=42869 (accessed December 20, 2021).
13. Henry CJ, Kaur B, Quek RYC. Chrononutrition in the management of diabetes. *Nutr Diab.* (2020) 10:6. doi: 10.1038/s41387-020-0109-6
14. Khaper N, Bailey CDC, Ghugre NR, Reitz C, Awosanmi Z, Waines R, et al. Implications of disturbances in circadian rhythms for cardiovascular health: A new frontier in free radical biology. *Free Radic Biol Med.* (2018) 119:85–92. doi: 10.1016/j.freeradbiomed.2017.11.006
15. Chellappa SL, Qian J, Vujovic N, Morris CJ, Nedeltcheva A, Nguyen H, et al. Daytime eating prevents internal circadian misalignment and glucose intolerance in night work. *Sci Adv.* (2021) 7:9910. doi: 10.1126/SCIADV.ABG9910
16. Boege HL, Bhatti MZ, St-Onge MP. Circadian rhythms and meal timing: impact on energy balance and body weight. *Curr Opin Biotechnol.* (2021) 70:1–6. doi: 10.1016/J.COPBIO.2020.08.009
17. Kaneko H, Itoh H, Kiriyama H, Kamon T, Fujiu K, Morita K, et al. Possible association between eating behaviors and cardiovascular disease in the general population: Analysis of a nationwide epidemiological database. *Atherosclerosis.* (2021) 320:79–85. doi: 10.1016/j.atherosclerosis.2021.01.022
18. Imai S, Kajiyama S, Hashimoto Y, Yamane C, Miyawaki T, Ozasa N, et al. Divided consumption of late-night-dinner improves glycemic excursions in patients with type 2 diabetes: A randomized cross-over clinical trial. *Diabetes Res Clin Pract.* (2017) 129:206–12. doi: 10.1016/j.diabres.2017.05.010

19. Imai S, Saito Y, Kajiyama S, Nitta A, Miyawaki T, Matsumoto S, et al. Late-night-dinner deteriorates postprandial glucose and insulin whereas consuming dinner dividedly ameliorates them in patients with type 2 diabetes: a randomized crossover clinical trial. *Asia Pac J Clin. Nutr.* (2020) 29:68–76. doi: 10.6133/apjcn.202003_29(1).0010
20. Patterson RE, Sears DD. Metabolic effects of intermittent fasting. *Annu Rev Nutr.* (2017) 37:371–93. doi: 10.1146/annurev-nutr-071816-064634
21. Most J, Tosti V, Redman LM, Fontana L. Calorie restriction in humans: an update. *Ageing Res Rev.* (2017) 39:36–45. doi: 10.1016/j.arr.2016.08.005
22. Pellegrini M, Cioffi I, Evangelista A, Ponzo V, Goitre I, Ciccone G, et al. Effects of time-restricted feeding on body weight and metabolism. A systematic review and meta-analysis. *Rev Endocr Metab Disord.* (2020) 21:17–33. doi: 10.1007/s11154-019-09524-w
23. Trichopoulou A, Lagiou P. Healthy traditional Mediterranean diet: An expression of culture, history, and lifestyle. *Nutr Rev.* (1997) 55:383–9. doi: 10.1111/j.1753-4887.1997.tb01578.x
24. Bower A, Marquez S, de Mejia EG. The health benefits of selected culinary herbs and spices found in the traditional mediterranean diet. *Crit Rev Food Sci Nutr.* (2016) 56:2728–46. doi: 10.1080/10408398.2013.805713
25. Kołłajtis-Dołowy A, Pietruszka B, Kałuza J, Pawliñiska-Chmara R, Broczek K, Mossakowska M. The nutritional habits among centenarians living in Warsaw. *Rocz Państwowego Zakładu Hig.* (2007) 58:279–86.
26. McPhee JS, French DP, Jackson D, Nazroo J, Pendleton N, Degens H. Physical activity in older age: perspectives for healthy ageing and frailty. *Biogerontology.* (2016) 17:567–80. doi: 10.1007/s10522-016-9641-0
27. Kirk-Sanchez N, McGough E. Physical exercise and cognitive performance in the elderly: current perspectives. *Clin Interv Aging.* (2014) 9:51. doi: 10.2147/CIA.S39506

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 28 March 2022
doi: 10.3389/fnut.2022.837207

Circulating Vitamin D Levels and the Risk of Atrial Fibrillation: A Two-Sample Mendelian Randomization Study

*Shengyi Yang[1], Hong Zhi[2], Ying Sun[3] and Lina Wang[1]**

[1] Key Laboratory of Environmental Medicine Engineering, Ministry of Education, Department of Epidemiology and Biostatistics, School of Public Health, Southeast University, Nanjing, China, [2] Department of Cardiology, Zhongda Hospital, Southeast University, Nanjing, China, [3] Zhalongkou Community Healthcare Center, Hangzhou, China

Aim: We performed a two-sample Mendelian randomization (MR) analysis to evaluate the association between serum vitamin D levels and atrial fibrillation (AF) risks.

Methods: Data on the single-nucleotide polymorphisms (SNPs) related to vitamin D, 25-hydroxyvitamin D, and AF outcome were obtained from a UK Biobank study, SUNLIGHT consortium, and the latest meta-analysis of genome-wide association studies GWASs with six independent cohorts, respectively. MR analysis was performed to obtain the estimates, followed by the use of inverse variance weighted (IVW) method, weighted median method, maximum likelihood, MR-egger method, and MR-PRESSO methods.

Results: The IVW estimate showed that genetically predicted vitamin D and 25-hydroxyvitamin D levels were not causally associated with the risk of AF with two models. The association was consistent in complementary analyses.

Conclusions: Our MR finding suggested that no genetic evidence of serum vitamin D levels was significantly associated with AF risk. Further researches are necessary to explore the potential role and mechanisms of circulating serum vitamin D levels on AF.

Keywords: 25-hydroxyvitamin D, vitamin D, atrial fibrillation, Mendelian randomization, cause-effect

OPEN ACCESS

Edited by:
Francesco Sofi,
Università degli Studi di Firenze, Italy

Reviewed by:
Omer Iqbal,
Loyola University Chicago, United States
Liangkai Chen,
Huazhong University of Science and Technology, China

****Correspondence:***
Lina Wang
lnwang@seu.edu.cn

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *16 December 2021*
Accepted: *14 February 2022*
Published: *28 March 2022*

Citation:
Yang S, Zhi H, Sun Y and Wang L (2022) Circulating Vitamin D Levels and the Risk of Atrial Fibrillation: A Two-Sample Mendelian Randomization Study. Front. Nutr. 9:837207. doi: 10.3389/fnut.2022.837207

INTRODUCTION

Atrial fibrillation (AF) is a common arrhythmia contributing to substantial social and medical burdens with significant health and socioeconomic impact (1). The Global Burden of Disease project estimated a worldwide prevalence of AF in about 46.3 million individuals in 2016 (2). The prevalence of AF is estimated to rise to 16 million by 2050 in the United States and 14 million by 2060 in the European Union (3). AF is associated with high healthcare system utilization, low quality of life, and increased risk for hospitalization, heart failure, stroke, and death (4).

Vitamin D is an essential fat-soluble vitamin that undergoes 2 hydroxylation steps to produce the active form. The first of these produces 25-hydroxyvitamin D, which can be measured to determine vitamin D status (5). Vitamin D deficiency has become a pandemic health problem in the world (6). In recent decades, the focus has been on vitamin D deficiency and nonskeletal diseases risks, including various cardiovascular diseases (7, 8). However, unlike for the skeletal disease, the association between vitamin D deficiency or 25-hydroxyvitamin D levels and AF risks has been inconclusive. Two dose-response meta-analyses (9, 10) indicated that circulating

vitamin D deficiency was associated with an increased risk of AF in the general population, which were not consistent with another meta-analysis of randomized controlled trials (11). Conclusions about causality cannot be drawn merely based on the presence of an association in an observational design, which was retrospective or cross-sectional in design with limited sample sizes and confounders.

To investigate the causal association between circulating vitamin D and AF risks is challenging due to the reverse causation and confounding. Mendelian randomization (MR) has emerged as a powerful method for identifying the causation between risk factors and diseases using genetic variants as instrument variables (IVs) (12). MR analysis can largely overcome the confounders with random assignment of an individual's genetic variants at conception. Moreover, the risk of reverse causation could also be minimized since the presence of a disease could not affect individuals' genotypes (13).

In our study, we applied a two-sample MR analysis to identify the potential causal association between circulating serum vitamin D levels (including serum vitamin D and its metabolite, 25-hydroxyvitamin D) and risk of AF using the summary statistics from the publicly available genome-wide association studies (GWAS) data.

METHODS

Data Resources and Study Design

We searched GWAS to extract leading single-nucleotide polymorphisms (SNPs) as genetic instrumental variables. Summary statistic data for vitamin D levels were derived from a meta-analyzed GWAS for 35 biomarkers in the UK Biobank (UKB) in 304,818 participants of White British European ancestry (14). UK Biobank is a prospective cohort which recruited more than 500,000 men and women aged 40–96 years between 2006 and 2010, and their health is being followed on a long-term (15). Summary statistic data for 25-hydroxyvitamin D was drawn from the most recent GWAS on serum 25-hydroxyvitamin D from the SUNLIGHT consortium with 79,366 European-ancestry participants including 31 studies (16). This study identified 142 independent risk variants at 111 loci and prioritized 151 functional candidate genes likely to be involved in atrial fibrillation (16). Data for AF was obtained from the latest meta-analysis of GWASs for AF with six independent cohorts (The Nord-Trøndelag Health Study, Michigan Genomics Initiative, DECODE, UK Biobank, DiscovEHR Collaboration Cohort, and AF Gen Consortium) with more than 1,000,000 subjects of European ancestry, including 60,620 cases with AF and 970,216 controls (17). The details are presented in **Table 1**.

We designed a two-sample Mendelian randomization analysis to estimate the causal effects of circulating serum vitamin D and 25-hydroxyvitamin D levels (recommended biomarker for vitamin D levels, **Figure 1A**) on AF risks with two models (**Figure 1B**). Model 2 was performed by extracting SNPs that were associated with any potential confounders on AF risks, while Model 1 was not.

Selection of Genetic Instrumental Variables

All genetic variants reaching genome-wide significance ($p < 5 \times 10^{-8}$) were selected as instruments for the MR analysis. The corresponding linkage disequilibrium was tested to confirm if there were any SNPs in linkage disequilibrium and whether the SNPs were independent by pruning SNPs within a 10,000 kb window with an $r^2 < 0.001$ threshold (18). Then, the SNPs were extracted that were associated with any potential confounders of the outcomes. In this study, blood pressure, blood glucose, BMI, chronic nephropathy, coronary artery disease (CAD), and C-reactive protein were identified as confounding factors when AF was identified as the outcome (http://www.phenoscanner.medschl.cam.ac.uk/) (19). SNP harmonization was conducted to correct the orientation of the alleles. Finally, we used 62 SNPs and 56 SNPs (3 SNPs were associated with BMI: rs56675301, rs35635959, and rs1229984 and 3 SNPs were associated with CAD: rs2207132, rs2229742, and rs2539986) as instrument variables for Vitamin D levels in model 1 and model 2, 6 SNPs and 5 SNPs (1 SNP was associated with white blood cell: rs10745742) for 25-hydroxyvitamin D levels in model 1 and model 2, respectively **(Supplementary Tables 1–4)**. F statistics for every instrument-exposure effect ranged from 31.678 to 169.767, demonstrating the small possibility of weak instrumental variable bias **(Table 1)**. In another directional MR, we used 13 SNPs and 42 SNPs for AF on vitamin D and 25-hydroxyvitamin D levels, respectively, and no SNP was associated with confounding factors when AF was identified as the exposure.

Statistical Analysis

T To obtain an MR estimate, an inverse variance weighted (IVW) meta-analysis of each Wald Ratio (20) was performed. When there was no evidence of directional pleiotropy (P for MR-Egger intercept > 0.05) among the selected IVs, the IVW method was considered with the most reliability (21).

Complementary analyses using the weighted median method (22), maximum likelihood (23) and MR-egger method (22), and MR Robust adjusted profile score (MR.RAPS) were utilized as supplements to IVW. The weighted median analysis can generate consistent estimates if at least 50% of the weight in the analysis comes from valid instrumental variables (24). Cochran's Q test was applied to assess heterogeneity between individual genetic variants estimates. If the p-value of Cochran's Q test was < 0.05, the final results of MR were referred to a multiplicative random-effects model of IVW; otherwise, a fixed-effects model was used (25). To examine whether there was a violation of the main MR assumptions due to directional pleiotropy, the MR-Egger test for directional pleiotropy was performed (22), where the intercept estimates the average pleiotropic effect across the genetic variants and can be a useful indicator of whether directional horizontal pleiotropy is driving the results of an MR analysis (26). The possible directional pleiotropy was were also examined by observing asymmetry in thefunnel plots to gauge the reliability of the current MR analyses. Finally, MR-PRESSO

Abbreviations: AF, atrial fibrillation; MR, Mendelian randomization; SNP, single nucleotide polymorphisms; GWAS, genome-wide association; IVW, inverse variance weighted; IVs, instrument variables; UKB, UK Biobank; MR-PRESSO, MR pleiotropy residual sum and outlier test.

TABLE 1 | Details of studies included and predictive strength of IVs in Mendelian randomization analyses (two-sided $\alpha = 0.05$).

Exposures/outcomes	Consortium	Ethnicity	Sample sizes	Model	R-squared % (of variance in Exposure)	F-statistic (total)
Vitamin D	UK Biobank	European	304,818	Model 1	0.546	31.578
				Model 2	0.521	33.279
25-hydroxyvitamin D	SUNLIGHT	European	79,366	Model 1	1.095	146.428
				Model 2	1.059	169.767
Atrial fibrillation	HUNT, DECODE, MGI, DiscovEHR, UK Biobank, and AFGen Consortium	European	1,030,836	NA	NA	NA

AF, atrial fibrillation; HUNT, The Nord-Trøndelag Health Study; DECODE, DiscovEHR, Collaborative analysis of Diagnostic criteria in Europe study; MGI, Michigan Genomics Initiative; AF Gen, Atrial Fibrillation Genetics. Model 1, SNPs were not extracted which were associated with any potential confounders of AF; Model 2, SNPs were extracted which were associated with any potential confounders of AF.

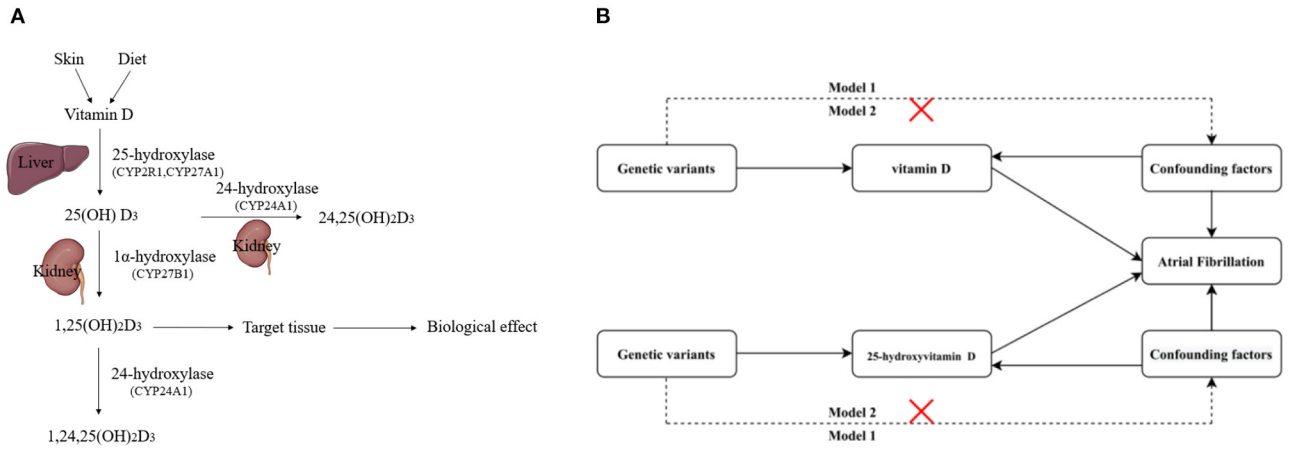

FIGURE 1 | (A) Process of vitamin D metabolism. **(B)** Schematic overview of the present study design. Model 1, SNPs were not extracted which were associated with any potential confounders of AF; Model 2, SNPs were extracted which were associated with any potential confounders of AF. $25(OH)D_3$, 25-hydroxyvitamin D; $24,25(OH)_2D_3$, 24,25-Dihydroxyvitamin D3; $1,25(OH)_2D_3$, 1,25-Dihydroxyvitamin D3; $1,24,25(OH)_2D_3$, 1,24,25-Dihydroxyvitamin D3.

was performed to support the results by IVW method, which detects and corrects the effects from outliers, yielding causal estimates that were robust to heterogeneity (27). The leave-one-out sensitivity analyses were implemented by removing a single SNP each time to assess whether the variant was driving the association between the exposure and the outcome variable. To improve the visualization of the IVW and MR-Egger estimates, we performed IVW radial variants and MR-Egger radial variants models, which were similar to the conventional IVW and MR-Egger regression models, but regressed the product of the Wald Ratio estimate and the square root of the weighting for each genetic variant upon the square root of the genetic variants weighting (28). *R*-squared was calculated to estimate the proportion of variance in outcomes, and F-statistic value was calculated to predict the strength of IVs.

A two-sided *P*-value of < 0.05 was considered suggestive for significance. All analyses were performed using the package "Two-Sample-MR" (version 0.5.6), "MR-PRESSO" (version 1.0), and "Radial MR" (version 1.0) in R (version 4.0.5).

RESULTS

Association of Serum Vitamin D Levels With AF Risks

Figure 2 reported the MR estimated for vitamin D levels on AF. In model 1, the fixed-model IVW estimate showed that genetically predicted vitamin D levels were not significantly associated with AF risks ($N = 53$ SNPs, OR: 1.028, 95% CI: 0.962–1.099, $p = 0.408$). After extracting 6 SNPs, the result was consistent ($N = 48$ SNPs, OR: 1.011, 95% CI: 0.945–1.082, $P = 0.751$). The association was consistent in complementary analyses using weighted-median method, maximum likelihood, MR-egger, and MR-RAPS method.

There were potential heterogeneities but no directional pleiotropies for the analysis results **(Supplementary Table 5)**. Radial plots showed there were outlines in Model 1 and Model 2 (**Figures 3A,B**). To ensure the robustness of our results, MR-PRESSO was also conducted with outliner correction which

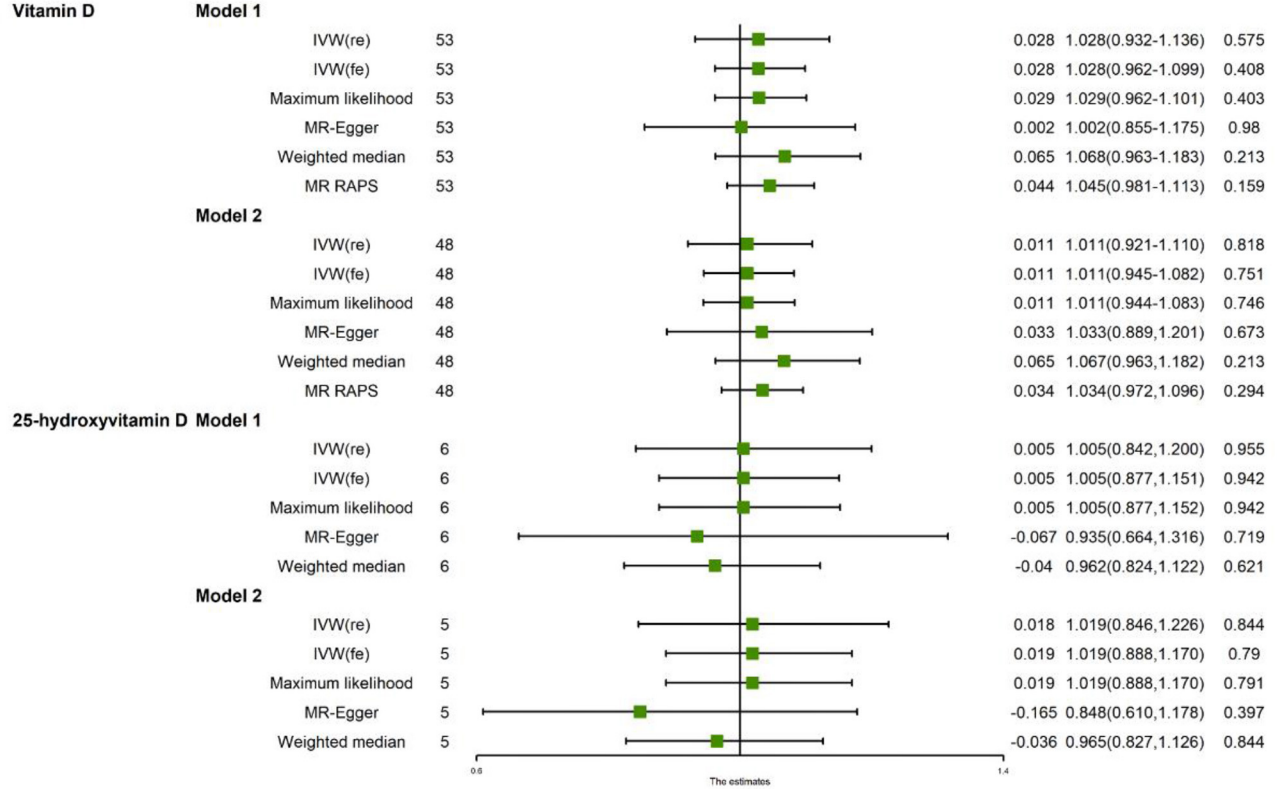

FIGURE 2 | Associations of vitamin D and 25-hydroxyvitamin D levels with AF in two-sample Mendelian randomization analysis. SNPs, single nucleotide polymorphisms; IVW, inverse variance weighted; OR, odds ratio; RAPS, robust adjusted profile score.

showed consistent results that vitamin D levels had no effect on the risk of AF (**Table 2**).

The scatter plots and forest plots are displayed in **Supplementary Figures 1A,B, 2A,B**. The funnel plots were symmetrical **(Supplementary Figures 3A,B)**, and the leave-one-out analysis revealed that no individual SNP was substantially driving the association between vitamin D and AF **(Supplementary Figures 4A,B).**

Association of Serum 25-Hydroxyvitamin D Levels With AF Risks

In model 1, the random-model IVW estimate showed that genetically predicted 25-hydroxyvitamin D levels were not significantly associated with AF risks ($N = 6$ SNPs, OR: 1.005, 95% CI: 0.842–1.200, $P = 0.955$). After extracting 1 SNPs, the result was consistent ($N = 5$ SNPs, OR: 1.019, 95% CI: 0.846–1.226, $P = 0.621$). The association was consistent in complementary analyses by weighted median method, maximum likelihood, and MR-egger method, while MR-RAPS method was not applicable for limited SNPs. There were no potential heterogeneities and no directional pleiotropies for the analysis results **(Supplementary Table 5**). Radial plots showed there were no outlines both in model 1 and model 2 (**Figures 3C,D**). To ensure the robustness of our results, MR-PRESSO was also conducted with outliner correction, which showed similar results that vitamin D levels were not associated with the risk of AF (**Table 2**).

The scatter plots, forest plots, and funnel plots aredisplayed in **Supplementary Figures 1C,D, 2C,D, 3C,D**, and the leave-one-out analysis indicated that no individual SNP was substantially driving the association between them **(Supplementary Figures 4C,D).**

Association of Serum AF With and Vitamin D and 25-Hydroxyvitamin D Levels

The IVW method estimate showed that genetically predicted AF was not significantly associated with vitamin D and 25-hydroxyvitamin D levels risks ($N = 13$ SNPs, OR: 1.032, 95% CI: 0.977–1.075, $p = 0.057$; $N = 42$ SNPs, OR: 0.997, 95% CI: 0.989–1.006, $P = 0.527$, **Supplementary Table 5**). The association was consistent in MR-PRESSO (**Supplementary Table 8**). The scatter plots, forest plots and funnel plots were displayed in **Supplementary Figures 1E,F, 2E,F, 3E,F**, and the leave-one-out analysis indicated that no individual SNP was substantially driving the association between them **(Supplementary Figures 4E,F).**

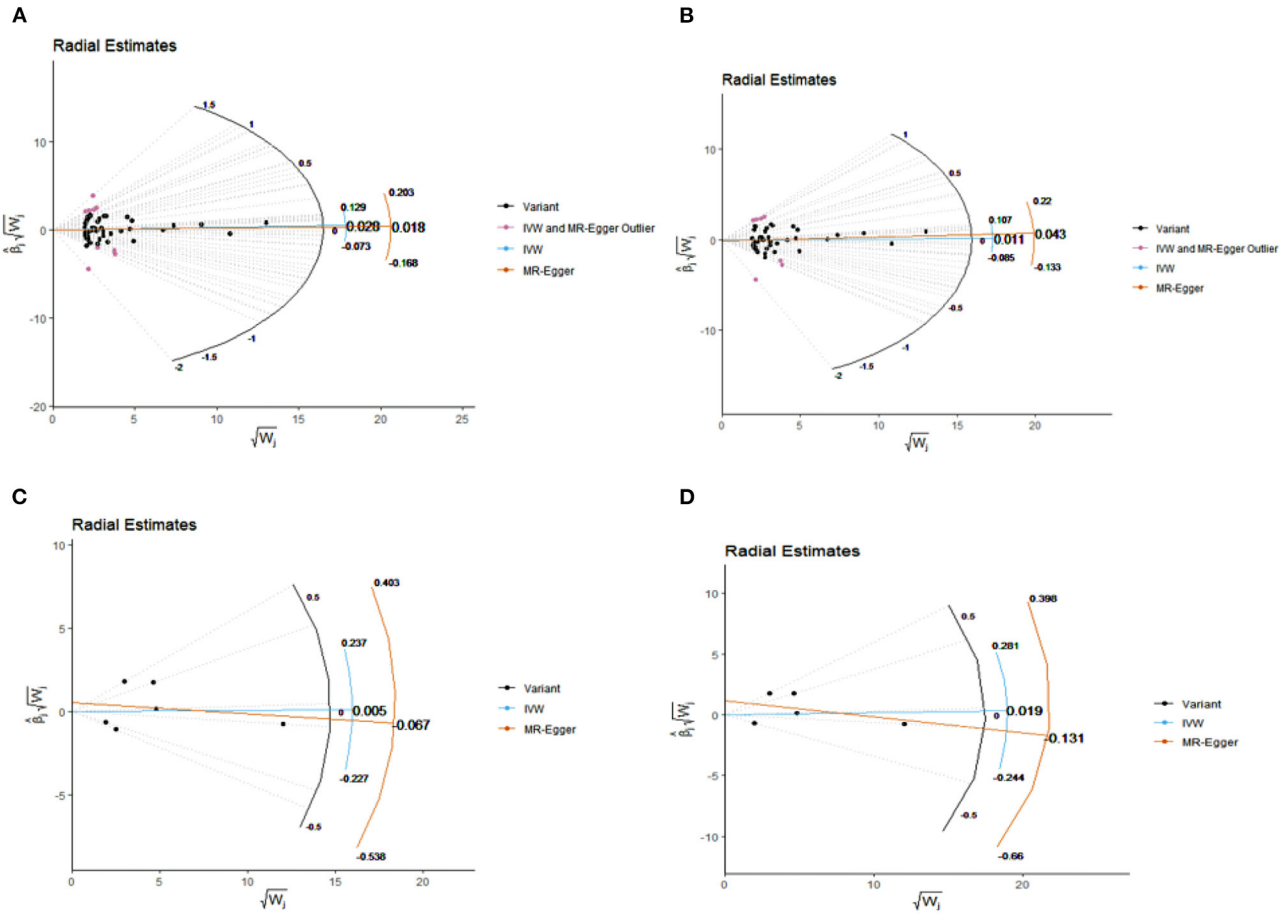

FIGURE 3 | Radial plots to visualize individual outlier single nucleotide polymorphisms (SNPs) in the Mendelian randomization (MR) estimates for association between vitamin D with AF by model 1 **(A)** and model 2 **(B)** or association between 25-hydroxyvitamin D with AF by model 1 **(C)** and model 2 **(D)**. Black dots show valid SNPs and purple dots display invalid outlier SNPs. There is no significant outlier SNP in present plots C and D. IVW, indicates inverse-variance weighted.

TABLE 2 | MR-PRESSO for causal effect between vitamin D and AF.

Exposure	Model	Raw estimates				Outlier corrected estimates				Distortion test
		nSNP	Beta	OR (95%CI)	*P*-value	nSNP	Beta	OR (95%CI)	*P*-value	*P*-value
Vitamin D	Model 1	53	0.039	1.040 (0.954,1.133)	0.368	50	0.05	1.051(0.978,1.130)	0.173	0.741
	Model 2	48	0.021	1.021 (0.942,1.107)	0.613	47	0.03	1.031(0.958,1.108)	0.422	0.812
25-hydroxyvitamin D	Model 1	6	0.005	1.005 (0.842,1.199)	0.958	6	NA	NA	NA	NA
	Model 2	5	0.019	1.019 (0.846,1.226)	0.853	5	NA	NA	NA	NA

AF, atrial fibrillation; SNP, single-nucleotide polymorphisms; OR, odds ratio.

DISCUSSION

In this two-sample MR study, we found no significant causal relationship between serum vitamin D levels and AF risks.

There is consistent evidence to show that low serum 25-hydroxyvitamin D levels are associated with increased risk of cardiovascular diseases, including hypertension, coronary artery disease, ischemic heart disease, and stroke (7, 29–32). However, the causal relationship between vitamin D and AF is inconclusive. Previous retrospective studies investigated the positive relationship between vitamin D and AF risks. For example, Chen et al. (33) found that the serum 25(OH)D level was significantly lower in the AF group than in the nonAF group. However, this trial was not randomized, prospective, and blinded, and low vitamin D levels could be presented in those without AF, so that a mechanistic cause of low vitamin D was not proven. Other two studies (34, 35) also showed the preventive role of vitamin D in patients with AF. These two studies enrolled AF

patient with hypertension and chronic heart failure, which are risk factors of AF, respectively. It seemed that positive results observed in these studies were amplified by confounding factors, including the other cardiovascular diseases.

Several prospective cohort study and RCTs have been performed to investigate the cause–effect of vitamin D supplementation on AF. The Rotterdam Study (36), the Multi-Ethnic Study of Atherosclerosis (MESA) (37) and the Cardiovascular Health Study (CHS) (37) all showed vitamin D deficiency was not associated with the occurrence of AF. A latest meta-analysis suggested that vitamin D deficiency was modestly associated with the occurrence of AF on a pooled analysis of case–control studies, while there appeared to be no association on pooled analysis of cohort studies (10). The discrepancy among the findings of many observational studies is likely due to the residual confounding. Our results are in accordance with the most recent meta-analysis of randomized controlled trials, which showed that serum vitamin D might not to play a major role in the development of new-onset AF (11).

Different from the other CVDs, AF is a complex arrhythmia that could be the outcome of various pathophysiological processes (38). The pathophysiology of AF included the basic electrophysiological and structural changes within the left atrium, the genetics of AF, and wider systemic and metabolic perturbations (38, 39). At present, the association between serum vitamin-D levels and AF has several potential pathophysiological mechanisms. Firstly, 1,25[OH]D, the activated form of vitamin D, inhibits the renin–angiotensin–aldosterone system (RAAS) (40, 41). RAAS plays a role in both structural and electrical remodeling of the atrium, suppresses cardiac myocyte hypertrophy and reduces inflammation (42). It can be inferred vitamin D deficiency may impair the prevention of AF by inhibiting RAAS. Secondly, vitamin D was associated with an inflammatory milieu and could increase the synthesis of C-reactive protein (CRP) directly or indirectly, which was crucial for the pathogenesis of AF (43). However, studies have suggested that vitamin D deficiency may be a consequence, not a cause of inflammation (44). In a word, the potential mechanisms of vitamin D and AF are still not fully illuminated and in dispute.

Our analysis has several strengths. Firstly, data from a large genetic consortium for serum vitamin D (n = 304,818), 25-hydroxyvitamin D levels (n = 79, 366), and AF (n = 1,030,836) allowed to increase the statistical power to detect small effects in complex phenotypes (45). Secondly, MR study avoided the potential biases based on the three core assumptions (46). Thirdly, the genetic variants used as the IVs were located in different chromosomes, the potential gene–gene interaction might have little effect on the estimated value (47). Furthermore, the sensitivity analysis with different MR methods showed consistent effects, including the radial plots and MR-PRESSO process. All the results showed no significant causal effects of serum vitamin D levels on AF risks.

There are some limitations in our study. Firstly, there were some heterogeneities in the study. Due to the GWAS data, any potential factors related to health status, age, and sex might contribute to the heterogeneities. Secondly, our study could not rule out the effect of canalization (i.e., dilution of the gene-exposure association), and thus the estimate might be inflated (48). Thirdly, the directional pleiotropy cannot be excluded, which is almost completely mediated through other causal pathways. Fourthly, the association between vitamin D deficiency and different AF subtypes was not explored because of the limited data, especially paroxysmal AF. Fifthly, our datasets included the European populations which limited applicability of results to non-European populations. Finally, there are potential biases in our studies caused by overlapping use of UK Biobank data. More studies are needed to verify the applicability of these results in other populations and other ethnicities in the future.

CONCLUSION

Our MR study did not find the association between circulating vitamin D levels and the AF risks. Further studies in different ethnicities are necessary to explore the potential role and mechanisms of circulating serum vitamin D levels on AF.

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

SY wrote the manuscript, performed quality assessment, and statistic analysis. LW designed the project and edited the manuscript. HZ helped revised the manuscript for language and checked the results. YS checked the results. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the National Natural Science Foundation of China (81673259), Natural Science Foundation of Jiangsu Province, China (BK20161435), and Jiangsu Commission of Health (H2019079).

ACKNOWLEDGMENTS

We are grateful for Feihong Chen for her helpful advice of methodology and statistics.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.837207/full#supplementary-material

REFERENCES

1. Chung MK, Refaat M, Shen WK, Kutyifa V, Cha YM, Di Biase L, et al. Atrial fibrillation: JACC council perspectives. *J Am Coll Cardiol.* (2020) 75:1689–713. doi: 10.1016/j.jacc.2020.02.025
2. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, et al. Heart disease and stroke statistics-2019 update: a report from the American Heart Association. *Circulation.* (2019) 139:e56–e528. doi: 10.1161/CIR.0000000000000659
3. Kornej J, Börschel CS, Benjamin EJ, Schnabel RB. Epidemiology of atrial fibrillation in the 21st century: novel methods and new insights. *Circ Res.* (2020) 127:4–20. doi: 10.1161/CIRCRESAHA.120.316340
4. Georgiopoulos G, Ntritsos G, Stamatelopoulos K, Tsioufis C, Aimo A, Masi S, et al. The relationship between blood pressure and risk of atrial fibrillation: a Mendelian randomization study. *Eur J Prev Cardiol.* (2021)28:14. doi: 10.1093/eurjpc/zwab005
5. Ong JS, Dixon-Suen SC, Han X, An J, Liyanage U, Dusingize JC, et al. A comprehensive re-assessment of the association between vitamin D and cancer susceptibility using Mendelian randomization. *Nat Commun.* (2021) 12:246. doi: 10.1038/s41467-020-20368-w
6. Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? *J Steroid Biochem Mol Biol.* (2014) 144:138–45. doi: 10.1016/j.jsbmb.2013.11.003
7. Pilz S, Verheyen N, Grübler MR, Tomaschitz A, März W. Vitamin D and cardiovascular disease prevention. *Nat Rev Cardiol.* (2016) 13:404–17. doi: 10.1038/nrcardio.2016.73
8. de la Guía-Galipienso F, Martínez-Ferran M, Vallecillo N, Lavie CJ, Sanchis-Gomar F, Pareja-Galeano H. Vitamin D and cardiovascular health. *Clin Nutr.* (2021) 40:2946–57. doi: 10.1016/j.clnu.2020.12.025
9. Liu X, Wang W, Tan Z, Zhu X, Liu M, Wan R, et al. The relationship between vitamin D and risk of atrial fibrillation: a dose-response analysis of observational studies. *Nutr J.* (2019) 18:73. doi: 10.1186/s12937-019-0485-8
10. Zhang Z, Yang Y, Ng CY, Wang D, Wang J, Li G, et al. Meta-analysis of Vitamin D deficiency and risk of atrial fibrillation. *Clin Cardiol.* (2016) 39:537–43. doi: 10.1002/clc.22563
11. Huang WL, Yang J, Yang J, Wang HB, Yang CJ, Yang Y. Vitamin D and new-onset atrial fibrillation: a meta-analysis of randomized controlled trials. *Hellenic J Cardiol.* (2018) 59:72–7. doi: 10.1016/j.hjc.2017.11.006
12. Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. *JAMA.* (2017) 318:1925–6. doi: 10.1001/jama.2017.17219
13. Sheehan NA, Didelez V, Burton PR, Tobin MD. Mendelian randomisation and causal inference in observational epidemiology. *PLoS Med.* (2008) 5:e177. doi: 10.1371/journal.pmed.0050177
14. Sinnott-Armstrong N, Tanigawa Y, Amar D, Mars N, Benner C, Aguirre M, et al. Genetics of 35 blood and urine biomarkers in the UK Biobank. *Nat Genet.* (2021) 53:185–94. doi: 10.1038/s41588-020-00757-z
15. Collins R. What makes UK Biobank special? *Lancet.* (2012) 379:1173–4. doi: 10.1016/S0140-6736(12)60404-8
16. Jiang X, O'Reilly PF, Aschard H, Hsu YH, Richards JB, Dupuis J, et al. Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. *Nat Commun.* (2018) 9:260. doi: 10.1038/s41467-017-02662-2
17. Nielsen JB, Thorolfsdottir RB, Fritsche LG, Zhou W, Skov MW, Graham SE, et al. Biobank-driven genomic discovery yields new insight into atrial fibrillation biology. *Nat Genet.* (2018) 50:1234–9. doi: 10.1038/s41588-018-0171-3
18. Park S, Lee S, Kim Y, Lee Y, Kang MW, Kim K, et al. Atrial fibrillation and kidney function: a bidirectional Mendelian randomization study. *Eur Heart J.* (2021) 42:2816–23. doi: 10.1101/2020.07.31.20166207
19. Staley JR, Blackshaw J, Kamat MA, Ellis S, Surendran P, Sun BB, et al. PhenoScanner: a database of human genotype-phenotype associations. *Bioinformatics.* (2016) 32:3207–9. doi: 10.1093/bioinformatics/btw373
20. Yang J, Ferreira T, Morris AP, Medland SE, Madden PA, Heath AC, et al. Conditional and joint multiple-SNP analysis of GWAS summary statistics identifies additional variants influencing complex traits. *Nat Genet.* (2012) 44:369–75. doi: 10.1038/ng.2213
21. Holmes MV, Ala-Korpela M, Smith GD. Mendelian randomization in cardiometabolic disease: challenges in evaluating causality. *Nat Rev Cardiol.* (2017) 14:577–90. doi: 10.1038/nrcardio.2017.78
22. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol.* (2016) 40:304–14. doi: 10.1002/gepi.21965
23. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization *via* the zero modal pleiotropy assumption. *Int J Epidemiol.* (2017) 46:1985–98. doi: 10.1093/ije/dyx102
24. Burgess S, Bowden J, Fall T, Ingelsson E, Thompson SG. Sensitivity analyses for robust causal inference from Mendelian randomization analyses with multiple genetic variants. *Epidemiology.* (2017) 28:30–42. doi: 10.1097/EDE.0000000000000559
25. Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan N, Thompson J, et al. A framework for the investigation of pleiotropy in two-sample summary data Mendelian randomization. *Stat Med.* (2017) 36:1783–802. doi: 10.1002/sim.7221
26. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol.* (2015) 44:512–25. doi: 10.1093/ije/dyv080
27. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet.* (2018) 50:693–8. doi: 10.1038/s41588-018-0099-7
28. Sanderson E, Davey Smith G, Windmeijer F, Bowden J. An examination of multivariable Mendelian randomization in the single-sample and two-sample summary data settings. *Int J Epidemiol.* (2019) 48:713–27. doi: 10.1093/ije/dyy262
29. Saponaro F, Marcocci C, Zucchi R. Vitamin D status and cardiovascular outcome. *J Endocrinol Invest.* (2019) 42:1285–90. doi: 10.1007/s40618-019-01057-y
30. Skaaby T, Thuesen BH, Linneberg A. Vitamin D, cardiovascular disease and risk factors. *Adv Exp Med Biol.* (2017) 996:221–30. doi: 10.1007/978-3-319-56017-5_18
31. Zittermann A, Pilz S. Vitamin D and cardiovascular disease: an update. *Anticancer Res.* (2019) 39:4627–35. doi: 10.21873/anticanres.13643
32. Zittermann A, Trummer C, Theiler-Schwetz V, Lerchbaum E, März W, Pilz S. Vitamin D and cardiovascular disease: an updated narrative review. *Int J Mol Sci.* (2021) 22:2896. doi: 10.3390/ijms22062896
33. Chen WR, Liu ZY, Shi Y, Yin DW, Wang H, Sha Y, et al. Relation of low vitamin D to nonvalvular persistent atrial fibrillation in Chinese patients. *Ann Noninvasive Electrocardiol.* (2014) 19:166–73. doi: 10.1111/anec.12105
34. Ozcan OU, Gurlek A, Gursoy E, Gerede DM, Erol C. Relation of vitamin D deficiency and new-onset atrial fibrillation among hypertensive patients. *J Am Soc Hypertens.* (2015) 9:307–12. doi: 10.1016/j.jash.2015.01.009
35. Belen E, Aykan AC, Kalaycioglu E, Sungur MA, Sungur A, Cetin M. Low-level vitamin D is associated with atrial fibrillation in patients with chronic heart failure. *Adv Clin Exp Med.* (2016) 25:51–7. doi: 10.17219/acem/34690
36. Vitezova A, Cartolano NS, Heeringa J, Zillikens MC, Hofman A, Franco OH, et al. Vitamin D and the risk of atrial fibrillation–the Rotterdam Study. *PLoS ONE.* (2015) 10:e0125161. doi: 10.1371/journal.pone.0125161
37. Mathew JS, Sachs MC, Katz R, Patton KK, Heckbert SR, Hoofnagle AN, et al. Fibroblast growth factor-23 and incident atrial fibrillation: the Multi-Ethnic Study of Atherosclerosis (MESA) and the Cardiovascular Health Study (CHS). *Circulation.* (2014) 130:298–307. doi: 10.1161/CIRCULATIONAHA.113.005499
38. Wijesurendra RS, Casadei B. Mechanisms of atrial fibrillation. *Heart.* (2019) 105:1860–7. doi: 10.1136/heartjnl-2018-314267
39. Lau DH, Linz D, Sanders P. New findings in atrial fibrillation mechanisms. *Card Electrophysiol Clin.* (2019) 11:563–71. doi: 10.1016/j.ccep.2019.08.007
40. Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W, Kong J. Vitamin D: a negative endocrine regulator of the renin-angiotensin system and blood pressure. *J Steroid Biochem Mol Biol.* (2004) 89–90:387–92. doi: 10.1016/j.jsbmb.2004.03.004
41. Forman JP, Williams JS, Fisher ND. Plasma 25-hydroxyvitamin D and regulation of the renin-angiotensin system in humans. *Hypertension.* (2010) 55:1283–8. doi: 10.1161/HYPERTENSIONAHA.109.148619
42. van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. *J Steroid Biochem Mol Biol.* (2005) 97:93–101. doi: 10.1016/j.jsbmb.2005.06.002

43. Shea MK, Booth SL, Massaro JM, Jacques PF, D'Agostino RB Sr, Dawson-Hughes B, et al. Vitamin K and vitamin D status: associations with inflammatory markers in the Framingham Offspring Study. *Am J Epidemiol.* (2008) 167:313–20. doi: 10.1093/aje/kwm306
44. Bie L. The status and research progress on vitamin D deficiency and atrial fibrillation. *Braz J Cardiovasc Surg.* (2019) 34:605–9. doi: 10.21470/1678-9741-2018-0322
45. Manousaki D, Mokry LE, Ross S, Goltzman D, Richards JB. Mendelian randomization studies do not support a role for vitamin D in coronary artery disease. *Circ Cardiovasc Genet.* (2016) 9:349–56. doi: 10.1161/CIRCGENETICS.116.001396
46. Zheng J, Baird D, Borges MC, Bowden J, Hemani G, Haycock P, et al. Recent developments in Mendelian randomization studies. *Curr Epidemiol Rep.* (2017) 4:330–45. doi: 10.1007/s40471-017-0128-6
47. Wang T, Xu L. Circulating vitamin E levels and risk of coronary artery disease and myocardial infarction: a Mendelian randomization study. *Nutrients.* (2019) 11:2153. doi: 10.3390/nu11092153
48. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol.* (2003) 32:1–22. doi: 10.1093/ije/dyg070

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 13 April 2022
doi: 10.3389/fnut.2022.852398

Assessment of Sustainable Elimination Criteria for Iodine Deficiency Disorders Recommended by International Organizations

Lijun Fan[1], *Fangang Meng*[2], *Qihao Sun*[3], *Yuqian Zhai*[3] *and Peng Liu*[3]*

[1] National Health Commission and Education Bureau of Heilongjiang Province, Key Laboratory of Etiology and Epidemiology, Harbin Medical University, Harbin, China, [2] Heilongjiang Provincial Key Laboratory of Trace Elements and Human Health, Harbin Medical University, Harbin, China, [3] Endemic Disease Control Center, Chinese Center for Disease Control and Prevention, Harbin Medical University, Harbin, China

OPEN ACCESS

Edited by:
Francesco Sofi,
Università degli Studi di Firenze, Italy

Reviewed by:
Xin Sun,
The First Affiliated Hospital of Soochow University, China
Creswell John Eastman,
Westmead Hospital, Australia

***Correspondence:**
Peng Liu
liup7878@163.com

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: 11 January 2022
Accepted: 28 February 2022
Published: 13 April 2022

Citation:
Fan L, Meng F, Sun Q, Zhai Y and Liu P (2022) Assessment of Sustainable Elimination Criteria for Iodine Deficiency Disorders Recommended by International Organizations. Front. Nutr. 9:852398. doi: 10.3389/fnut.2022.852398

Enormous efforts have been made to evaluate the worldwide prevention and control of iodine deficiency disorders (IDDs). This study evaluated China's achievements in IDD prevention and control against WHO criteria for sustainable elimination of IDD. The study sample consisted of 556,390 school-aged children and 271,935 pregnant women enrolled in the 2018 China National IDD Surveillance. As a result, at the national level, median urine iodine concentration (MUIC) was 206.1 and 163.5 μg/l in children and in pregnant women, respectively. The proportion of households consuming adequate iodized salt (PHCAIS) was 90.2%. The prevalence rates of goiter in children and thyroid disease in pregnant women were 2.0 and 0.8%, respectively. MUIC showed significant non-linear increasing trends with increasing PHCAIS in both children and pregnant women. The prevalence of thyroid disease in pregnant women had a sharp decreasing trend with increasing PHCAIS. Of note, the prevalence of goiter in children and thyroid disease in pregnant women against MUIC both presented as significant U-shaped curves, with the lowest prevalence at 100–300 μg/l of MUIC in children and 150–250 μg/l in pregnant women. PHCAIS, MUIC, and the programmatic indicators at the national level were all above their cut-offs proposed in the 2007 Criteria. Evaluation by adding the prevalence of goiter (<5%) yielded the different results at the county level. Sustainable elimination of IDD has been achieved nationally. 2018 Chinese surveillance data support the expansion of global cut-offs for optimal iodine status in school-age children from 100–199 to 100–299 μg/l as recommended by others and the lower limit of MUIC (150 μg/l) in pregnant women also seems justified. Inclusion of goiter prevalence <5% in our analysis reduced the number of municipalities and counties which had achieved sustainable elimination of IDD.

