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Dietary and nutritional indices and chronic diseases, 2nd Edition

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Dietary and nutritional indices and chronic diseases, 2nd Edition

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Editorial: Dietary and nutritional indices and chronic diseases

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KEYWORDS

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Editorial on the Research Topic
Dietary and nutritional indices and chronic diseases

Introduction

Regular or disease-specific diets are composed of different components, each of which has different properties such as high and low inflammatory, oxidative, acidogenic, glycemic and insulin load, etc. Therefore, the cumulative health effects of the food items may significantly differ on the body. Each of these food properties can be used as a specific index that can be linked to proper health outcomes. To measure each index, the attribute is calculated for all food components and then summed up to constitute the general characteristics of a diet in that aspect. In this regard, we established our Research Topic on March 28, 2022, and invited researchers interested in the benefits, concerns, or harms associated with diets of different characteristics with the greatest emphasis on prevention, control, and management of chronic diseases.

Frontiers in Nutrition published 20 articles that evaluated the association of dietary indices ($n = 8$), nutritional indices ($n = 2$), dietary patterns ($n = 2$), and specific nutrients or food groups ($n = 8$) with various health outcomes. Most of the studies were prospective cohorts or derived from cohort studies with large sample sizes.

Dietary indices and health outcomes

Valle-Hita et al. in a prospective cohort study on 9,513 older adults with metabolic syndrome found that higher dietary acid load was associated with lower kidney function after 1 year of follow-up.

Nie et al., in a cohort study on 23,109 adult people, reported that individuals with the highest Healthy Eating Index 2015 (HEI-2015) score had a 12.2% reduced risk of gout and a 2.2% reduced risk of hyperuricemia than individuals with the lowest HEI-2015 score.

Moludi et al., in a study derived from the RaNCD cohort survey involving 9,824 individuals, reported that a high intake of a pro-inflammatory diet was related to a higher incidence of chronic kidney disease.

Nutritional indices and health outcomes

Wang J. et al., in a study on 13,871 adults, found a positive association between visceral adiposity index with urolithiasis and the prevalence of kidney stones.

Han et al., in a retrospective cohort study on 4,411 patients with heart failure, showed that a high triglyceride glucose index was significantly correlated with the risk of in-hospital mortality in the patients, independent of type 2 diabetes mellitus and coronary artery disease.

Dietary patterns and health outcomes

Llaha et al., in a prospective cohort study on 450,000 individuals from nine European countries, concluded that high adherence to the Mediterranean diet was not strongly associated with differentiated thyroid cancer risk after 14.1 years of follow-up. The authors found that low meat and moderate alcohol intake were associated with lower differentiated thyroid cancer risk.

Wang Y. B. et al., in a cross-sectional study on 1,792 community-dwelling adults, showed that a plant-sourced nutrient pattern was strongly and independently related to lower systemic inflammation, particularly in men and obese individuals.

Specific nutrients or food groups and health outcomes

Tao et al., in a Mendelian randomization study on 5,575 participants, found a causal relationship between n-3 PUFAs, n-6 PUFAs, the ratio of n-3 PUFAs to total fatty acids, the ratio of n-6 PUFAs to n-3 PUFAs with estimated bone mineral density. The authors also showed an association between n-3 PUFAs with forearm and lumbar spine with bone mineral density and fracture.

Li Q.-H. et al., in a study on 655 gout patients, determined the high intake of sugar-sweetened beverages as the main dietary risk factor for gout in early-onset patients and found a direct association between sugar-sweetened beverages with serum urate level and obesity.

Peng et al., in a study of 7,725 participants, concluded that intake of a diet with a low percentage of energy from fat appears to be beneficial in the prevention of osteoarthritis risk.

Huang et al., in a study on 2,533 normotensive individuals, reported that serum vitamin C was adversely associated with both systolic and diastolic blood pressure.

All the investigations, except one, comprised in this Research Topic reinforced a link between healthy diet/nutrition status and improvement of health consequences. Overall, the findings of the articles published in this Research Topic may be beneficial in understanding that the dietary/nutritional indices could be effectively used as predictive biomarkers of health outcomes, particularly in patients with chronic diseases. Further, the evaluation of the indices would be beneficial in providing the necessary recommendations for the promotion of dietary status and in the consequent improvement of the health status of people, either at the clinical or community level.