Keywords: iodine deficiency disorders, urine iodine concentration, goiter prevalence, iodized salt, sustainable elimination

INTRODUCTION

Iodine is an essential element required to produce thyroid hormones and plays a major role in human growth and tissue development (1). It is well-known that reduced iodine intake is associated with iodine deficiency disorders (IDDs), namely, endemic goiter, hypothyroidism, and cretinism (2), a decreased fertility rate, an increased incidence of perinatal death, and infant mortality (3).

In the 1990s, the WHO recommended universal salt iodization (USI) as the first-line strategy for IDD prevention and control (4, 5). The WHO, the United Nations International Children's Emergency Fund, and the International Council for Control of Iodine Deficiency Disorders (currently the Iodine Global Network) developed the guideline "Assessment of iodine deficiency disorders and monitoring their elimination: A guide for program managers, 3rd edition." Chapter 6 of this guide on "Indicators of the sustainable elimination of IDD" includes "technical criteria" for the sustainable elimination of IDD with regards to salt iodization and population iodine status (Table 10) and 10 programmatic indicators, hereafter referred to as the "2007 criteria" (2).

The USI has been implemented in China for over two decades. The latest Chinese surveillance data showed that iodine nutritional status of the general population and pregnant women has been greatly improved (6). A series of programmatic indicators which are similar to those in the 2007 criteria were established in China. By 2000, IDD had been essentially eliminated in China (7).

Since the publication of the 2007 guideline "Assessment of iodine deficiency disorders and monitoring their elimination: A guide for program managers, 3rd edition," expansion of the range of median of urinary iodine concentration (MUIC) considered optimal in school-age children has been suggested (8, 9) and the necessity to achieve >90% proportion of households consuming adequate iodized salt (PHCAIS) has been questioned (10). This article also suggests the inclusion of the prevalence of goiter >5% as criteria against which to assess the sustainable elimination of IDD. At present, considerable efforts have been made worldwide to evaluate the effect of USI on the sustainable elimination of IDD. Therefore, the revision and application of the 2007 criteria are more important than ever.

This study aims to assess the achievement of the sustainable elimination of IDD in China based on the technical and programmatic indicators in the 2007 criteria and the Chinese criteria and to provide evidence for revision of the guidelines. This aim was achieved by analyzing the 2018 China National IDD Surveillance data (6).

METHODS

Data Source and Sampling Method

In the 2018 China National IDD Surveillance, a multistage sampling procedure was used with the county being the sampling unit. Each county was divided into five sampling areas: east, west, south, north, and middle, and one town or district was randomly sampled from each area. Out of 2,879 counties, we analyzed data from 2,827 counties having water iodine concentration (WIC) < 100 μg/l, with 52 counties having WIC ≥ 100 μg/l excluded.

Children of ages 8–10 years ($n = 40$) from local primary schools and pregnant women ($n = 20$) from communities around the schools were randomly selected from each of the five sampling areas in each county. A total of 200 children and 100 pregnant women were recruited from each county. The inclusion criteria were as follows: residents living in the sampling site for at least half a year; an equal number of girls and boys; pregnant women who were diagnosed as being pregnant in a medical institution.

Information on geographic regions, coastal living, and capita income was collected from the State Statistical Bureau. Information on date of birth, sex, ethnicity, gestational weeks, and history of thyroid disease (including thyroid nodules, clinical and subclinical hyperthyroidism, clinical and subclinical hypothyroidism, thyroiditis, and thyroid cancer) was obtained using a questionnaire. Spot urine specimens were collected from all participants, and salt samples were collected from their households. We also analyzed the data from the National Survey on Iodine in Drinking Water (11). WIC at the county level was used to establish the inclusion criteria for the current study.

The study protocol was approved by the ethics committee of the Harbin Medical University. Written informed consent was obtained from all participants or their guardians.

Laboratory Analysis

The direct iodine titration method was used to assay the iodine content of salt samples (12). Salt consumed was classified as non-, low-, qualified, and high-iodized salt according to the iodine contents as shown in **Supplementary Table 1**. In China, provinces are allowed to select one/two of three options for salt iodine content—20, 25, or 30 mg/kg with a variation of ±30%, according to the Chinese standard (GB 26878) (13). At present, 14 provinces selected 25 (14–29) mg/kg, 12 provinces selected 30 (17–35) mg/kg, and 5 provinces selected 25 mg/kg for the general population and 30 mg/kg for pregnant women (13). As^{3-}-Ce^{4+} catalytic spectrophotometry was used to measure urinary iodine concentration (36), and the iodine nutritional status at different levels was evaluated according to the international standard (2). The thyroid volume of children was measured using B-ultrasonography. Goiter was diagnosed as thyroid volume being above the Chinese standard (37).

Assessment of Elimination of IDD

The 2018 China National IDD Surveillance data have four levels, i.e., national, provincial, municipal, and county. Then for each level, elimination of IDD was assessed based on individual technical indicators and multiple technical indicators in 2007 criteria plus programmatic indicators in both the 2007 criteria and the Chinese criteria. Chinese criteria for evaluating the sustainable elimination of IDD (a scoring table) are shown in **Supplementary Table 2**. Individual items were scored based on IDD elimination achievements in the past 3 years, and then individual scores were added up as a total score. Areas with a total score ≥ 85 along with relevant technical indicators were classified as sustainable elimination of IDD.

TABLE 1 | Descriptive data of the study sample.

Variable	Children						Pregnant women			
	TV (mL)		Goiter prevalence		MUIC (μg/L)		TD prevalence		MUIC (μg/L)	
	N	Median	*N*	(%)	*N*	Median	*N*	(%)	*N*	Median
Children age (year)										
8	70,110	2.41*	70,110	2.15*	150,914	203.10*	—	—	—	—
9	100,793	2.60	100,793	1.96	217,410	205.20	—	—	—	—
10	87,701	2.84	87,701	1.27	188,006	209.70	—	—	—	—
Pregnant stage										
1st-	—	—	—	—	—	—	537	0.94*	56,306	166.30*
2nd-	—	—	—	—	—	—	926	0.79	115,577	165.20
3rd-	—	—	—	—	—	—	772	0.77	100,052	159.20
Type of iodized salt										
Non-iodized	8,678	2.91*	8,678	3.09*	18,895	163.10*	238	2.98*	7,972	125.59*
Low-iodized	10,772	2.68	10,772	2.40	24,239	195.30	79	0.85	9,270	158.00
Qualified iodized	240,571	2.61	240,571	1.70	510,150	198.40	1,566	0.76	206,245	166.50
High-iodized	2,613	2.65	2,613	1.95	5,187	214.70	—	—	2,274	164.34
Water iodine (μg/L)										
<10	232,348	2.60*	232,348	1.72	482,599	194.50*	1,968	0.83	237,588	161.60*
10∼	26,841	2.71	26,841	2.16	57,010	205.90	248	0.83	29,619	168.00
40∼	2,210	2.86	2,210	3.53	9,998	251.25	—	—	3,489	206.00
100∼	3,248	2.90	3,248	2.00	6,723	281.60	—	—	3,291	223.53
Coastal region										
Yes	20,546	2.63*	20,546	1.81*	36,774	174.50*	453	2.47*	18,116	131.19*
No	160,519	2.61	160,519	1.72	349,317	199.03	1,278	0.74	172,804	165.60
Landform										
Plain	62,523	2.69*	62,523	2.11*	135,671	197.80*	753	1.11*	67,640	162.50
Hilly	48,006	2.58	48,006	1.61	113,612	196.10	556	0.99	55,920	159.94
Mountain	66,293	2.57	66,293	1.51	126,975	196.10	382	0.61	62,390	165.40
Capita income										
<¥20,000	170,756	2.63	170,756	1.70*	260,690	197.10	1,009	0.56*	178,258	165.50*
¥20,000–¥40,000	73,755	2.66	73,755	1.91	153,655	197.91	725	0.80	75,696	160.30
>¥40,000	20,136	2.64	20,136	2.13	41,232	197.41	501	2.49	20,033	152.40
Nationwide	264,740	2.61	264,740	2.00	570,271	206.10	274,929	0.80	274,578	163.50

*TV, thyroid volume; MUIC, median urine iodine concentration; TD, thyroid disease; *p < 0.05 for trend.*

Statistical Analysis

All the survey data were entered into the IDD Information Management System (a standard data management system). Statistical analyses were performed using SAS version 9.1 (SAS Institute, Incorporation, Cary, North Carolina, USA). The differences in continuous and categorical variables between groups were tested using the ANOVA/Wilcoxon's test and the chi-squared test, respectively. The curve between MUIC and PHCAIS in children and pregnant women and the curve of MUIC with the prevalence of goiter in children and prevalence of thyroid disease in pregnant women were fitted by polynomial regression analysis models.

RESULTS

Descriptive Data

Descriptive data of the study sample are given in **Table 1**. The variables showed significant increasing or decreasing trends across most subgroups. In the total sample of the 2018 China National IDD Surveillance, MUIC was 206.1 and 163.5 μg/l in children and in pregnant women, respectively; PHCAIS was 90.2%; the prevalence rates of goiter in children and thyroid disease in pregnant women were 2.0 and 0.8%, respectively.

Associations Between PHCAIS, MUIC, and Thyroid Disease

In **Figure 1**, PHCAIS was divided into the 15 groups (<30% and groups with an interval of 5% from 30% onward) for **Figures 1A,C,D**. There were significant non-linear increasing trends of MUIC with increasing PHCAIS in children (**Figure 1A**) and pregnant women (**Figure 1C**). It is notable that MUIC of school-age children was > 130 μg/l in all the PHCAIS groups, including those <90% and even those groups <40%. This implies sources of iodine other than household salt. The prevalence of thyroid disease in pregnant women slightly increased and then sharply decreased with increasing PHCAIS (**Figure 1D**).

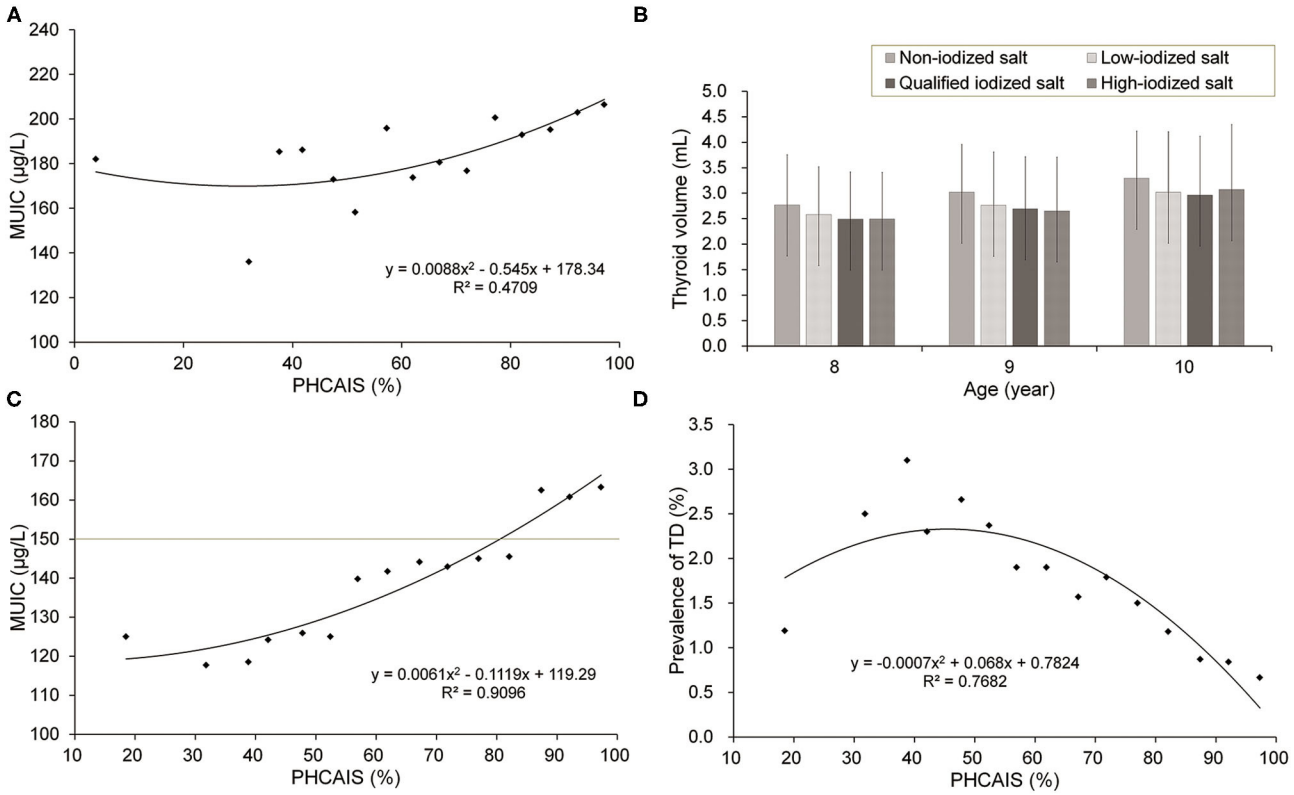

FIGURE 1 | The influence of iodized salt on thyroid disease and iodine nutrition. MUIC, median urine iodine concentration; PHCAIS, proportion of households consuming adequately iodized salt; TD, thyroid disease. **(A,B)** children and **(C,D)** pregnant women. $p < 0.0001$ for trend in all 3 age groups in **(B)**.

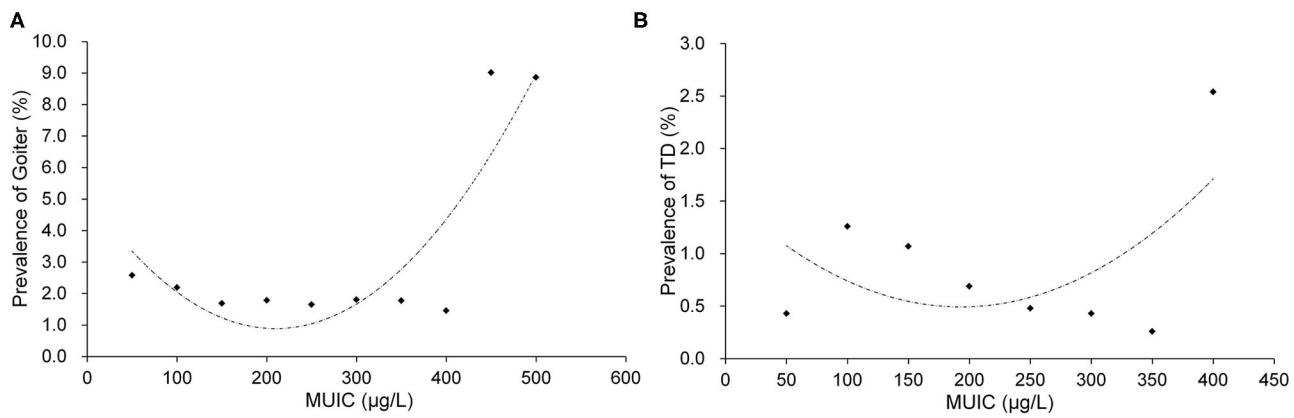

FIGURE 2 | The relationships of MUIC with goiter in children **(A)** and TD in pregnant women **(B)**. MUIC, median urine iodine concentration; TD, thyroid disease. **(A)** $R^2 = 0.7763$ and **(B)** $R^2 = 0.3059$.

In **Figure 1B**, thyroid volume showed significant and consistent decreasing trends across the four subgroups consuming different types of iodized salt within age groups ($p < 0.0001$ for the trend in all three age groups). The thyroid volume was the smallest in the qualified iodized salt group in age groups of 8 and 10 years and the largest in the non-iodized salt group for all the age groups.

Figure 2 presents the relationships of MUIC with goiter in children (**Figure 2A**) and thyroid disease in pregnant women (**Figure 2B**). MUIC was divided into 10 groups (<100 μg/l, groups with an interval of 50 and ≥500 μg/l) in **Figure 2A** and 8 groups (<100 μg/l, groups with an interval of 50 and ≥ 400 μg/l) in **Figure 2B**. The prevalence rates of goiter in children and

TABLE 2 | Evaluation results of IDD in China.

Unit	Total	Number of units assessed by individual indicators				Rates, *n* (%)			
		PHCAIS (>90%)	MUIC[a] (100–299 μg/L)	MUIC[b] (150–249 μg/L)	GP (<5%)	Method 1	Method 2	Method 3	Method 4
Nation	1	1	1	1	1	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
Province	31	22	31	22	31	17 (54.8)	17 (54.8)	17 (54.8)	17 (54.8)
Municipality	366	280	359	245	350	194 (53.0)	194 (53.0)	188 (51.4)	188 (51.4)
County	2,827	2,125	2,700	1,717	2,639	1,257 (44.5)	1,257 (44.5)	1,185 (41.9)	1,184 (41.9)

PHCAIS, proportion of households consuming adequately iodized salt; MUIC, median urine iodine concentration; GP, goiter prevalence.
[a]Children and [b]pregnant women.
Method 1 represents the results evaluated by technical indicators of PHCAIS (>90%), MUIC (100–299 μg/l) in children and MUIC (150–249 μg/l) in pregnant women in the 2007 criteria.
Method 2 represents the results evaluated by the indicators in Method 1 plus the programmatic indicators in the 2007 criteria.
Method 3 represents the results evaluated by the indicators in Method 1 plus GP < 5%.
Method 4 represents the results evaluated by the indicators in Method 3 plus a score >85 of programmatic indicators in the Chinese criteria.
Rate (%) = the number of areas meeting the criteria/total number of areas × 100.

thyroid disease in pregnant women both showed a significant U-shaped tendency with increasing MUIC. The prevalence of goiter in children (**Figure 2A**) and the prevalence of thyroid disease in pregnant women (**Figure 2B**) were the lowest around 200 μg/l of MUIC.

Evaluation of Sustainable Elimination of IDD in China

Achievement of the sustainable elimination of IDD in China was assessed by four different methods using the 2018 China National IDD Surveillance data, as shown in **Table 2**. The rates at the national level were 100%. The rates by methods 1 and 2 were higher than those by methods 3 and 4 (53.0 vs. 51.4% at the municipal level and 44.5 vs. 41.9% at the county level). When more indicators were used for evaluation (method 3, 4), the rates of elimination of IDD became lower at the municipal and county levels.

DISCUSSION

In this study, we assessed the IDD elimination status in China of 2018 based on the 2007 criteria and the Chinese criteria using the 2018 China National IDD Surveillance data. Since China has a vast territory with a huge population, we evaluated the rates of IDD elimination at various levels from nation to the county to examine variation in the attainment of IDD elimination among areas and regions. The 2018 China National IDD Surveillance provided an excellent opportunity to evaluate the IDD elimination status in China. The 2018 surveillance system covers all counties in China and has a huge sample size with data available on thyroid volume in school-age children and a history of thyroid disease in pregnant women. Therefore, we took these advantages to conduct comprehensive analyses to assess the IDD elimination status in China for the purpose of providing convincing evidence for the revision of current international and domestic guidelines.

Goiter has been recognized as a historical indicator for assessing the severity of IDD (38). A prevalence of goiter ≥5% in children is a signal of a public health problem (2). Instead of adults, children have a very low prevalence of autoimmune thyroiditis and other thyroid disease (14, 39). Besides, the cutoff point of 5% allows both for some margin of error of goiter assessment, and for goiter that may occur in iodine-replete populations due to other causes such as goitrogens and autoimmune thyroid diseases based on the WHO's guideline (2). However, the prevalence of goiter was not included as an indicator in the 2007 criteria (2). The major reason might be concerns regarding limitations of B-ultrasound (high cost, electricity usage, and requirement for special training) 14 years ago, especially in some developing countries and regions (15, 16). The results from this study showed that method 3 (the 2007 criteria + prevalence of goiter < 5%) identified 72 counties less than method 1 (the 2007 criteria alone) in evaluating the elimination (**Table 2**). The difference in the number of counties meant that these 72 counties might be at high risk for IDD due to insufficient iodine intake that resulted in a higher prevalence of goiter. Based on the observation of this study, the inclusion of the prevalence of goiter <5% as a technical indicator is recommended for the revision of the 2007 criteria. Furthermore, a goiter prevalence ≥5% in school-aged children showed as a signal of a public health problem (2), while a goiter prevalence <5% reflects a long-term endeavor to reduce IDD prevalence in a country. Under a long-term intervention of USI, goiter prevalence should be <5% in all areas. Once goiter prevalence rises to more than 5% in an area, this area might be a high-risk area that may suffer IDD again. Therefore, although goiter prevalence might be not a timely indicator for "iodine nutrition status," it should still be an indicator for the inadequacy of "IDD prevention and control measures" for a period past, which identified as to whether the prevention measures have been "sustained" in place, correspondence to "sustained" elimination. Furthermore, at the individual level, thyroid volume changes inversely in response to alterations in iodine intake, with a lag interval that varies between a few months and several years. However, goiter prevalence is a population indicator, and because of the sampling method, participants for each year's surveillance are different. It means if the persons in one group consume iodized salt more than 50%, MUIC will be appropriate. However,

even 5% of people consume non-iodized salt for more than 6 months, goiter prevalence can be more than 5%. Therefore, goiter prevalence is even more sensitive than MUIC when iodine deficiency, as only a few individuals present goiter, it could be caught by the surveillance under the long-term USI surroundings. Besides, although ultrasound scan is a precise, safe, and non-invasive measurement of thyroid volume and the limitations of B-ultrasound have been substantially reduced, along with the improvement in the economy worldwide, it may still not be feasible in some countries and regions due to the poverty, and in these areas, it can be replaced by palpation. Goiter examination by palpation is not as accurate as by B-ultrasound, however, the error could be lessened by training. Chinese IDD surveillance data (17–19) also showed the results by palpation were acceptable (Chinese IDD surveillance data from 1997 to 2002, $r = 0.88$, $p < 0.0001$; shown as **Supplementary Figure 1**). Therefore, we suggested that under extreme conditions, when thyroid volume assessment using B-ultrasound is not feasible, it might be replaced by palpation.

The proportion of households consuming adequate iodized salt (PHCAIS) (>90%) is one of the technical indicators proposed in the 2007 criteria (2). However, a previous study reported that MUIC in children tended to be adequate, and prevalence of IDD was low in some areas with PHCAIS < 90% in China, and then a question was raised, i.e., whether it is necessary to require PHCAIS > 90% in these areas (10). We found in this study that with the increase of PHCAIS, MUIC in both children and pregnant women showed an upward trend (**Figures 1A,C**). And with increasing PHCAIS, the prevalence of thyroid disease in pregnant women showed a sharp decrease (**Figure 1D**). The association between PHCAIS and MUIC indicates that PHCAIS is a key factor to maintain iodine nutritional health and a crucial indicator for the evaluation of IDD elimination. Although MUIC in children was adequate in subgroups with PHCAIS <90%, MUIC in pregnant women was below 150 μg/l in almost all these subgroups, suggesting that PHCAIS > 90% is necessary to ensure adequate iodine nutrition levels, especially for pregnant women.

It showed that as well as the lowest thyroid volume, school-age children who consumed qualified and higher iodized salt had the highest MUIC values (**Table 1** and **Figure 1B**), which proved that intake of qualified iodized salt is very important. However, it should also be noted that MUIC in children was higher than 130 μg/l in all subgroups including those with PHCAIS < 90% in this study (**Figure 1A**) and children who consumed non-iodized salt still had adequate iodine status (**Table 1**). This phenomenon was also seen in the Chinese population in samples/cohorts research that was different from ours (10, 20, 21). Multiple possible reasons may be responsible for these observations. First, part of school-aged children has lunch in school dining rooms where iodized salt is strictly required for cooking. However, the salt in this survey only came from children's households. This might contribute in part to the high MUIC in children in the counties with low PHCAIS in China. Second, other dietary sources of iodine such as milk, eggs, and processed foods containing added iodized salt may account for a proportion of total iodine intake in children. A study on processed food (including instant noodles, biscuits, smoked meat, puffed food, canned meat, milk products, etc.) was performed in six provinces in China, and the results showed that processed foods accounted for 4.0% (10.9 μg/d) of the total iodine intake (22). Third, PHCAIS is defined as the proportion of households consuming "qualified" salt, not including high-iodized salt. Children who consumed high-iodized salt were also classified in the subgroups with low PHCAIS (<90%). Although the situation of iodine intake from school dining room and processed food might be special in China, concerning global status, in this study, it was suggested that proportion of food cooked in school dining rooms and processed food made with iodized salt should be considered as supplementary items in the surveillance to define PHCAIS, especially in urban areas where people more often dine out and have more intake of iodine from the diet such as processed food (23, 24).

We found in this study that the prevalence of goiter in children presented a marked U-shaped relationship with MUIC, with the prevalence being the lowest around 200 μg/l of MUIC (**Figure 2A**). This observation is consistent with the findings in children from previous studies (25–27). Furthermore, the prevalence of thyroid disease in pregnant women also showed a substantially similar U-shaped relationship to that noted in children, with the prevalence being the lowest at the same value of MUIC (around 200 μg/l) (**Figure 2B**). Other studies have also reported similar results in pregnant women (28–30). The relation between them could be explained from an etiological aspect. However, although the tendency of the "U" curve is no doubt when MUIC is particularly high, a few points may not fit well with the curve, sometimes, they might be higher or even lower. This wave-like rise at the high side of the curve might be caused by four reasons: first, the thyroid atrophy and fibrosis following enlargement due to extremely high iodine intake occur in some of the subjects (31, 32); second, the wave-like rise might be caused by the less and less sample size from middle to the high end, although the total sample size is huge, it is uneven between groups; third, as the extremely skewed distribution of urinary iodine, logarithm transformation, and grouping analysis methods are normally used to correct the "U" curve, but sometimes the curve is not very symmetrical, with a right tail; last, the sensitivity is different of individual suffered high iodine damage. Therefore, possibly, these points may not be outliers. This phenomenon could also be seen in other published articles (33, 34). Although these points weaken the stability and symmetry of the curve, they were valuable and made the results more reasonable and meaningful. In the fitting model of MUIC with the prevalence of goiter in children, the points were basically around the fitting curve, and the R^2 was satisfied with the value around 0.8. In pregnant women, it does not fit as good as in children, but the result was also acceptable. The findings in children and pregnant women of this study provided additional evidence to support the range of MUIC in children (100–299 μg/l) and pregnant women (150–249 μg/l) as the cut-off values of these two indicators proposed in the 2007 criteria (2).

We evaluated the IDD elimination status in China using the 2018 China National IDD Surveillance data by four different methods. The 2007 criteria were recommended to evaluate the IDD elimination status at the national level, and in China, PHCAIS, MUIC in children and pregnant women, and the programmatic indicators at the national level were all above their cutoffs proposed in the 2007 criteria (2). To further assess the IDD elimination in local areas, the 2007 criteria were creatively applied for evaluation in individual provinces, municipalities, and counties in this study. The IDD elimination did not reach the criteria in all provinces, municipalities, and counties. The influencing factor was mainly the MUIC in pregnant women. In most areas under elimination in China, MUIC in pregnant women was slightly lower than 150 μg/l. However, the international organization mentioned that where USI has been effective for at least 2 years, with the PHCAIS more than 90%, it can be reasonably expected that the iodine needs of pregnant women are covered by their diet, and that the iodine stored in the gland is sufficient to ensure adequate hormone synthesis and secretion (35). The sustainable elimination of IDD is a long-term goal. Improvement in technical and programmatic indicators has implications for assessing programs and maintaining the sustainability of the elimination. In the 2007 criteria, the programmatic indicators cover 10 framework aspects which are basal and general requirements but do not have details for every aspect. To overcome these shortcomings, China established its system of programmatic indicators based on the 2007 criteria. The comparison with the 2007 criteria showcased the characteristics of the Chinese programmatic indicators (**Supplementary Table 3**). A scoring system is more applicable in China. It facilitates strengthening the capacity of teams for IDD prevention and control. Chinese programmatic indicators may provide useful information for the update of the 2007 criteria.

The 2018 China National IDD Surveillance data have strengths for the evaluation of IDD elimination, including the huge sample size and national representativeness. There are a few limitations to this study. Although we discussed the possible reasons for the high MUIC in children in areas with PHCAIS < 90% in China, no corresponding investigation such as a detailed questionnaire survey on dining in school or processed food adding iodized salt was conducted in this study. Further research should focus on this phenomenon in the areas with low PHCAIS and high frequency of dining out and consuming processed food adding iodized salt in China. In addition, children with thyroid disease were not excluded in this research, and individual thyroid diseases in pregnant women were not analyzed separately. Last, although the polynomial regression model may be affected by potential "outliers," information was still provided for reference.

In conclusion, IDD elimination has been essentially achieved in China evaluated by the 2007 criteria and the Chinese criteria; based on the 2018 China National IDD Surveillance data, recommendations and suggestions for revision of the 2007 criteria were made concerning adding the prevalence of goiter (<5%), keeping the ranges of MUIC (100–299 μg/l) in children and MUIC (>150 μg/l) in pregnant women, and the requirement of PHCAIS > 90%, especially for pregnant women. This study has importance and implications for providing evidence for the revision of international and domestic guidelines for IDD elimination and thus promoting global public health.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because they are the national surveillance data. Requests to access the datasets should be directed to Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention.

ETHICS STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by Ethics Committee of Harbin Medical University, the code is ZZXM2018011. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

PL conceived and designed the study. LF wrote the original draft. FM, QS, and YZ performed the statistical analysis. All authors contributed to the acquisition, analysis, interpretation of the data, and approved the final version of the manuscript.

FUNDING

This study was supported by the 2019 National Health Standards Project (20190502) and in part supported by the Natural Science Foundation of China (NSFC 81773370 and NSFC 82173638).

ACKNOWLEDGMENTS

We thank the staff who participated in the 2018 China National IDD Surveillance in the Center for Disease Control and Prevention and the Institute for Endemic Disease Control and Prevention in all provinces in China.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.852398/full#supplementary-material

Supplementary Figure 1 | The correlation of goiter prevalence in provinces by B-ultrasound and by palpation (the Chinese IDD surveillance data from 1997 to 2002).

Supplementary Table 1 | Classification of salt consumed in China based on iodine content.

Supplementary Table 2 | Programmatic indicators for monitoring progress toward sustainable elimination of iodine deficiency disorders (IDDs) in China.

Supplementary Table 3 | The comparison between the 2007 criteria and the Chinese criteria.

REFERENCES

1. Morreale de EG, Obregon MJ, Escobar del RF. Role of thyroid hormone during early brain development. *Eur J Endocrinol.* (2014) 151(Suppl. 3):U25–U37. doi: 10.1530/eje.0.151u025
2. WHO/UNICEF/ICCIDD. *Assessment of Iodine Deficiency Disorders Monitoring Their Elimination, A Guide for Programme Managers, 3rd Edn.* (2007). Available online at: https://www.ign.org/p142003094.html (accessed October 07, 2021).
3. Zimmermann MB, Boelaert K. Iodine deficiency and thyroid disorders. *Lancet Diabetes Endocrinol.* (2015) 3:286–95. doi: 10.1016/S2213-8587(14)70225-6
4. WHO/UNICEF/ICCIDD. *Indicators for Assessing Iodine Deficiency Disorders and Their Control Programme*. (1994). Available online at: https://www.ign.org/cm_data/1993_WHO_Indicators_for_assessing_IDD_WHO_NUT_93.1.pdf (accessed October 07, 2021).
5. Zimmermann MB. Assessing iodine status and monitoring progress of iodized salt programs. *J Nutr.* (2004) 134:1673–7. doi: 10.1093/jn/134.7.1673
6. Liu P, Fan L, Meng F, Su X, Sun D. Prevention and control of iodine deficiency disorders - China, 1995-2020. *China CDC Weekly.* (2020) 2:345–9. doi: 10.46234/ccdcw2020.090
7. Sun D, Codling K, Chang S, Zhang S, Shen H, Su X, et al. Eliminating iodine deficiency in China: achievements, challenges and global implications. *Nutrients.* (2017) 9:361. doi: 10.3390/nu9040361
8. UNICEF. *Guidance on the Monitoring of Salt Iodization Programmes and Determination of Population Iodine Status*. (2018). Available online at: https://www.ign.org/p142003099.html (accessed October 07, 2021).
9. Liu P, Su X, Li M, Shen H, Yu J, Kelly PJ, et al. Should urinary iodine concentrations of school-aged children continue to be used as proxy for different populations? Analysis of data from Chinese national surveys. *Br J Nutr.* (2016) 116:1068–76. doi: 10.1017/S0007114516002828
10. Fan L, Su X, Shen H, Liu P, Meng F, Yan J, et al. Iodized salt consumption and iodine deficiency status in China: a cross-sectional study. *Glob Health J.* (2017) 1:23–37. doi: 10.1016/S2414-6447(19)30076-4
11. Chinese National Bureau for Disease Control and Prevention. *The Report of the National Survey on Iodine in Drinking Water*. (2019). Available online at: http://www.nhc.gov.cn/jkj/s5874/201905/bb1da1f5e47040e8820b9378e6db4bd3.shtml (accessed October 07, 2021) (in Chinese).
12. General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China. *General Test Method in Salt Industry: Determination of Iodine GB GB/T 13025.7-2012*. (2012). Available online at: http://c.gb688.cn/bzgk/gb/showGb?type=online&hcno=85D289F3BCE6F6A31822CD0BDAAA7579 (accessed October 07, 2021) (a Chinese national standard).
13. General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, Standardization Administration of People's Republic of China. *National Standard for Food Safety: Iodine Content in Edible Salt*. GB26878-2011 (2011). Available online at: http://down.foodmate.net/standard/sort/3/28365.html (accessed October 07, 2021) (a Chinese national food standard).
14. Khan L. Thyroid disease in children and adolescents. *Pediatr Ann.* (2021) 50:e143–e7. doi: 10.3928/19382359-20210322-01
15. Ito Y, Amino N, Miyauchi A. Thyroid ultrasonography. *World J Surg.* (2010) 34:1171–80. doi: 10.1007/s00268-009-0211-3
16. Stewart KA, Navarro SM, Kambala S, Tan G, Poondla R, Lederman S, et al. Trends in ultrasound use in low and middle income countries: a systematic review. *Int J MCH AIDS.* (2020) 9:103–20. doi: 10.21106/ijma.294
17. Chen J, Li Z, Xu H, Hao Y. *China National Iodine Deficiency Disorders Surveillance Report 1997.* Beijing: People's Medical Publishing House (1999).
18. Chen X, Li Z, Hao Y, Xu H. *China National Iodine Deficiency Disorders Surveillance Report 1999*. People's Medical Publishing House (2001).
19. Chen X, Sun D, Liu S. *China National Iodine Deficiency Disorders Surveillance Report 2002*. People's Medical Publishing House (2003).
20. Wang Z, Zang J, Shi Z, Zhu Z, Song J, Zou S, et al. Iodine status of 8 to 10 years old children within 20 years following compulsory salt iodization policy in Shanghai China. *Nutr J.* (2019) 18:63. doi: 10.1186/s12937-019-0491-x
21. Lou X, Wang X, Mao G, Zhu W, Mo Z, Wang Y, et al. Geographical influences on the iodine status in pregnant women, neonates, and school-age children in China. *Nutr J.* (2020) 19:7. doi: 10.1186/s12937-020-0525-4
22. The Endemic Disease Control Center of Chinese Center for Disease Control and Prevention. *Investigation Report on the Assessment of Iodine Deficiency Diseases Elimination.* A National Health Report in China (2020).
23. Spohrer R, Garrett GS, Timmer A, Sankar S, Kar B, Rasool F, et al. Processed foods as an integral part of universal salt iodization programs: a review of global experience and analyses of Bangladesh and Pakistan. *Food Nutr Bull.* (2012) 33:S272–80. doi: 10.1177/15648265120334S303
24. Knowles J, van der Haar F, Shehata M, Gerasimov G, Bimo B, Cavenagh B, et al. Iodine intake through processed food: case studies from Egypt, Indonesia, the Philippines, the Russian Federation and Ukraine, 2010-2015. *Nutrients.* (2017) 9:797. doi: 10.20944/preprints201707.0056.v1
25. Zimmermann MB, Aeberli I, Andersson M, Assey V, Yorg JAJ, Jooste P, et al. Thyroglobulin is a sensitive measure of both deficient and excess iodine intakes in children and indicates no adverse effects on thyroid function in the UIC range of 100-299 μg/L: a UNICEF/ICCIDD study group report. *J Clin Endocrinol Metab.* (2013) 98:1271–80. doi: 10.1210/jc.2012-3952
26. Cui SL, Liu P, Su XH, Liu SJ. Surveys in areas of high risk of iodine deficiency and iodine excess in China, 2012-2014: current status and examination of the relationship between urinary iodine concentration and goiter prevalence in children aged 8-10 years. *Biomed Environ Sci.* (2017) 30:88–96. doi: 10.3967/bes2017.012
27. Wang H, Yu D, Tan Z, Hu R, Zhang B, Yu J. Estimation of thyroid volume from scintigraphy through 2D/3D registration of a statistical shape model. *Phys Med Biol.* (2019) 64:095015. doi: 10.1088/1361-6560/ab186d
28. Moreno-Reyes R, Glinoer D, Van Oyen H, Vandevijvere S. High prevalence of thyroid disorders in pregnant women in a mildly iodine-deficient country: a population-based study. *J Clin Endocrinol Metab.* (2013) 98:3694–701. doi: 10.1210/jc.2013-2149
29. Taylor PN, Okosieme OE, Dayan CM, Lazarus ZH. Therapy of endocrine disease: Impact of iodine supplementation in mild-to-moderate iodine deficiency: systematic review and meta-analysis. *Eur J Endocrinol.* (2014) 170:R1–R15. doi: 10.1530/EJE-13-0651
30. Abel MH, Korevaar T, Erlund I, Villanger GD, Caspersen IH, Arohonka P, et al. Iodine intake is associated with thyroid function in mild to moderately iodine deficient pregnant women. *Thyroid.* (2018) 28:1359–71. doi: 10.1089/thy.2018.0305
31. Shang L, Zhang Q, Zhou Y, Wang P, Chen Z, Wu M, et al. Thyroid volume among 8∼10-year-old school children exposed to iodine excess. *Chin J Endemiol.* (2007) 26:327–9.
32. Shen H. *Prevention and Control of Water-borne Iodine Excess in China*. Beijing: People's Medical Publishing House (2019) (in Chinese).
33. Liu P, Liu SJ, Su XH, Zhang SB, Ji XH. Relationship between urinary iodine and goiter prevalence: results of the Chinese national iodine deficiency disorders survey. *J Endocrinol Invest.* (2010) 33:26–31. doi: 10.1007/BF03346545
34. Song J, Zou SR, Guo CY, Zang JJ, Zhu ZN, Mi M, et al. Prevalence of thyroid nodules and its relationship with iodine status in Shanghai: a population-based study. *Biomed Environ Sci.* (2016) 29:398–407. doi: 10.3967/bes2016.052
35. *Iodine Requirements in Pregnancy and Infancy*. IDD Newsletter (2007). Available online at: https://www.ign.org/newsletter/idd_nl_feb07_pregnancy.pdf (accessed October 07, 2021).
36. National Health and Family Planning Commission of the People's Republic of China. *Determination of Iodine in Urine - Part I: As3+-Ce4+ Catalytic Spectrophotometry*. WS/T107.1-2016 (2016). Available online at: http://www.doc88.com/p-7738454013156.html (accessed October 07, 2021) (a Chinese health industry standard).
37. Ministry of Health of the People's Republic of China. *Diagnostic Criterion of Endemic Goiter*. WS276-2007 (2007). Available online at: https://www.renrendoc.com/paper/86282115.html (accessed October 07, 2021) (a Chinese health industry standard).
38. Smyth PP, Darke C, Parkes AB, Smith DF, John R, Hetherton AM, et al. Assessment of goiter in an area of endemic iodine deficiency. *Thyroid.* (1999) 9:895–901. doi: 10.1089/thy.1999.9.895

39. Hanley P, Lord K, Bauer AJ. Thyroid disorders in children and adolescents: a review. *JAMA Pediatrics.* (2016) 170:1008–19. doi: 10.1001/jamapediatrics.2016.0486

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 27 April 2022
doi: 10.3389/fnut.2022.803985

Association of Serum 25(OH)D, Cadmium, CRP With All-Cause, Cause-Specific Mortality: A Prospective Cohort Study

Yan Liu[1], *Donghui Yang*[1], *Fang Shi*[1], *Fang Wang*[2], *Xiaoxue Liu*[1], *Haoyu Wen*[1], *Sumaira Mubarik*[1] *and Chuanhua Yu*[1]*

[1] School of Public Health, Wuhan University, Wuhan, China, [2] School of Public Health, Xuzhou Medical University, Xuzhou, China

Introduction: To explore the relationship between serum 25(OH)D, cadmium, and CRP with all-cause mortality among people in diabetic and non-diabetic.

Methods: This study used data from the NHANES (2001–2010). Cox regression was used to analyze the relationship between Serum 25(OH)D, cadmium, CRP, and all-cause, cause-specific mortality. We used restricted cubic splines to explore the dose-response relationship between serum 25(OH)D, cadmium, CRP, and all-cause mortality.

Results: During a mean follow-up of 9.1 years, the study included 20,221 participants, 2,945 people with diabetes, and 17,276 people without diabetes. Compared with serum 25(OH)D deficiency group in diabetic patients, the sufficient serum 25(OH)D group was associated with lower all-cause mortality (HR = 0.41, 95%CI 0.28-0.60, $P < 0.001$) and cardiovascular mortality (HR = 0.46, 95%CI 0.22–0.95, $P = 0.04$). Compared with the low cadmium group, the high cadmium group was associated with higher all-cause mortality (HR = 1.49, 95%CI 1.06–2.09, $P = 0.02$). Compared with the low CRP group, the high CRP group was associated with higher all-cause mortality (HR = 1.65, 95%CI 1.24–2.19, $P = 0.001$) and cancer mortality (HR = 3.25, 95%CI 1.82–5.80, $P < 0.001$). Restricted cubic splines analysis showed a significant nonlinear association between serum 25(OH)D (P-nonlinearity $P < 0.001$), cadmium (P-nonlinearity = 0.002), CRP (P-nonlinearity = 0.003), and HR for all-cause mortality risk in diabetic patients. The results were similar among non-diabetic patients, but with different levels of risk. Sensitivity analysis and subgroup analysis presented the results of population studies with different follow-up times, different genders and ages.

Conclusions: In diabetic patients, serum 25(OH)D, cadmium, and CRP were related to all-cause mortality; serum 25(OH)D was related to cardiovascular mortality; CRP was related to cancer mortality. The results were similar among non-diabetic patients, but with different levels of risk.

Keywords: serum 25(OH)D, cadmium, C-reactive protein, mortality, diabetic

OPEN ACCESS

Edited by:
Francesco Sofi,
Università degli Studi di Firenze, Italy

Reviewed by:
Dongming Wang,
Huazhong University of Science and Technology, China
William B. Grant,
Sunlight Nutrition and Health Research Center, United States

***Correspondence:**
Chuanhua Yu
yuchua@whu.edu.cn

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *28 October 2021*
Accepted: *24 February 2022*
Published: *27 April 2022*

Citation:
Liu Y, Yang D, Shi F, Wang F, Liu X, Wen H, Mubarik S and Yu C (2022) Association of Serum 25(OH)D, Cadmium, CRP With All-Cause, Cause-Specific Mortality: A Prospective Cohort Study. Front. Nutr. 9:803985. doi: 10.3389/fnut.2022.803985

INTRODUCTION

International Diabetes Federation (IDF) estimated the global diabetes prevalence in 2019 was estimated to be 9.3% (463 million people), it is expected to reach 10.9% (700 million) by 2045 (1). Cardiovascular disease (CVD) was the main cause of death and morbidity in patients with diabetes, especially type 2 diabetes (2). Therefore, it was of great significance to explore the causes of diabetes, prevent or control the complications of diabetes and reduce the risk of death.

Vitamin D was believed to prevent common chronic diseases, including CVD and cancer (3, 4). In a meta-analysis of randomized controlled trials, vitamin D supplementation significantly reduced overall cancer mortality (5). However, clinical data examining the effect of vitamin D supplementation on mortality was still inconclusive (6, 7). The relationship between vitamin D status and the risk of death in diabetic patients was not consistent (8, 9). This may be related to the different sample size, the definition of vitamin D deficiency, and the different adjustment of covariates in the detection method. There was increasing interest in the possible protective effects of vitamin D in health outcomes.

Cadmium (Cd) is a toxic heavy metal that causes various diseases and increases mortality. Diet is the main source of Cd exposure for most people. A survey from NHANES in 2007–2012 showed that the food groups with the most Cd were grains and breads, leafy vegetables, potatoes, beans and nuts, and stem/root vegetables (10). The cadmium content in food is related to geographic location, bioavailability of cadmium in soil, crop genetics and agronomy (11). Cadmium exposure was associated with kidney disease (12), osteoporosis and fractures (13), atherosclerosis (14) and CVD (15). The relationship between epidemiological studies of low-level cadmium exposure and the risk of cancer and cardiovascular disease was limited, and there are usually fewer cohort studies based on small sample sizes, case-control, or cross-sectional designs. A meta-analysis showed that cadmium appears to be associated with increased all-cause mortality and cardiovascular mortality even at low levels of exposure (16). But this meta-analysis contains only 9 original studies, and the heterogeneity was great. In addition, we also separated the diabetic and non-diabetic for analyses.

C-reactive protein (CRP) was a protein synthesized in the liver, and the inflammatory response of tissue damage was related to the level of CRP (17). Despite the large number of studies on CRP, the convincing evidence on associations and causal effects was very limited (18). A study using the China Longitudinal Study of Health and Retirement (CHARLS) showed that plasma CRP was a predictor of all-cause mortality in the middle-aged and elderly population in China (19). A cohort study in Brazil also reached the same conclusion (20). Meta-analysis showed that CRP levels were associated with all-cause mortality in patients with type 2 diabetes and the risk of cardiovascular mortality was higher (21). Our data with larger samples and longer follow-up time. In addition, we also separated the diabetic and non-diabetic for analyses.

This research attempted to explore the relationship between serum 25(OH)D, cadmium, and CRP with all-cause mortality among people in diabetic and non-diabetic.

METHODS

Study Population

This study used data from the NHANES (2001–2010). The National Health and Nutrition Examination Survey (NHANES) is a program of studies designed to assess the health and nutritional status of adults and children in the United States. NHANES is a major program of the National Center for Health Statistics (NCHS). 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. The survey examines a nationally representative sample of about 5,000 persons each year. These persons were located in counties across the country, 15 of which were visited each year. The NHANES interview includes demographic, socioeconomic, dietary, and health-related questions. The examination component consists of medical, dental, and physiological measurements, as well as laboratory tests administered by highly trained medical personnel.

We collated and merged the data from 2001 to 2010 and obtained 52,195 respondents. After the deletion of missing research variables (serum vitamin D, blood cadmium, C-reactive protein) and covariates (age, gender, education, marital status, race, ratio of family income to poverty, BMI, drinking, smoking, physical activity), 20,221 respondents were finally obtained. In the end, there were 2,945 diabetic people and 17,276 non-diabetic people. Diabetes is defined as self-reported as diabetes when asked by a doctor, using insulin or oral hypoglycemic drugs, fasting blood glucose $\geq$7.0 mmol/L, or glycosylated hemoglobin A1c (HbA1c) $\geq$6.5%. Gestational diabetes is excluded.

Measurement of Serum 25(OH)D

NHANES 2001–2006, serum 25(OH)D concentration was measured by DiaSorin radioimmunoassay kit (Stillwater, MN), but the data was converted to equivalent 25(OH)D measured value from LC-MS/MS method by regression method. From 2007 to 2010, serum 25(OH)D concentration was measured by a standardized liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The CDC LC-MS/MS method has better analytical specificity and sensitivity compared to immunoassay methods, and fixed analytical goals for imprecision ($\leq$10%) and bias ($\leq$5%).

Measurement of Serum Cadmium

We used data of blood cadmium. Whole blood samples were processed, stored under frozen (−30°C) conditions, and shipped to the Ministry of Laboratory Science, the National Center for Environmental Health, and the Centers for Disease Control and Prevention for analysis. Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the concentration of cadmium (Cd) in whole blood.

Measurement of Serum C-Reactive Protein

C-reactive protein was considered to be one of the best measures of acute-phase response to infectious diseases or other tissue damage and inflammation. The blood samples are processed, stored, and shipped to the University of Washington. Quantify CRP by latex-enhanced turbidimetry. The CRP concentration is calculated by using a calibration curve. Data reduction of the signals was performed by using a storable logit-log function for the calibration curve performed data reduction of the signals. These measurements were performed on the Behring Nephelometer for quantitative CRP measurement.

Ascertainment of Mortality

NCHS has linked various surveys with death certificate records from the National Death Index (NDI). The restricted-use Linked Mortality File (LMF) has been updated with mortality follow-up data through December 31, 2015. All-cause mortality, cardiovascular disease, and cancer mortality were determined by correlation with the National Death Index. ICD-10 is used to determine deaths from specific diseases. Cancer mortality was defined as ICD-10 codes C00–C97, and CVD mortality was defined as ICD-10 codes I00–I09, I11, I13, I20–I51, or I60–I69.

Assessment of Covariates

The covariates we included age, gender, education, marital status, race, the ratio of family income to poverty, BMI, drinking, smoking, physical activity. Education level was categorized as less than high school, more than high school. Marital status was categorized as married or living with a partner; widowed, divorced, separated; never married. The race was classified as Hispanic, non-Hispanic-White, non-Hispanic-Black, non-Hispanic other. The family income-to-poverty ratio was classified as 0–1.0, 1.0–3.0, and >3.0. BMI was categorized as <25.0 kg/m^2, 25.0–29.9 kg/m^2, and ≥ 30 kg/m^2. Drinking status was categorized as never drinker (less than 12 alcohol drinks/lifetime), Ever drinker (had at least 12 alcohol drinks/1 yr and no drink alcohol over past 12 months), current drinker (drink alcohol over past 12 months). Smoking was categorized as never smoker (smoked less than 100 cigarettes in life), ever smoker (smoked at least 100 cigarettes in life and no smoke now), current smoker (smoked at least 100 cigarettes in life and smoke now). Physical activity was categorized as <150 min MVPA(moderate to vigorous physical activity), ≥150 min MVPA 0. 75 min of vigorous physical activity is equal to 150 min of moderate physical activity (22). In addition, strict procedures were applied for collection and analysis in the whole blood, and the details were described in the NHANES laboratory.

Statistical Analysis Method

This study describes the basic situation of objects. We generated a new weight for 2001–2010 = wtmec2yr/5. Cox regression analysis was used to analyze the relationship between serum 25(OH)D, cadmium, CRP, and all-cause and cause-specific mortality. According to the clinical practice guidelines of the Endocrine Society, serum 25(OH)D status is divided into four groups: severe deficiency (<25.0 nmol/L), moderate deficiency (25.0–49.9 nmol/L), insufficient (50.0–74.9 nmol/L), and sufficient (>75.0 nmol/L) (23). Cadmium and CRP were also divided into four groups according to the interquartile range. We conducted a subgroup analysis based on age and gender. At the same time, we conducted sensitivity analyses by excluding subjects whose follow-up time was less than 2 years.

We also used restricted cubic splines (RCS) with three knots (5th, 50th, and 75th) to explore the dose-response relationship between serum 25(OH)D, cadmium, CRP, and all-cause mortality. At the same time, we adjusted multiple covariates mentioned above. The essence of restrictive cubic splines is regression splines with additional requirements. The regression spline is a piecewise polynomial that requires continuous and second-order derivable in each piece. Restricted cubic splines require regression splines: the spline function was a linear function at the extreme ends of the independent variable data range.

All of the analyses were conducted using Stata 15.0. $P > 0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics

The study included 20,221 participants, 2,945 people with diabetes, and 17,276 people without diabetes. The average age of the diabetic population is 61.6 years old, and the average age of the non-diabetic population is 47.4 years old. The specific baseline characteristics of the study population were shown in **Table 1**. People with diabetes were more likely to be older, non-Hispanic black, low-educated, obese people who currently drink alcohol and do not exercise enough.

All-Cause and Cause-Specific Mortality

In the follow-up of people with diabetes, 808 deaths were recorded, including 198 deaths from CVD and 154 deaths from cancer. In the Cox regression analysis, Model 1 adjusts age and gender; Model 2 further adjusted education, marital status, race, the ratio of family income to poverty based on model 1; Model 3 adjusted BMI, drinking, smoking, physical activity based on Model 2. We used the severe serum 25(OH)D deficiency group as the reference group, the sufficient serum 25(OH)D group was associated with lower all-cause mortality (HR = 0.41, 95%CI 0.28–0.60, $P < 0.001$) and cardiovascular mortality (HR = 0.46,95%CI 0.22–0.95, $P = 0.04$). We divided diabetic patients into 4 groups based on the interquartile range of cadmium concentration in the blood. At the same time, the low-concentration group (<0.24 μg/l) was used as the control group, the results showed that higher blood cadmium concentration group (>0.6 μg/l) was associated with higher all-cause mortality (HR = 1.49, 95%CI 1.06–2.09, $P = 0.02$). We divided diabetic patients into 4 groups based on the interquartile range of CRP concentration in the blood. At the same time, the low concentration group (<0.08 mg/dL) was used as the control group, the higher CRP concentration group (>0.49 mg/dL) was

TABLE 1 | Baseline characteristics of participants in the NHANES 2001–2010.

Baseline characteristics	Total	Diabetes		Non-diabetes	
		Male	Female	Male	Female
Sample size (%)	20,221	1,565 (7.7)	1,380 (6.8)	8,330(41.2)	8,946(44.2)
Age (years) (%)					
<65	15,189	843 (5.6)	747 (4.9)	6,467 (42.6)	7,132 (47.0)
≥65	5,032	722 (14.3)	633 (12.6)	1,863 (37.0)	1,814 (36.0)
Race (%)					
Hispanic	5,095	454 (8.9)	430 (8.4)	2,009 (39.4)	2,202 (43.2)
Non-hispanic white	10,597	724 (6.8)	536 (5.1)	4,498 (42.4)	4,839 (45.7)
Non-hispanic black	3,759	341 (9.1)	357 (9.5)	1,503 (40.0)	1,558 (41.4)
Non-hispanic other	770	46 (6.0)	57 (7.4)	320 (41.6)	347 (45.1)
Education level (%)					
Less than high school degree	5,636	616 (10.9)	577 (10.2)	2,261 (40.1)	2,182 (38.7)
High school degree	4,739	334 (7.0)	334 (7.0)	2,064 (43.6)	2,007 (42.4)
More than high school degree	9,776	615 (6.3)	469 (4.8)	4,005 (41.0)	4,687 (47.9)
Marital status (%)					
Married or living with partner	12,678	1,116 (8.8)	664 (5.2)	5,652 (44.6)	5,246 (41.4)
Widowed, divorced, separated	4,414	333 (7.5)	619 (14.0)	1,181 (26.8)	2,281 (51.7)
Never married	3,129	116 (3.7)	97 (3.1)	1,497 (47.8)	1,419 (45.3)
Family income-poverty ratio (%)					
0–1.0	3,792	270 (7.1)	354 (9.3)	1,430 (37.7)	1,738 (45.8)
1.1–3.0	8,478	727 (8.6)	693 (8.2)	3,378 (39.8)	3,680 (43.4)
>3.0	7,951	568 (7.1)	333 (4.2)	3,522 (44.3)	3,528 (44.4)
BMI,kg/m^2					
<25.0	5,989	228 (3.8)	174 (2.9)	2,487 (41.5)	3,100 (51.8)
25.0–29.9	7,097	532 (7.5)	353 (5.0)	3,454 (48.7)	2,758 (38.9)
≥30	7,135	805 (11.3)	853 (12.0)	2,389 (33.5)	3,088 (43.3)
Drinking status (%)					
Never drinker	2,792	129 (4.6)	418 (15.0)	568 (20.3)	1,677 (60.1)
Ever drinker	4,089	531 (13.0)	448 (11.0)	1,478 (36.1)	1,632 (39.9)
Current drinker	13,340	905 (6.8)	514 (3.9)	6,284 (47.1)	5,637 (42.3)
Smoking Status (%)					
Never smoker	10,445	567 (5.4)	809 (7.7)	3,630 (34.8)	5,439 (52.1)
Ever smoker	5,332	689 (12.9)	373 (7.0)	2,481 (46.5)	1,789 (33.6)
Current smoker	4,444	309 (7.0)	198 (4.5)	2,219 (49.9)	1,718 (38.7)
Physical activity (%)					
<150 min MVPA	11,375	990 (8.7)	1,051 (9.2)	3,958 (34.8)	5,376 (47.3)
≥150 min MVPA	8,846	575 (6.5)	329 (3.7)	4,372 (49.4)	3,570 (40.4)

Values are n (percentage).

associated with higher all-cause mortality (HR 1.65, 95%CI 1.24–2.19, $P = 0.001$) and cancer mortality (HR = 3.25, 95%CI 1.82–5.80, $P < 0.001$). Other details were presented in **Table 2**.

In the follow-up of people with non-diabetes, 1981 deaths were recorded, including 400 deaths from CVD and 472 deaths from cancer. In the Cox regression analysis, Model 1 adjusts age and gender; Model 2 further adjusts education, marital status, race, the ratio of family income to poverty based on Model 1; Model 3 adjusts BMI, drinking, smoking, physical activity based on Model 2. We used the serum 25(OH)D deficiency group as the reference group, the sufficient serum 25(OH)D group was associated with lower all-cause mortality (HR = 0.67,95%CI = 0.48–0.93, $P = 0.02$) and cardiovascular mortality (HR = 0.50,95%CI = 0.28–0.91, $P = 0.02$). We divided non-diabetic patients into 4 groups based on the interquartile range of cadmium concentration in the blood. At the same time, the low-concentration group (<0.21 μg/l) was used as the control group, the results showed that higher blood cadmium concentration group (>0.6 μg/l) was associated with higher all-cause mortality (HR = 1.70, 95%CI = 1.34–2.15, $P < 0.001$). We divided non-diabetic patients into 4 groups based on the interquartile range of CRP concentration in the blood. At the same time, the low concentration group (<0.08 mg/dL) was used as the control group, the higher CRP concentration group (>0.46 mg/dL) was

TABLE 2 | HR (95%CIs) for all-cause and cause-specific mortality according to serum 25 (OH)D, Cadmium and CRP among participants with diabetes.

Life styles	Deaths	Total population	Model 1[a]		Model 2[b]		Model 3[c]	
			HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*–value
25 (OH)D								
All-cause mortality								
<25.0 (nmol/L)	65	188	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
25.0–49.9 (nmol/L)	302	1,118	0.56 (0.39- 0.80)	0.002	0.54 (0.37–0.78)	0.001	0.55 (0.39–0.79)	0.001
50.0–74.9 (nmol/L)	303	1,132	0.39 (0.28–0.54)	<0.001	0.36 (0.25–0.52)	<0.001	0.40 (0.27–0.58)	<0.001
>75.0 (nmol/L)	138	507	0.39 (0.28–0.55)	<0.001	0.38 (0.26–0.55)	<0.001	0.41 (0.28–0.60)	<0.001
CVD mortality								
<25.0 (nmol/L)	20	188	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
25.0–49.9 (nmol/L)	68	1,118	0.47 (0.24–0.92)	0.03	0.46 (0.24–0.86)	0.02	0.45 (0.24–0.58)	0.01
50.0–74.9 (nmol/L)	69	1,132	0.29 (0.16–0.53)	<0.001	0.30 (0.17–0.51)	<0.001	0.31 (0.17–0.55)	<0.001
>75.0 (nmol/L)	41	507	0.44 (0.20–0.99)	0.048	0.46 (0.22–0.98)	0.04	0.46 (0.22–0.95)	0.04
Cancer mortality								
<25.0 (nmol/L)	6	188	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
25.0–49.9 (nmol/L)	76	1,118	1.96 (0.74–5.20)	0.17	1.89 (0.70–5.07)	0.21	2.16 (0.78–5.95)	0.13
50.0–74.9 (nmol/L)	49	1,132	0.85 (0.32–2.26)	0.74	0.76 (0.28–2.02)	0.57	1.00 (0.37–2.68)	0.99
>75.0 (nmol/L)	23	507	1.00 (0.35–2.86)	0.99	0.89 (0.30–2.67)	0.84	1.23 (0.40–3.77)	0.71
Cadmium								
All-cause mortality								
<0.24 (ug/l)	132	733	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
0.24–0.39 (ug/l)	169	769	0.90 (0.64–1.27)	0.55	0.94 (0.66–1.32)	0.70	0.91 (0.66–1.25)	0.56
0.39–0.6 (ug/l)	267	767	1.32 (0.99–1.77)	0.06	1.28 (0.94–1.74)	0.11	1.21 (0.90–1.62)	0.20
>0.6 (ug/l)	240	676	1.96 (1.45–2.64)	<0.001	1.82 (1.33–2.50)	<0.001	1.49 (1.06–2.09)	0.02
CVD mortality								
<0.24 (ug/l)	30	733	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
0.24–0.39 (ug/l)	44	769	1.21 (0.70–2.11)	0.496	1.22 (0.69–2.17)	0.496	1.16 (0.65–2.07)	0.61
0.39–0.6 (ug/l)	69	767	1.46 (0.75–2.85)	0.26	1.38 (0.71–2.68)	0.33	1.26 (0.65–2.46)	0.49
>0.6 (ug/l)	55	676	2.12 (1.06–4.28)	0.04	1.92 (0.94–3.93)	0.07	1.55 (0.70–3.45)	0.28
Cancer mortality								
<0.24 (ug/l)	28	733	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
0.24–0.39 (ug/l)	35	769	0.88 (0.40–1.95)	0.75	0.92 (0.42–2.05)	0.85	0.89 (0.43–1.87)	0.76
0.39–0.6 (ug/l)	43	767	0.82 (0.43–1.58)	0.55	0.81 (0.42–1.58)	0.54	0.79 (0.41–1.52)	0.48
>0.6 (ug/l)	48	676	1.95 (0.95–3.98)	0.07	1.85 (0.91–3.77)	0.09	1.21 (0.57–2.59)	0.61
CRP								
All-cause mortality								
<0.08 (mg/dL)	176	727	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
0.08–0.21 (mg/dL)	216	775	1.36 (1.01–1.84)	0.045	1.32 (0.97–1.77)	0.07	1.26 (0.94–1.68)	0.12
0.21–0.49 (mg/dL)	206	708	1.53 (1.21–1.95)	0.001	1.44 (1.12–1.84)	0.01	1.38 (1.08–1.77)	0.01
>0.49 (mg/dL)	210	735	1.90 (1.44–2.52)	<0.001	1.76 (1.34–2.32)	<0.001	1.65 (1.24–2.19)	0.001
CVD mortality								
<0.08 (mg/dL)	44	727	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
0.08–0.21 (mg/dL)	53	775	1.33 (0.84–2.10)	0.21	1.35 (0.81–2.25)	0.25	1.37 (0.81–2.31)	0.24
0.21–0.49 (mg/dL)	56	708	1.66 (0.84–3.30)	0.14	1.58 (0.76–3.28)	0.22	1.65 (0.76–3.56)	0.20
>0.49 (mg/dL)	45	735	1.37 (0.76–2.47)	0.29	1.26 (0.68–2.34)	0.46	1.24 (0.67–2.31)	0.49
Cancer mortality								
<0.08 (mg/dL)	25	727	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
0.08–0.21 (mg/dL)	40	775	2.65 (1.46–4.79)	0.002	2.51 (1.40–4.49)	0.002	2.13 (1.18–3.84)	0.01
0.21–0.49 (mg/dL)	44	708	2.98 (1.71–5.17)	<0.001	2.77 (1.58–4.83)	0.001	2.23 (1.27–3.92)	0.01
>0.49 (mg/dL)	45	735	4.25 (2.28–7.90)	<0.001	4.06 (2.23–7.42)	<0.001	3.25 (1.82–5.80)	<0.001

[a]*Model 1: adjusted for age and sex.*

[b]*Model 2: Model 1, additionally adjusted for education, marital status, race, the ratio of family income to poverty.*

[c]*Model 3: Model 2, additionally adjusted for BMI, drinking, smoking, physical activity.*

TABLE 3 | HR(95%CIs) for all-cause and cause-specific mortality according to serum 25(OH)D,Cadmium and CRP among participants without diabetes.

Life styles	Deaths	Total population	Model 1[a]		Model 2[b]		Model 3[c]	
			HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
Serum 25 (OH)D								
All-cause mortality								
<25.0 (nmol/L)	88	710	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
25.0–49.9 (nmol/L)	624	5,118	0.77 (0.60–0.98)	0.03	0.88 (0.68–1.13)	0.30	0.97 (0.75–1.25)	0.79
50.0–74.9 (nmol/L)	810	6,881	0.51 (0.39–0.68)	<0.001	0.63 (0.47–0.86)	0.004	0.73 (0.54–0.99)	0.04
>75.0 (nmol/L)	459	4,567	0.44 (0.32–0.60)	<0.001	0.57 (0.40–0.80)	0.001	0.67 (0.48–0.93)	0.02
CVD mortality								
<25.0 (nmol/L)	18	710	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
25.0–49.9 (nmol/L)	128	5,118	0.86 (0.51–1.46)	0.58	0.92 (0.52–1.62)	0.77	0.98 (0.55–1.72)	0.93
50.0–74.9 (nmol/L)	167	6,881	0.51 (0.29–0.87)	0.02	0.56 (0.32–1.00)	0.05	0.62 (0.35–1.11)	0.10
>75.0 (nmol/L)	87	4,567	0.38 (0.22–0.67)	0.001	0.44 (0.24–0.80)	0.01	0.50 (0.28–0.91)	0.02
Cancer mortality								
<25.0 (nmol/L)	26	710	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
25.0–49.9 (nmol/L)	168	5,118	0.64 (0.40–1.03)	0.07	0.78 (0.49–1.25)	0.31	0.88 (0.54–1.44)	0.62
50.0–74.9 (nmol/L)	170	6,881	0.38 (0.22–0.65)	0.001	0.52 (0.30–0.90)	0.02	0.63 (0.36–1.10)	0.10
>75.0 (nmol/L)	108	4,567	0.33 (0.19–0.56)	<0.001	0.47 (0.27–0.83)	0.01	0.58 (0.33–1.03)	0.06
Cadmium								
All-cause mortality								
<0.21 (ug/l)	200	4,247	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
0.21–0.37 (ug/l)	338	4,506	1.05 (0.82–1.34)	0.71	1.05 (0.83–1.34)	0.68	1.01 (0.80–1.29)	0.92
0.37–0.6 (ug/l)	677	4,273	1.35 (1.06–1.73)	0.02	1.31 (1.03–1.65)	0.03	1.18 (0.94–1.49)	0.15
>0.6 (ug/l)	766	4,250	2.47 (1.99–3.06)	<0.001	2.15 (1.74–2.66)	<0.001	1.70 (1.34–2.15)	<0.001
CVD mortality								
<0.21 (ug/l)	42	4,247	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
0.21–0.37 (ug/l)	73	4,506	0.89 (0.58–1.38)	0.60	0.90 (0.59–1.39)	0.64	0.88 (0.58–1.35)	0.56
0.37–0.6 (ug/l)	141	4,273	1.17 (0.75–1.81)	0.49	1.14 (0.74–1.77)	0.55	1.04 (0.68–1.62)	0.83
>0.6 (ug/l)	144	4,250	1.67 (1.06–2.63)	0.03	1.49 (0.95–2.34)	0.08	1.26 (0.77–2.08)	0.35
Cancer mortality								
<0.21 (ug/l)	47	4,247	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
0.21–0.37 (ug/l)	87	4,506	1.46 (0.93–2.31)	0.10	1.45 (0.93–2.27)	0.10	1.35 (0.86–2.12)	0.20
0.37–0.6 (ug/l)	141	4,273	1.66 (1.10–2.51)	0.02	1.61 (1.07–2.41)	0.02	1.34 (0.89–2.02)	0.16
>0.6 (ug/l)	197	4,250	3.37 (2.30–4.96)	<0.001	3.04 (2.06–4.49)	<0.001	2.08 (1.35–3.22)	0.001
CRP								
All-cause mortality								
<0.08 (mg/dL)	327	4,156	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
0.08–0.2 (mg/dL)	525	4,705	1.03 (0.89–1.20)	0.66	1.02 (0.88–1.19)	0.78	1.03 (0.88–1.21)	0.69
0.2–0.46 (mg/dL)	539	4,175	1.22 (1.03–1.45)	0.02	1.19 (0.99–1.43)	0.07	1.18 (0.98–1.43)	0.08
>0.46 (mg/dL)	590	4,240	1.77 (1.49–2.11)	<0.001	1.65 (1.38–1.97)	<0.001	1.62 (1.34–1.96)	<0.001
CVD mortality								
<0.08 (mg/dL)	70	4,156	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
0.08–0.2 (mg/dL)	112	4,705	1.01 (0.72–1.44)	0.94	1.00 (0.71–1.43)	0.98	0.98 (0.68–1.42)	0.91
0.2–0.46 (mg/dL)	101	4,175	0.99 (0.74–1.31)	0.92	0.97 (0.72–1.29)	0.82	0.92 (0.68–1.24)	0.58
>0.46 (mg/dL)	117	4,240	1.61 (1.12–2.33)	0.01	1.52 (1.05–2.19)	0.03	1.41 (0.97–2.04)	0.07
Cancer mortality								
<0.08 (mg/dL)	74	4,156	1.00 [Reference]		1.00 [Reference]		1.00 [Reference]	
0.08–0.2 (mg/dL)	116	4,705	1.05 (0.74–1.48)	0.80	1.03 (0.73–1.44)	0.88	1.00 (0.71–1.40)	0.99
0.2–0.46 (mg/dL)	127	4,175	1.18 (0.87–1.16)	0.28	1.13 (0.83–1.55)	0.44	1.06 (0.76–1.48)	0.73
>0.46 (mg/dL)	155	4,240	1.98 (1.44–2.71)	<0.001	1.79 (1.31–2.47)	<0.001	1.62 (1.15–2.28)	0.01

[a]*Model 1: adjusted for age and sex.*

[b]*Model 2: Model 1, additionally adjusted for education, marital status, race, the ratio of family income to poverty.*

[c]*Model 3: Model 2, additionally adjusted for BMI, drinking, smoking, physical activity.*

associated with higher all-cause mortality (HR = 1.62, 95%CI = 1.34–1.96, $P < 0.001$) and cancer mortality (HR = 1.62, 95%CI = 1.15–2.28, $P = 0.006$). Other details were presented in **Table 3**.

Sensitivity Analyses

In this study, sensitivity analyses were performed on diabetic and non-diabetic patients, excluding the follow-up time of less than 2 years. In diabetic patients, serum 25(OH)D and CRP were associated with all-cause mortality. Cadmium and CRP were associated with all-cause mortality in non-diabetics. Details were in the **Supplementary Tables 1, 2**.

Subgroup Analyses

We conducted subgroup analyses of diabetic and non-diabetic people based on age (based on whether the group was older than 65) and gender. serum 25(OH)D and CRP were associated with all-cause mortality in male diabetic patients; serum 25(OH)D, cadmium, and CRP were associated with all-cause mortality in female diabetic patients (**Supplementary Table 3**). Serum 25(OH)D was associated with all-cause mortality in diabetic patients younger than 65; serum 25(OH)D, cadmium, and CRP were associated with all-cause mortality in diabetic patients older than 65 (**Supplementary Table 4**). Cadmium and CRP were associated with all-cause mortality in male non-diabetic patients; serum vitamin D, cadmium, and CRP were associated with all-cause mortality in female non-diabetic patients (**Supplementary Table 5**). Serum 25(OH)D, cadmium, and CRP were associated with all-cause mortality in non-diabetic patients younger than 65; cadmium and CRP were associated with all-cause mortality in non-diabetic patients older than 65 (**Supplementary Table 6**).

The Dose-Response Relationship

After adjusting for multiple covariates, RCS analysis showed that there was a significant nonlinear association between serum 25(OH)D (P-nonlinearity < 0.001, **Figure 1A**), cadmium (P-nonlinearity $= 0.002$, **Figure 1B**), CRP (P-nonlinearity $= 0.003$, **Figure 1C**), and HR for all-cause mortality risk in diabetic patients. When the serum 25(OH)D concentration is 54.7 nmol/L, HR changes reached a plateau (**Figure 1**). As the concentration of CRP and cadmium increased, the HR of all-cause mortality increased. There was a significant nonlinear association between serum vitamin D (P-nonlinearity < 0.001, **Figure 2A**), cadmium (P-nonlinearity < 0.001, **Figure 2B**), CRP (P-nonlinearity $P< 0.001$, **Figure 2C**) and HR for all-cause mortality risk in non-diabetic patients. When the serum

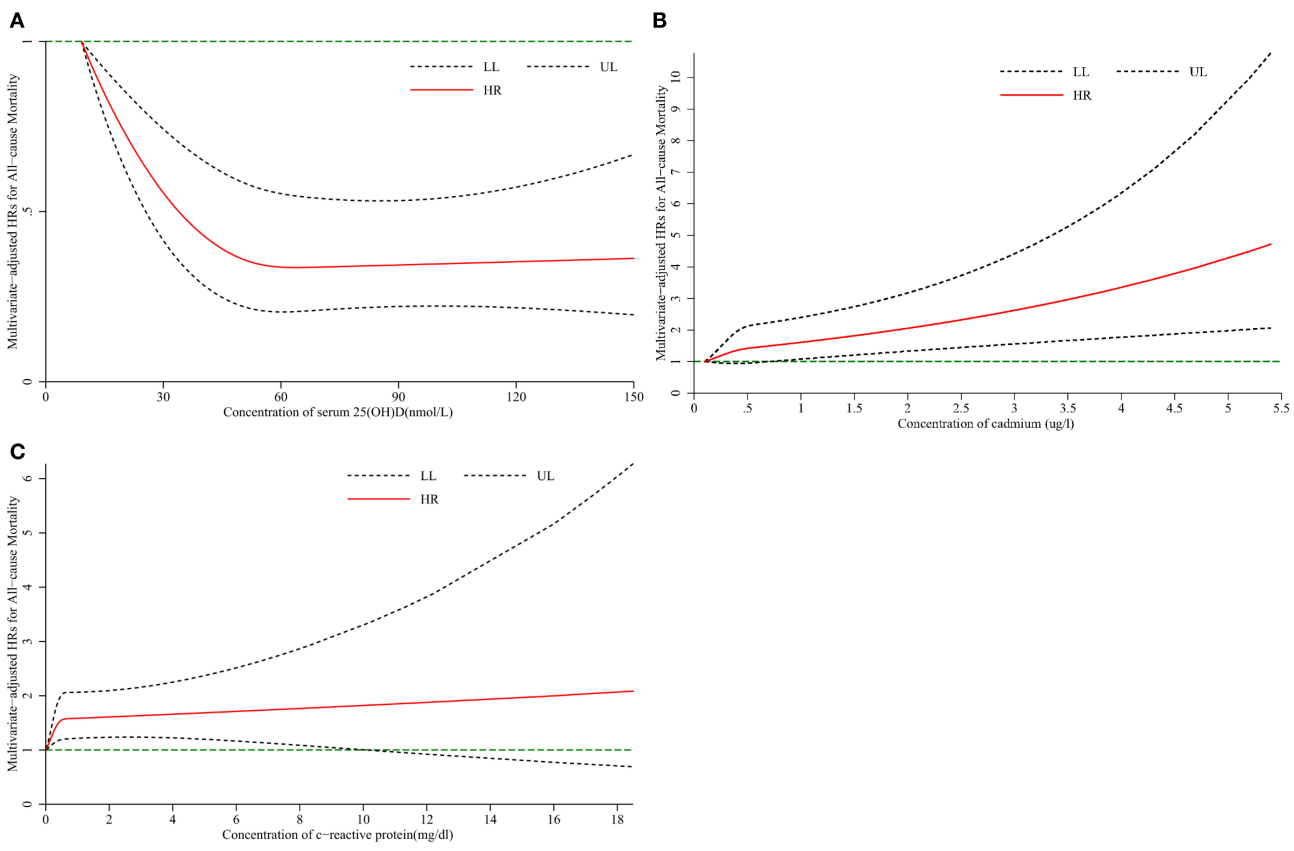

FIGURE 1 | The restricted cubic spline for the association between serum 25(OH)D **(A)**, cadmium **(B)**, C-reactive protein **(C)** and risk of all-cause mortality among adults with diabetes. Knots were placed at the 5th, 50th, and 75th percentiles of the serum 25(OH)D, cadmium, C-reactive protein distribution. Adjustment factors were age, sex, education, marital status, race, the ratio of family income to poverty, BMI, drinking, smoking, physical activity. HR, hazard ratio; LL, lower limit; UL, upper limit.