Definitely, the number of 20 studies included in the present Research Topic is not able to fully cover all aspects of the Research Topic. The areas that were less addressed in the current Research Topic are:

- Interventional studies confirming the causal relationship between dietary/nutritional indices-health outcomes,
- Mechanistic pathways linking dietary/nutritional indices and chronic diseases,
- Studies linking dietary/nutritional indices with metabolic status, acid-base balance, inflammatory and oxidative stress conditions in the body, circulating biomarkers, and risk factors.

Author contributions

SK prepared initial draft of the article. MA involved in the revision of the article. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Sugar-Sweeten Beverage Consumption Is Associated With More Obesity and Higher Serum Uric Acid in Chinese Male Gout Patients With Early Onset

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Background: Early onset gout has received increasing interest from researchers. Previous studies have reported that serum urate (sUA) levels and prevalence of obesity are higher in early onset gout patients than in later-onset gout patients. We explored the dietary habits of early onset and later-onset gout patients and their association with clinical features.

Materials and Methods: Gout patients completed a 10-item food frequency questionnaire. Early onset gout patients were defined as gout onset before the age of 40, and onset after age 40 was classified as later-onset. Associations between dietary factors, obesity, and sUA level of ≥600 μmol/L were assessed using logistic regression.

Results: Among the 655 gout patients, 94.6% were males, and 59.1% presented with early onset gout. All early onset patients were males. sUA level was significantly higher in the early onset group than in the later-onset group (550.7 vs. 513.4 μmol/L). The proportion of patients with a sUA level of ≥ 600 μmol/L (40.3 vs. 26.2%) and obesity (27.6 vs. 10.7%) was higher in the early onset group than in the later-onset group (all $p < 0.05$). The early onset group consumed more red meat (101–200 g/day: 43.6 vs. 26.0%), sugar-sweetened beverages (>4 times/week: 27.9 vs. 7.7%), and milk and milk products (1–2 times/week: 28.5 vs. 16.6%), but less alcohol (>84 g/day: 8.5 vs. 21.5%) and tea (>4 times/week: 35.7 vs. 52.4%; all $p < 0.05$). Sugar-sweetened beverage intake was positively correlated with sUA level of ≥600 μmol/L (compared with <once/week [reference], >4 times/week: adjusted odds ratio = 2.2, 95% confidence interval: 1.4, 3.7) and obesity (compared with <once/week [reference], >4 times/week: adjusted odds ratio = 2.2, 95% confidence interval: 1.2, 3.7). These correlations remained significant for early onset gout patients.

Conclusion: Sugar-sweetened beverage intake replaced alcohol as the main dietary risk factor for gout in early onset patients, and this change was associated with a greater prevalence of obesity and higher sUA level. Clinicians should provide specific dietary education for different generations of gout patients. The epidemic of sugar-sweetened beverage consumption should be considered for the development of public health policies for the prevention of gout.

Keywords: early onset gout, food frequency questionnaire, sugar-sweeten beverages, obesity, serum urate

INTRODUCTION

Gout is a chronic disease caused by long-term hyperuricemia, which leads to the deposition of monosodium urate crystals in the joints, adjacent structures, and other tissues. Genetic and environmental factors are involved in the development of hyperuricemia. Genetic analysis has identified approximately 30 loci associated with hyperuricemia (1). A recent meta-analysis found that single nucleotide polymorphisms contribute to nearly one-quarter of the variance of serum urate (sUA) level (2). Dietary factors, such as alcohol, red meat, and seafood consumption, are well-established risk factors of hyperuricemia and gout flares (3). Moreover, fructose intake is strongly positively correlated with sUA level (4) and gout risk (5, 6). Fructose is also an important risk factor for obesity and type 2 diabetes mellitus (T2DM), which are common comorbidities in gout patients (7).