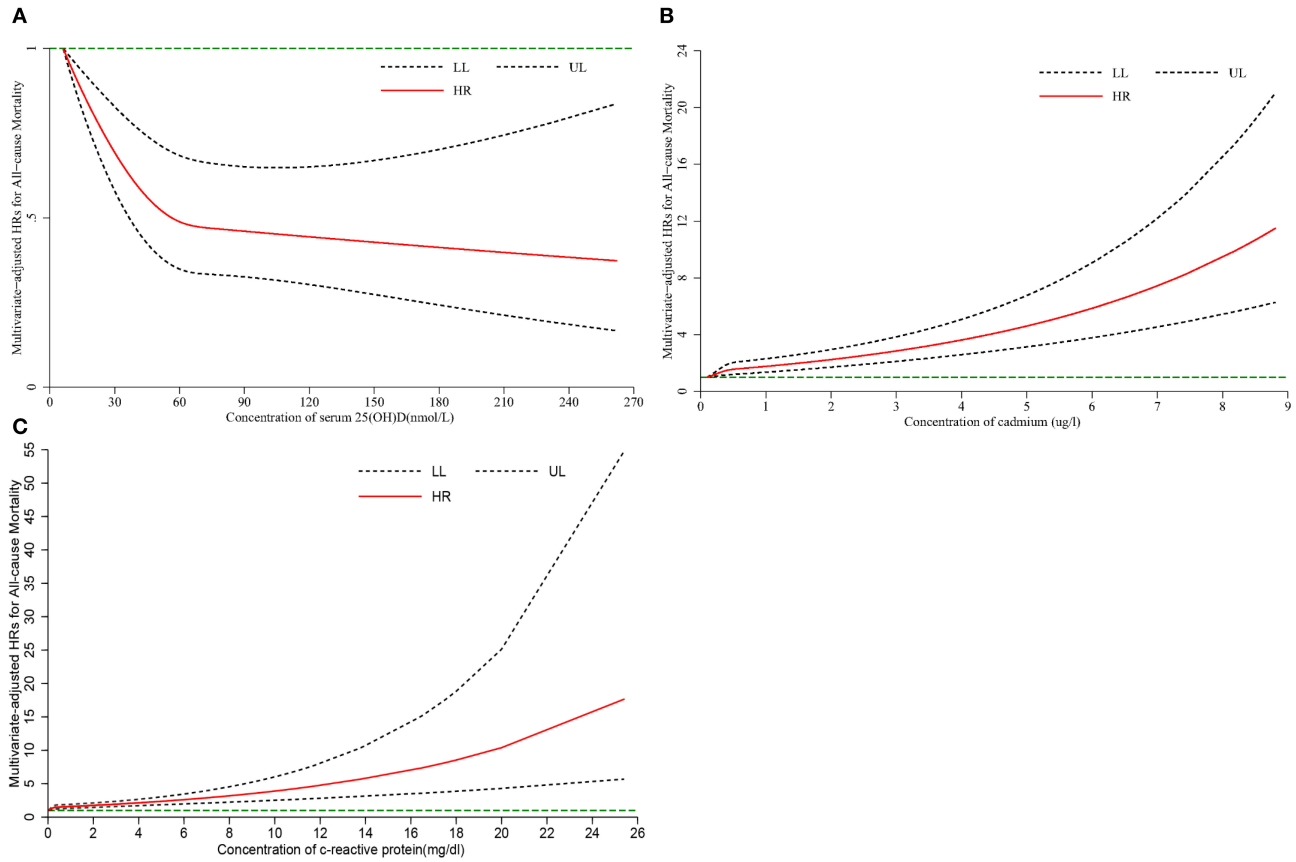

FIGURE 2 | The restricted cubic spline for the association between serum 25(OH)D **(A)**, cadmium **(B)**, C-reactive protein **(C)** and risk of all-cause mortality among adults non-diabetes. Knots were placed at the 5th, 50th, and 75th percentiles of the serum 25(OH)D, cadmium, C-reactive protein distribution. Adjustment factors were age, sex, education, marital status, race, the ratio of family income to poverty, BMI, drinking, smoking, physical activity. HR, hazard ratio; LL, lower limit; UL, upper limit.

25(OH)D concentration is 60 nmol/L, HR changes reached a plateau (**Figure 2**). As the concentration of CRP and cadmium increased, the HR of all-cause mortality increased.

DISCUSSION

In diabetic patients, serum 25(OH)D, cadmium, and CRP were associated with all-cause mortality; serum 25(OH)D was associated with cardiovascular mortality; CRP was associated with cancer mortality. The same conclusions were obtained in non-diabetic patients, but with different levels of risk. Subgroup analyses and sensitivity analyses obtained slightly different results. The results of RCS analyses showed that serum 25(OH)D, cadmium, and CRP had a nonlinear dose-response relationship with the HR of all-cause mortality.

Stratified Mendelian randomization analysis demonstrated a causal relationship between 25(OH)D concentrations and mortality in individuals with low vitamin D status (24). A large cohort study from the UK Biobank, which included 365,530 participants, the results showed that a higher 25(OH)D concentration was non-linearly associated with a lower risk of all-cause, CVD, and cancer mortality. A threshold of 60 nmol/L of 25(OH)D for all-cause and CVD deaths and at 45 nmol/L for cancer deaths, so 45 to 60 nmol/L may represent an intervention goal to reduce the overall risk of premature death (25). In our study, the threshold for diabetic people is 54.7 nmol/L, and the threshold for non-diabetic people is 60 nmol/L. Studies have shown that daily vitamin D supplementation to maintain serum 25(OH)D levels $\geq$100 nmol/L is a promising approach to reduce the risk of diabetes in adults with prediabetes (26). Studies have shown that in CVD patients, elevated serum 25(OH)D levels are associated with a reduced risk of all-cause and cause-specific mortality (27). Studies have shown that in patients with vitamin D deficiency and no history of myocardial infarction, maintaining (25-OH)D levels at 30 ng/mL at the time of treatment is associated with a lower risk of myocardial infarction (28). A large cohort study in Germany obtained the same results. There was a strong association between 25-hydroxyvitamin D concentration and mortality from all-cause, cardiovascular, cancer, and respiratory diseases (29). The subjects of the two research were the whole population. When we divided the subjects into diabetic and non-diabetic

subjects, no correlation was found between serum 25(OH)D and cancer mortality. A study specifically for diabetic patients also confirmed our results. A study from the Third National Health and Nutrition Examination Survey (NHANES III) and NHANES 2001–2014 included 6,329 adult diabetic patients. The results showed that serum 25(OH)D was associated with all-cause mortality and CVD mortality (30). Our conclusions need to be validated by a standardized and large-scale RCT (31).

The Korean National Health and Nutrition Examination Survey (KNHANES), a cross-sectional study based on 10,626 participants aged 20–59 years, found that high blood cadmium levels may be related to stroke and hypertension prevalent in people under 60 (32). A study using data from the National Health and Nutrition Examination Survey (NHANES 1999–2014) showed that there was a potential positive correlation between the concentration of heavy metal mixtures (including cadmium) and overall, CVD, and cancer mortality (33). But the method used is poisson regression analysis and the method we used was cox regression analysis. At the same time, the study emphasizes the effect of multiple metal mixtures on all-cause mortality and specific-cause mortality. Our study paid more attention to the individual effects of cadmium in serum. A study with data from NHANES found that zinc intake may change the association between cadmium and mortality. In addition, the Cd/Zn ratio was positively correlated with all-cause mortality, cancer, and CVD mortality (34). Our research paid more attention to the relationship between serum 25(OH)D, cadmium, and CRP with mortality among people in diabetic and non-diabetic.

A meta-analysis showed that CRP has a non-linear relationship with all-cause mortality and CVD mortality and a linear relationship with cancer and non-cardiovascular mortality (35). Our study also found that CRP had a non-linear relationship with all-cause mortality. A meta-analysis based on a cohort study showed that CRP levels can predict the risk of all-cause mortality and cardiovascular mortality in the general population (36). The indicator calculated by two meta-analyses were RR and our study used HR, which may be the reason for the difference. A study from NHANES (1999–2011) showed that higher levels of CRP were associated with lower overall survival rates and CVD survival rates (37). Our research result was that CRP was associated with all-cause mortality and cancer mortality. The reason may be that we divide the study subjects into diabetic and non-diabetic patients, or the overall level of CRP in this study is low.

This study had the following advantages: Firstly, the study was prospective study design with a larger sample (20,221 participants) and the follow-up time was longer (NAHNEAS 2001–2010, follow-up to 31 December, 2015). Secondly, we analyzed the diabetic and non-diabetic separately. Thirdly, we did both subgroup analyses and sensitivity analyses. Fourthly, we also analyzed the effects of serum 25(OH)D, cadmium, and CRP on all-cause mortality and specific-cause mortality. Several limitations should also be noted. First of all, some statistical indicators were obtained by self-reporting and had bias. Secondly, although we adjusted many confounding factors, other unknown confounding factors may also have an impact on the research results. Finally, this research was designed for observational research, so causality cannot be determined.

CONCLUSION

In diabetic patients, serum 25(OH)D, cadmium, and CRP were related to all-cause mortality; serum 25(OH)D was related to cardiovascular mortality; CRP was related to cancer mortality. The similar conclusions were obtained in non-diabetic patients, but with different risk levels. Our conclusions needed to be confirmed and supplemented by follow-up studies.

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 human participants were reviewed and approved by the NCHS Research Ethics Review Board (ERB). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

YL acquired the data, performed the analysis of data, and wrote the manuscript. DY acquired the data and contributed to the analysis of data. FS, FW, XL, SM, and HW contributed to the coding of the statistical analysis. CY designed and evaluated the whole work. All authors read and approved the final manuscript.

FUNDING

This research was funded by the National Natural Science Foundation of China (Grant Nos. 81773552 and 82173626).

ACKNOWLEDGMENTS

Thanks to NHANES for providing open data resources.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.803985/full#supplementary-material

REFERENCES

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, 9(th) edition. *Diabetes Res Clin Pract.* (2019) 157:107843. doi: 10.1016/j.diabres.2019.107843
2. Dal Canto E, Ceriello A, Rydén L, Ferrini M,Hansen TB, Schnell O, et al. Diabetes as a cardiovascular risk factor: an overview of global trends of macro and micro vascular complications. *Eur J Prev Cardiol.* (2019) 26:25–32. doi: 10.1177/2047487319878371
3. Wang L, Manson JE, Song Y, Sesso HD. Systematic review: vitamin D and calcium supplementation in prevention of cardiovascular events. *Ann Intern Med.* (2010) 152:315–23. doi: 10.7326/0003-4819-152-5-201003020-00010
4. Dimitrakopoulou VI, Tsilidis KK, Haycock PC, Dimou NL, Al-Dabhani K, Martin RM, et al. Circulating vitamin D concentration and risk of seven cancers: mendelian randomisation study. *BMJ.* (2017) 359:j4761. doi: 10.1136/bmj.j4761
5. Keum N, Lee DH, Greenwood DC, Manson JE, Giovannucci E. Vitamin D supplementation and total cancer incidence and mortality: a meta-analysis of randomized controlled trials. *Ann Oncol.* (2019) 30:733–43. doi: 10.1093/annonc/mdz059
6. Zhang Y, Fang F, Tang J, Jia L, Feng Y, Xu P, et al. Association between vitamin D supplementation and mortality: systematic review and meta-analysis. *BMJ.* (2019) 366:l4673. doi: 10.1136/bmj.l4673
7. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, et al. Vitamin D supplementation for prevention of mortality in adults. *Cochrane Database Syst Rev.* (2021) 2014:CD007470. doi: 10.1002/14651858.CD007470.pub3
8. Joergensen C, Gall MA, Schmedes A, Tarnow L, Parving HH, Rossing P. Vitamin D levels and mortality in type 2 diabetes. *Diabetes Care.* (2010) 33:2238–2243. doi: 10.2337/dc10-0582
9. Jennersjö P, Guldbrand H, Björne S, Länne T, Fredrikson M, Lindström T, et al. A prospective observational study of all-cause mortality in relation to serum 25-OH vitamin D3 and parathyroid hormone levels in patients with type 2 diabetes. *Diabetol Metab Syndr.* (2015) 7:53. doi: 10.1186/s13098-015-0049-9
10. Kim K, Melough MM, Vance TM, Noh H, Koo SI, Chun OK. Dietary cadmium intake and sources in the US. *Nutrients.* (2018) 11:2. doi: 10.3390/nu11010002
11. Schaefer HR, Dennis S, Fitzpatrick S. Cadmium: mitigation strategies to reduce dietary exposure. *J Food Sci.* (2020) 85:260–267. doi: 10.1111/1750-3841.14997
12. Nordberg G, Jin T, Wu X, Lu J, Chen L, Liang Y, et al. Kidney dysfunction and cadmium exposure–factors influencing dose-response relationships. *J Trace Elements Med.* (2012) 26:197–200. doi: 10.1016/j.jtemb.2012.03.007
13. James KA, Meliker JR. Environmental cadmium exposure and osteoporosis: a review. *Int J Public Health.* (2013) 58:737–45. doi: 10.1007/s00038-013-0488-8
14. Messner B, Knoflach M, Seubert A, Ritsch A, Pfaller K, Henderson B, et al. Cadmium is a novel and independent risk factor for early atherosclerosis mechanisms and in vivo relevance. *Arterioscler Thromb Vasc Biol.* (2009) 29:1392–8. doi: 10.1161/ATVBAHA.109.190082
15. Lee MS, Park SK, Hu H, Lee S. Cadmium exposure and cardiovascular disease in the 2005 Korea National Health and Nutrition Examination Survey. *Environ Res.* (2011) 111:171–6. doi: 10.1016/j.envres.2010.10.006
16. Larsson SC, Wolk A. Urinary cadmium and mortality from all causes, cancer and cardiovascular disease in the general population: systematic review and meta-analysis of cohort studies. *Int J Epidemiol.* (2016) 45:782–791. doi: 10.1093/ije/dyv086
17. Rajab IM, Hart PC, Potempa LA. How C-reactive protein structural isoforms with distinctive bioactivities affect disease progression. *Front Immunol.* (2020) 11:2126. doi: 10.3389/fimmu.2020.02126
18. Markozannes G, Koutsioumpa C, Cividini S, Monori G, Tsilidis KK, Kretsavos N, et al. Global assessment of C-reactive protein and health-related outcomes: an umbrella review of evidence from observational studies and Mendelian randomization studies. *Eur J Epidemiol.* (2021) 36:11–36. doi: 10.1007/s10654-020-00681-w
19. Shen Y, Zhang Y, Xiong S, Zhu X, Ke C. High-sensitivity C-reactive protein and cystatin C independently and jointly predict all-cause mortality among the middle-aged and elderly Chinese population. *Clin Biochem.* (2019) 65:7–14. doi: 10.1016/j.clinbiochem.2018.12.012
20. Maluf CB, Barreto SM, Giatti L, Ribeiro AL, Vidigal PG, Azevedo DRM, et al. Association between C reactive protein and all-cause mortality in the ELSA-Brasil cohort. *J Epidemiol Commun Health.* (2020) 74:421–7. doi: 10.1136/jech-2019-213289
21. Tian R, Tian M, Wang L, Qian H, Zhang S, Pang H, et al. C-reactive protein for predicting cardiovascular and all-cause mortality in type 2 diabetic patients: A meta-analysis. *Cytokine.* (2019) 117:59–64. doi: 10.1016/j.cyto.2019.02.005
22. Bull FC, Al-Ansari SS, Biddle S, Borodulin K, Buman MP, Cardon G, et al. World Health Organization 2020 guidelines on physical activity and sedentary behaviour. *Br J Sports Med.* (2020) 54:1451–62. doi: 10.1136/bjsports-2020-102955
23. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* (2011) 96:1911–30. doi: 10.1210/jc.2011-0385
24. Emerging Risk Factors Collaboration/EPIC-CVD/Vitamin D Studies Collaboration. Estimating dose-response relationships for vitamin D with coronary heart disease, stroke, and all-cause mortality: observational and Mendelian randomisation analyses. *Lancet Diabetes Endocrinol.* (2021) 9:837–46. doi: 10.1016/S2213-8587(21)00263-1
25. Fan X, Wang J, Song M, Giovannucci EL, Ma H, Jin G, et al. Vitamin D status and risk of all-cause and cause-specific mortality in a large cohort: results from the UK biobank. *J Clin Endocrinol Metab.* (2020) 105:432. doi: 10.1210/clinem/dgaa432
26. Dawson-Hughes B, Staten MA, Knowler WC, Nelson J, Vickery EM, LeBlanc ES, et al. Intratrial exposure to vitamin D and new-onset diabetes among adults with prediabetes: a secondary analysis from the vitamin D and type 2 diabetes (D2D) study. *Diabetes Care.* (2020) 43:2916–22. doi: 10.2337/dc20-1765
27. Dai L, Liu M, Chen L. Association of serum 25-hydroxyvitamin D concentrations with all-cause and cause-specific mortality among adult patients with existing cardiovascular disease. *Front Nutr.* (2021) 8:740855. doi: 10.3389/fnut.2021.740855
28. Acharya P, Dalia T, Ranka S, Sethi P, Oni OA, Safarova MS, et al. The effects of vitamin D supplementation and 25-Hydroxyvitamin D levels on the risk of myocardial infarction and mortality. *J Endocr Soc.* (2021) 5:bvab124. doi: 10.1210/jendso/bvab124
29. Schöttker B, Haug U, Schomburg L, Köhrle J, Perna L, Müller H, et al. Strong associations of 25-hydroxyvitamin D concentrations with all-cause, cardiovascular, cancer, and respiratory disease mortality in a large cohort study. *Am J Clin Nutr.* (2013) 97:782–93. doi: 10.3945/ajcn.112.047712
30. Wan Z, Guo J, Pan A, Chen C, Liu L, Liu G. Association of serum 25-hydroxyvitamin D concentrations with all-cause and cause-specific mortality among individuals with diabetes. *Diabetes Care.* (2021) 44:350–7. doi: 10.2337/dc20-1485
31. Pilz S, Trummer C, Theiler-Schwetz V, Grübler MR, Verheyen ND, Odler B, et al. Critical appraisal of large vitamin D randomized controlled trials. *Nutrients.* (2022) 14:303. doi: 10.3390/nu14020303
32. Jeong J, Yun SM, Kim M, Koh YH. Association of blood cadmium with cardiovascular disease in Korea: from the Korea National Health and Nutrition Examination Survey 2008-2013 and 2016. *Int J Environ Res Public Health.* (2020) 17:288. doi: 10.3390/ijerph17176288
33. Duan W, Xu C, Liu Q, Xu J, Weng Z, Zhang X, et al. Levels of a mixture of heavy metals in blood and urine and all-cause, cardiovascular disease and cancer mortality: a population-based cohort study. *Environ Pollution.* (2020) 263(Pt A):114630. doi: 10.1016/j.envpol.2020.114630
34. Kim K, Melough MM, Sakaki JR, Ha K, Marmash D, Noh H, et al. Association between urinary cadmium to zinc intake ratio with adult mortality in a follow-Up Study of NHANES 1988-1994 and 1999-2004. *Nutrients.* (2019) 12:56. doi: 10.3390/nu12010056
35. Ni P, Yu M, Zhang R, Cheng C, He M, Wang H, et al. Dose-response association between C-reactive protein and risk of all-cause and cause-specific mortality: a systematic review and meta-analysis of cohort studies. *Ann Epidemiol.* (2020) 51:20–27.e11. doi: 10.1016/j.annepidem.2020.07.005

36. Li Y, Zhong X, Cheng G, Zhao C, Zhang L, Hong Y, et al. Hs-CRP and all-cause, cardiovascular, and cancer mortality risk: a meta-analysis. *Atherosclerosis*. (2017) 259:75–82. doi: 10.1016/j.atherosclerosis.2017.02.003
37. Liu J, Zhang Y, Lavie CJ, Tabung FK, Xu J, Hu Q, et al. Associations of C-reactive protein and fibrinogen with mortality from all-causes, cardiovascular disease and cancer among U.S. adults. *Prevent Med*. (2020) 139:106044. doi: 10.1016/j.ypmed.2020.106044

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 12 July 2022
doi: 10.3389/fnut.2022.946277

Serum Serine and the Risk of All-Cause Mortality: A Nested Case-Control Study From the China Stroke Primary Prevention Trial (CSPPT)

*Qiangqiang He[1,2†], Nan Zhang[3], Qiongyue Liang[4], Zhuo Wang[5], Ping Chen[6], Yun Song[7,8], Ziyi Zhou[1,2], Yaping Wei[5], Yong Duan[9,10], Binyan Wang[2,11], Peiwu Qin[12], Xianhui Qin[11] and Xiping Xu[5,11]**

[1] Graduate School at Shenzhen, Tsinghua University, Shenzhen, China, [2] Shenzhen Evergreen Medical Institute, Shenzhen, China, [3] Department of Cardiology, Peking University First Hospital, Beijing, China, [4] State Key Laboratory of Natural Medicines, Research Center of Biostatistics and Computational Pharmacy, China Pharmaceutical University, Nanjing, China, [5] Key Laboratory of Precision Nutrition and Food Quality, Ministry of Education, Department of Nutrition and Health, College of Food Sciences and Nutritional Engineering, China Agricultural University, Beijing, China, [6] College of Pharmacy, Jinan University, Guangzhou, China, [7] AUSA Research Institute, Shenzhen AUSA Pharmed Co. Ltd., Shenzhen, China, [8] Institute of Biomedicine, Anhui Medical University, Hefei, China, [9] Yunnan Key Laboratory of Laboratory Medicine, Kunming, China, [10] Department of Clinical Laboratory, the First Affiliated Hospital of Kunming Medical University, Kunming, China, [11] National Clinical Research Center for Kidney Disease, State Key Laboratory for Organ Failure Research, Guangdong Provincial Key Laboratory of Renal Failure Research, Guangzhou Regenerative Medicine and Health, Guangdong Laboratory, Division of Nephrology, Nanfang Hospital, Southern Medical University, Guangzhou, China, [12] Institute of Biopharmaceutical and Health Engineering, Shenzhen International Graduate School, Tsinghua University, Shenzhen, China

OPEN ACCESS

Edited by:
Francesco Sofi,
Università degli Studi di Firenze, Italy

Reviewed by:
Hanping Shi,
Capital Medical University, China
Huihui Bao,
Second Affiliated Hospital of Nanchang University, China

***Correspondence:**
Xiping Xu
xipingxu126@126.com

†ORCID:
Qiangqiang He
orcid.org/0000-0002-1384-9033

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: 17 May 2022
Accepted: 20 June 2022
Published: 12 July 2022

Citation:
He Q, Zhang N, Liang Q, Wang Z, Chen P, Song Y, Zhou Z, Wei Y, Duan Y, Wang B, Qin P, Qin X and Xu X (2022) Serum Serine and the Risk of All-Cause Mortality: A Nested Case-Control Study From the China Stroke Primary Prevention Trial (CSPPT). Front. Nutr. 9:946277. doi: 10.3389/fnut.2022.946277

Background: Serine plays a key role in numerous cellular processes, the levels and metabolism is therefore of critical importance. However, few data are available to illustrate the association of serine with long-term health effects, especially, the predictive value for long-term mortality.

Objective: This study was conducted to evaluate the relationship between serum serine levels and all-cause mortality in general hypertensive patients in a longitudinal cohort, and to examine the potential effect modifiers.

Methods: A nested case-control (NCC) study was conducted utilizing 20702 hypertensive participants from the China Stroke Primary Prevention Trial (CSPPT), a randomized, double-blind, actively controlled trial conducted from May 2008 to August 2013 in China. The current study included 291 cases of all-cause mortality and 291 controls matched on age (≤1 year), sex and treatment group. All-cause mortality was the main outcome in this analysis, which included death due to any reason.

Results: With the increase in serum serine levels, the risk of all-cause mortality first increased before flattening. After adjusting for related variables, the risk of mortality increased significantly with the increase of serum serine levels. Compared with group Q1, the mortality risk of group Q2, Q3 and Q4 were significantly increased [ORs, 95% CI: Q2: 2.32, (1.32–4.07); Q3: 2.59, (1.48–4.54); and Q4: 1.85, (1.07–3.22)]. In the exploratory analysis, we observed three effect modifiers, total homocysteine,

5-Methyltetrahydrofolate, and estimated glomerular filtration rate significantly modified the serum serine and all-cause mortality association.

Conclusion: Serum serine levels were significantly associated with an increased risk of all-cause mortality in hypertensive patients. Our results and findings, if confirmed further, suggest that serum serine should be considered as a marker for screening risk factors of mortality.

Clinical Trial Registration: [https://www.clinicaltrials.gov/ct2/show/study/NCT00794885.], identifier [CSPPT, NCT00794885].

Keywords: serum serine, longitudinal cohort, all-cause mortality, hypertension, nutrition

INTRODUCTION

In addition to contributing to protein synthesis, amino acids support various bioenergetic and biosynthetic processes in mammalian cells. Serine, the main source of one-carbon donors (1), plays a key role in feeding one-carbon units to the tetrahydrofolate (THF) cycle and supports both nucleotide synthesis (2) and contributes to the S-adenosyl methionine (SAM) cycle (3) by providing formyl groups, thus, the dysregulation of serine metabolism has an impact on DNA methylation (4) and epigenetics (5).

Serine deficiency disorders are usually caused by defects in the synthesizing enzymes of the serine biosynthesis pathway, the biochemical hallmarks of synthesizing enzymes defects are low concentrations of serine in cerebrospinal fluid and plasma (6). However, aberrant elevated serine levels were observed in the type 1 diabetes subjects (7) and associated with decreased overall survival (OS) in head and neck cancer (HNC) patients (8). Besides, there are evidence that cancer cells usually demonstrate increased serine biosynthesis and uptake.

Different intake doses of some amino acids may be associated with the changes of the mortality (9), it was reported indispensable amino acids have a positive and some non-indispensable amino acids have a negative, independent, strong association with the risk of cardiovascular mortality (10). The prior study also demonstrated that plasma amino acid constellations are promising additional biomarkers for predicting mortality in end-stage liver disease (11). Numerous studies have focused on the mechanism of serine metabolism and its physiological function, however, few data are available to illustrate the association of serine with long-term health effects, especially, the predictive value for long-term mortality. We therefore, conducted a retrospective cohort nested case-control (NCC) study to investigate the associations of serum serine levels and the risk of all-cause death in a cohort of hypertensive adults.

Abbreviations: eGFR, estimated glomerular filtration rate; tHcy, total homocysteine; NCC, nested case-control; SAM, S-adenosyl methionine; CSPPT, the China Stroke Primary Prevention Trial; SD, standard deviation; OR, odds ratio; CI, confidence interval; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MTHFR, methylenetetrahydrofolate reductase; HDL-C, high density lipoprotein cholesterol; DM, diabetes mellitus.

METHODS

Study Population

The methods and major results of the CSPPT (NCT00794885) have been reported elsewhere (12). Briefly, the CSPPT was a multi-community, randomized, double-blind, controlled trial conducted from 19 May 2008 to 24 August 2013 in 32 communities in Anqing and Lianyungang of China. Eligible participants were men and women aged 45–75 years with hypertension, defined as seated resting systolic blood pressure (SBP) $\geq$140 mm Hg or diastolic blood pressure (DBP) $\geq$90 mm Hg at both the screening and recruitment visits or who were taking antihypertensive medications. The major exclusion criteria included a history of physician-diagnosed stroke, myocardial infarction, heart failure, postcoronary revascularization, or congenital heart disease.

Intervention and Follow-Up

In the CSPPT, a total of 20,702 eligible participants were randomly assigned, in a 1:1 ratio, to one of two treatment groups: a daily oral dose of one tablet containing 10 mg enalapril and 0.8 mg folic acid (the enalapril folic acid group), or a daily oral dose of one tablet containing 10 mg enalapril only (the enalapril-only group). Participants were followed up every 3 months for a median duration of 4.5 years. A total of 291 mortality cases from the CSPPT were analyzed in this study.

Outcomes Assessment

All-cause mortality, a prespecified endpoint of the CSPPT, was the primary outcome of this analysis. All-cause mortality included mortality due to any reason. Evidence for mortality included death certificates from hospitals or reports to the investigator from follow-up visits. Secondary outcomes included death from cardiovascular disease (CVD) including sudden cardiac death, death due to MI, heart failure, stroke, or cardiovascular invasive procedures, death due to cardiovascular hemorrhage, death due to other known vascular causes, and death from cancer including death as a direct result of cancer, or from a complication of cancer, or withdrawal of other therapies due to concerns relating to the poor prognosis associated with cancer. All the study outcomes were reviewed and adjudicated by an independent Endpoint Adjudication Committee, whose members were unaware of study-group assignments.

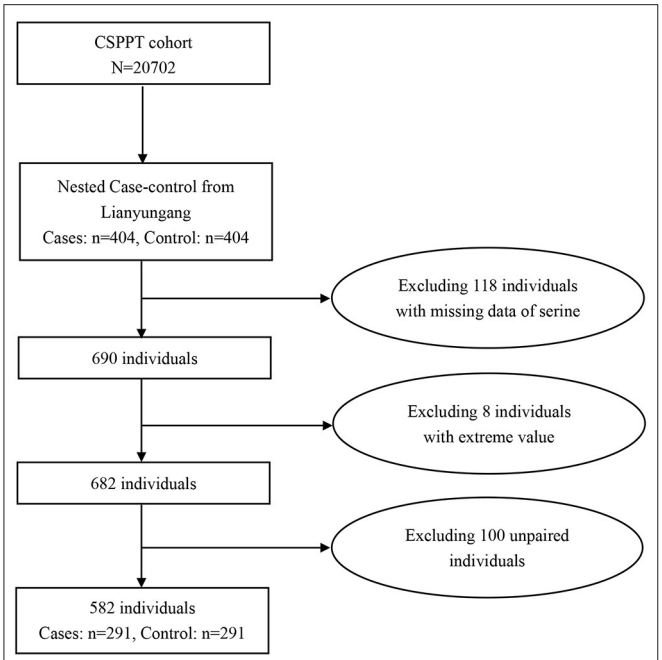

FIGURE 1 | Flow chart of the study participants.

Nested Case-Control Study

During a median treatment duration of 4.5 years, all-cause mortality occurred in 302 participants (2.9%) in the enalapril-folic acid group as compared to 320 participants (3.1%) in the enalapril group (HR, 0.94; 95% CI, 0.81–1.10; $P = 0.47$).

Using data from the CSPPT, we established a nested case-control study with a total of 291 incident cases and 291 matched controls within this cohort. Controls were randomly chosen from the baseline CSPPT participants who were alive during the follow-up and were matched for age (≤1 year), sex, treatment group, and study site with the cases on a 1:1 ratio. Our final analysis included 291 incident cases and matched them with 291 controls within this cohort from the study center Lianyungang, exclusions included 118 participants with missing data on serine, eight participants with extreme values of serine, and 100 unpaired participants (**Figure 1**).

The parent study (the CSPPT) and the current study were approved by the Ethics Committee of the Institute of Biomedicine, Anhui Medical University, Hefei, China (Federal wide assurance number: FWA00001263). All participants provided written informed consent.

Laboratory Assays

Overnight fasting venous blood samples were obtained from each study participant at baseline. Routine biochemical samples, including serum total homocysteine (tHcy), blood glucose, and lipid levels, were analyzed on an automatic clinical analyzer (Beckman Coulter) at the core laboratory of the National Clinical Research Center for Kidney Disease, Guangzhou, China. Serum folate and vitamin B12 were measured in a commercial laboratory using a chemiluminescent immunoassay (New Industrial, Shenzhen). The estimated glomerular filtration rate (eGFR) was calculated with the use of the Chronic Kidney Disease Epidemiology Collaboration equation (13). The stable-isotope-dilution liquid chromatography-tandem mass spectrometry (4500MD, AB SCIEX) was used to detect serine concentrations in the electrospray ionization (ESI +) mode. The mobile phase was 0.5% acetic acid-water (containing 10 m mol/L ammonium acetate)−95% acetonitrile-water (containing 0.5% acetic acid, 10 m mol/L ammonium acetate). The chromatographic column used was Waters ACQUITY UPLC ® BEH HILIC (2.1 × 100 mm, 1.7 μm). This method had excellent sensitivity (LOQ 1 ug / ml), precision (CV < 8%) and recovery (87–111%).

Statistical Analysis

Baseline characteristics were presented as means ± SDs or median (interquartile range, IQR) for continuous variables and proportions for categorical variables. Differences in baseline characteristics between cases and controls were compared using the Chi-square test for categorical variables and the Wilcoxon signed rank test for continuous variables.

Odds ratios (ORs) and 95% confidence intervals (95% CIs) for all-cause mortality in relation to serum serine levels were calculated using conditional logistic regression models, without and with adjustment for age, sex, body mass index (BMI), smoking status, alcohol drinking status, SBP, DBP, fasting blood glucose, total cholesterol (TC), triglycerides (TG), methylenetetrahydrofolate reductase (MTHFR) C677T genotype, treatment group, high-density lipoprotein cholesterol (HDL-C), eGFR, serum folate, total homocysteine (tHcy), vitamin B12 at baseline, as well as time-averaged SBP and time-averaged DBP during the treatment period.

As additional exploratory analyses, possible modifications of the relation of all-cause mortality with serum serine were also assessed for variables including sex, age (<64.5 [median] vs. ≥64.5 years), treatment group (enalapril vs. enalapril-folic acid), MTHFR C677T genotype (CC vs. CT vs. TT), tHcy (<13.7 [median] vs. ≥13.7 μ mol/L), serum folate (<6.5 [median] vs. ≥6.5 ng/mL), 5-Methyltetrahydrofolate (5-MTHF, <6.1 [median] vs. ≥6.1 ng/mL), and vitamin B12 (<374.7 [median] vs. ≥374.7 p g/mL).

A 2-tailed $P < 0.05$ was considered to be statistically significant in all analyses. R software (version 3.6.1; http://www.R-project.org) and Empower (R) (www.empowerstats.com, X&Y Solutions, Inc. Boston, MA) were used for all statistical analyses.

RESULTS

Baseline Characteristics

In this study, we analyzed 291 mortality cases, and 291 matched controls. **Table 1** describes the baseline characteristics of this population. There were no significant differences in age, sex, BMI, treatment group, or smoking and drinking status between the cases and the controls. The mean time-averaged systolic blood pressure and diastolic blood pressure of the cases (144.7/83.4 mmHg) were significantly higher than those of the controls (140.4/82.2 mmHg). In addition, no

TABLE 1 | Baseline characteristics of the study participants stratified by control and case.

Characteristics	Total ($N = 582$)	Mortality		*P*
		Controls ($n = 291$)	Cases ($n = 291$)	
Age,y	64.5 (57.4, 70.6)	64.5 (57.4, 70.6)	64.5 (57.4, 70.7)	0.978
Male, *n* (%)	328 (56.4)	164 (56.4)	164 (56.4)	1.000
BMI, kg/m^2	24.5 (22.2, 27.1)	24.9 (22.5, 27.3)	24.3 (21.9, 26.7)	0.060
Treatment group, *n* (%)				1.000
Enalapril	310 (53.3)	155 (53.3)	155 (53.3)	
Enalapril-folic acid	272 (46.7)	136 (46.7)	136 (46.7)	
BP, mmHg				
SBP at baseline	168.0 (156.0, 182.0)	165.3 (154.7, 180.7)	170.7 (158.0, 183.7)	0.073
DBP at baseline	94.0 (87.3, 101.3)	93.3 (87.7, 100.0)	95.3 (87.3, 102.0)	0.224
Time-averaged SBP	141.6 (134.7, 149.1)	140.4 (134.6, 146.9)	144.7 (135.0, 152.9)	0.001
Time-averaged DBP	82.6 (77.4, 88.3)	82.2 (77.5, 87.2)	83.4 (77.3, 89.8)	0.046
Laboratory results				
Fasting glucose, mmol/L	5.6 (5.2, 6.4)	5.6 (5.2, 6.3)	5.7 (5.2, 6.4)	0.450
Total cholesterol, mmol/L	5.6 (4.8, 6.3)	5.6 (4.9, 6.3)	5.5 (4.6, 6.2)	0.410
Triglycerides, mmol/L	1.4 (1.1, 2.0)	1.5 (1.0, 2.1)	1.4 (1.1, 1.9)	0.352
HDL cholesterol, mmol/L	1.3 (1.1, 1.5)	1.3 (1.1, 1.5)	1.3 (1.0, 1.6)	0.878
tHcy, μmol/L	13.7 (11.0, 18.8)	13.6 (10.8, 18.2)	14.0 (11.1, 19.3)	0.240
Folate, n g/mL	6.5 (4.8, 9.2)	6.8 (4.7, 9.3)	6.2 (4.9, 8.8)	0.848
5-MTHF, ng/mL	6.1 (3.4, 9.6)	6.7 (3.7, 10.3)	5.6 (3.1, 9.0)	0.008
Vitamin B12, pmol/L	374.7 (313.1, 460.2)	373.7 (312.7, 471.1)	374.8 (313.3, 448.9)	0.859
Serine, μmol/L	313.5 (254.2, 397.8)	302.7 (237.9, 395.6)	325.5 (275.1, 405.8)	0.001
eGFR, mL/(min per 1.73 m^2)	91.2 (82.5, 99.2)	91.2 (82.4, 98.9)	91.3 (83.6, 99.7)	0.966
MTHFR genotype, *n* (%)				0.679
CC	141 (24.2)	73 (25.1)	68 (23.4)	
CT	292 (50.2)	148 (50.9)	144 (49.5)	
TT	149 (25.6)	70 (24.1)	79 (27.1)	
Smoking, *n* (%)				0.962
Never	316 (54.3)	158 (54.3)	158 (54.3)	
Former	70 (12.0)	36 (12.4)	34 (11.7)	
Current	196 (33.7)	97 (33.3)	99 (34.0)	
Drinking, *n* (%)				0.564
Never	332 (57.0)	163 (56.0)	169 (58.1)	
Former	65 (11.2)	30 (10.3)	35 (12.0)	
Current	185 (31.8)	98 (33.7)	87 (29.9)	

Values are presented as median (Q1, Q3) or n (%). Differences between cases and controls in baseline characteristics were compared with the use of the Chi-square test for categorical variables and the Wilcoxon signed rank test for continuous variables. BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; tHcy, total homocysteine; 5-MTHF, 5-methyl-tetrahydrofolate; eGFR, estimated glomerular filtration rate; MTHFR, methylenetetrahydrofolate reductase.

significant differences were found in laboratory parameters including fasting blood glucose, blood lipids, baseline Hcy levels, baseline serum folate levels, baseline vitamin B12 levels, and eGFR between the two groups, nor was there any difference in MTHFR C677T genotypes between the two groups. But cases have significantly higher serum 5-MTHF concentrations compared to controls ($P = 0.008$). The median values of serum serine concentrations in mortality cases and control subjects were 325.5 μ mol/L (IQR: 275.1–405.8) and 320.7 μ mol/L (IQR: 237.9–395.6), respectively, the levels for cases were significantly higher than those in the controls ($P = 0.001$).

Association of Baseline Serine Levels With the Risk of All-Cause Mortality

Figure 2 shows the relationship between serum serine and the risk of all-cause mortality. After adjusting for the related variables, as serine levels increased, the risk of mortality increased first and then tended to flatten. The results of logistic regression analyses are shown in **Table 2**, in the unadjusted model, with the increase of serum serine levels, the risk of all-cause mortality showed an overall upward trend. After adjusting for related variables, the risk of mortality increased significantly with the increase in serum serine levels. Serine was assessed as quartiles, compared with group the lowest quartile Q1, < 254.2 μ mol/L),

the adjusted ORs (95% CI) for mortality of group Q2 (254.2–313.5 μ mol/L), Q3 (313.5–397.8 μ mol/L) and Q4 (≥ 397.8 μ mol/L) were significantly increased [ORs: Q2, 2.32 (1.32–4.07); Q3, 2.59 (1.48–4.54); Q4, 1.85 (1.07–3.22). *P* for trend =0.020]. When combined, the subjects in the up three quartiles [quartiles 2–4 (≥ 254.2 μ mol/L): the adjusted OR: 2.24; 95% CI: 1.52–3.30] showed a significantly higher risk of all-cause mortality compared with Q1 ($P < 0.001$).

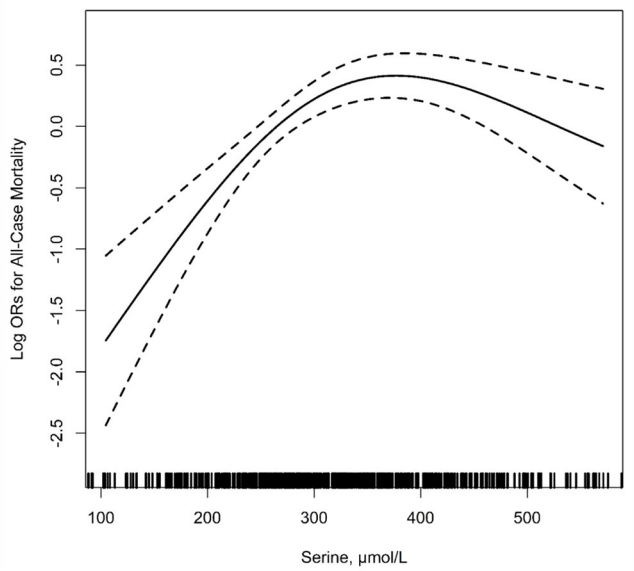

FIGURE 2 | The relationship of serum serine with the risk of all-cause mortality[1]. [1]Adjusted for age, sex, body mass index (BMI), treatment group, *MTHFR* C677T genotypes, smoking, alcohol drinking, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), estimated glomerular filtration rate (eGFR) at baseline, folate, total homocysteine (tHcy), vitamin B12 as well as mean SBP and DBP during the treatment period.

Stratification Analysis

Table 3 shows the results of the stratification analyses in various subgroups with serine modeled as a quartile variable, and compared Q1 to combined Q2–Q4(<254.2 vs. ≥254.2 μ mol/L). As shown in the table, after adjusting for the related variables, the majority of the variables including age, sex, BMI, smoking and drinking status, blood pressure, treatment group, MTHFR C677T genotype, blood lipids, HDL, diabetes status, baseline serum folate and baseline vitamin B12 levels have no modifiable effects on the relationship between serine and mortality.

We observed interactions between serum serine and baseline tHcy levels [<13.7 (median) vs. ≥13.7 μ mol/L] (*P* for interaction=0.012), baseline 5-MTHF levels [<6.1 (median) vs. ≥6.1 ng/mL] (*P* for interaction=0.048), as well as eGFR levels [<91.2 (median) vs. ≥91.2 mL/(min per 1.73 m^2)] (*P* for interaction =0.018) on all-cause mortality (**Table 3**). Higher serum serine levels were significantly associated with a higher risk of all-cause mortality in those with eGFR <91.2 mL/(min per 1.73 m^2) (OR, 4.42; 95% CI, 2.25–8.69), tHcy ≥13.7 μ mol/L (OR, 4.76; 95% CI, 2.43–9.32), and 5-MTHF <6.1 ng/mL (OR, 4.10; 95% CI, 2.09–8.05).

DISCUSSION

In this cohort of hypertensive Chinese adults, we found that the risk of all-cause mortality first increased sharply, and then tended to flatten. After adjusting for possible related confounders, the risk of mortality still increased significantly with the increase in serine levels. Compared with group Q1, the mortality risks of groups Q2, Q3 and Q4 were significantly increased [ORs, 95% CI: Q2: 2.32, (1.32–4.07); Q3: 2.59, (1.48–4.54); and Q4: 1.85, (1.07–3.22)]. To the best of our knowledge, this study is the first to illustrate the potential correlations between serum serine levels and all-cause mortality in a longitudinal cohort.

There are few studies concerning serum serine levels predicting the risk of mortality. Teymoori et al. evaluated the association between dietary serine intakes and hypertension

TABLE 2 | The risk of all-cause mortality with serum serine concentrations[1].

	N	Cases (%)	Crude model		Adjusted model[2]	
			OR(95%CI)	*P*-value	OR(95%CI)	*P*-value
Serine, μmol/L	582	291(50.0)	1.24(1.03,1.50)	0.022	1.18(0.96,1.44)	0.112
Quartile						
Q1 (<254.2)	146	50 (34.2)	1.00 (1.00,1.00)	Ref	1.00 (1.00,1.00)	Ref
Q2 (254.2–313.5)	145	81 (55.9)	2.35 (1.44,3.83)	<0.001	2.32 (1.32,4.07)	0.004
Q3 (313.5–397.8)	145	83 (57.2)	2.43 (1.51,3.92)	<0.001	2.59 (1.48,4.54)	<0.001
Q4 (≥397.8)	146	77 (52.7)	2.00 (1.25,3.19)	0.004	1.85 (1.07,3.22)	0.029
P for trend				0.003		0.020
Categories						
Q1 (<254.2)	146	50 (34.2)	1.00 (1.00,1.00)	Ref	1.00 (1.00,1.00)	Ref
Q2–Q4 (≥254.2)	436	241 (55.3)	2.24 (1.52,3.30)	<0.001	2.22 (1.41,3.50)	<0.001

[1]*ORs of all-cause mortality in relation to serum concentrations of serine quartiles were calculated with the use of conditional logistic regression models. Q, quartile; Ref, reference.*
[2]*Adjusted for age, sex, body mass index, treatment group, MTHFR C677T, smoking status, alcohol drinking status, systolic blood pressure, diastolic blood pressure, fasting blood glucose, total cholesterol, triglycerides, HDL-C, eGFR, total homocysteine at baseline, vitamin B12, serum folate at baseline, and time-averaged BP, during the treatment period.*

TABLE 3 | The association between serum serine and the risk of all-cause mortality in various subgroups.

Subgroups	Serine, μ mol/L				OR(95%CI)	*P* for interaction
	Q1 (<254.2)		Q2–Q4 (≥254.2)			
	N	Cases (%)	*N*	Cases (%)		
Age, y						0.199
<64.5 (median)	69	28 (40.6)	222	117 (52.7)	1.83 (0.98, 3.41)	
≥64.5	77	22 (28.6)	214	124 (57.9)	3.12 (1.67, 5.81)	
Sex						0.509
Female	58	21 (36.2)	196	106 (54.1)	2.17 (1.10, 4.28)	
Male	88	29 (33.0)	240	135 (56.2)	2.82 (1.57, 5.07)	
BMI, kg/m^2						0.141
<24.5 (median)	71	29 (40.8)	220	126 (57.3)	1.97 (1.06, 3.66)	
≥24.5	75	21 (28.0)	216	115 (53.2)	3.96 (2.04, 7.67)	
Smoking						0.777
Never	75	26 (34.7)	241	132 (54.8)	2.36 (1.31, 4.27)	
Former	26	8 (30.8)	44	26 (59.1)	5.54 (1.20, 25.50)	
Current	45	16 (35.6)	151	83 (55.0)	3.08 (1.27, 7.48)	
Alcohol drinking						0.848
Never	82	30 (36.6)	250	139 (55.6)	2.54 (1.42, 4.53)	
Former	15	5 (33.3)	50	30 (60.0)	3.51 (0.39, 31.57)	
Current	49	15 (30.6)	136	72 (52.9)	3.15 (1.36, 7.30)	
Systolic BP at baseline, mmHg						0.650
<168.0 (median)	64	20 (31.2)	223	111 (49.8)	3.19 (1.59, 6.37)	
≥168.0	82	30 (36.6)	213	130 (61.0)	2.57 (1.42, 4.65)	
Diastolic BP at baseline, mmHg						0.684
<94.0 (median)	71	24 (33.8)	215	111 (51.6)	2.37 (1.24, 4.54)	
≥94.0	75	26 (34.7)	221	130 (58.8)	2.53 (1.40, 4.58)	
Treatment group						0.286
Enalapril	84	25 (29.8)	226	130 (57.5)	3.30 (1.78, 6.10)	
Enalapril-folic acid	62	25 (40.3)	210	111 (52.9)	2.09 (1.08, 4.04)	
MTHFR C677T						0.182
CC	28	6 (21.4)	113	62 (54.9)	4.06 (1.32, 12.54)	
CT	71	22 (31.0)	221	122 (55.2)	2.99 (1.60, 5.57)	
TT	47	22 (46.8)	102	57 (55.9)	1.20 (0.51, 2.83)	
eGFR, mL/(min·1.73 m^2)						0.018
<91.2 (median)	73	19 (26.0)	211	121 (57.3)	4.42 (2.25, 8.69)	
≥91.2	70	30 (42.9)	215	112 (52.1)	1.32 (0.72, 2.44)	
Total cholesterol, mmol/L						0.570
<5.6 (median)	76	25 (32.9)	208	118 (56.7)	2.81 (1.53, 5.17)	
≥5.6	67	24 (35.8)	218	115 (52.8)	2.27 (1.18, 4.37)	
Triglycerides, mmol/L						0.084
<1.4 (median)	65	27 (41.5)	223	121 (54.3)	1.80 (0.97, 3.36)	
≥1.4	80	23 (28.7)	209	117 (56.0)	3.35 (1.81, 6.22)	
HDL cholesterol, mmol/L						0.807
<1.3 (median)	75	27 (36.0)	208	115 (55.3)	2.38 (1.29, 4.38)	
≥1.3	70	23 (32.9)	225	124 (55.1)	2.39 (1.29, 4.45)	
Diabetes						0.878
Non-DM	121	41 (33.9)	358	191 (53.4)	2.46 (1.54, 3.93)	
DM	23	8 (34.8)	69	43 (62.3)	1.67 (0.36, 7.83)	
tHcy, μmol/L						0.012
<13.7 (median)	65	27 (41.5)	220	110 (50.0)	1.38 (0.74, 2.58)	
≥13.7	78	22 (28.2)	208	125 (60.1)	4.76 (2.43, 9.32)	

(Continued)

TABLE 3 | Continued

Subgroups	Serine, μ mol/L				OR(95%CI)	*P* for interaction
	Q1 (<254.2)		Q2–Q4 (≥254.2)			
	N	Cases (%)	*N*	Cases (%)		
Folate, ng/mL						0.056
<6.5 (median)	63	19 (30.2)	220	125 (56.8)	3.58 (1.84, 6.95)	
≥6.5	78	29 (37.2)	206	107 (51.9)	1.48 (0.82, 2.68)	
Vitamin B12, pg/mL						0.753
<374.7 (median)	78	27 (34.6)	205	112 (54.6)	2.55 (1.39, 4.67)	
≥374.7	63	21 (33.3)	221	120 (54.3)	3.08 (1.56, 6.08)	
5-MTHF, ng/mL						0.048
<6.1 (median)	68	23 (33.8)	219	135 (61.6)	4.10 (2.09, 8.05)	
≥6.1	74	26 (35.1)	214	105 (49.1)	1.83 (0.98, 3.40)	

Adjusted, if not stratified, for age, sex, body mass index, treatment group, MTHFR C677T genotype, smoking status, alcohol drinking status, systolic blood pressure, diastolic blood pressure, fasting blood glucose, total cholesterol, triglycerides, HDL-C, eGFR, total homocysteine at baseline, total folate at baseline, vitamin B12, and time-averaged BP during the treatment period. DM, diabetes mellitus.

incidents, and observed that 10% of the cohort subjects (429) incident cases of hypertension were ascertained after 3 years of follow-up. The OR of the highest quartile of serine intake was 1.43 (95% CI: 1.05–1.95; *P* for trend: 0.03) compared to the lowest adjusted for age and sex (14). Studies have reported that majorly depressed (15) and schizophrenic patients have significantly increased plasma serine levels compared to normal control groups (16), these psychiatric disorders may contribute to all-cause mortality in the long term. Gu et al. reported that in the serum samples, serine levels and their metabolites were elevated in the colorectal cancer group compared to that of the control group (17). Cadoni et al. found higher serine was significantly associated with decreased overall survival and increased risk of the advanced stage in head and neck cancer (8).

However, the previous findings are inconsistent. Kinny-Köster et al. demonstrated that plasma branched-chain and aromatic amino acids are promising additional biomarkers to determine the increased risk of mortality in patients with end-stage liver disease, but serine showed no significant correlations in survival analysis (11), besides, Mustafa et al. investigated the serum amino acids profiles, demonstrated that serum serine levels were significantly decreased in patients with renal cell carcinoma (RCC) compared to the age and sex matched controls (18), they assumed that the underlying reason would be that kidney tumors might be affecting the reabsorption of amino acids by affecting overall renal function, and the declined serine levels is a consequent result of the RCC.

The clinical characteristics of the study population may account for the aforementioned inconsistent epidemiological research observations, therefore, it is necessary to further examine whether reported associations exist stably in different race and ethnic groups.

Serine can be derived from four possible sources: dietary intake; biosynthesis from the glycolytic intermediate 3-phosphoglycerate; from glycine; and by protein and phospholipid degradation (19), a study showed that subjects belonging to different habitual diet groups have significantly different plasma concentrations of many amino acids, but the plasma serine concentrations were less marked (20). Actually, the *de novo* synthesis of serine is critical, as dietary serine contributes little or nothing to serine metabolism (19), thus it is insufficient to meet the demands of whole body serine homeostasis (21). We speculated that the disturbance of serine homeostasis may attribute to its aberrant biosynthesis pathway, rather than dietary habit or food components of people, this may the different characteristic of serine, a non-essential amino acid, distinguishes from essential amino acids.

A possible biological explanation for our finding would be the roles of serine in the pathological process. Serine is a critically important "input" of one-carbon metabolism and nucleotide biosynthesis, as a hub of one-carbon metabolism and therefore, its overexpression is an important feature of different malignancies (22, 23). The enzyme of serine biosynthesis, phosphoglycerate dehydrogenase (PHGDH) is overexpressed in various types of cancer (24). It seems that cancer cells have a high demand for serine, the flux toward serine synthesis is up-regulated in breast cancer has been observed (25, 26). A study demonstrated that the flux of serine synthesis from 3-phosphoglycerate exhibits a positive correlation with the proliferation rate of tumor-derived cell lines (27). Additionally, it was proved the synthesis and transport pathways genes of serine were overexpressed or up-regulated in various types of cancer (24–26, 28). Combined with the chronic disease, hypertension, aberrant metabolism in the pathological conditions accompanied by the increased serum serine levels may be the reasons promote to the development of diseases and, in the long term, the increased mortality risk.

Some interesting findings of particular note are the potential effect modifiers (**Table 3**): baseline tHcy and 5-MTHF, a plausible biological explanation for the interaction from tHcy and 5-MTHF is that higher tHcy, which is usually resulted from lower circulating folate and 5-MTHF, is associated with many

dangerous and lethal diseases, such as cardiovascular disease (29), stroke (30), and Alzheimer's disease (31). Besides, we found that eGFR negatively modified the effect of serine on the all-cause mortality, previous research showed a significantly greater risk for both all-cause and cardiovascular mortality in the lower eGFR group compared with the stable group over a median of 7.2 years after the last eGFR measure (32) in elderly treated hypertensive patients. If these results are further confirmed, maintaining low tHcy, high 5-MTHF, high eGFR, and low serine levels could be a highly effective strategy for reducing the risk of mortality.

One advantage of this study is we were able to obtain an accurate measurement of the serum serine, as compared with the fortified amino acid dietary intervention method utilized in a study by Verhoef et al. (33), the serum serine levels measured in our study are more directly and precisely reflect the actual available amount for physiological activities or molecular mechanisms, while the amount of serine obtained from dietary intake may be influenced by the food matrix, the bio-accessibility and bioavailability of the amino acid. Meanwhile, several limitations should be noted. First, the existence of the enzyme DL-serine racemase (EC 5.1.1.10) has been reported to directly convert L-serine into D-serine (34), two enantiomers may possess different effects on the health and disease status (35), however the serine levels we determined in this study only present the total serine. Second, our current study was conducted in a Chinese hypertensive population, thus whether the observed findings can be extrapolated or applicable to other populations needs further investigation. Third, due to a lack of data on specific causes of death, the present study could not further explore cause-specific mortality. In addition, despite the comprehensive adjustment for confounders, we cannot exclude the possibility of residual confounding by related dietary factors or other variables. This study provides a key piece of evidence toward the importance of evaluating serum serine levels when assessing the risk of mortality.

CONCLUSION

In summary, our study observed for the first time that the levels of baseline serum serine are a risk factor in increasing all-cause mortality. To explore these correlations in greater depth, further experimental studies and clinical trials are required. Our results suggest that serine levels should be considered as a potential marker for screening risk factors of mortality, in both clinical practice and public health settings, but this finding needs to be validated and confirmed in future investigations with larger populations.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s'.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Institute of Biomedicine, Anhui Medical University, Hefei, China (FWA assurance number FWA00001263). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XX, XQ, QH, and PQ conceived and designed the experiments. XX, YD, BW, XQ, and YS conducted the study. PC performed the quantification of serum samples. PQ, QL, NZ, ZZ, and YW collected and analyzed the data. QH, NZ, ZW, and ZZ drafted the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by National Key Research and Development Program grants 2016YFE0205400 (to XX), 2018ZX09739010 (to XX), and 2018ZX09301034003 (to XX); Science and Technology Planning Project of Guangzhou, China grant 201707020010 (to XX); Science, Technology and Innovation Committee of Shenzhen grants GJHS20170314114526143 (to XX) and JSGG20180703155802047 (to XX); and Economic, Trade and Information Commission of Shenzhen Municipality grants 20170505161556110 (to XX), 20170505160926390 (to XX), and 201705051617070 (to XX).

ACKNOWLEDGMENTS

We acknowledge the contribution of all the staff members who participated in this study as well as the study participants.

REFERENCES

1. Davis SR, Stacpoole PW, Williamson J, Kick LS, Quinlivan EP, Coats BS, et al. Tracer-derived total and folate-dependent homocysteine remethylation and synthesis rates in humans indicate that serine is the main one-carbon donor. *Am J Physiol-Endocrinol Metab.* (2004) 286:E272–9. doi: 10.1152/ajpendo.00351.2003
2. Snell K, Natsumeda Y, Weber G. The modulation of serine metabolism in hepatoma 3924A during different phases of cellular proliferation in cluture. *Biochemical Journal.* (1987) 245:609–12. doi: 10.1042/bj2450609
3. Maddocks OD, Labuschagne CF, Adams PD, Vousden KH. Serine metabolism supports the methionine cycle and DNA/RNA methylation through de novo ATP synthesis in cancer cells. *Mol Cell.* (2016) 61:210–21. doi: 10.1016/j.molcel.2015.12.014
4. Zeng JD, Wu WK, Wang HY Li XX. Serine and one-carbon metabolism, a bridge that links mTOR signaling and DNA methylation in cancer. *Pharmacol Res.* (2019) 149:104352. doi: 10.1016/j.phrs.2019.104352
5. Parker SJ, Metallo CM. Chasing one-carbon units to understand the role of serine in epigenetics. *Mol Cell.* (2016) 61:185–6. doi: 10.1016/j.molcel.2016.01.006

6. van der Crabben SN, Verhoeven-Duif NM, Brilstra EH, Van Maldergem L, Coskun T, Rubio-Gozalbo E, et al. *An update on serine deficiency disorders. J Inherit Metab Dis.* (2013) 36:613–9. doi: 10.1007/s10545-013-9592-4
7. Mathew AV, Jaiswal M, Ang L, Michailidis G, Pennathur S, Pop-Busui R, et al. *Impaired Amino Acid and TCA Metabolism and Cardiovascular Autonomic Neuropathy Progression in Type 1 Diabetes. Diabetes.* (2019) 68:2035–44. doi: 10.2337/db19-0145
8. Cadoni G, Giraldi L, Chiarla C, Gervasoni J, Persichilli S, Primiano A, et al. *Prognostic Role of Serum Amino Acids in Head and Neck Cancer. Dis Markers.* (2020) 2020:1–8. doi: 10.1155/2020/2291759
9. Dong Z, Gao X, Chinchilli V, Muscat J, Richie J. Higher intake of sulfur amino acids is associated with greater mortality among US adults: NHANESIII cohort study (P18-061-19*). Current Developments in Nutrition.* (2019) 3(Suppl 1):nzz039–P18. doi: 10.1093/cdn/nzz039.P18-061-19
10. Tharrey M, Mariotti F, Mashchak A, Barbillon P, Delattre M, Huneau JF, et al. Patterns of amino acid intake are strongly associated with cardiovascular mortality, independently of the sources of protein. *Int J Epidemiol.* (2020) 49:312–21. doi: 10.1093/ije/dyz194
11. Kanda T, Kinny-Köster B, Bartels M, Becker S, Scholz M, Thiery J, et al. Plasma Amino Acid Concentrations Predict Mortality in Patients with End-Stage Liver Disease. *PLoS ONE.* (2016) 11:e0159205. doi: 10.1371/journal.pone.0159205
12. Huo Y, Li J, Qin X, Huang Y, Wang X, Gottesman RF, et al. Efficacy of folic acid therapy in primary prevention of stroke among adults with hypertension in China: the CSPPT randomized clinical trial. *JAMA.* (2015) 313:1325–35. doi: 10.1001/jama.2015.2274
13. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro III AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* (2009) 150:604–12. doi: 10.7326/0003-4819-150-9-200905050-00006
14. Teymoori F, Asghari G, Jalali SM, Mirmiran P, Azizi F. Dietary serine intake and higher risk of hypertension: Tehran lipid and glucose study. *Nutr Food Sci Res.* (2017) 4:7–14. doi: 10.18869/acadpub.nfsr.4.2.2
15. Hashimoto K, Yoshida T, Ishikawa M, Fujita Y, Niitsu T, Nakazato M, et al. *Increased serum levels of serine enantiomers in patients with depression. Acta Neuropsychiatr.* (2016) 28:173–8. doi: 10.1017/neu.2015.59
16. Sumiyoshi T, Anil AE, Jin D, Jayathilake K, Lee M, Meltzer HY, et al. Plasma glycine and serine levels in schizophrenia compared to normal controls and major depression: relation to negative symptoms. *Int J Neuropsychopharmacol.* (2004) 7:1–8. doi: 10.1017/S1461145703003900
17. Gu J, Xiao Y, Shu D, Liang X, Hu X, Xie Y, et al. Serum from Patients with Colorectal Polyp and Colorectal Cancer by 1H-NMR Spectrometry. *Dis Markers.* (2019) 2019:1–14. doi: 10.1155/2019/3491852
18. Mustafa A, Gupta S, Hudes GR, Egleston BL, Uzzo RG, Kruger WD, et al. *Serum Amino Acid Levels as a Biomarker for Renal Cell Carcinoma. J Urol.* (2011) 186:1206–12. doi: 10.1016/j.juro.2011.05.085
19. Koning TJd, Fuchs SA, Klomp LWJ. *Serine, Glycine, and Threonine.* Handbook of Neurochemistry and Molecular Neurobiology, (2007) 23–45. doi: 10.1007/978-0-387-30373-4_2
20. Schmidt JA, Rinaldi S, Scalbert A, Ferrari P, Achaintre D, Gunter MJ, et al. Plasma concentrations and intakes of amino acids in male meat-eaters, fish-eaters, vegetarians and vegans: a cross-sectional analysis in the EPIC-Oxford cohort. *Eur J Clin Nutr.* (2015) 70:306–12. doi: 10.1038/ejcn.2015.144
21. Tabatabaie L, Klomp LW, Berger R, De Koning TJ. L-serine synthesis in the central nervous system: a review on serine deficiency disorders. *Mol Genet Metab.* (2010) 99:256–62. doi: 10.1016/j.ymgme.2009.10.012
22. Shuvalov O, Petukhov A, Daks A, Fedorova O, Vasileva E, Barlev NA, et al. One-carbon metabolism and nucleotide biosynthesis as attractive targets for anticancer therapy. *Oncotarget.* (2017) 8:23955–77. doi: 10.18632/oncotarget.15053
23. Jain M, Nilsson R, Sharma S, Madhusudhan N, Kitami T, Souza AL, et al. Metabolite Profiling Identifies a Key Role for Glycine in Rapid Cancer Cell Proliferation. *Science.* (2012) 336:1040–4. doi: 10.1126/science.1218595
24. Frezza C. *Addicted to serine. Nat Chem Biol.* (2016) 12:389–90. doi: 10.1038/nchembio.2086
25. Pollari S, Käkönen SM, Edgren H, Wolf M, Kohonen P, Sara H, et al. Enhanced serine production by bone metastatic breast cancer cells stimulates osteoclastogenesis. *Breast Cancer Res Treat.* (2011) 125:421–30. doi: 10.1007/s10549-010-0848-5
26. Possemato R, Marks KM, Shaul YD, Pacold ME, Kim D, Birsoy K, et al. Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. *Nature.* (2011) 476:346–50. doi: 10.1038/nature10350
27. Tedeschi PM, Markert EK, Gounder M, Lin H, Dvorzhinski D, Dolfi SC, et al. Contribution of serine, folate and glycine metabolism to the ATP, NADPH and purine requirements of cancer cells. *Cell Death and Disease.* (2013) 4:e877–e877. doi: 10.1038/cddis.2013.393
28. Mattaini KR, Sullivan MR, Vander Heiden MG. *The importance of serine metabolism in cancer. J Cell Biol.* (2016) 214:249–57. doi: 10.1083/jcb.201604085
29. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ.* (2002) 325:1202–8. doi: 10.1136/bmj.325.7374.1202
30. Casas JP, Bautista LE, Smeeth L, Sharma P, Hingorani AD. Homocysteine and stroke: evidence on a causal link from mendelian randomisation. *Lancet.* (2005) 365:224–32. doi: 10.1016/S0140-6736(05)70152-5
31. Yu JT, Xu W, Tan CC, Andrieu S, Suckling J, Evangelou E, et al. Evidence-based prevention of Alzheimer's disease: systematic review and meta-analysis of 243 observational prospective studies and 153 randomised controlled trials. *J Neurol Neurosurg Psychiatry.* (2020) 91:1201–9. doi: 10.1136/jnnp-2019-321913
32. Chowdhury EK, Langham RG, Ademi Z, Owen A, Krum H, Wing LMH, et al. Rate of change in renal function and mortality in elderly treated hypertensive patients. *Clin J Am Soc Nephrol.* (2015) 10:1154–61. doi: 10.2215/CJN.07370714
33. Verhoef P, Steenge GR, Boelsma E, Van Vliet T, Olthof MR. Katan MB. Dietary serine and cystine attenuate the homocysteine-raising effect of dietary methionine: a randomized crossover trial in humans. *Am J Clin Nutr.* (2004) 80:674–9. doi: 10.1093/ajcn/80.3.674
34. Wolosker H, Sheth KN, Takahashi M, Mothet JP, Brady Jr RO, Ferris CD, et al. Purification of serine racemase: biosynthesis of the neuromodulator D-serine. *Proc Nat Acad Sci.* (1999) 96:721–5. doi: 10.1073/pnas.96.2.721
35. Madeira C, Lourenco MV, Vargas-Lopes C, Suemoto CK, Brandão CO, Reis T, et al. d-serine levels in Alzheimer's disease: implications for novel biomarker development. *Transl Psychiatry.* (2015) 5:e561–e561. doi: 10.1038/tp.2015.52

Conflict of Interest: YS was employed by Shenzhen AUSA Pharmed Co Ltd.

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.

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 18 July 2022
doi: 10.3389/fnut.2022.871768

Interaction Analysis Based on Shapley Values and Extreme Gradient Boosting: A Realistic Simulation and Application to a Large Epidemiological Prospective Study

Nicola Orsini[1]*, *Alex Moore*[2] and *Alicja Wolk*[3,4]

[1] Department of Global Public Health, Karolinska Institutet, Stockholm, Sweden, [2] Managed Self Ltd T/A Klarity, Bournemouth, United Kingdom, [3] Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, [4] Department of Surgical Sciences, Orthopaedics, Uppsala University, Uppsala, Sweden

Background: SHapley Additive exPlanations (SHAP) based on tree-based machine learning methods have been proposed to interpret interactions between exposures in observational studies, but their performance in realistic simulations is seldom evaluated.

Methods: Data from population-based cohorts in Sweden of 47,770 men and women with complete baseline information on diet and lifestyles were used to inform a realistic simulation in 3 scenarios of small ($OR_M = 0.75$ vs. $OR_W = 0.70$), moderate ($OR_M = 0.75$ vs. $OR_W = 0.65$), and large ($OR_M = 0.75$ vs. $OR_W = 0.60$) discrepancies in the adjusted mortality odds ratios conferred by a healthy diet among men and among women. Estimates were obtained with logistic regression (L-OR_M; L-OR_W) and derived from SHAP values (S-OR_M; S-OR_W).

Results: The sensitivities of detecting small, moderate, and large discrepancies were 28, 83, and 100%, respectively. The sensitivities of a positive sign (L-OR_W > L-OR_M) in the 3 scenarios were 93, 100, and 100%, respectively. Similarly, the sensitivities of a positive discrepancy based on SHAP values (S-OR_W > S-OR_M) were 86, 99, and 100%, respectively.

Conclusions: In a realistic simulation study, the ability of the SHAP values to detect an interaction effect was proportional to its magnitude. In contrast, the ability to identify the sign or direction of such interaction effect was very high in all the simulated scenarios.

Keywords: extreme gradient boosting, Shapley values, interaction, simulation study, prospective cohort design

OPEN ACCESS

Edited by:
Megan A. McCrory, Boston University, United States

Reviewed by:
Jean-Baptiste Tondji, The University of Texas Rio Grande Valley, United States
Massimo Cavallaro, University of Warwick, United Kingdom

***Correspondence:**
Nicola Orsini
nicola.orsini@ki.se

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *08 February 2022*
Accepted: *09 June 2022*
Published: *18 July 2022*

Citation:
Orsini N, Moore A and Wolk A (2022) Interaction Analysis Based on Shapley Values and Extreme Gradient Boosting: A Realistic Simulation and Application to a Large Epidemiological Prospective Study. Front. Nutr. 9:871768. doi: 10.3389/fnut.2022.871768

INTRODUCTION

SHapley Additive exPlanations (SHAP) values have been recently proposed to facilitate the explanation of results obtained from supervised machine learning algorithms (1). Interaction between the predictors of an outcome is often of interest in epidemiological and public health research. For example, in nutritional epidemiology, the association of a dietary factor with the future occurrence of a particular disease may vary according to another factor. However, this is not a secondary analysis. It is the main research question of substantial interest.

An appealing feature of SHAP methods is that an assessment of interaction can be based on visualizations rather than complex numerical derivations (2). This facilitates a graphical illustration of how the association between one exposure and the outcome may vary along the distribution of another factor. SHAP values are computed on an individual level to explain the importance of the predictors (3). In epidemiological research, the possibility to utilize SHAP values to derive a concise numerical summary at the population-level, which is also capable of expressing the direction and magnitude of interaction effects, would be helpful for interpreting results obtained from machine learning methods.

The field of nutritional epidemiology—characterized by a lack of randomly assigned exposures, relatively modest associations, and possibly complex dependencies between genetic, lifestyle, environmental, and socio-demographic factors—can represent an ideal setting to evaluate the insights provided by SHAP methods derived from popular tree-based machine learning algorithms. If health-related decisions or public recommendations are going to be based on applications of these methods, then it is important to understand their performance in controlled, yet realistic, scenarios. It is important to evaluate the ability of SHAP methods to pinpoint a specific aspect of the data generating mechanism that underlies the observed outcomes, that is, a genuine variation of an exposure-outcome association across levels of another factor.

Data from a large population-based Swedish Mammography Cohort and a Cohort of Swedish Men were used to inform a realistic Monte-Carlo simulation focusing on interaction effects. To complement standard SHAP-based visualizations of dependencies between predictors, we derived a summary measure of exposure effect from SHAP values to facilitate comparisons with conditional odds ratios estimated in multivariable logistic regression models**.** This simulation study was used to evaluate the ability of SHAP methods to correctly indicate an interaction between healthy diet and female sex when predicting all-cause mortality.

MATERIALS AND METHODS

Study Population

This study included participants from two large population-based cohorts of Swedish men and women, the Cohort of Swedish Men (COSM) and the Swedish Mammography Cohort (SMC) (4). Briefly, a total of 48,850 men and 39,227 women responded to the 1997 questionnaires and were included in this study. We excluded participants with diabetes, cancer, or cardiovascular disease at baseline. Furthermore, participants with any missing data on healthy diet, sleep duration, daily walking, alcohol consumption, smoking, cohabitation, body mass index, waist circumference, and educational level were automatically excluded from the analysis. The analytical sample was based on 47,770 participants (23,045 women and 24,725 men), aged 45–83 years. Descriptive statistics of the participants are presented in the **Supplementary Material**.

Predictors of All-Cause Mortality

Diet was assessed using a 96-item food frequency questionnaire. Quality of diet was assessed by recommended food score based on 36 items and non-recommended food score based on 16 items (5). A binary indicator for a healthy diet was obtained by combining recommended food items (top quartiles) and non-recommended food items (bottom quartiles); otherwise not healthy. Age (<65; 65+ years), sex (woman; man), sleep duration (7 h; either <7 or >7 h), daily walking (never or <20 min/day; >20 min/day), smoking status (never; former or current), moderate total alcohol (including wine, beer, and spirits) intake (5–10 g/day for women and 5–20 g/day for men; either below or above such intervals), living with someone (yes; no), body mass index ($\geq$20; <20 kg/m^2), small waist circumference (<88 cm for women and <102 cm for men), and educational level (high school/university; primary) were also assessed at baseline with a self-administered questionnaire.

Case Ascertainment and Follow-Up

Data on death was collected through linkage of the COSM and SMC data to the Swedish Cause of Death Register at the National Board of Health and Welfare (6). Over 20 years of follow-up, from January 1, 1998 to December 31, 2017, 21,978 deaths (9,566 in women and 12,412 in men) were documented in the analytical sample size of 47,770 participants.

Monte-Carlo Simulation

The characteristics of the COSM and SMC data were used to inform the parameters underlying a Monte-Carlo simulation of a prospective cohort study. Descriptive statistics are provided in the **Supplementary Material**.

The interaction mechanism of interest is that the association of a healthy diet with decreased mortality, as measured by the odds ratio, is stronger among women (denoted as OR_W) than men (denoted OR_M), while accounting for possible differences with respect to age, body mass index, waist circumference, physical activity, smoking, alcohol consumption, education, cohabitation, and sleeping time. Given a fixed sample size of 47,770 persons, we considered 3 scenarios: small ($OR_M = 0.75$ vs. $OR_W = 0.70$), moderate ($OR_M = 0.75$ vs. $OR_W = 0.65$), and large ($OR_M = 0.75$ vs. $OR_W = 0.60$) discrepancy by sex in the adjusted inverse association of healthy diet with mortality risk.

To summarize the estimates obtained in 1,000 replications under the 3 scenarios of a genuine interaction effect, the first quantity of interest was the fraction of sample realizations that are correctly indicated as incompatible with the hypothesis of no interaction effect. This is the simulated sensitivity for a certain discrepancy (also known as statistical power). Ignoring the precise magnitude of the discrepancy and focusing only on its sign, the second quantity of interest was the fraction of studies in which the estimated mortality adjusted odds ratio conferred by a healthy diet is correctly estimated to be greater among women than men. This is the simulated sensitivity of a positive discrepancy.

Data Analysis

The association between healthy diet (yes/no) and mortality risk according to sex (men/women) while adjusting for other important predictors was estimated using a traditional logistic regression model and by SHAP values based on extreme gradient boosting.

Extreme Gradient Boost (XGBoost) is a powerful supervised learning method that is well suited to tabular datasets (7). XGBoost chains together decision trees, with each tree trained to predict the previous tree's residuals, commonly known as gradient boosting. There are several hyperparameters controlling XGBoost. To maximize the accuracy of XGBoost these hyperparameters must be optimized. In this study the following hyperparameters were optimized before training our XGBoost model: the number of estimators (decision trees), the maximum depth of a given decision tree, the minimum child weight in a decision tree. The objective logistic link function was specified in the XGBoost classifier.

Shapley values originated as a concept in 1953 from cooperative game theory (8). Early surveys by Tijs et al. (9), Roth (10), and Winter (11) offer a review of the large number of studies that has grown out from the Shapley's seminal paper. Recently, Algaba et al. (12) and the references therein provide a volume devoted to the modern development and applications of the Shapley value in game theory and operations research, decision-making, and applied socio-economics research in various fields (13). In line with this growing literature, Lundberg et al. (1), Molnar (14), and Molnar et al. (15) propose applying the Shapley value in machine learning.

SHAP values facilitate the explanation of highly non-linear models, such as XGBoost, breaking down the impact of input features on prediction (1, 3, 14). SHAP values can be calculated by observing the change in a model's output when each feature is added sequentially. By considering all possible combinations of features, this approach ensures that complex interactions between inputs are captured (3). These interactions explain why two individuals with identical feature values may have different SHAP values associated with those features.

Adjusted Odds Ratios Based on Logistic Regression

The adjusted mortality odds ratio conferred by a healthy diet among men (L-OR_M) was obtained by taking the exponential value of the regression coefficient of healthy diet in a logistic regression model. An estimate of the adjusted mortality odds ratio conferred by healthy diet among women (L-OR_W) was obtained by taking the exponential value of the estimated regression coefficient of healthy diet, plus the estimated regression coefficient of the product term between healthy diet and female sex. A two-sided Wald-type statistical test for the hypothesis of no interaction effect—that is, a regression coefficient of the interaction term equal to zero—was conducted with reference to a standard normal distribution. The result of this statistical test, as a measure of compatibility between data and hypothesis, was used to evaluate the sensitivity of the certain discrepancies previously described.

Adjusted Odds Ratios Based on SHAP Values

Since individual SHAP values are represented as changes in the unit of log-odds, relative to an expected referent (16), a summary of such values may complement graphical illustrations based on dependence plots. The average of the individual SHAP values was first computed for each of the four possible combinations of healthy diet and sex. The SHAP-based adjusted mortality odds ratio comparing healthy diet vs. not-healthy diet was defined as the exponential value of the difference between the average SHAP values of healthy diet and the average SHAP values of not a healthy diet among men (S-OR_M) and women (S-OR_W), respectively.

RESULTS

Simulation Study

The results of 1,000 Monte-Carlo simulated studies according to low, moderate, and large discrepancy in the effect of healthy diet on mortality risk by female sex, while adjusting for all other relevant factors, are shown in **Figure 1**.

Based on estimates obtained with a multivariable logistic regression model, the sensitivities of a small, moderate, and large discrepancy were 28, 83, and 100%, respectively. Graphically, this phenomenon is indicated by an increasing separation in the frequencies of estimated adjusted mortality odds ratios conferred by a healthy diet among men and among women. The sensitivity of a positive discrepancy in adjusted mortality odds ratios for healthy diet comparing women vs. men in scenarios of small, moderate, and large interaction effects were 93, 100, and 100%, respectively.

The small interaction effect underlying **Figure 1A** is 25 and 30% lower adjusted mortality odds ratio conferred by a healthy diet among men and women, respectively. The percentage of sample realizations where the Wald-type test is correctly rejecting the hypothesis of no interaction effect was 28%. In 93% of the simulated studies, the estimated adjusted mortality odds ratio conferred by a healthy diet was correctly greater among women than men. Regarding the large interaction effect underlying **Figure 1C**, the sensitivity of a such large discrepancy as well as the sensitivity of a positive discrepancy were both 100%.

The second column of **Figure 1** shows the individual SHAP values estimated based on XGBoost on a random sample drawn from the 3 interaction mechanisms described above. The fact that the distributions of SHAP values conferred by a healthy diet (right cloud) are consistently lower than the SHAP values conferred by a not-healthy diet (left cloud) indicates an adjusted inverse association of a healthy diet with mortality risk. The increasing magnitude of the interaction effect when moving from **Figures 1A–C** can be visually appreciated by the increasing vertical distinction in blue (men)/red (women) colors. The stronger protective effect of a healthy diet in women is indicated by the greater distance between the red points among those with and without a healthy diet. This is better appreciated in **Figure 1C** where the distance between red points (women) is consistently greater than the distance between blue points (men).

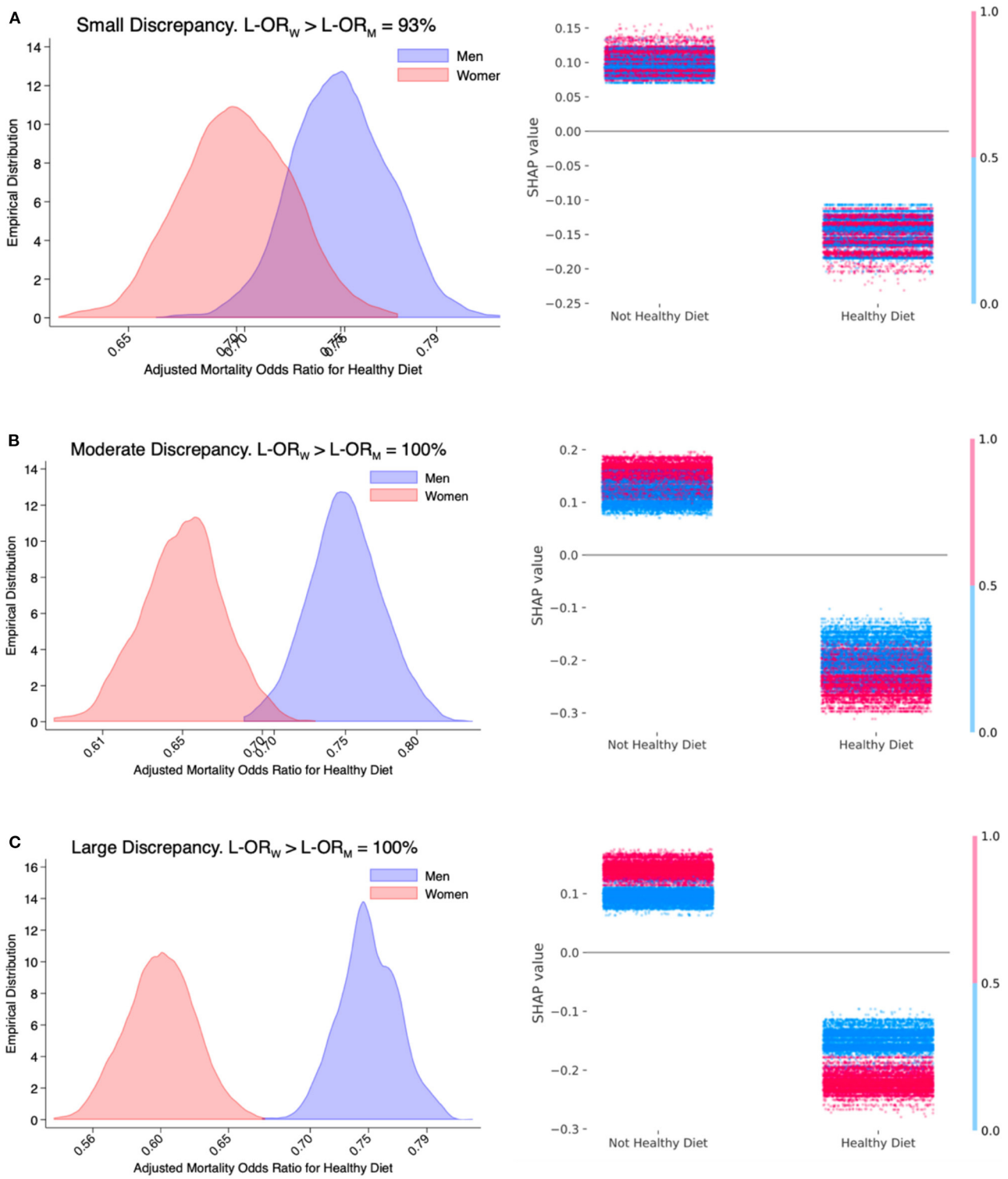

FIGURE 1 | Simulation study—small (**A**; Power = 28%), moderate (**B**; Power = 83%), and large (**C**; Power = 100%) discrepancy by sex in the adjusted mortality odds ratio for healthy diet estimated with a logistic regression model. The left column shows the sampling distribution of the adjusted mortality odds ratio (on the log-scale) conferred by a healthy diet among men and women based on 1,000 simulated studies. The three numbers shown on the x-axis log scale are the 2.5th, 50th, and 97.5th percentiles of the simulated sampling distribution based on a logistic regression model. The percentage indicated in the title is the fraction of studies in which the estimated adjusted mortality odds ratio among women (L-OR$_W$) is greater than men (L-OR$_M$). The right column shows individual SHAP values derived from extreme gradient boosting on one random sample drawn from the interaction mechanisms that are presented on the left column.

Figure 2 provides the simulated sampling distribution of the SHAP-based adjusted mortality odds ratios conferred by a healthy diet among men and women. The sensitivity of a positive discrepancy in small, moderate, and large interaction effects were 86, 99, and 100%, respectively. In contrast to **Figure 1**, the sampling distributions of SHAP-based adjusted mortality odds ratios conferred by a healthy diet are far from being approximated (on a log scale) by a normal distribution, and the magnitude of the interaction effect, as separation in central tendency, tends to be lower.

Performance of the Simulation Algorithms

In terms of execution speed, to conduct one simulation study (MacBook Pro 2019, 2.6 GHz 6-Core Intel Core i7) using logistic regression model took about 0.15 s, whereas using XGBoost took about 13 s. Conducting 1,000 simulations required between 3.5 and 4 h for each scenario. In the 1,000 simulated studies using the logistic regression model the bias—defined as the average distance between each simulated interaction effect relative to its true value—was 0.004, −0.002, and 0.001 for the three scenarios of low, moderate, and large discrepancies, respectively. It was difficult to assess bias about the interaction effect derived from XGBoost simply because data were generated according to a conditional probabilistic model, while the SHAP-based odds ratio are marginal effects. The algorithm converged in all the simulated studies and scenarios. The code written in Python is available in the **Supplementary Material**.

Empirical Study

Among men, the estimated adjusted mortality odds conferred by a healthy diet was 25% lower (L-OR_M = 0.75, 95% CI = 0.70, 0.80). Among women, the estimated adjusted mortality odds conferred by healthy diet was 30% lower (L-OR_W = 0.70; 95% CI = 0.65, 0.75). The result of the Wald-type test indicates a compatibility between this sample of data and the hypothesis of no interaction effects between sex and healthy diet in predicting mortality risk upon adjustment for all the relevant factors ($z = -1.42$, p-value = 0.156). The p-value larger than the nominal 0.05, however, should not be taken, in itself, as a strong indication of the absence of interaction because the ability to detect an adjusted discrepancy of this magnitude (**Figure 1A**) has been shown to be quite low in the corresponding simulation study. A table of estimates of the estimated multivariable logistic regression model (Area Under Curve = 0.80) is presented in the **Supplementary Material**.