The prevalence of gout has continued to rise globally (8). Approximately 40 years ago, gout was considered a rare disease in China; no gout was reported in a survey on health screening across four large cities in 1980 (9). However, a recent meta-analysis has shown that the prevalence of gout has increased to 1.4% between 2011 and 2014 in China (10), and a recent cross-sectional study found that nearly one-third of all college students are diagnosed with hyperuricemia in China (11). Several studies to date have focused on early onset gout (11–15). Nearly 60% of Chinese gout patients at a tertiary hospital in Beijing experienced an initial gout flare before the age of 40 between 2008 and 2014 (13). Younger gout patients are characterized by a higher sUA level and body mass index (BMI) than those of older patients (13, 15).

Genetic variants contribute to higher sUA levels in early onset gout patients. Among the genetic variants that are related to heightened sUA levels, the ABCG2 rs2231142 T-allele is associated with gout onset before the age of 40 (16). Although dietary factors are well-established risk factors for gout, differences in dietary habits between early onset and later-onset gout patients have not been reported. Furthermore, the influence of dietary factors on the clinical features of early onset gout patients has also yet to be ascertained. Therefore, we conducted a questionnaire survey on food intake frequency in Chinese gout patients to evaluate dietary habits and clinical features in gout patients.

MATERIALS AND METHODS

Patients and Grouping

Consecutive patients who met the 2015 gout classification criteria (17) were recruited from August 2017 to July 2021 from the Department of Rheumatology, Sun Yat-sen Memorial Hospital. Exclusion criteria were as follows: patients had already received urate-lowering therapy, pyrazinamide, azathioprine, or cyclosporine within the 1 month before study enrolment; gout onset before the age of 16, and gout secondary to single gene disorders, malignancy, and hematological proliferative diseases. Patients who experienced an initial gout attack before the age of 40 were classified as early onset patients (16), and all other patients were classified as later-onset. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Sun Yat-sen Memorial Hospital (SYSEC-KY-KS-022). All patients provided written informed consent before participating in the study.

Clinical and Laboratory Assessments

Demographic and disease assessment data were collected from all patients. Gout assessment included age of onset, disease duration, counts of ever-involved joints, tophi, and nephrolithiasis, gout attack times during the preceding year, and family history of gout. Subjects were categorized by BMI as obese (BMI $\geq$ 28 kg/m^2), overweight (24 kg/m^2 $\leq$ BMI $<$ 28 kg/m^2), normal weight (18.5 kg/m^2 $\leq$ BMI $<$ 24 kg/m^2), and underweight (BMI $<$ 18.5 kg/m^2) (18). Central obesity was defined as a waist circumstance of $\geq$90 cm for men and $\geq$85 cm for women (18).

Overnight fasting blood samples were collected from basilic vein to measure sUA level, serum creatinine (SCr), plasma glucose (FPG), and lipid profiles using the Hitachi 7600-110 Chemistry Autoanalyzer. Ultrasonography was used to evaluate fatty liver disease and nephrolithiasis. Comorbidities of the gout patients were recorded, which included obesity, hypertension, T2DM, dyslipidemia, metabolic syndrome, coronary heart disease, chronic kidney disease (CKD), urolithiasis, and fatty liver disease. Diagnoses of hypertension, T2DM, dyslipidemia, and metabolic syndrome were made according to the latest local guidelines (19–21). Estimated glomerular filtration rate (eGFR) was calculated using the following equation: eGFR (ml/min/1.73 m^2) = 175$\times$SCr (mg/dl)$^{-1.234}\times$age$^{-0.179}\times$0.79 (if female) (22). CKD was defined as an eGFR of $<$60 ml/min/1.73 m^2.

Food Frequency Questionnaire and Judgment Criteria

A simplified food frequency questionnaire was adapted from the food frequency questionnaire of the China National Nutrition and Health Survey (23). Foods and food groups were selected according to the 2012 American College of Rheumatology guidelines for the management of gout (24) and included animal offal, red meat, seafood, alcohol, sugar-sweetened beverages (SSB), coffee, and milk and milk products. SSB included soda, fruit juice, fruit-flavored drink, cordials, sport drinks and herbal tea which were sweetened by high fructose corn syrup or sucrose. Hotpot, slow-cooking soup, and tea were also included because these popular Chinese foods may influence sUA level. The final questionnaire comprised 10 items. All patients completed the questionnaire in approximately 10–15 min.

Trained investigators administered the questionnaire to outpatients in the clinical room and inpatients at