Figure 3 shows the adjusted beneficial effect of a healthy diet on mortality risk based on the SHAP values computed after one run of XGBoost on the empirical data (Area Under Curve = 0.74). This indication emerged by the fact that the cloud of SHAP values among those with a healthy diet are consistently lower, meaning lower mortality, than the SHAP values among those with a not healthy diet. Since one may distinguish a cluster of blue dots (men) at the bottom of the not healthy diet (left cloud) and a cluster of red dots (women) at the bottom of the healthy diet (right cloud), **Figure 3** suggests a slightly stronger adjusted protective effect of healthy diet on mortality risk among women, in comparison to men. The derived SHAP-based adjusted mortality odds ratio among women (S-OR_W = 0.72) was slightly greater than men (S-OR_M = 0.74). An

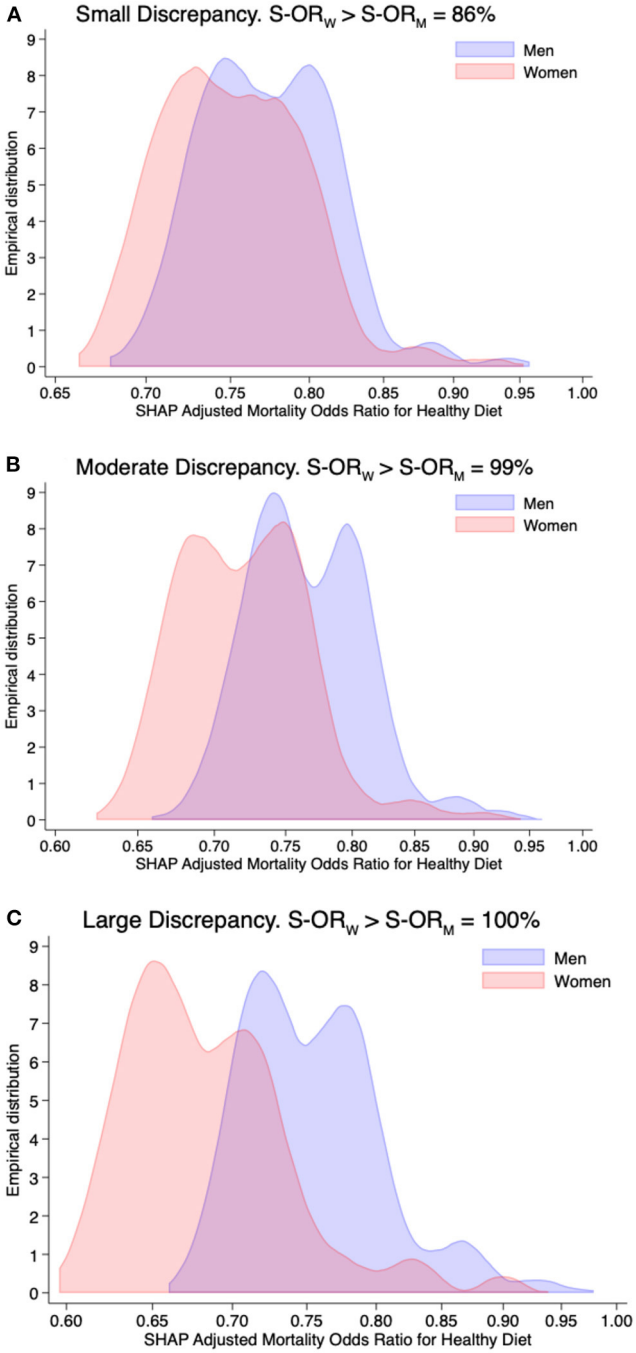

FIGURE 2 | Simulation study—small **(A)**, moderate **(B)**, and large **(C)** discrepancies by sex in the adjusted mortality adjusted odds ratio (on the log-scale) for healthy diet computed in 1,000 studies, each of size of 47,770 individuals and with SHAP values derived from extreme gradient boosting. The x-axis is on the log scale. The percentage indicated in the title is the fraction of times in which the SHAP-based adjusted mortality odds ratio among women (S-OR_W) is greater than men (S-OR_M).

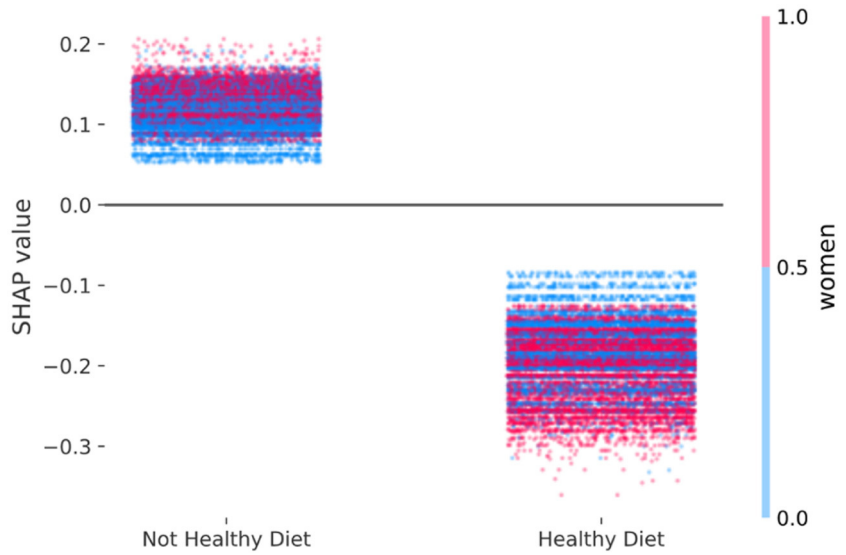

FIGURE 3 | Empirical study—multivariable adjusted association of a healthy diet with mortality risk according to female sex estimated with the SHAP values derived from extreme gradient boosting based on 47,770 participants of the population-based cohorts SMC and COSM. The computed SHAP-based adjusted mortality odds ratio among women (S-$OR_W = 0.72$) is greater than men (S-$OR_M = 0.74$).

attempt to locate the empirical SHAP values shown in **Figure 3** into simulated scenarios shown on the left column of **Figure 1** suggests that the sensitivity to discern an interaction effect of a similar size to the one observed was low, but the sensitivity of a positive discrepancy was high.

DISCUSSION

The ability of SHAP methods based on extreme gradient boosting to indicate the presence of an interaction effect of a certain size was proportional to the sensitivity or traditional power of a statistical test in a logistic regression model. Conversely, the ability of SHAP methods to correctly identify the sign or direction of an interaction was very high in all the scenarios, characterized by either small or large interaction effects. The results obtained with the empirical data appeared to be in line with a small interaction effect and a slightly stronger inverse adjusted association between healthy diet and mortality risk among women.

The strength of this study was the simulation of a complex interaction mechanism grounded in the specific characteristics of a real, large population-based prospective epidemiological study. The simulation study allowed us to appreciate the ability of a statistical model or a machine learning algorithm to pinpoint the true data generating mechanism underlying the outcome observations. The stronger the magnitude of the interaction effect, the easier it was for the chosen method, when applied to a particular sample, to correctly indicate the presence of such interaction effect. Since sample size corresponding to the actual analytical sample was fixed, the sensitivity to detect an interaction effect increased with its size.

Consistency between insights provided by visualizations of SHAP values and prior literature is often highlighted in support of the application of machine learning methods. By deriving and describing a numerical summary measure, such as an adjusted mortality odds ratio, we were able to complement the graphical intuitions provided by increasingly popular dependence plots of individual SHAP values (3, 17). The sensitivity of correctly identifying the sign or direction of an interaction effect was very high in all scenarios using either a logistic regression model or XGBoost. It should be emphasized that the interaction term (between healthy diet and sex) was explicitly specified when defining the logistic regression model, whereas the XGBoost was trained without any interaction term. Therefore, the very good performance of the logistic regression model was not surprising, since the model was specified in perfect agreement with the data generating mechanism underlying the outcome observations. In other words, a logistic regression model without including the right interaction term would not be able to uncover what the combination of SHAP and XGBoost uncovered without including any prior knowledge.

The adjusted mortality odds ratios estimated with a logistic regression model and derived from SHAP-values were numerically similar and pointed in the same direction. It should be noted, however, that they are conceptually and mathematically different. A logistic regression model is parametrized directly in terms of the parameter of interest. SHAP values reflect the relative importance of each predictor through its marginal contribution.

Our focused simulation study, in line with the characteristics of a real epidemiological study, provided a reasonable background to carefully interpret visualizations of SHAP values based on machine learning methods computed from the

data at hand. Recognizing the difficulty in discerning a genuine interaction effect of a certain size of substantial importance can help the investigator to avoid binary claims (presence/absence of interaction) about plausible, yet unknown, mechanisms underlying the data.

A limitation of our simulation study was that the distribution of all the predictors were dichotomized to simplify the analysis and coding. There is no doubt that different categorizations of the predictors or modeling them as quantitative values, possibly considering non-linearities, would have led to different estimates in our empirical study. Since our goal was to conduct a simulation study focusing on the interaction effect between healthy diet and sex, dichotomization of predictors greatly simplified its implementation and analysis. Our simplified model, however, presented a relatively high ability to discriminate the mortality outcomes. Another limitation was that hyperparameters of XGBoost were obtained by means of two-fold cross validation, which might be suboptimal and explain why the area under the curve from XGBoost was, at least in the empirical data, slightly lower than that from logistic regression. However, performing predictions was not a primary concern in our simulation study.

In conclusion, in this realistic simulation study we found that the ability of the SHAP values to detect an interaction effect was proportional to its magnitude. In contrast, the ability to identify the sign or direction of such interaction effect was very high in all the simulated scenarios. The results obtained with the empirical data appeared to be in line with a small interaction effect.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: original individual data that inspired the simulation study cannot be shared. Algorithm to simulate individual data is available in the **Supplementary Material**. Requests to access these datasets should be directed to alicja.wolk@ki.se.

AUTHOR CONTRIBUTIONS

NO, AM, and AW defined the question, conceptualize the simulation study, and drafted the manuscript. NO and AM wrote the code to simulate and analyze a realistic large prospective study.

FUNDING

This study was supported by grants from the Swedish Research Council (grant no 2017-06100). We acknowledge the national research infrastructure SIMPLER (www.simpler4health.se) for providing data. SIMPLER receives funding from the Swedish Research Council (grant no 2017-00644).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.871768/full#supplementary-material

REFERENCES

1. Lundberg SM, Lee SI. A unified approach to interpreting model predictions. *Adv Neural Inf Process Syst*. (2017) 30:4768–77. doi: 10.5555/3295222.3295230
2. Moncada-Torres A, van Maaren MC, Hendriks MP, Siesling S, Geleijnse G. Explainable machine learning can outperform Cox regression predictions and provide insights in breast cancer survival. *Sci Rep*. (2021) 11:6968. doi: 10.1038/s41598-021-86327-7
3. Lundberg SM, Erion G, Chen H, DeGrave A, Prutkin JM, Nair B, et al. From local explanations to global understanding with explainable AI for trees. *Nat Mach Intell*. (2020) 2:56–67. doi: 10.1038/s42256-019-0138-9
4. Harris H, Hkansson N, Olofsson C, Julin B, Kesson A, Wolk A. The Swedish mammography cohort and the cohort of Swedish men: study design and characteristics of 2 population-based longitudinal cohorts. *OA Epidemiology*. (2013) 1:16. doi: 10.13172/2053-079X-1-2-943
5. Kaluza J, Håkansson N, Brzozowska A, Wolk A. Diet quality and mortality: a population-based prospective study of men. *Eur J Clin Nutr*. (2009) 63:451–7. doi: 10.1038/sj.ejcn.1602968
6. Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, Ekbom A. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. *Eur J Epidemiol*. (2009) 24:659–67. doi: 10.1007/s10654-009-9350-y
7. Chen T, Guestrin C. XGBoost: a scalable tree boosting system. In: *Proc 22nd ACM SIGKDD Int Conf Knowl Discov Data Min*. (2016). p. 785–94.
8. Shapley LS. A value for n-person games. In: Kuhn HW, Tucker AW, editors. *Contributions to the Theory of Games (AM-28), Volume II*. Princeton University Press (1953). p. 307—18. Available online at: http://www.jstor.org/stable/j.ctt1b9x1zv
9. Tijs SH, Driessen TSH. Game theory and cost allocation problems. *Manag Sci*. (1986) 32:1015–28. doi: 10.1287/mnsc.32.8.1015
10. Roth AE. The Shapley value: essays in honor of Lloyd S. Shapley. Cambridge University Press (1988). p. 342. doi: 10.1017/CBO9780511528446
11. Winter E. The shapley value. In: Aumann RJ, Hart S, editors. *Handbook of Game Theory with Economic Applications*. North Holland: Elsevier (2002). p. 2025–54.
12. Algaba E, Fragnelli V, Sánchez-Soriano J. *Handbook of the Shapley Value*. CRC Press (2019). p. 472 p.
13. Pongou R, Tondji JB. Valuing inputs under supply uncertainty: the Bayesian Shapley value. *Games Econ Behav*. (2018) 108:206–24. doi: 10.1016/j.geb.2017.08.005
14. Molnar C. *Interpretable Machine Learning*. Available online at: https://christophm.github.io/interpretable-ml-book/ (accessed May 18, 2022).
15. Molnar C, Casalicchio G, Bischl B. *Interpretable Machine Learning – A Brief History, State-of-the-Art and Challenges*. (2020). p. 417–31. Available online at: http://arxiv.org/abs/2010.09337 (accessed May 26, 2022).
16. Ariza-Garzón MJ, Arroyo J, Caparrini A, Segovia-Vargas MJ. Explainability of a machine learning granting scoring model in peer-to-peer lending. *IEEE Access*. (2020) 8:64873–90. doi: 10.1109/ACCESS.2020.2984412
17. Li R, Shinde A, Liu A, Glaser S, Lyou Y, Yuh B, et al. Machine learning–based interpretation and visualization of non-linear interactions in prostate cancer survival. *JCO Clin Cancer Inform*. (2020) 4:637–46. doi: 10.1200/CCI.20.00002

Conflict of Interest: AM was employed by Managed Self Ltd T/A Klarity, Bournemouth, UK.

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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 22 July 2022
DOI 10.3389/fnut.2022.877917

OPEN ACCESS

EDITED BY
Megan A. McCrory,
Boston University, United States

REVIEWED BY
Marek Kieliszek,
Warsaw University of Life Sciences, Poland
Tommaso Filippini,
University of Modena and Reggio Emilia, Italy

*CORRESPONDENCE
Zewu Zhu
zhuzev@163.com
Hequn Chen
chenhequnxy@126.com

SPECIALTY SECTION
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

RECEIVED 22 April 2022
ACCEPTED 01 July 2022
PUBLISHED 22 July 2022

CITATION
Liu M, Cui Z, Chen J, Gao M, Zhu Z and Chen H (2022) Dietary selenium intake and the risk of kidney stones in adults, an analysis of 2007–2018 National Health and Nutrition Examination Survey, a cross-sectional study. *Front. Nutr.* 9:877917. doi: 10.3389/fnut.2022.877917

Dietary selenium intake and the risk of kidney stones in adults, an analysis of 2007–2018 National Health and Nutrition Examination Survey, a cross-sectional study

Minghui Liu[1,2], Zhongxiao Cui[1,2], Jinbo Chen[1,2], Meng Gao[1,2], Zewu Zhu[1,2]* and Hequn Chen[1,2]*

[1]Department of Urology, Xiangya Hospital, Central South University, Changsha, China, [2]National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, China

Purpose: To evaluate the association between dietary selenium intake and the risk of kidney stones in adults.

Materials and methods: We performed a cross-sectional analysis using data from 2007 to 2018 National Health and Nutrition Examination Survey (NHANES). Dietary intake information of 30,184 participants was obtained using first 24-h dietary recall interview, and kidney stones were presented by a standard questionnaire. The quartile analysis, stratified analysis and non-linearity analysis were used to estimate the association between dietary selenium intake and kidney stones after an adjustment for potential confounders.

Results: The multiple logistic regression indicated that the fourth quantile (Q4) of dietary selenium intake had a lower risk of kidney stones than the first quantile (Q1) in Model 3 (OR 0.82, $P < 0.05$). The stratified analyses indicated there were statistical differences between dietary selenium intake and kidney stones among younger (age < 50) (OR 0.65, $P < 0.01$), male (OR 0.73, $P < 0.01$) and overweight/obese (BMI ≥ 25.0) (OR 0.80, $P < 0.05$) individuals in Model 3. The non-linear relationship was founded between dietary selenium intake and kidney stones in all participants, younger, male and overweight/obese individuals after adjusting for confounding factors.

Conclusion: Our study revealed an inverse relation between the level of dietary selenium intake and the risk of kidney stones for the United States population, especially for younger (age < 50), male and overweight/obese (BMI ≥ 25.0) individuals. The study provides preliminary guidance on dietary selenium intake for the prevention of kidney stones in different populations. Further studies are required to confirm our findings and clarified the biological mechanisms.

KEYWORDS

dietary selenium, kidney stones, National Health and Nutrition Examination Survey, calcium oxalate, Randall plaques

Introduction

Nephrolithiasis is a common disease, with a lifetime incidence of about 10% and a 5-year recurrence rate of 50% (1, 2). Furthermore, the prevalence of nephrolithiasis has been rising during the last decades, resulting in increased economic burden on the health care system (1, 3). The incidence of urolithiasis is related to geographical, climatic, ethnic, genetic and dietary factors. Urinary stone incidence depends on Urine becomes excessively supersaturated with urine mineral or certain relatively insoluble drugs, resulting in crystal formation, growth, aggregation and retention. Calcium oxalate (CaOx) is the main constituent in about 80% of kidney stones (4), many of which grow over depositions of calcium phosphate (CaP) called Randall plaques (RP), which are attached to the renal papillary surface. Infection stones are typically caused by infection with urease-producing bacteria. Hypercalciuria caused by Hyperparathyroidism and Cushing's disease may cause kidney stone by boosting supersaturation of calcium oxalate or phosphate (5, 6). Obesity, hypertension, diabetes, and metabolic syndrome are also risk factors for stone formation (4). Nowadays, dietary habits are considered crucial factors in the formation of kidney stones (7), such as intakes of fluid, calcium, sodium and animal proteins. Urinary stone formation can be reduced by improving fluid intake. Excessive intake of animal protein, calcium and sodium should be avoided to prevent calcium stone formation. Excessive intake of oxalate may increase the incidence of calcium oxalate stones (8).

Selenium was considered a toxin until 1957 when Schwartz and Foltz discovered the protective effect of selenium on liver in rats and was later proved to be an essential trace element in human body by numerous experiments. Products with rich selenium content include fish, meat, offal, cereals and plants of the Brassica genus. Taking supplements containing organic selenium is a quick and effective way (9). Selenium reserves accumulate during the first half of our lives and the daily requirement mainly depends on age and state of our body. Combining selenium with vitamin E can strengthen the antioxidant protection and antagonize heavy metal toxicity. It is essential to identify the status and consumption of selenium for a specific community duo to the highly variable levels of selenium between diverse populations and regions (10).

Selenium has multiple and complex effects on human health (11). It has been well-researched that selenium deficiency primarily affects heart muscle, joints, nephropathy and neurological diseases (12). Excess of selenium may cause gastrointestinal upsets, infertility, hair loss, skin rash, nervous system disorders and so on (9). However, recent evidence suggests that high selenium intake through food or dietary supplements does not prevent cancer in humans and may even increase the risk of type 2 diabetes (13–15). The biological functions of dietary selenium are achieved by selenoproteins whose active center is selenocysteine, and the best known is the antioxidant glutathione peroxidase (GPX) (11). In recent years, the role of selenium preventing atherosclerosis and CVDs has attracted significant attention. Several findings have shown an association between kidney stones and CVDs (16), although the causation has not been definitively established. Moreover, some animal experiments have suggested negative relationships between selenium and CaOx stones (17–19).

Based on these findings, we supposed that dietary selenium intake might be associated with the risk of kidney stones in humans. This study aimed to confirm the hypothesis with participants from the 2007 to 2018 National Health and Nutrition Survey (NHANES).

Abbreviations: CaOx, Calcium oxalate; CaP, calcium phosphate; RP, Randall plaques; CVDs, cardiovascular diseases; GPX, glutathione peroxidase; NHANES, National Health and Nutrition Survey; BMI, body mass index; Q1, the first quantile; Q2, the second quantile; Q3, the third quantile; Q4, the fourth quantile; OS, oxidative stress; OPN, osteopontin; ROS, reactive oxygen species; SeP, Selenoprotein P; GPX1, Glutathione peroxidase 1; GPX3, Glutathione peroxidase 3; *FABP4,* Fatty acid binding protein 4.

Materials and methods

Study population

For the current analysis, the six consecutive 2-year cycles of NHANES 2007–2008, 2009–2010, 2011–2012, 2013–2014, 2015–2016, and 2017–2018 were collected, as the questions about kidney stones were responded by participants during these cycles. There were 59,842 participants aged 0–80 years in NHANES 2007–2018. The exclusion criteria were as follows: (a) participants who had not completed the kidney stones survey (n = 25,163) (all participants aged less than 20 and average age is 8.3); (b) unknown selenium (n = 3,963) (these participants did not receive 24-h dietary recall interview and we failed to obtain any dietary data); (c) pregnancy (n = 267); (d) unknown body mass index (BMI) (n = 318); (e) unknown diabetes (n = 15); (f) unknown hypertension (n = 39); (g) unknown recreational activities (n = 7); (h) unknown smoking data (n = 14); (i) abnormal selenium (n = 1501). And a total of 28,555 participants were included in the study.

Study variables and outcome

The dietary intake data were obtained from a 24-h dietary recall interview with all participants. The intakes of selenium and other components from foods and beverages were calculated using the United States Department of Food and Nutrient Database for Dietary Studies (FNDDS). The first 24-h dietary recall interview (Dietary Interview - Total Nutrient Intakes, First Day) was used in the present study.

Based on the previous studies on dietary intake and kidney stones (20, 21), we included the following covariates: age (<50 years and ≥50 years), sex (male/female), race (Mexican American, other Hispanic, Non-Hispanic white, Non- Hispanic black and other), marital status (married and unmarried), education level (less than 11th grade, high school or equivalent, some college or AA degree, and college graduate or above), vigorous and moderate recreational activities, annual family income ($0–$19,999, $20,000–$44,999, $45,000–$74,999, ≥ $75,000 and other), hypertension, diabetes, BMI (< 25.0 kg/m^2 and ≥ 25.0 kg/m^2), smoking, daily intake of total energy, water, caffein, alcohol, calcium, phosphate, potassium, sodium, magnesium, and vitamins A, B6, C, D, E and K.

The NHANES 2007-2018 (Kidney Conditions – Urology) provides personal interview data on kidney stones for participants aged 20 years and older. And we considered participants who answered "Yes" to the question "Have you ever had kidney stones?" (KIQ026) as having a history of nephrolithiasis.

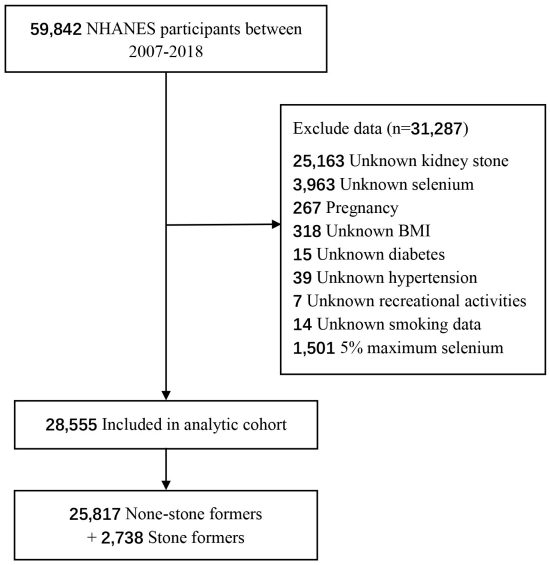

FIGURE 1
Flowchart of the participants from National Health and Nutrition Examination Survey (NHANES) in the present study.

Statistical analysis

Data were described as mean ± standard deviation (SD) for continuous variables and percentages (%) for categorical variables. The Kruskal Wallis test was used to evaluated continuous variables because of the non-normal distribution for dietary intake data, and chi-square ($\chi 2$) tests were used to analyze the categorical variables. We calculated quartiles based on the participants without kidney stone and calculated quartiles in each subgroup and analyzed to better match different populations. Three different multiple logistic regression models were used to calculate odds ratios (ORs) and 95% confidence interval (CI) for each quartile of selenium to kidney stones. Model 1 was the crude model, and we adjusted for age, sex and race in Model 2. And in Model 3, we further adjusted for the covariates of marital status, education level, vigorous and moderate recreational activities, annual family income, hypertension, diabetes, BMI, smoking, daily intake of total energy, water, caffein, alcohol, calcium, phosphate, potassium, sodium, magnesium, and vitamins A, B6, C, D, E, and K.

After an adjustment for potential confounders, we performed non-linear correlation by smoothing plot with three knots located at the 5th, 50th, and 95th percentiles of selenium intake based on the relevant study (22). If the non-linear correlation was founded, we further performed a two-piecewise linear regression model to calculate the threshold effect of the dietary selenium intake on kidney stones according to the smoothing plot. The inflection point was automatically calculated by recursive method when the relationship between the two became obvious in smoothed curve (23). All statistical analyses were performed using the software EmpowerStats.[1] Two-tailed P values < 0.05 was considered as a statistically significant difference.

Results

Participant characteristics

We included 28,555 participants from NHANES 2007 to 2018 according to the inclusion and exclusion criteria, including 25,817 (90.4%) cases without kidney stones and 2,738 (9.6%) cases with kidney stones (Figure 1). Characteristics of participants are presented as two groups in Table 1. There are significant statistical differences among several variables, including age ($P < 0.01$), sex ($P < 0.01$), race ($P < 0.01$), marital status ($P < 0.01$), vigorous recreational activities ($P < 0.01$), moderate recreational activities ($P < 0.01$), education level ($P < 0.01$), hypertension ($P < 0.01$), diabetes ($P < 0.01$), BMI ($P < 0.01$), smoked at least 100 cigarettes in life ($P < 0.01$), total water drank ($P < 0.01$), daily intake of alcohol ($P < 0.01$), caffeine ($P < 0.01$), selenium ($P = 0.02$), phosphorus ($P = 0.01$), magnesium ($P < 0.01$), Vitamin B6 ($P < 0.01$) and Vitamin C ($P < 0.01$). Obviously, participants with kidney stones were more likely to be male (54.3%), aged more than 50 years (66.4%), non-Hispanic white (55.1%), married (57.2%), less vigorous recreational activities (85.3%),

1 http://www.empowerstats.com

TABLE 1 Characteristics of participants in National Health and Nutrition Examination Survey (NHANES) 2007–2018.

Characteristic	None-stone No. (%)	Stone No. (%)	*P*-value
Total participants	25817 (90.41)	2738 (9.59)	
Age			< **0.01**
Mean (SD)	49.43 (17.69)	56.44 (15.98)	
< 50 years	13059 (50.58)	919 (33.57)	
≥ 50 years	12758 (49.42)	1819 (66.44)	
Sex			< **0.01**
Male	12020 (46.56)	1487 (54.31)	
Female	13797 (53.44)	1251 (45.69)	
Race			< **0.01**
Mexican American	3900 (15.11)	338 (12.34)	
Other Hispanic	2660 (10.30)	321 (11.72)	
Non-Hispanic White	10492 (40.64)	1509 (55.11)	
Non-Hispanic Black	5765 (22.33)	361 (13.19)	
Other	3000 (11.62)	209 (7.63)	
Marital status			< **0.01**
Married	13036 (50.49)	1567 (57.23)	
Unmarried	12781 (49.51)	1171 (42.77)	
Vigorous recreational activities			< **0.01**
Yes	5680 (22.00)	402 (14.68)	
No	20137 (78.00)	2336 (85.32)	
Moderate recreational activities			< **0.01**
Yes	10448 (40.47)	975 (35.61)	
No	15369 (59.53)	1763 (64.39)	
Education			< **0.01**
Less than 11th grade	6249 (24.21)	691 (25.24)	
High school or equivalent	5938 (23.00)	617 (22.54)	
Some college or AA degree	7590 (29.40)	867 (31.67)	
College graduate or above	6040 (23.40)	563 (20.56)	
Annual family income			0.34
$0–$19 999	6005 (23.62)	653 (24.19)	
$20 000 to $44 999	8096 (31.84)	888 (32.90)	
$45 000 to $74 999	4466 (17.56)	479 (17.75)	
≥$ 75 000	5939 (23.36)	593 (21.97)	
Other	921 (3.62)	86 (3.19)	
Hypertension			< **0.01**
Yes	9151 (35.45)	1398 (51.06)	
No	16666 (64.55)	1340 (48.94)	
Diabetes			< **0.01**
Yes	3205 (12.41)	616 (22.50)	
No/Borderline	22612 (87.59)	2122 (77.50)	
BMI (kg/m^2)			< **0.01**
Mean (SD)	29.17 (7.01)	30.57 (6.87)	
< 25.0	7552 (29.25)	527 (19.25)	
≥ 25.0	18265 (70.75)	2211 (80.75)	
Smoked at least 100 cigarettes in life (%)			< **0.01**
Yes	11253 (43.59)	1382 (50.48)	
No	14564 (56.41)	1356 (49.53)	
Daily intake [Mean (SD)]			
Total energy (kcal)	2001.23 (858.47)	1969.50 (827.45)	0.15
Total water drank (g)	1088.02 (1172.16)	1035.39 (1171.61)	< **0.01**
Alcohol (g)	9.77 (27.36)	6.93 (26.28)	< **0.01**
Caffeine (mg)	146.93 (200.39)	168.77 (234.70)	< **0.01**
Selenium (mcg)	103.02 (47.18)	100.44 (45.36)	**0.02**
Calcium (mg)	879.99 (530.57)	860.99 (517.00)	0.10
Phosphorus (mg)	1266.65 (572.53)	1238.11 (556.52)	**0.01**
Sodium (mg)	3261.34 (1570.66)	3227.70 (1519.60)	0.43
Potassium (mg)	2500.64 (1143.26)	2465.67 (1139.89)	0.08
Magnesium (mg)	282.93 (135.43)	272.33 (131.32)	< **0.01**
Vitamin A (mcg)	580.94 (620.27)	583.20 (621.08)	0.26
Vitamin B6 (mg)	1.96 (1.55)	1.87 (1.31)	< **0.01**
Vitamin C (mg)	81.67 (93.34)	75.42 (90.60)	< **0.01**
Vitamin D (mcg)	4.22 (4.97)	4.18 (4.84)	0.51
Vitamin E (mg)	7.78 (5.87)	7.70 (5.84)	0.42
Vitamin K (mcg)	108.34 (192.91)	99.32 (136.27)	0.07

SD, standard deviation; BMI, body mass index. The bold values refer to $P < 0.05$, indicating significant statistical differences.

less moderate recreational activities (64.4%), some college or AA degree, hypertension-positive, diabetes-positive and BMI ≥ 25.0 kg/m^2 (80.8%).

Quartile analysis and non-linearity analysis

Multiple logistic regression models indicated that the fourth quantile (Q4) of dietary selenium intake had a lower risk of nephrolithiasis than the first quantile (Q1) in Model 1 (OR 0.87, 95% CI 0.78–0.97, $P < 0.05$), Model 2 (OR 0.85, 95% CI 0.75–0.95, $P < 0.01$) and Model 3 (OR 0.82, 95% CI 0.70–0.97, $P < 0.05$), while there were no statistical differences between the second quantile (Q2)/the third quantile (Q3) and Q1 (Table 2). The results showed that in stratified analysis by age, we found statistical differences between Q4 and Q1 for participants aged less than 50 in Model 1 (OR 0.75, 95% CI 0.62–0.91, $P < 0.01$), Model 2 (OR 0.73, 95% CI 0.60–0.89 $P < 0.01$) and Model 3 (OR 0.65, 95% CI 0.49–0.86, $P < 0.01$). But there was no positive correlation between selenium and kidney stones in cases aged more than 50. In stratified analysis by sex, there were statistical differences between Q4 and Q1 for male participants in Model 1 (OR 0.69, 95% CI 0.59–0.81, $P < 0.01$), Model 2 (OR 0.78, 95% CI 0.66–0.92 $P < 0.01$) and Model 3 (OR 0.73, 95% CI 0.58–0.91, $P < 0.01$). But we failed to find statistical significance for female participants. In stratified analysis by BMI, there were

TABLE 2 Multivariate analysis of kidney stones by quartiles of selenium intake, National Health and Nutrition Examination Survey (NHANES) 2007–2018.

	Cutoff (mcg)	None-stone No. (%)	Stone No. (%)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Overall						
Q1	< 67.8	6432 (90.0)	718 (10.0)	1.00	1.00	1.00
Q2	67.8–97.2	6458 (90.2)	701 (9.8)	0.97 (0.87–1.09)	0.95 (0.85–1.06)	0.94(0.83–1.06)
Q3	97.2–133.8	6461 (90.3)	693 (9.7)	0.96 (0.86–1.07)	0.93 (0.83–1.05)	0.92 (0.80–1.05)
Q4	≥ 133.8	6466 (91.2)	626 (8.8)	0.87 (0.78–0.97)*	0.85 (0.75–0.95)**	0.82 (0.70–0.97)*
P value				0.01	< 0.01	0.02
Age						
< 50 years						
Q1	<72.1	3320 (92.5)	262 (7.5)	1.00	1.00	1.00
Q2	72.1–103.3	3269 (93.3)	234 (6.7)	0.89 (0.73–1.06)	0.87 (0.72–1.04)	0.83 (0.68–1.02)
Q3	103.3–141.2	3273 (93.7)	221 (6.3)	0.83 (0.69–1.00)	0.81 (0.67–0.98)	0.77 (0.62–0.97)
Q4	≥ 141.2	3297 (94.2)	202 (5.8)	0.75 (0.62–0.91)**	0.73 (0.60–0.89)**	0.65 (0.49–0.86)**
P trend				< 0.01	<0.01	< 0.01
≥ 50 years						
Q1	< 63.8	3201 (88.0)	436 (12.0)	1.00	1.00	1.00
Q2	63.8–91.6	3211 (88.0)	437 (12.0)	1.00 (0.87–1.15)	0.93 (0.81–1.08)	0.93 (0.80–1.09)
Q3	91.6–125.5	3148 (86.5)	491 (13.5)	1.15 (1.00–1.32)	1.02 (0.89–1.18)	1.02 (0.86–1.20)
Q4	≥ 125.5	3198 (87.5)	455 (12.5)	1.05 (0.91–1.20)	0.87 (0.75–1.01)	0.86 (0.70–1.05)
P trend				0.31	0.12	0.22
Gender						
Male						
Q1	< 79.5	2961 (88.0)	404 (12.0)	1.00	1.00	1.00
Q2	79.5–112.4	2961 (87.5)	423 (12.5)	1.05 (0.91–1.21)	1.06 (0.91–1.23)	1.02 (0.87–1.20)
Q3	112.4–149.8	3009 (89.1)	369 (10.9)	0.90 (0.77–1.04)	0.94 (0.81–1.09)	0.90 (0.75–1.08)
Q4	≥ 149.8	3089 (91.4)	291 (8.6)	0.69 (0.59–0.81)**	0.78 (0.66–0.92)**	0.73 (0.58–0.91)**
P trend				< 0.01	<0.01	< 0.01
Female						
Q1	< 60	3410 (90.8)	347 (9.2)	1.00	1.00	1.00
Q2	60.1–85.4	3455 (92.0)	301 (8.0)	0.86 (0.73–1.01)	0.86 (0.73–1.02)	0.84 (0.71–1.00)*
Q3	85.5–116.5	3462 (91.9)	304 (8.1)	0.86 (0.74–1.01)	0.89 (0.76–1.05)	0.85 (0.71–1.03)
Q4	≥ 116.5	3470 (92.1)	299 (7.9)	0.85 (0.72–1.00)*	0.93 (0.79–1.09)	0.86 (0.68–1.09)
P trend				0.07	0.52	0.30
BMI (kg/m^2)						
< 25.0						
Q1	<66.9	1880 (93.1)	140 (6.9)	1.00	1.00	1.00
Q2	66.9–96.7	1871 (92.8)	145 (7.2)	1.04 (0.82–1.32)	1.04 (0.81–1.33)	1.09 (0.84–1.42)
Q3	96.7–133.8	1892 (93.7)	127 (6.3)	0.90 (0.70–1.16)	0.95 (0.74–1.23)	0.98 (0.72–1.32)
Q4	≥ 133.8	1909 (94.3)	115 (5.7)	0.81 (0.63–1.04)	0.88 (0.67–1.15)	0.95 (0.65–1.38)
P trend						
≥ 25.0						
Q1	< 67.9	4533 (88.6)	582 (11.4)	1.00	1.00	1.00
Q2	67.9–97.0	4570 (89.3)	548 (10.7)	0.93 (0.83–1.06)	0.91 (0.80–1.03)	0.89 (0.78–1.02)
Q3	97.0–133.1	4550 (89.0)	562 (11.0)	0.96 (0.85–1.09)	0.92 (0.81–1.04)	0.90 (0.78–1.05)
Q4	≥ 133.1	4612 (89.9)	519 (10.1)	0.88 (0.77–0.99)*	0.84 (0.73–0.95)**	0.80 (0.66–0.96)*
P trend				0.06	0.01	0.03

Model 1: no covariates were adjusted.
Model 2: adjusted for gender, age and race.
Model 3: adjusted for gender, age, race, marital status, vigorous and moderate recreational physical activity, education level, annual family income, hypertension, diabetes, BMI (body mass index), smoking, energy, water, dietary intakes of alcohol, caffeine, calcium, phosphate, sodium, potassium, magnesium, vitamins A, C, D, E, K.
*$P < 0.05$, **$P < 0.01$.

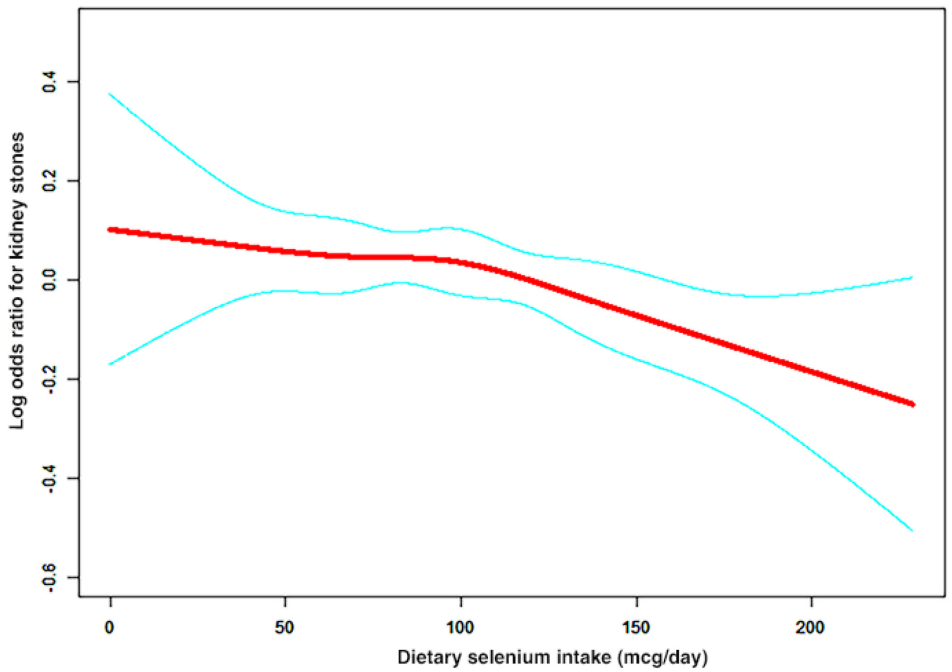

FIGURE 2
The association curve between dietary selenium intakes and the risk of kidney stones. The solid red line represents the smooth curve fit between variables and the blue line represents the 95% of confidence interval from the fit.

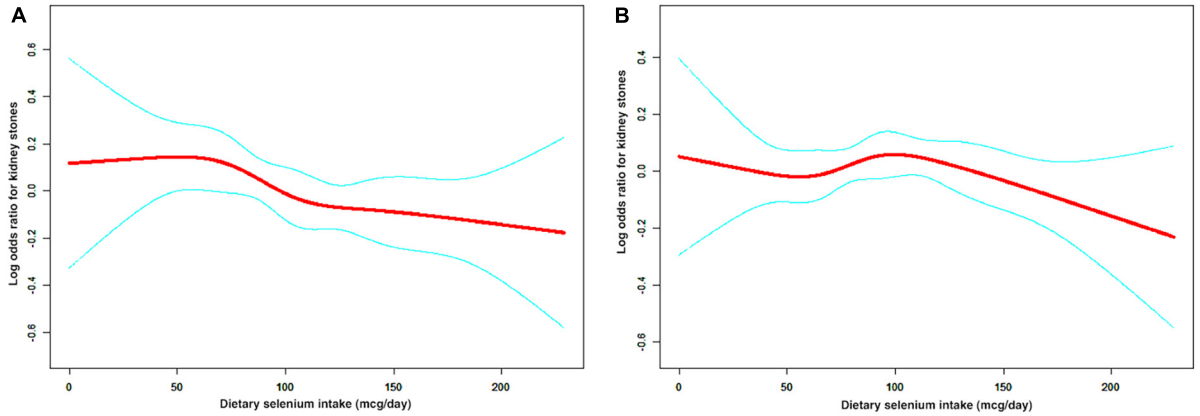

FIGURE 3
The non-linear relationship between dietary selenium intakes and the risk of kidney stones in stratified analysis by age. **(A)** age < 50 years group, **(B)** age ≥ 50 years group.

statistical differences between Q4 and Q1 for individuals with a BMI ≥ 25.0 in Model 1 (OR 0.88, 95% CI 0.77–0.99, $P < 0.05$), Model 2 (OR 0.84, 95% CI 0.73–0.95 $P < 0.01$) and Model 3 (OR 0.80, 95% CI 0.66–0.96, $P < 0.05$). But there were no statistical differences for individuals with a BMI < 25.0.

A non-linear relationship between dietary selenium intake and kidney stones was founded in all participants (Figure 2), younger (Figure 3A), male (Figure 4A) and overweight/obese (Figure 5B) individuals after adjusting for confounding factors. But the non-linear relationship can not be founded in older (Figure 3B), female (Figure 4B) and people with a BMI < 25.0 (Figure 5A). The two-piecewise linear regression model was used to calculate threshold effect and we founded the inflection points were 99.2, 36.3, 57.8, and 47.5 (mcg/day) in all participants, younger, male and overweight/obese individuals, respectively, after an adjustment for potential confounders (Table 3).

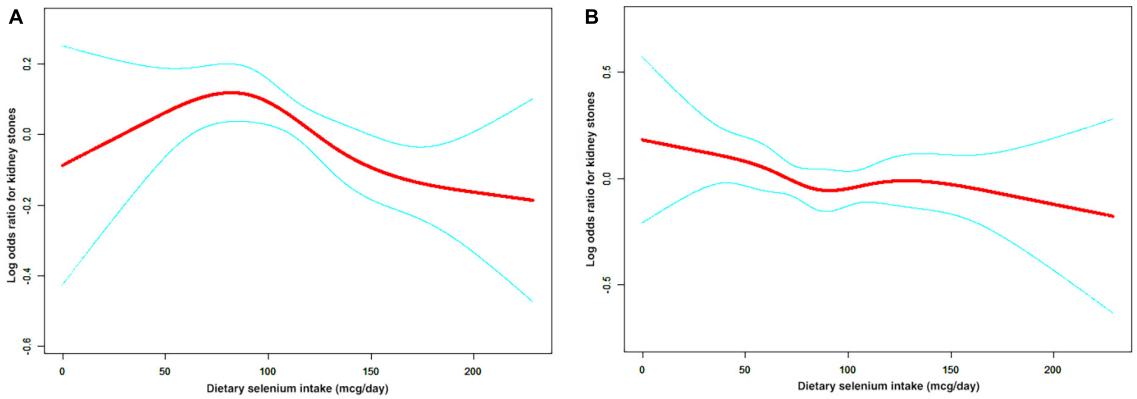

FIGURE 4
The non-linear relationship between dietary selenium intakes and the risk of kidney stones in stratified analysis by sex. **(A)** male group, **(B)** female group.

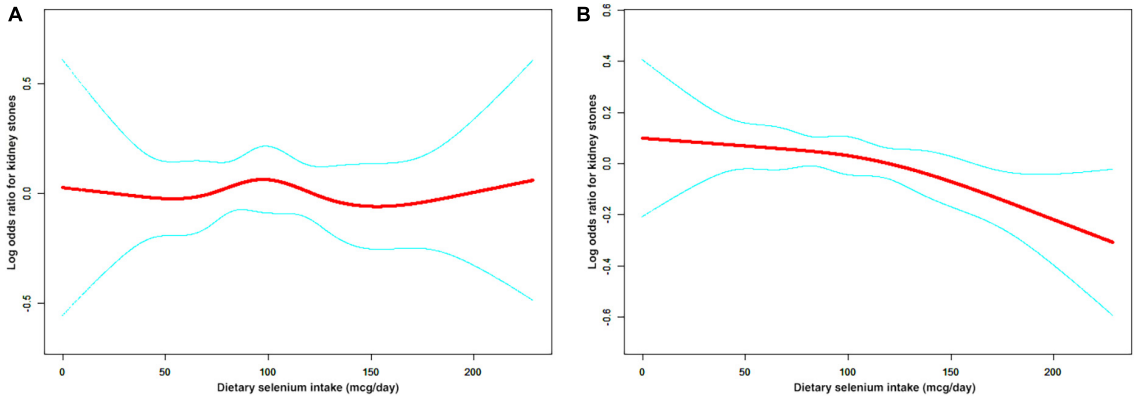

FIGURE 5
The non-linear relationship between dietary selenium intakes and the risk of kidney stones in stratified analysis by BMI. **(A)** BMI < 25.0 group **(B)** BMI ≥ 25.0 group.

Discussion

We confirmed dietary selenium intake was inversely associated with the incidence of kidney stones in adults from the 2007 to 2018 NHANES, which is the first study to discovery that dietary selenium can prevent kidney stones in humans. Furthermore, the stratified analyses indicated there were statistical differences between dietary selenium intake and kidney stones among younger (age < 50), male and overweight/obese (BMI ≥ 25.0) individuals.

With the development of modern endoscopy techniques, the correlation between RP and CaOx stones has been well documented. According to the vascular theory, renal papillary circulation is apt to injury because of turbulent flow, relative hypoxia, and hyperosmolarity environment, which increases the likelihood of atherosclerotic-like reaction and precipitates plaque formation during the repair (24). Experimental studies showed that exposure to high oxalate, CaOx or CaP crystals can induce inflammatory response and biomineralization in renal cells and lots of crystallization modulators and inhibitors participant in crystal nucleation, growth, aggregation and retention. Then nearby cells react to the foreign body by producing reactive oxidative stress (ROS) (25). Once the generation of ROS is uncontrolled or the endogenous antioxidant capacity is decreased, oxidative stress (OS) will be created and may result in inflammation and injury, which increases intracellular levels of antioxidants (25).

Previous studies revealed that RP were a process of biomineralization, sharing similarities with atherosclerotic plaques (24, 26). Plenty of clinical and laboratory studies suggests that OS is also an important feature in the pathogenesis of atherosclerosis. Risk factors for atherosclerosis such as hypertension, diabetes and hypercholesterolemia increase the production of ROS in the arterial wall, leading to oxidative stress (27). Some experiments *in vitro* and vivo support

TABLE 3 Threshold effect analysis of dietary selenium intake on the prevalence of kidney stones using piece-wise linear regression.

Inflection points (mcg/day)	Adjusted* OR (95% CI)	*P*-value
All participants		
<99.2	1.000 (0.997, 1.002)	0.747
≥ 99.2	0.998 (0.996, 0.999)	**0.012**
Age		
<50 years		
<36.3	1.014 (0.992, 1.035)	0.216
≥36.3	0.998 (0.995, 0.999)	**0.041**
≥50 years		
<114.8	1.001 (0.999, 1.003)	0.428
≥114.8	0.997 (0.995, 1.000)	0.053
Gender		
Male		
<57.8	1.006 (0.998, 1.015)	0.149
≥57.8	0.998 (0.996, 0.999)	**0.010**
Female		
<69	0.996 (0.991, 1.001)	0.110
≥69	1.000 (0.997, 1.002)	0.716
BMI (kg/m^2)		
<25.0		
<83.1	1.001 (0.995, 1.007)	0.763
≥83.1	0.999 (0.995, 1.002)	0.539
≥25.0		
<47.5	1.004 (0.996, 1.012)	0.368
≥47.5	0.998 (0.997, 0.999)	**0.010**

*Adjusted for gender, age, race, marital status, vigorous and moderate recreational physical activity, education level, annual family income, hypertension, diabetes, BMI (body mass index), smoking, energy, water, dietary intakes of alcohol, caffeine, calcium, phosphate, sodium, potassium, magnesium, vitamins A, C, D, E, K. The bold values refer to $P < 0.05$, indicating significant statistical differences.

the anti-atherosclerosis effect of selenium mainly owing to its antioxidant properties. As the biologically active form of selenium, selenoproteins can prevent atherosclerosis and CVDs by inhibiting OS, inflammation reaction, endothelial dysfunction and vascular calcification (27). And a meta-analysis of 25 studies showed a significant inverse association between blood selenium status and coronary heart disease risk (28).

It is reasonable to speculate that selenium may inhibit the formation of kidney stones through a similar pathway. In addition, the protective effect of selenium is more evident in young individuals, because the incidence of CVDs in elderly patients is significantly increased, indicating that the ability to resist ectopic calcification is greatly weakened, and the protective effect of selenium on kidney stones is covered up.

Only three animal experiments so far have suggested inverse associations between selenium and CaOx stones. Sakly et al. reported for the first time that intraperitoneal injection of selenium greatly inhibited CaOx deposition in rats (18). Another study indicated that supplementation of vitamin E and selenium reduced the level of renal lipid peroxidation and the activities of oxalate biosynthetic enzymes in rats with feeding calculi producing diet (17). And the lipid peroxide production was associated with renal cell damage that was caused by oxalate and CaOx crystals (29). A recent study in dogs further suggested that hyperoxaluria caused the excessive osteopontin (OPN) expression, and dietary selenium may inhibit CaOx stones by downregulating OPN expression (19). The previous studies concluded that OPN is able to inhibit the formation and retention of CaOx crystal in the kidney *in vivo* (30).

We discovered a relation between kidney stones and dietary selenium intake in men, but not in women. A potential explanation is that sex greatly influences the metabolism of ROS in the body. Several studies found that estrogen and estrogen receptor signaling pathways might suppress oxidative stress-induced renal cell injury (31, 32). Zhu et al. recently reported estrogen receptor β signals may inhibit renal CaOx crystal deposition by reducing OS in tubular cells (33). Compared with males, females mitochondria produced significantly less hydrogen peroxide and higher amounts of GPX, reduced glutathione and manganese superoxide dismutase (34). Therefore, the complex anti-oxidative mechanisms in female might explain our failure to discover any association between kidney stones and dietary selenium. Contrary to estrogen, androgen and androgen receptor signaling might influence the anabolism of ROS, leading to oxidative stress-induced renal injury and further renal CaOx crystal deposition (35). But selenoproteins can prevent renal CaOx deposition in males by inhibiting OS. Selenium has been known to support the production of testosterone in laboratory and animal models (36), not in humans. And which mechanism dominates remains to be proved.

Furthermore, some animal studies indicated sexual differences in selenium distribution and selenoprotein expression in various organs are distinctly different, which vary with selenium status and individual age (37, 38). Selenoprotein P (SeP) participates in the storage and transport of about 60% of plasma selenium (39). Lutz et al discovered that renal SeP mRNA concentrations were 1.7-fold higher in male mice than in female mice, and the difference is sustained with age. Meanwhile, renal mRNA concentrations of Glutathione peroxidase 1 (GPX1) and Glutathione peroxidase 3 (GPX3) displayed no significant sex differences in both young and old mice (37).

We also discovered that dietary selenium intake was inversely associated with kidney stones in overweight/obese individuals. Numerous findings have confirmed that BMI is related to the incidence of urolithiasis in the last decade (40–42). The accumulation of visceral fat is a risk factor for nephrolithiasis (43), and mounting evidence links adipose cells to urinary stone formation (44, 45). Fatty acid binding protein 4 (*FABP4*), mainly expressed in adipocytes and macrophages, is involved in lipid transfer and transport and significantly

correlates with plasma lipid levels (46). A recent study demonstrated that lipid metabolism in renal papillary tissue containing RP was impaired, which was associated with the downregulation of *FABP4* based on immunohistochemistry of human renal tissue, microarray analysis of nephrocalcinosis model mice and *FABP4* knockout mice (47).

Previous studies observed a positive correlation between plasma selenium levels and total, TG and LDL cholesterol (46, 48), although there were inconsistent for HDL cholesterol in some studies (49). Galan-Chilet et al. found statistical interactions of selenium status with genetic variation (such as *FABP4*) in lipid metabolic pathways, indicating potentially interconnected pathways in selenium and lipid transport and transfer (46). Another study showed a positive association between *FABP4* and plasma selenium levels and a negative association between *FABP4* and GPX3 activity in Indonesian men with visceral obesity, suggesting selenium status may play different roles in obese people, such as in OS condition and inflammatory process (50).

To our knowledge, this is the first population-based study to examine the association between dietary selenium intake and the risk of kidney stones. Using data from large and consecutive nationally representative surveys, we can assess the associations between selenium and kidney stones by both quartile analysis and dose-response analysis after adjusting potential confounding variables. However, the limitations of the study deserve mention. Firstly, causality cannot be proved because of the cross-sectional survey study. Secondly, selection bias cannot be completely avoided because the formation of kidney stones is affected by numerous factors. Thirdly, a person's long-term intake habits may not be set in stone, and cannot be accurately described by a single 24-h dietary recall, meaning that kidney stones may have occurred before participants changed their dietary habits. However, data from NHANES has been used for decades in epidemiological studies and health sciences research. Fourthly, our findings are only representative of the United States population. Because human dietary selenium intake varies by country, depending on soil and geography (12). Fifthly, no information is available on stone composition that may further illuminate the relationship between selenium and kidney stones. Sixthly, we can't know whether dietary selenium intakes can prevent phosphate stones, urate stones and cystine stones, relieve renal colic, or affect the recurrence rate of kidney stones in children and in heredity. Finally, despite the biological plausibility, additional longitudinal and laboratory studies are needed to confirm our results and elucidate the potential mechanisms.

Conclusion

Our study revealed an inverse relation between the level of dietary selenium intake and the risk of kidney stones for the United States population, especially for younger (age < 50), male and overweight/obese (BMI ≥ 25.0) individuals. Increasing dietary selenium intake might be meaningful to the prevention of kidney stones. Further studies are required to confirm our findings and clarified the biological mechanisms.

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.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board of the National Center for Health Statistics (NCHS). The patients/participants provided their written informed consent to participate in this study.

Author contributions

ML: project development, data analysis, and manuscript writing. ZC, JC, and MG: data collection and analysis. ZZ and HC: project development and manuscript editing. All authors contributed to the article and approved the submitted version.

Funding

Funding was provided by the National Natural Science Foundation of China (82170781 to HC), Natural Science Foundation of Hunan Province (2021JJ31050 to HC), and the Fundamental Research Funds for the Central Universities of Central South University (2022ZZTS0294 to ML).

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

References

1. Scales CD, Smith AC, Hanley JM, Saigal CS. Prevalence of kidney stones in the United States. *Eur Urol.* (2012). 62:160–5. doi: 10.1016/j.eururo.2012.03.052

2. Fink HA, Wilt TJ, Eidman KE, Garimella PS, MacDonald R, Rutks IR, et al. Medical management to prevent recurrent nephrolithiasis in adults: a systematic review for an American college of physicians clinical guideline. *Ann Intern Med.* (2013) 158:535–43. doi: 10.7326/0003-4819-158-7-201304020-00005

3. Lotan Y. Economics and cost of care of stone disease. *Adv Chron Kidney Dis.* (2009) 16:5–10. doi: 10.1053/j.ackd.2008.10.002

4. Khan SR, Pearle MS, Robertson WG, Gambaro G, Canales BK, Doizi S, et al. Kidney stones. *Nat Rev Dis Prim.* (2016) 2:16008. doi: 10.1038/nrdp.2016.8

5. Faggiano A, Pivonello R, Melis D, Filippella M, Di Somma C, Petretta M, et al. Nephrolithiasis in Cushing's disease: prevalence, etiopathogenesis, and modification after disease cure. *J Clin Endocrinol Metab.* (2003) 88:2076–80. doi: 10.1210/jc.2002-021494

6. Bilezikian JP, Bandeira L, Khan A, Cusano NE. Hyperparathyroidism. *Lancet.* (2018) 391:168–78. doi: 10.1016/S0140-6736(17)31430-7

7. Ferraro PM, Taylor EN, Gambaro G, Curhan GC. Dietary and lifestyle risk factors associated with incident kidney stones in men and women. *J Urol.* (2017) 198:858–63. doi: 10.1016/j.juro.2017.03.124

8. Skolarikos A, Straub M, Knoll T, Sarica K, Seitz C, Petřík A, et al. Metabolic evaluation and recurrence prevention for urinary stone patients: EAU guidelines. *Eur Urol.* (2015) 67:750–63. doi: 10.1016/j.eururo.2014.10.029

9. Kieliszek M, Błażejak S. Current knowledge on the importance of selenium in food for living organisms: a review. *Molecules.* (2016) 21:609. doi: 10.3390/molecules21050609

10. Kieliszek M, Bano I, Zare H. A comprehensive review on selenium and its effects on human health and distribution in Middle Eastern countries. *Biol Trace Elem Res.* (2022) 200:971–87. doi: 10.1007/s12011-021-02716-z

11. Kieliszek M, Błażejak S. Selenium: significance, and outlook for supplementation. *Nutrition.* (2013) 29:713–8. doi: 10.1016/j.nut.2012.11.012

12. Rayman MP. Selenium and human health. *Lancet.* (2012) 379:1256–68. doi: 10.1016/S0140-6736(11)61452-9

13. Vinceti M, Filippini T, Del Giovane C, Dennert G, Zwahlen M, Brinkman M, et al. Selenium for preventing cancer. *Cochrane Database Syst Rev.* (2018) 1:CD005195. doi: 10.1002/14651858.CD005195.pub4

14. Steinbrenner H, Duntas LH, Rayman MP. The role of selenium in type-2 diabetes mellitus and its metabolic comorbidities. *Redox Biol.* (2022) 50:102236. doi: 10.1016/j.redox.2022.102236

15. Vinceti M, Filippini T, Wise LA, Rothman KJ. A systematic review and dose-response meta-analysis of exposure to environmental selenium and the risk of type 2 diabetes in nonexperimental studies. *Environ Res.* (2021) 197:111210. doi: 10.1016/j.envres.2021.111210

16. Alexander RT, Hemmelgarn BR, Wiebe N, Bello A, Samuel S, Klarenbach SW, et al. Kidney stones and cardiovascular events: a cohort study. *Clin J Am Soc Nephrol.* (2014) 9:506–12. doi: 10.2215/CJN.04960513

17. Santhosh Kumar M, Selvam R. Supplementation of vitamin E and selenium prevents hyperoxaluria in experimental urolithic rats. *J Nutr Biochem.* (2003) 14:306–13. doi: 10.1016/S0955-2863(03)00033-0

18. Sakly R, Chaouch A, el Hani A, Najjar MF. Effects of intraperitoneally administered vitamin E and selenium on calcium oxalate renal stone formation: experimental study in rat. *Ann Urol.* (2003) 37:47–50. doi: 10.1016/S0003-4401(03)00007-X

19. Liu Y, Xu H, Zhong W, Shen Q, Zhuang T, Huang K. Organic selenium alleviated the formation of ethylene glycol-induced calcium oxalate renal calculi by improving osteopontin expression and antioxidant capability in dogs. *Biol Trace Elem Res.* (2015) 168:392–400. doi: 10.1007/s12011-015-0373-9

20. Ferraro PM, Curhan GC, Gambaro G, Taylor EN. Total, dietary, and supplemental vitamin C intake and risk of incident kidney stones. *Am J Kidney Dis.* (2016) 67:400–7. doi: 10.1053/j.ajkd.2015.09.005

21. Sun Y, Wang Y, Wang D, Zhou Q. Dietary zinc intake, supplemental zinc intake and serum zinc levels and the prevalence of kidney stones in adults. *J Trace Elem Med Biol.* (2020) 57:126410. doi: 10.1016/j.jtemb.2019.126410

22. Desquilbet L, Mariotti F. Dose-response analyses using restricted cubic spline functions in public health research. *Stat Med.* (2010) 29:1037–57. doi: 10.1002/sim.3841

23. Liu S, Wang X, Lu Y, Li T, Gong Z, Sheng T, et al. The effects of intraoperative cryoprecipitate transfusion on acute renal failure following orthotropic liver transplantation. *Hepatol Int.* (2013) 7:901–9. doi: 10.1007/s12072-013-9457-9

24. Khan SR, Canales BK, Dominguez-Gutierrez PR. Randall's plaque and calcium oxalate stone formation: role for immunity and inflammation. *Nat Rev Nephrol.* (2021) 17:417–33. doi: 10.1038/s41581-020-00392-1

25. Khan SR. Reactive oxygen species as the molecular modulators of calcium oxalate kidney stone formation: evidence from clinical and experimental investigations. *J Urol.* (2013) 189:803–11. doi: 10.1016/j.juro.2012.05.078

26. Khan SR, Gambaro G. Role of osteogenesis in the formation of Randall's plaques. *Anat Rec.* (2016) 299:5–7. doi: 10.1002/ar.23275

27. Liu H, Xu H, Huang K. Selenium in the prevention of atherosclerosis and its underlying mechanisms. *Metallomics.* (2017) 9:21–37. doi: 10.1039/C6MT00195E

28. Flores-Mateo G, Navas-Acien A, Pastor-Barriuso R, Guallar E. Selenium and coronary heart disease: a meta-analysis. *Am J Clin Nutr.* (2006) 84:762–73. doi: 10.1093/ajcn/84.4.762

29. Thamilselvan S, Hackett RL, Khan SR. Lipid peroxidation in ethylene glycol induced hyperoxaluria and calcium oxalate nephrolithiasis. *J Urol.* (1997) 157:1059–63. doi: 10.1016/S0022-5347(01)65141-3

30. Wesson JA, Johnson RJ, Mazzali M, Beshensky AM, Stietz S, Giachelli C, et al. Osteopontin is a critical inhibitor of calcium oxalate crystal formation and retention in renal tubules. *J Am Soc Nephrol.* (2003) 14:139–47. doi: 10.1097/01.ASN.0000040593.93815.9D

31. Aufhauser DD, Wang Z, Murken DR, Bhatti TR, Wang Y, Ge G, et al. Improved renal ischemia tolerance in females influences kidney transplantation outcomes. *J Clin Investig.* (2016) 126:1968–77. doi: 10.1172/JCI84712

32. Park KM, Kim JI, Ahn Y, Bonventre AJ, Bonventre JV. Testosterone is responsible for enhanced susceptibility of males to ischemic renal injury. *J Biol Chem.* (2004) 279:52282–92. doi: 10.1074/jbc.M407629200

33. Zhao Z, Mai Z, Ou L, Duan X, Zeng G. Serum estradiol and testosterone levels in kidney stones disease with and without calcium oxalate components in naturally postmenopausal women. *PLoS One.* (2013) 8:e75513. doi: 10.1371/journal.pone.0075513

34. Viña J, Sastre J, . Pallardó F, Borrás C. Mitochondrial theory of aging: importance to explain why females live longer than males. *Antioxid Redox Signal.* (2003) 5:549–56. doi: 10.1089/152308603770310194

35. Liang L, Li L, Tian J, Lee SO, Dang Q, Huang CK, et al. Androgen receptor enhances kidney stone-CaOx crystal formation *via* modulation of oxalate biosynthesis & oxidative stress. *Mol Endocrinol.* (2014) 28:1291–303. doi: 10.1210/me.2014-1047

36. Seale LA, Ogawa-Wong AN, Berry MJ. Sexual dimorphism in selenium metabolism and selenoproteins. *Free Radic Biol Med.* (2018) 127:198–205. doi: 10.1016/j.freeradbiomed.2018.03.036

37. Schomburg L, Riese C, Renko K, Schweizer U. Effect of age on sexually dimorphic selenoprotein expression in mice. *Biol Chem.* (2007) 388:1035–41. doi: 10.1515/BC.2007.128

38. Pinto RE, Bartley W. Changes in glutathione reductase and glutathione peroxidase activities in rat liver related to age and sex. *Biochem J.* (1968) 109:34. doi: 10.1042/bj1090034Pa

39. Bodnar M, Konieczka P, Namiesnik J. The properties, functions, and use of selenium compounds in living organisms. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev.* (2012) 30:225–52. doi: 10.1080/10590501.2012.705164

40. Taylor EN, Stampfer MJ, Curhan GC. Obesity, weight gain, and the risk of kidney stones. *JAMA.* (2005) 293:455–62. doi: 10.1001/jama.293.4.455

41. Yoshimura E, Sawada SS, Lee IM, Gando Y, Kamada M, Matsushita M, et al. Body mass index and kidney stones: a cohort study of Japanese men. *J Epidemiol.* (2016) 26:131–6. doi: 10.2188/jea.JE20150049

42. Siener R, Glatz S, Nicolay C, Hesse A. The role of overweight and obesity in calcium oxalate stone formation. *Obes Res.* (2004) 12:106–13. doi: 10.1038/oby.2004.14

43. Fujimura M, Sakamoto S, Sekita N, Mikami K, Ichikawa T, Akakura K. Visceral fat accumulation is a risk factor for urinary stone. *Int J Urol.* (2014) 21:1184–5. doi: 10.1111/iju.12556

44. Zuo L, Tozawa K, Okada A, Yasui T, Taguchi K, Ito Y, et al. A paracrine mechanism involving renal tubular cells, adipocytes and macrophages promotes kidney stone formation in a simulated metabolic syndrome environment. *J Urol.* (2014) 191:1906–12. doi: 10.1016/j.juro.2014.01.013

45. Ichikawa J, Okada A, Taguchi K, Fujii Y, Zuo L, Niimi K, et al. Increased crystal-cell interaction *in vitro* under co-culture of renal tubular

cells and adipocytes by *in vitro* co-culture paracrine systems simulating metabolic syndrome. *Urolithiasis.* (2014) 42:17–28. doi: 10.1007/s00240-013-0612-5

46. Galan-Chilet I, Guallar E, Martin-Escudero JC, De Marco G, Dominguez-Lucas A, Gonzalez-Manzano I, et al. Do genes modify the association of selenium and lipid levels? *Antioxid Redox Signal.* (2015) 22:1352–62. doi: 10.1089/ars.2015.6248

47. Taguchi K, Chen L, Usawachintachit M, Hamamoto S, Kang M, Sugino T, et al. Fatty acid-binding protein 4 downregulation drives calcification in the development of kidney stone disease. *Kidney Int.* (2020) 97:1042–56. doi: 10.1016/j.kint.2020.01.042

48. Laclaustra M, Stranges S, Navas-Acien A, Ordovas JM, Guallar E. Serum selenium and serum lipids in US adults: national health and nutrition examination survey (NHANES) 2003-2004. *Atherosclerosis.* (2010) 210:643–8. doi: 10.1016/j.atherosclerosis.2010.01.005

49. Salonen JT, Salonen R, Seppänen K, Kantola M, Parviainen M, Alfthan G, et al. Relationship of serum selenium and antioxidants to plasma lipoproteins, platelet aggregability and prevalent ischaemic heart disease in Eastern Finnish men. *Atherosclerosis.* (1988) 70:155–60. doi: 10.1016/0021-9150(88)90109-8

50. Mutakin, Meiliana A, Wijaya A, Kobayashi K, Yamazaki C, Kameo S, et al. Association between selenium nutritional status and metabolic risk factors in men with visceral obesity. *J Trace Elem Med Biol.* (2013) 27:112–6. doi: 10.1016/j.jtemb.2012.09.006

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 22 July 2022
DOI 10.3389/fnut.2022.889303

OPEN ACCESS

EDITED BY
Francesco Sofi,
Università degli Studi di Firenze, Italy

REVIEWED BY
Yujun Shi,
Sichuan University, China
Zaleha Md Isa,
National University of Malaysia, Malaysia
Fatemeh Mohammadi-Nasrabadi,
National Nutrition and Food Technology Research Institute, Iran

*CORRESPONDENCE
Guo-Chao Zhong
gczhong1991@stu.cqmu.edu.cn
Jun-Hua Gong
2019010025@stu.cqmu.edu.cn

SPECIALTY SECTION
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

RECEIVED 04 March 2022
ACCEPTED 29 June 2022
PUBLISHED 22 July 2022

CITATION
Zhong G-C, Zhu Q, Gong J-P, Cai D, Hu J-J, Dai X and Gong J-H (2022) Fried food consumption and the risk of pancreatic cancer: A large prospective multicenter study. *Front. Nutr.* 9:889303. doi: 10.3389/fnut.2022.889303

Fried food consumption and the risk of pancreatic cancer: A large prospective multicenter study

Guo-Chao Zhong[1]*, Qian Zhu[2], Jian-Ping Gong[1], Dong Cai[1], Jie-Jun Hu[1], Xin Dai[1] and Jun-Hua Gong[1]*

[1]Department of Hepatobiliary Surgery, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China, [2]Department of Epidemiology and Biostatistics, School of Public Health and Management, Chongqing Medical University, Chongqing, China

Background and aims: Whether fried food consumption is associated with the risk of pancreatic cancer remains elusive. We aimed to examine this association in a US population.

Methods: A population-based cohort of 101,729 US adults was identified. Fried food consumption was assessed with a validated food frequency questionnaire. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated. Explanatory analyses were conducted to identify main contributor(s) to the observed association.

Results: During an average follow-up of 8.86 years (900871.2 person-years), 402 pancreatic cancer cases occurred. High consumption of total fried foods (deep-fried plus pan-fried foods; $HR_{quartile4\ vs.\ 1}$ 0.71, 95% CI 0.51–0.99, P_{trend} = 0.047) and deep-fried foods ($HR_{quartile\ 4\ vs.\ 1}$ 0.64, 95% CI 0.47–0.88, P_{trend} = 0.011), but not pan-fried foods ($HR_{quartile\ 4\ vs.\ 1}$ 0.98, 95% CI 0.73–1.32; P_{trend} = 0.815), was found to be associated with a reduced risk of pancreatic cancer in a non-linear dose–response manner, which was not modified by predefined stratification factors and persisted in sensitivity analyses. In explanatory analyses, only chip consumption was found to be inversely associated with the risk of pancreatic cancer; consistently, the initial significant associations between total fried food and deep-fried food consumption and the risk of pancreatic cancer changed to be non-significant after omitting or further adjusting for chip consumption.

Conclusion: Consumption of deep-fried foods, but not pan-fried foods, is inversely associated with the risk of pancreatic cancer in this US population. The role of deep-fried foods in reducing the risk of pancreatic cancer appears to be mainly attributable to chips. More studies are needed to confirm our findings in other populations and settings.

KEYWORDS

fried foods, chips, pancreatic cancer, prospective study, nutritional epidemiology

Introduction

Frying is a commonly used cooking method in Western countries and makes foods more palatable and crunchy. Frying changes the composition of foods and their frying media through polymerization, hydrogenation, and oxidation (1), which produce some compounds thought to be potentially carcinogenic (e.g., acrylamide and heterocyclic amines). The potential carcinogenic role of fried foods has been suggested by several case-control studies, all of which revealed a positive association between fried food consumption and the risk of cancer, including pharyngolaryngeal cancer (2, 3), gastric cancer (4), gallbladder cancer (5), and prostate cancer (6). Moreover, individuals with high fried food consumption were found to be at elevated risks of obesity and type 2 diabetes (7, 8), two well-known risk factors for cancer (9). Together, these data suggest that fried foods are potentially carcinogenic.

Pancreatic cancer is the third most common cause of cancer-related mortality in the US, with an estimated 48,220 cancer deaths in 2021 (10). Diets have been indicated to play an important role in the etiology of this cancer (11), which is in accordance with our recent findings on dietary behaviors and pancreatic cancer (12–14). The association of fried food consumption with the risk of pancreatic cancer has been evaluated in an early case-control study in Canada, with a positive association observed (15). However, this study has a small sample size (418 individuals), which makes it be prone to small study effects (i.e., small studies tend to show larger risk estimates and are performed with less methodological rigor than large studies) (16). More importantly, case–control studies are susceptible to recall bias and cannot establish a temporal association.

Clarifying the potential association between fried food consumption and the risk of pancreatic cancer is critical for public health, considering the dismal prognosis of this cancer and that more than one-third of Americans patronize fast food restaurants daily where fried foods comprise the majority of items sold (8, 17). Therefore, we performed a prospective multicenter cohort study to test an *a priori* hypothesis that high fried food consumption is associated with an increased risk of pancreatic cancer in a US population.

Materials and methods

Our results were reported following the Strengthening the Reporting of Observational Studies in Epidemiology statement (18).

Study population

The study population was derived from the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, which was developed for determining the potential beneficial roles of selected screening tests in reducing mortality from PLCO cancers. The PLCO Cancer Screening Trial is a multicenter randomized clinical study, whose study design has been described elsewhere (19). Briefly, between November 1993 and September 2001, individuals 55–74 years of age were invited to participate in this trial in ten screening centers across the US. As shown in **Supplementary Table 1**, there were significant differences in some sociodemographic characteristics (e.g., sex and ethic group) between study centers, indicating that study population of the PLCO Cancer Screening Trial was heterogeneous at the level of study center. A total of 154,887 individuals were finally enrolled based on the predefined eligibility criteria (**Figure 1**). Enrolled individuals were individually randomized to the intervention group or the control group in equal proportions, with those in the intervention group receiving screening tests for PLCO cancers while those in the control group receiving usual care. The PLCO Cancer Screening Trial was approved by institutional review boards of the US National Cancer Institute and each screening center. All participants provided written informed consent.

In this study, following participants were further excluded: (1) 36,076 participants failing to complete a diet history questionnaire (DHQ); (2) 5,364 participants with an invalid DHQ, which refers to death date prior to DHQ completion date, more than eight missing frequency responses, missing the date of completion, and/or extreme values of energy intake (the first or last percentile); (3) 1,940 participants failing to complete a baseline questionnaire; (4) 115 participants with outcome events (incident pancreatic cancer, loss to follow-up, or death) observed between randomization and DHQ completion; (5) 9,644 participants receiving a diagnosis of cancer before DHQ completion; and (6) 19 participants with a diagnosis of endocrine pancreatic cancer. Finally, a total of 101,729 participants were qualified for inclusion (**Figure 1**). Notably, a comparison between included and excluded populations found that standardized differences of most sociodemographic characteristics were less than 0.1 (20), indicating that the potential for non-participation bias resulting from the exclusion of a huge number of participants was low (**Supplementary Table 2**).

Dietary assessment

Dietary assessment was performed at baseline using the aforementioned DHQ (version 1.0, National Cancer Institute), which is a 124-item self-reported food frequency questionnaire used for evaluating the frequency and portion size of an individual's food consumption during the past year. The Eating at America's Table Study had compared the performance of the DHQ with four 24-h dietary recalls in a nationally representative sample of 1,640 subjects; the corresponding results showed that the DHQ had good performance in the evaluation of dietary

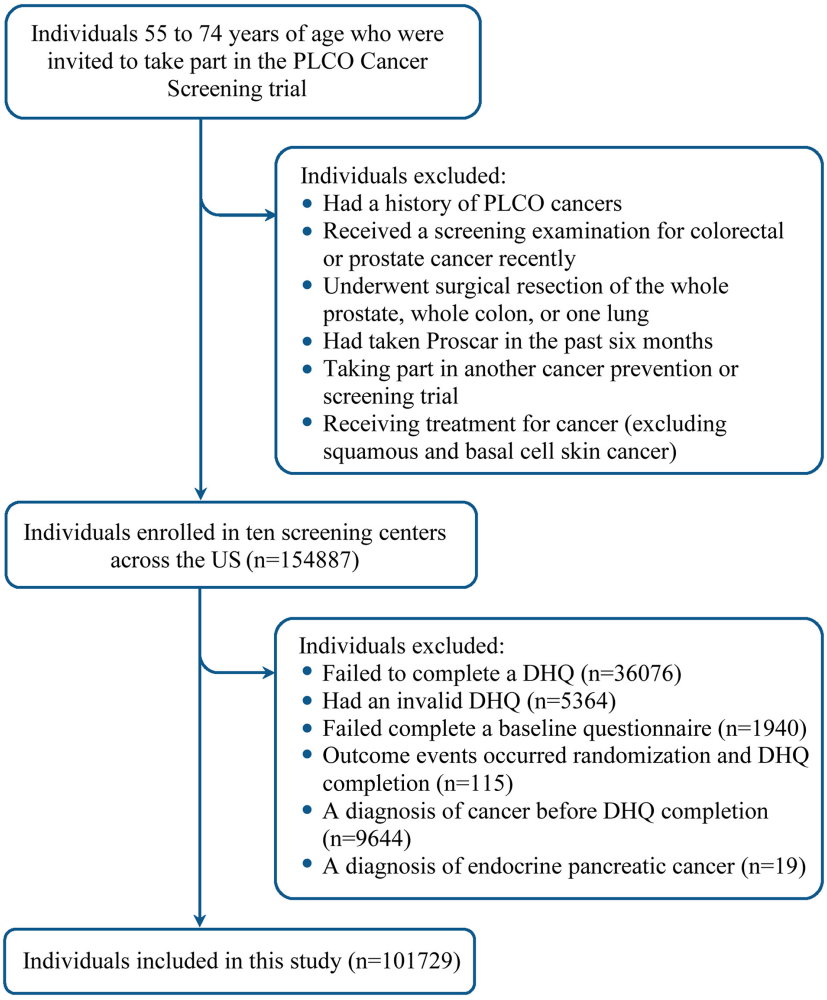

FIGURE 1
Flow chart identifying participants included in this study. PLCO, Prostate, Lung, Colorectal, and Ovarian; DHQ, diet history questionnaire.

intake (21). Daily food consumption was approximated by multiplying food frequency by portion size; daily intakes of nutrients and energy were estimated on the basis of two nutrient databases, namely Nutrition Data Systems for Research (22) and US Department of Agriculture's 1994–1996 Continuing Survey of Food Intakes by Individuals (23). Healthy Eating Index-2015, a frequently used index reflecting an individual's diet quality, was computed as described previously (24). To eliminate the extraneous variation of dietary data caused by energy intake, food consumption and nutrient intake were adjusted for energy intake using the residual method before the formal statistical analyses (25).

Fried foods in the DHQ were categorized into deep-fried and pan-fried foods. A given fried food was assumed to be deep-fried if its frying type was not mentioned (26). Thus, in this study, deep-fried chicken, fried fish (including fried seafood or shellfish), fried potato, and chips were classified into deep-fried foods, while pan-fried bacon, pan-fried chicken, pan-fried hamburger, pan-fried pork chops, pan-fried sausage, and pan-fried steak were classified into pan-fried foods. Here, fried potatoes referred to French fries, home fries, tater tots, or hash browns; chips referred to potato chips, tortilla chips, or corn chips. To investigate the potential impacts of doneness degree, we further divided pan-fried foods into the following three categories: just done, well done, and very well done. Total fried food consumption was calculated as the sum of consumption of deep-fried and pan-fried foods.

Ascertainment of pancreatic cancer

Pancreatic cancer was ascertained predominantly through an annual study update form, which was mailed to participants by each screening center for inquiring whether they were diagnosed with pancreatic cancer, and if so, the date and location of diagnosis and contact information of their healthcare

providers. Cancers reported on the annual study update form were further ascertained by scrutinizing any available medical records. In addition, death certificates and family reports were used as **Supplementary Material** for cancer ascertainment. To reduce the heterogeneity of pancreatic cancer cases, only participants with a diagnosis of exocrine pancreas cancer (ICD-O-2 codes: C25.0-C25.3 and C25.7-C25.9) were considered.

Assessment of non-dietary variables

Sex, ethnicity, educational degree, body weight, height, smoking status, pack-years, history of diabetes, family history of pancreatic cancer, and aspirin use, were assessed with a self-reported baseline questionnaire. Body mass index was computed by dividing body weight (kg) by height squared (m2). Physical activity level was expressed as total time of moderate-to-vigorous activity each week, which was assessed through a self-reported supplemental questionnaire. Age at DHQ completion and alcohol consumption were assessed with the above DHQ.

Statistical analysis

To reduce the potential biases and increase the statistical power, we used the following methods to impute missing values. Specifically, for variables with less than 5% missing values, we used the modal value and the median to impute missing values of categorical and continuous variables, respectively; for the variable "physical activity level" that had 25.69% missing values, we assumed that these values were missing at random and then used multiple imputation with chained equations to impute them, with the number of imputations set at 25 (27). The distribution of variables with missing values before and after imputation is shown in **Supplementary Table 3**. To examine the potential influence of data imputation on our results, main statistical analyses were repeated in the population with complete covariate data.

Cox proportional hazards regression was used to compute hazard ratios (HRs) with 95% confidence intervals (CIs) of the association of fried food consumption with the risk of pancreatic cancer, with follow-up length as time metric. Follow-up length was computed from the date of DHQ completion to the date of pancreatic cancer diagnosis, study dropout, death, or the end of follow-up (December 31, 2009), whichever occurred first (**Figure 2**). In regression analyses, fried food consumption was split into quartiles and the first quartile was set as the reference group. A P for linear trend across quartiles was computed by modeling the median of fried food consumption in each quartile as a continuous variable. The proportional hazards assumption of Cox regression was found to be satisfied after examination of Schoenfeld residuals (P for global test > 0.05) (28). As recommended, covariate selection for multivariable analyses was based on our causal knowledge of the existing literature rather than statistical criteria (29). Specifically, model 1 adjusted for age and sex; model 2 further adjusted for well-known factors associated with the risk of pancreatic cancer, namely smoking status, alcohol consumption, body mass index, aspirin use, history of diabetes, family history of pancreatic cancer, and energy intake from diet. To reflect how robust the observed association was to the unmeasured confounding, the E-value was computed using an online calculator[1] (30). The E-value estimates what the minimum HR would have to be for any unmeasured confounder to negate the observed association of fried food consumption with the risk of pancreatic cancer (30).

Restricted cubic spline regression with knots located at the 10th, 50th, and 90th percentiles were used to explore the potential dose–response association between fried food consumption and the risk of pancreatic cancer, with the reference level set at 0 g/day. Of note, the choice of the number of knots was based on the Akaike's information criterion in this study (31), with the lowest value indicating the best fitted model. To eliminate the potential impacts of extreme values, participants with extreme fried food consumption (top 2.5% or bottom 2.5%) were excluded from the dose–response analysis. A P for non-linearity was computed by testing whether the estimated value of the second spline equals 0.

To determine the potential effect modifiers of the observed association of fried food consumption with the risk of pancreatic cancer, prespecified subgroup analyses were performed after stratifying for age ($\geq$ 65 vs. $<$ 65 years), sex (male vs. female), body mass index ($\geq$ 25 vs. $<$ 25), aspirin use (yes vs. no), smoking status (current or past vs. never), alcohol consumption ($\geq$ median vs. $<$ median), and trial group (intervention vs. control groups). A P for interaction was computed by comparing models with and without interaction terms prior to conducting the formal subgroup analyses to avert possibly spurious subgroup differences.

The following sensitivity analyses were performed to evaluate the robustness of our results: (1) excluded participants with extreme values of energy intake, defined as $<$ 800 or $>$ 4,000 kcal/day for males and $<$ 500 or $>$ 3,500 kcal/day for females (32); (2) excluded participants with extreme fried food consumption as defined as above; (3) excluded pancreatic cancer cases observed within the first 2 years of follow-up to test the potential influence of reverse causation; (4) excluded participants receiving a diagnosis of pancreatic cancer following other cancer diagnoses; (5) repeated the analysis with energy-unadjusted fried food consumption; (6) repeated the analysis with sex-specific quartiles, as the distribution of fried food consumption was found to be different by sex; (7) further adjusted for Healthy Eating Index-2015 on model 2 to test whether the observed association was mediated by diet

1 www.evalue-calculator.com/

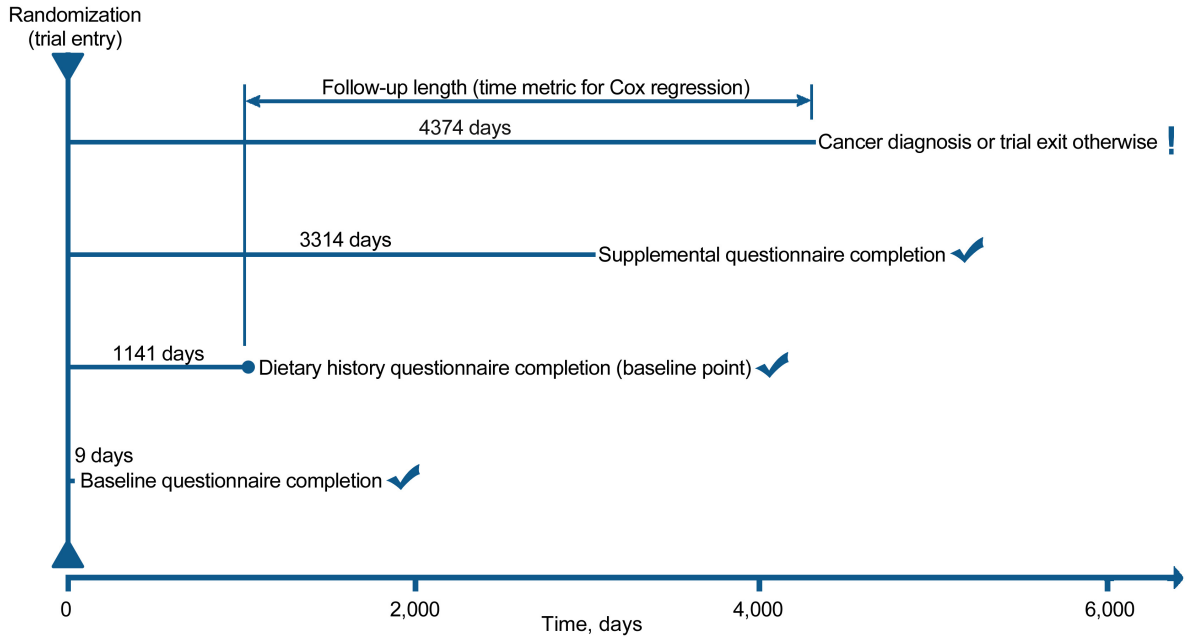

FIGURE 2
The timeline and follow-up scheme of our study.

quality; and (8) further adjusted for physical activity level and consumption of fruits, vegetables, red and processed meat, and coffee on model 2.

To identify the main contributor(s) to the observed association, the following explanatory analyses were performed: (1) examined the association of individual fried food consumption with the risk of pancreatic cancer; (2) examined the association of fried food consumption with the risk of pancreatic cancer after further adjusting for or omitting an individual fried food. A statistical significance level of $P < 0.05$ was used under a two-tailed test. All statistical analyses were performed using STATA software (version 12.0, StataCorp LP, College Station, Texas).

Results

Participant characteristics

The mean (standard deviation) energy-adjusted consumption of total fried foods, deep-fried foods, and pan-fried foods in the whole study population was 24.3 (28.7) g/day, 17.2 (23.2) g/day, and 7.1 (11.9) g/day, respectively. Baseline characteristics of study population are shown in Table 1. Compared with participants in the lowest quartile of total fried consumption, those in the highest quartile were more likely to be male, be current or past smokers, and be aspirin users, had lower levels of education and physical activity and lower Healthy Eating Index-2015 but had higher body mass index, alcohol consumption, and energy intake from diet, and were more likely to have a history of diabetes. Moreover, participants in the highest vs. the lowest quartiles of total fried food consumption had higher consumption of vegetables, red processed meat, coffee, and nuts but lower consumption of fruits. When study population was classified by deep-fried food or pan-fried food consumption, a similar phenomenon was observed (data not shown).

Fried food consumption and the risk of pancreatic cancer

During an average (standard deviation) follow-up of 8.86 (1.91) years (900871.2 person-years), a total of 402 pancreatic cancer cases were observed, with the overall incidence rate of 4.46 cases per 10,000 person-years. The results of Cox regression in the whole study population are summarized in Table 2. In the fully adjusted model (model 2), participants in the highest quartile of total fried food consumption were found to have a 29% lower risk of pancreatic cancer than those in the lowest quartile ($HR_{quartile\ 4vs.\ 1}$ 0.71, 95% CI 0.51–0.99, P_{trend} = 0.047, *E*-value = 2.17). Similar results were obtained for deep-fried foods ($HR_{quartile\ 4vs.\ 1}$ 0.64, 95% CI 0.47–0.88, P_{trend} = 0.011, *E*-value = 2.50). However, no significant association was found for pan-fried food consumption and the risk of pancreatic cancer ($HR_{quartile\ 4vs.\ 1}$ 0.98, 95% CI 0.73–1.32, P_{trend} = 0.815, *E*-value = 1.16), which did

10.3389/fnut.2022.889303

TABLE 1 Characteristics of study population based on quartiles of energy-adjusted total fried food consumption[a].

Characteristics	Quartiles of energy-adjusted total fried food consumption, range (mean), g/day				
	<6.10 (2.6)	6.1 – <15.1 (10.2)	15.1 – ≤31.8 (22.2)	>31.8 (61.9)	*P* for differences across quartiles
Number of participants	25,433	25,432	25,432	25,432	
Age (years)	66.5 ± 5.9	65.8 ± 5.7	65.3 ± 5.6	64.4 ± 5.5	<0.001
Male	7,526 (29.6)	10,577 (41.6)	13,503 (53.1)	17,875 (70.3)	<0.001
Ethnic group					
Non-Hispanic white	23,111 (90.9)	23,253 (91.4)	23,211 (91.3)	22,937 (90.2)	<0.001
Non-Hispanic black	754 (3.0)	677 (2.7)	830 (3.3)	1,089 (4.3)	
Hispanic	364 (1.4)	351 (1.4)	352 (1.4)	428 (1.7)	
Others[b]	1,204 (4.7)	1,151 (4.5)	1,039 (4.1)	978 (3.8)	
Educational degree					
College below	14,643 (57.6)	15,995 (62.9)	16,624 (65.4)	17,673 (69.5)	<0.001
College graduate	4,945 (19.4)	4,590 (18.0)	4,357 (17.1)	3,950 (15.5)	
Postgraduate	5,845 (23.0)	4,847 (19.1)	4,451 (17.5)	3,809 (15.0)	
Body mass index[c]	26.0 ± 4.6	27.0 ± 4.7	27.6 ± 4.7	28.3 ± 4.8	<0.001
Physical activity (min/week)[d]	131.4 ± 127.3	119.3 ± 119.8	118.1 ± 120.8	119.4 ± 122.7	<0.001
Smoking status					
Current					<0.001
>60 Pack-years	322 (1.3)	525 (2.1)	847 (3.3)	1,210 (4.8)	
30–60 Pack-years	672 (2.6)	1,006 (4.0)	1,172 (4.6)	1,430 (5.6)	
<30 Pack-years	482 (1.9)	567 (2.2)	582 (2.3)	584 (2.3)	
Past					
>60 Pack-years	959 (3.8)	1,195 (4.7)	1,434 (5.6)	2,052 (8.1)	
30–60 Pack-years	2,522 (9.9)	2,838 (11.2)	3,135 (12.3)	3,617 (14.2)	
<30 Pack-years	6,747 (26.5)	6,464 (25.4)	6,514 (25.6)	6,283 (24.7)	
Never	13,729 (54.0)	12,837 (50.5)	11,748 (46.2)	10,256 (40.3)	
Alcohol consumption (g/day)	7.1 ± 20.1	8.6 ± 23.2	9.9 ± 25.7	12.6 ± 30.6	<0.001
Healthy Eating Index-2015	71.4 ± 8.9	67.8 ± 9.0	65.2 ± 9.1	61.8 ± 9.1	<0.001
Energy intake from diet (kcal/day)	1407.8 ± 567.3	1,516.4 ± 580.2	1,754.5 ± 629.3	2,275.9 ± 816.8	<0.001
History of diabetes	1,401 (5.5%)	1,573 (6.2%)	1,725 (6.8%)	2,104 (8.3%)	<0.001
Family history of pancreatic Cancer	696 (2.7%)	699 (2.7%)	687 (2.7%)	518 (2.0%)	<0.001
Aspirin user	11,655 (45.8)	11,702 (46.0)	12,118 (47.6)	12,319 (48.4)	<0.001
Food consumption					
Fruits (g/day)	320.9 ± 245.2	266.5 ± 202.9	257.7 ± 203.4	250.5 ± 213.7	<0.001
Vegetables (g/day)	282.9 ± 207.7	255.6 ± 167.7	271.8 ± 169.9	325.8 ± 193.5	<0.001
Whole grain (servings/day)	1.2 ± 0.8	1.1 ± 0.8	1.1 ± 0.8	1.3 ± 0.9	<0.001
Red processed meat (g/day)	4.7 ± 8.2	8.7 ± 10.6	13.3 ± 13.9	22.9 ± 22.3	<0.001
Coffee (g/day)	694.2 ± 698.4	795.7 ± 746.4	880.3 ± 795.4	1015.3 ± 893.3	<0.001
Dairy (servings/day)	1.3 ± 1.1	1.3 ± 1.1	1.4 ± 1.1	1.5 ± 1.2	<0.001
Nuts (g/day)	5.9 ± 14.8	5.9 ± 13.0	6.7 ± 13.9	8.4 ± 16.1	<0.001

[a]Values are mean ± standard deviation or counts (percentage) as indicated.
[b]"Others" refers to Asian, Pacific Islander, or American Indian.
[c]Weight (kg)/height $(m)^2$.
[d]Total time of moderate-to-vigorous physical activity per week.

not alter materially when the association was investigated by doneness degree (Supplementary Table 4). When Cox regression analyses were repeated in 97,822 participants with complete covariate data, similar results were obtained (Supplementary Table 5).

Based on restricted cubic spline regression models, consumption of total fried foods ($P_{\text{non-linearity}}$ = 0.043) and deep-fried foods ($P_{\text{non-linearity}}$ = 0.013) was found to be inversely related to the risk of pancreatic cancer in a non-linear dose–response manner, whereas such a relationship was not found for

TABLE 2 Hazard ratios of the association between energy-adjusted fried food consumption and the risk of pancreatic cancer.

Quartile of energy-adjusted fried food consumption (g/day)	Number of cases	Person-years	Crude incidence rate per 10,000 person-years	Hazard ratio (95% confidence interval)		
				Unadjusted	Model 1[a]	Model 2[b]
Total fried foods						
<6.10	106	227785.6	4.65	1.00 (reference)	1.00 (reference)	1.00 (reference)
6.10–15.06	98	225485.2	4.35	0.94 (0.71–1.23)	0.92 (0.70–1.22)	0.89 (0.68–1.18)
15.07–31.83	114	224597.6	5.08	1.09 (0.84–1.42)	1.06 (0.81–1.39)	1.00 (0.76–1.31)
>31.83	84	223002.8	3.77	0.81 (0.61–1.08)	0.78 (0.58–1.06)	0.71 (0.51–0.99)
P_{trend}				0.171	0.116	0.047
Deep-fried foods						
<3.39	117	227376.1	5.15	1.00 (reference)	1.00 (reference)	1.00 (reference)
3.39–9.47	97	225228.3	4.31	0.84 (0.64–1.10)	0.83 (0.64–1.09)	0.81 (0.62–1.07)
9.48–21.60	107	224773.1	4.76	0.93 (0.71–1.20)	0.91 (0.69–1.19)	0.87 (0.67–1.15)
>21.60	81	223493.7	3.62	0.71 (0.53–0.94)	0.68 (0.51–0.92)	0.64 (0.47–0.88)
P_{trend}				0.030	0.023	0.011
Pan-fried foods						
<0.56	98	227399.0	4.31	1.00 (reference)	1.00 (reference)	1.00 (reference)
0.56–2.95	88	225599.6	3.90	0.91 (0.68–1.21)	0.90 (0.68–1.20)	0.87 (0.65–1.16)
2.96–8.66	111	225124.1	4.93	1.15 (0.87–1.50)	1.11 (0.85–1.46)	1.06 (0.81–1.40)
>8.66	105	222748.5	4.71	1.10 (0.83–1.44)	1.05 (0.80–1.40)	0.98 (0.73–1.32)
P_{trend}				0.316	0.494	0.815

[a]Adjusted for age (years) and sex (male, female).
[b]Adjusted for age (years), sex (male, female), smoking status [current (> 60 pack-years, 30–60 pack-years, < 30 pack-years), former (> 60 pack-years, 30–60 pack-years, < 30 pack-years), never], alcohol consumption (g/day), body mass index (kg/m^2), aspirin use (yes, no), history of diabetes (yes, no), family history of pancreatic cancer (yes, no), and energy intake from diet (kcal/day).

pan-fried food consumption ($P_{non\text{-}linearity}$ = 0.900) (Figure 3). The initial associations of total fried food, deep-fried food, and pan-fried food consumption with the risk of pancreatic cancer were found to be not modified by the predefined stratification factors (all $P_{interaction}$ > 0.05) (Supplementary Table 6) and did not alter substantially in a series of sensitivity analyses (Supplementary Table 7).

Explanatory analyses to identify the main contributor(s) to the observed association

In this study population, main food items contributing to fried food consumption were fried potatoes (35.8%), followed by fried fish (20.5%) and chips (10.6%) (Figure 4). To identify the main contributor(s) to the observed association, we first examined the association between individual fried food consumption and the risk of pancreatic cancer. Among 10 fried foods, only chip consumption was found to be inversely associated with the risk of pancreatic cancer ($HR_{quartile\ 4vs.\ 1}$ 0.68, 95% CI 0.50–0.90, P_{trend} = 0.015) (Supplementary Table 8). We then examined the association of interest with further adjustment for individual fried food consumption. Consistently, only after further adjustment for chip consumption, the initial significant associations of consumption of total fried foods and deep-fried foods with the risk of pancreatic cancer changed to be non-significant (P_{trend} = 0.287 for total fried foods and P_{trend} = 0.133 for deep-fried foods, Supplementary Table 9). Finally, we examined the association of interest after ignoring an individual fried food in each turn. As expected, ignoring "chips" resulted in non-significant associations between consumption of total fried foods (P_{trend} = 0.122) and deep-fried foods (P_{trend} = 0.180) and the risk of pancreatic cancer, although ignoring "pan-fried chicken" (P_{trend} = 0.135) and "pan-fried steak" (P_{trend} = 0.161) also led to a similar phenomenon (Supplementary Table 10).

Discussion

Interestingly, contrary to our initial hypothesis, consumption of total fried foods and deep-fried foods was found to be inversely associated with the risk of pancreatic cancer in a non-linear dose–response manner, while no significant association was found for pan-fried food consumption and the risk of pancreatic cancer. Moreover, these observations were not

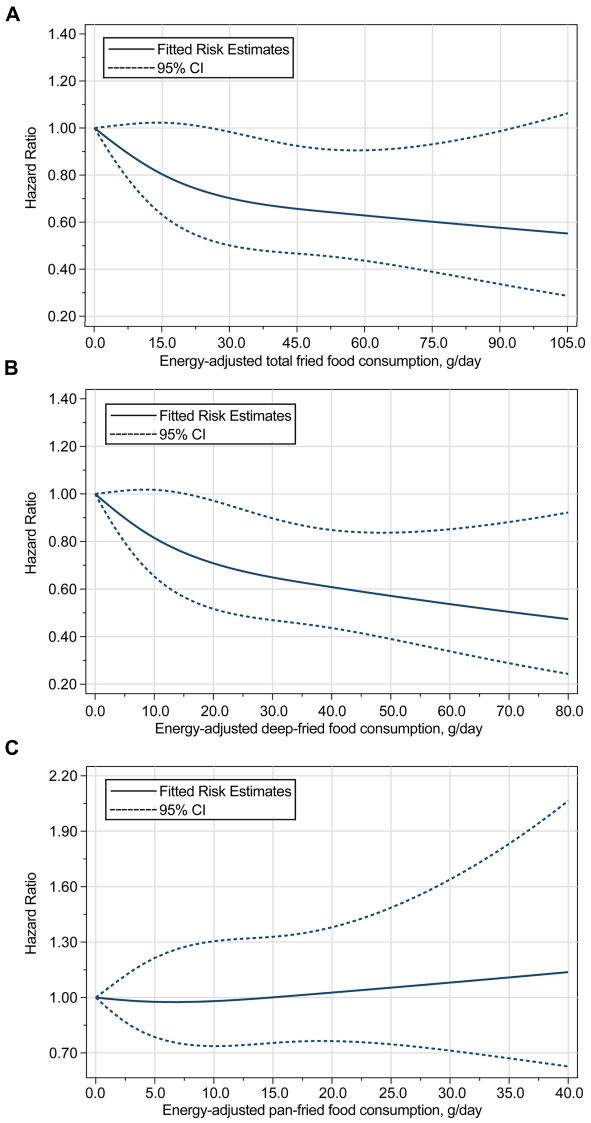

FIGURE 3
Dose–response analyses on the associations of energy-adjusted consumption of **(A)** total fried foods, **(B)** deep-fried foods, and **(C)** pan-fried foods with the risk of pancreatic cancer, with the reference level set at 0 g/day. Hazard ratios were adjusted for age, sex, smoking status, alcohol consumption, BMI, aspirin use, history of diabetes, family history of pancreatic cancer, and energy intake from diet. The P for non-linearity were 0.043 for total fried food consumption, 0.013 for deep-fried food consumption, and 0.900 for pan-fried food consumption.

modified by the predefined stratification factors and remained in a series of sensitivity analyses.

A growing number of evidence has suggested an important role of dietary habits in the etiology of pancreatic cancer (11). For example, a 2017 systematic review on dietary pattern and pancreatic cancer found that unfavorable patterns (e.g., western dietary pattern) were positively associated with while favorable patterns (e.g., prudent dietary pattern) were inversely associated with the risk of pancreatic cancer (33). However, whether fried food consumption is associated with the risk of pancreatic cancer remains to be elucidated. A1994 follow-up study of 9,990 Finnish men and women with only 29 pancreatic cancer cases revealed a null association between fried meat consumption and the risk of pancreatic cancer (relative risk for the highest vs. the lowest tertiles of fried meat consumption: 1.54; 95% CI: 0.53, 4.50) (34), whereas a later hospital-based case-control study of 629 patients in Iran found that deep-fried vegetable consumption conferred an increased risk of pancreatic cancer (odds ratio for consumers vs. non-consumers: 1.70; 95% CI: 1.16, 2.48) (35). Importantly, these studies had an inability to consider the potential antagonistic or synergistic effects among individual fried foods. A 1995 population-based case-control study of 418 individuals in Canada observed that more frequent consumption of fried foods conferred a higher risk of pancreatic

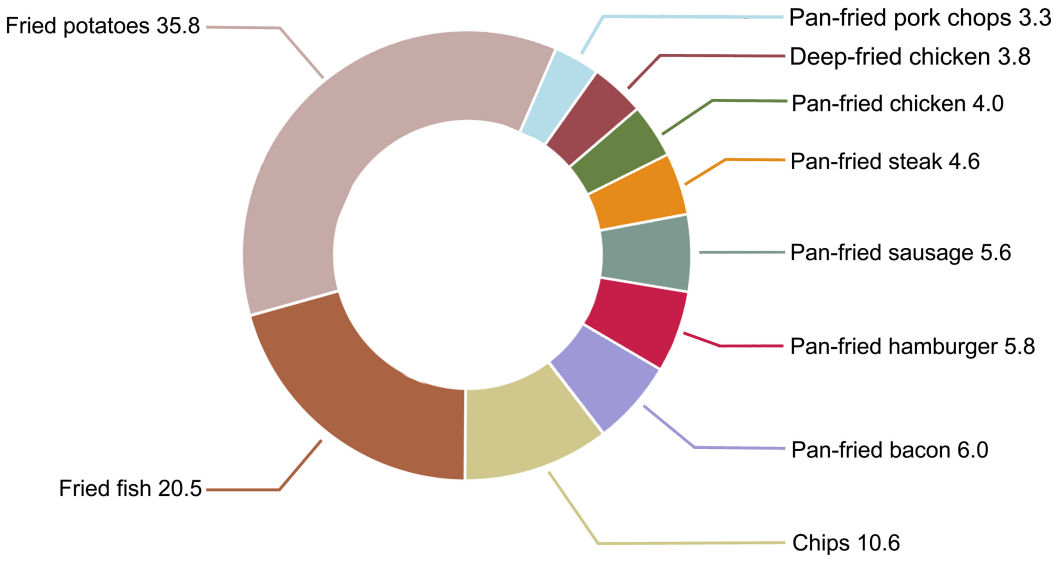

FIGURE 4
Proportion (%) of each fried food in total fried food consumption in our study population.

cancer (relative risk for often vs. never: 3.84; 95% CI: 1.74, 8.48) (15). By contrast, based on prospective data of 101,729 American adults, our study revealed inverse associations of total fried food and deep-fried food consumption with the risk of pancreatic cancer. Compared with some of previous studies in this field (15, 34, 35), our study has several advantages. First, our study design is a prospective study, which allows to establish a temporal association and make our results be free of recall bias. Second, we used dietary pattern approach to evaluate the association of fried food consumption with the risk of pancreatic cancer, enabling our results to account for the potential interactions among individual fried foods (36). Third, our study had a larger sample size and a longer follow-up length, thereby producing more pancreatic cancer cases, which make our study have higher power to detect the potential association of fried food consumption with the risk of pancreatic cancer. Lastly, we explored the potential dose–response associations between fried food consumption and the risk of pancreatic cancer using the well-developed method; the corresponding results provided a detailed description for the risk of pancreatic cancer across the indicated range of fried food consumption.

Consumers have own preferences for the degree of meat doneness in their daily life. A recent study found that well-done pork steak had higher energy content but lower retention ratios for iron and potassium than medium- or rare-done pork steak (37). In addition, another study found that percent fat and protein in beef increased with increase in the doneness degree (38). Moreover, it was found that the contents of heterocyclic aromatic amines, a class of mutagenic compounds formed during cooking of meats, also increased with increase in the doneness degree (39). Thus, doneness degree may impact the association of meat consumption with cancer risk. Indeed, observational studies have found that well-done meat consumption has a stronger positive association with the risk of colorectal cancer than rare- or medium-done meat consumption (40, 41). Given the above facts, we investigated the association of pan-fried food consumption with the risk of pancreatic cancer by doneness degree; nevertheless, the corresponding results indicated that the doneness degree of pan-fried foods had little influence on this association. The exact reasons behind this observation are unclear. One possible explanation is that doneness degree of fried foods does play minimal roles in the etiology of pancreatic cancer; also, another explanation may be that pan-fried food consumption by doneness is so small that the current study is incapable of detecting the potential influence of doneness on the association of interest. Of note, the association between fried food consumption and the risk of pancreatic cancer may be also affected by other factors, such as the type of oil used, whether the oil is reused, and the location where fried foods are eaten (away from home vs. at home). Unfortunately, in the PLCO Cancer Screening Trial, the information on these important factors was not collected, which precluded us to perform the relevant analyses to explore their potential impacts on the association of fried food consumption with the risk of pancreatic cancer. Nevertheless, in the US, fried foods are most often consumed at fast food restaurants, where corn oil is the most common frying medium and is frequently reused (26, 42). Overall, to obtain a better understanding for the association of fried food consumption with the risk of pancreatic cancer, more studies are needed to clarify the potential influence of these factors.

Interestingly, we observed that consumption of deep-fried foods, but not pan-fried foods, was inversely related to the risk of pancreatic cancer in our study population. The specific reasons for the differential association of deep-fried foods and pan-fried foods with the risk of pancreatic cancer are unknown. Considering the fundamental difference between deep-frying and pan-frying (i.e., foods are completely immersed in frying medium in deep-frying while only partially immersed in frying medium in pan-frying), the above-mentioned phenomenon may be due to that foods absorb different amounts of fat, such as saturated fatty acids and monounsaturated fatty acids. Indeed, a recent large prospective cohort study found that high dietary intakes of saturated fatty acids conferred an increased risk of pancreatic cancer ($HR_{quartile\ 4vs.\ 1}$ 1.05, 95% CI 1.01–1.09, P_{trend} = 0.01) while high dietary intakes of monounsaturated fatty acids exerted opposite effect ($HR_{quartile\ 4vs.\ 1}$ 0.92, 95% CI 0.86–0.99, P_{trend} = 0.04) (43). In addition, we noticed that deep-fried food consumption was much higher than pan-fried food consumption in our study population (the proportion in total fried food consumption: 70.7% for deep-fried foods vs. 29.3% for pan-fried foods, **Figure 4**). Thus, another explanation is that for a given participant, the potential influence of pan-fried food consumption on the risk of pancreatic cancer has been masked by the influence of deep-fried food consumption.

The inverse associations of total fried food and deep-fried food consumption with the risk of pancreatic cancer may be accounted by several mechanisms. Intuitively, the beneficial role of total fried foods or deep-fried foods in reducing the risk of pancreatic cancer may be attributable to individual fried foods. Consistent with this speculation, our explanatory analyses indicated that chips could be the main contributors to the observed associations. Potato chips are good sources of antioxidants; an animal study found that feeding potato chips elevated ascorbic acid levels and thus decreased reactive oxygen species levels in mouse tissues (44). Functionally, antioxidants protect cells from oxidative DNA damage, which plays a critical role in the initiation of pancreatic cancer (45); our recent work also showed that dietary antioxidant capacity was inversely related to the risk of pancreatic cancer (46). Meanwhile, potato chips contain abundant amounts of folate and magnesium, whose consumption has been inversely associated with the risk of pancreatic cancer (47, 48). In addition, a randomized crossover trial found that tortilla and corn chips could reduce serum levels of LDL cholesterol (49), which has been found to promote the proliferation of pancreatic cancer cells through activating STAT3 pathway (50). Nevertheless, it should be noted that the observed associations may be also partly attributable to the potential interactions between individual fried foods, given dietary pattern approach we used. In addition, in our study population, participants in the highest vs. the lowest quartiles of total fried food and deep-fried food consumption were found to consume more vegetables and coffee; therefore, it is possible that the inverse associations of total fried food and deep-fried food consumption with the risk of pancreatic cancer are actually mediated by these two factors, considering that both vegetable and coffee consumption have been inversely associated with the risk of pancreatic cancer (14, 51). However, the observation that the initial results did not change materially after adjusting for these two factors (**Supplementary Table 6**), makes this unlikely.

Our study has several limitations. First, dietary assessment was performed once at baseline in this study, and thus may be subject to non-differential bias, given that dietary habits could change over time. Nonetheless, it has been demonstrated that the approach only using baseline diet generally results in a weaker association than does that using the cumulative averages (52). In addition, like other food frequency questionnaires, data collection by the DHQ might be subject to recall bias. Nevertheless, the DHQ had been validated against four 24-h dietary recalls (21), which may attenuate this concern. Second, because the public perceives fried foods as being unhealthy, the possibility of under-reporting cannot be excluded. Nevertheless, under-reporting bias is non-differential, as this bias is not anticipated to be related to the future pancreatic cancer risk, and thus would bias risk estimates toward null, indicating that the true association between fried food consumption and the risk of pancreatic cancer would be stronger than that we observed. Third, as different countries or regions have different cooking traditions, which result in different frying practices and media, therefore, our findings in this US population may not be applicable to other populations. Fourth, as with any observational study, our results might be susceptible to residual confounding because of unrecognized or unmeasured confounders, though we had controlled for the potential confounders. Thus, we cannot rule out the possibility that our results were due to the lack of adjustment for variables associated with the risk of pancreatic cancer, such as serum glucose and lipids. Moreover, our results cannot establish a causal association of fried food consumption with the risk of pancreatic cancer, considering the observational nature of our study.

Conclusion

In conclusion, in this prospective multicenter cohort study of 101,729 US adults, high consumption of deep-fried foods, but not pan-fried foods, is found to be associated with a reduced risk of pancreatic cancer in a non-linear dose–response manner. The role of deep-fried foods in decreasing the risk of pancreatic cancer appears to be mainly attributable to chips. Our findings provide a novel and unique insight into the role of fried foods in the etiology of pancreatic cancer and may alleviate the people's concern that fried foods are potentially carcinogenic. Given the high popularity of fried foods, our findings have important public health implications. More studies are needed to confirm our findings in other populations and settings and to clarify the underlying biological mechanisms.

Data availability statement

The datasets presented in this article are not readily available because US National Cancer Institute's data policy. Requests to access the datasets should be directed to US National Cancer Institute.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Boards of the US National Cancer Institute and each screening center. The patients/participants provided their written informed consent to participate in this study.

Author contributions

G-CZ and J-HG developed the hypothesis, study design, and concept and other authors made useful suggestions and acted as guarantors for the integrity of the data and the accuracy of statistical analysis. G-CZ applied and acquired the original data from the US National Cancer Institute, drafted study protocol and the initial manuscript, and other authors made critical comments and revisions, and responsible for manuscript submission and had full access to the original data. QZ was responsible for statistical analyses. G-CZ attested that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. All authors interpreted the corresponding results together and approved the final version of the article, including the authorship list.

Funding

This study was supported by the China Postdoctoral Science Foundation (Project no. 2021M700638).

Acknowledgments

We express their appreciation to Yong Zhao and Jing-Jing Wu (Department of Nutrition and Food Hygiene, School of Public Health and Management, Chongqing Medical University) for their suggestions and help in statistical analyses. We also sincerely thank the National Cancer Institute for access to NCI's data collected by the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. The statements contained herein are solely those of the authors and do not represent or imply concurrence or endorsement by NCI.

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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.889303/full#supplementary-material

References

1. Dobarganes C, Márquez-Ruiz G. Possible adverse effects of frying with vegetable oils. *Br J Nutr.* (2015) 113(Suppl. 2):S49–57.

2. Uzcudun AE, Retolaza IR, Fernández PB, Sánchez Hernández JJ, Grande AG, García AG, et al. Nutrition and pharyngeal cancer: results from a case-control study in Spain. *Head Neck.* (2002) 24:830–40.

3. Bosetti C, Talamini R, Levi F, Negri E, Franceschi S, Airoldi L, et al. Fried foods: a risk factor for laryngeal cancer? *Br J Cancer.* (2002) 87:1230–3.

4. Pakseresht M, Forman D, Malekzadeh R, Yazdanbod A, West RM, Greenwood DC, et al. Dietary habits and gastric cancer risk in north-west Iran. *Cancer Causes Control.* (2011) 22:725–36. doi: 10.1007/s10552-011-9744-5

5. Jain K, Sreenivas V, Velpandian T, Kapil U, Garg PK. Risk factors for gallbladder cancer: a case-control study. *Int J Cancer.* (2013) 132:1660–6.

6. Li ML, Lin J, Hou JG, Xu L, Cui XG, Xu XX, et al. Environmental and psycho-social factors related to prostate cancer risk in the Chinese population: a case-control study. *Biomed Environ Sci.* (2014) 27:707–17. doi: 10.3967/bes2014.089

7. Guallar-Castillón P, Rodríguez-Artalejo F, Fornés NS, Banegas JR, Etxezarreta PA, Ardanaz E, et al. Intake of fried foods is associated with obesity in the cohort of Spanish adults from the European prospective investigation into cancer and nutrition. *Am J Clin Nutr.* (2007) 86:198–205. doi: 10.1093/ajcn/86.1.198

8. Cahill LE, Pan A, Chiuve SE, Sun Q, Willett WC, Hu FB, et al. Fried-food consumption and risk of type 2 diabetes and coronary artery disease: a prospective study in 2 cohorts of US women and men. *Am J Clin Nutr.* (2014) 100:667–75. doi: 10.3945/ajcn.114.084129

9. Kim DS, Scherer PE. Obesity, diabetes, and increased cancer progression. *Diabetes Metab J.* (2021) 45:799–812.

10. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin.* (2021) 71:7–33.

11. Salem AA, Mackenzie GG. Pancreatic cancer: a critical review of dietary risk. *Nutr Res.* (2018) 52:1–13.

12. Zhong GC, Li QJ, Yang PF, Wang YB, Hao FB, Wang K, et al. Low-carbohydrate diets and the risk of pancreatic cancer: a large prospective cohort study. *Carcinogenesis.* (2021) 42:724–32. doi: 10.1093/carcin/bgab006

13. Yu DW, Li QJ, Cheng L, Yang PF, Sun WP, Peng Y, et al. Dietary Vitamin K intake and the risk of pancreatic cancer: a prospective study of 101,695 American adults. *Am J Epidemiol.* (2021) 190:2029–41. doi: 10.1093/aje/kwab131

14. Huang Y, Liu F, Chen AM, Yang PF, Peng Y, Gong JP, et al. Type 2 diabetes prevention diet and the risk of pancreatic cancer: a large prospective multicenter study. *Clin Nutr.* (2021) 40:5595–604. doi: 10.1016/j.clnu.2021.09.037

15. Ghadirian P, Baillargeon J, Simard A, Perret C. Food habits and pancreatic cancer: a case-control study of the Francophone community in Montreal, Canada. *Cancer Epidemiol Biomarkers Prev.* (1995) 4:895–9.

16. Nüesch E, Trelle S, Reichenbach S, Rutjes AW, Tschannen B, Altman DG, et al. Small study effects in meta-analyses of osteoarthritis trials: meta-epidemiological study. *BMJ.* (2010) 341:c3515.

17. Powell LM, Nguyen BT, Han E. Energy intake from restaurants: demographics and socioeconomics, 2003-2008. *Am J Prev Med.* (2012) 43:498–504. doi: 10.1016/j.amepre.2012.07.041

18. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *PLoS Med.* (2007) 4:e296.

19. Prorok PC, Andriole GL, Bresalier RS, Buys SS, Chia D, Crawford ED, et al. Design of the prostate, lung, colorectal and ovarian (PLCO) cancer screening trial. *Control Clin Trials.* (2000) 21:273S–309S.

20. Brada LJH, Daamen LA, Magermans LG, Walma MS, Latifi D, van Dam RM, et al. Survival benefit associated with resection of locally advanced pancreatic cancer after upfront folfirinox Versus FOLFIRINOX only: multicenter propensity score-matched analysis. *Ann Surg.* (2021) 274:729–35. doi: 10.1097/SLA.0000000000005120

21. Subar AF, Thompson FE, Kipnis V, Midthune D, Hurwitz P, McNutt S, et al. Comparative validation of the block, willett, and national cancer institute food frequency questionnaires: the eating at America's table study. *Am J Epidemiol.* (2001) 154:1089–99. doi: 10.1093/aje/154.12.1089

22. Loth KA. Nutritional data systems for research. In: Wade T editor. *Encyclopedia of Feeding and Eating Disorders.* Singapore: Springer (2015). doi: 10.1007/978-981-287-087-2_6-1

23. Tippett KS, Cypel YS. *Design and Operation: the Continuing Survey of Food Intakes by Individuals and the Diet and Health Knowledge Survey, 1994–96. US Dept of Agriculture, Agriculture Research Service*. (2022). Available online at: https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/Design.pdf (accessed January 14, 2022)

24. Krebs-Smith SM, Pannucci TE, Subar AF, Kirkpatrick SI, Lerman JL, Tooze JA, et al. Update of the healthy eating index: HEI-2015. *J Acad Nutr Diet.* (2018) 118:1591–602.

25. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr.* (1997) 65:1220S–8S.

26. Sun Y, Liu B, Snetselaar LG, Robinson JG, Wallace RB, Peterson LL, et al. Association of fried food consumption with all cause, cardiovascular, and cancer mortality: prospective cohort study. *BMJ.* (2019) 364:k5420.

27. Spratt M, Carpenter J, Sterne JA, Carlin JB, Heron J, Henderson J, et al. Strategies for multiple imputation in longitudinal studies. *Am J Epidemiol.* (2010) 172:478–87.

28. Schoenfeld D. Chi-squared goodness-of-fit tests for the proportional hazards regression model. *Biometrika.* (1980) 67:145–53. doi: 10.1002/sim.7244

29. Hernán MA, Hernández-Díaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. *Am J Epidemiol.* (2002) 155:176–84.

30. Mathur MB, Ding P, Riddell CA, VanderWeele TJ. Web site and r package for computing E-values. *Epidemiology.* (2018) 29:e45–7. doi: 10.1097/EDE.0000000000000864

31. Akaike H. Likelihood of a model and information criteria. *J Econom.* (1981) 16:3–14.

32. Willett W. *Nutritional Epidemiology*. Oxford: Oxford University Press (2012).

33. Zheng J, Guinter MA, Merchant AT, Wirth MD, Zhang J, Stolzenberg-Solomon RZ, et al. Dietary patterns and risk of pancreatic cancer: a systematic review. *Nutr Rev.* (2017) 75:883–908.

34. Knekt P, Steineck G, Järvinen R, Hakulinen T, Aromaa A. Intake of fried meat and risk of cancer: a follow-up study in Finland. *Int J Cancer.* (1994) 59:756–60. doi: 10.1002/ijc.2910590608

35. Ghorbani Z, Hekmatdoost A, Zinab HE, Farrokhzad S, Rahimi R, Malekzadeh R, et al. Dietary food groups intake and cooking methods associations with pancreatic cancer: a case-control study. *Indian J Gastroenterol.* (2015) 34:225–32.

36. Schulz CA, Oluwagbemigun K, Nöthlings U. Advances in dietary pattern analysis in nutritional epidemiology. *Eur J Nutr.* (2021) 60:4115–30.

37. Jang A, Kim HJ, Kim D, Kim J, Lee SK. Effects of doneness on the microbial, nutritional, and quality properties of pork steak of different thicknesses. *Food Sci Anim Resour.* (2019) 39:756–67. doi: 10.5851/kosfa.2019.e63

38. Smith AM, Harris KB, Haneklaus AN, Savell JW. Proximate composition and energy content of beef steaks as influenced by USDA quality grade and degree of doneness. *Meat Sci.* (2011) 89:228–32. doi: 10.1016/j.meatsci.2011.04.027

39. Özsaraç N, Kolsarici N, Demirok Soncu E, Haskaraca G. Formation of heterocyclic aromatic amines in doner kebab cooked with different methods at varying degrees of doneness. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* (2019) 36:225–35. doi: 10.1080/19440049.2018.1562230

40. Cotterchio M, Boucher BA, Manno M, Gallinger S, Okey AB, Harper PA. Red meat intake, doneness, polymorphisms in genes that encode carcinogen-metabolizing enzymes, and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev.* (2008) 17:3098–107. doi: 10.1158/1055-9965.EPI-08-0341

41. de Batlle J, Gracia-Lavedan E, Romaguera D, Mendez M, Castaño-Vinyals G, Martín V, et al. Meat intake, cooking methods and doneness and risk of colorectal tumours in the Spanish multicase-control study (MCC-Spain). *Eur J Nutr.* (2018) 57:643–53. doi: 10.1007/s00394-016-1350-6

42. Rey-García J, Guallar-Castillón P, Donat-Vargas C, Moreno-Iribas C, Barricarte A, Rodriguez-Barranco M, et al. Fried-Food consumption does not increase the risk of stroke in the spanish cohort of the european prospective investigation into cancer and nutrition (EPIC) study. *J Nutr.* (2020) 150:3241–8.

43. Ghamarzad Shishavan N, Masoudi S, Mohamadkhani A, Sepanlou SG, Sharafkhah M, Poustchi H, et al. Dietary intake of fatty acids and risk of pancreatic cancer: golestan cohort study. *Nutr J.* (2021) 20:69. doi: 10.1186/s12937-021-00723-3

44. Kondo Y, Sakuma R, Ichisawa M, Ishihara K, Kubo M, Handa S, et al. Potato chip intake increases ascorbic acid levels and decreases reactive oxygen species in SMP30/GNL knockout mouse tissues. *J Agric Food Chem.* (2014) 62:9286–95. doi: 10.1021/jf502587j

45. Martinez-Useros J, Li W, Cabeza-Morales M, Garcia-Foncillas J. Oxidative stress: a new target for pancreatic cancer prognosis and treatment. *J Clin Med.* (2017) 6:29.

46. Zhong GC, Pu JY, Wu YL, Yi ZJ, Wan L, Wang K, et al. Total antioxidant capacity and pancreatic cancer incidence and mortality in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer Epidemiol Biomarkers Prev.* (2020) 29:1019–28. doi: 10.1158/1055-9965.EPI-19-1511

47. Fu H, Zeng J, Liu C, Gu Y, Zou Y, Chang H. Folate intake and risk of pancreatic cancer: a systematic review and updated meta-analysis of epidemiological studies. *Dig Dis Sci.* (2021) 66:2368–79. doi: 10.1007/s10620-020-06525-7

48. Dibaba D, Xun P, Yokota K, White E, He K. Magnesium intake and incidence of pancreatic cancer: the vitamins and lifestyle study. *Br J Cancer.* (2015) 113:1615–21. doi: 10.1038/bjc.2015.382

49. St-Onge MP, Aban I, Bosarge A, Gower B, Hecker KD, Allison DB. Snack chips fried in corn oil alleviate cardiovascular disease risk factors when substituted for low-fat or high-fat snacks. *Am J Clin Nutr.* (2007) 85:1503–10.

50. Jung YY, Ko JH, Um JY, Chinnathambi A, Alharbi SA, Sethi G, et al. LDL cholesterol promotes the proliferation of prostate and pancreatic cancer cells by activating the STAT3 pathway. *J Cell Physiol.* (2021) 236:5253–64. doi: 10.1002/jcp.30229

51. Nam SY, Jo J, Lee WK, Cho CM. The sex discrepancy effects of fruit and vegetable intake on pancreatic cancer risk; a large Korean cancer screening cohort study. *Dig Liver Dis.* (2022) 54:365–370.

52. Hu FB, Stampfer MJ, Rimm E, Ascherio A, Rosner BA, Spiegelman D, et al. Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. *Am J Epidemiol.* (1999) 149:531–40.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership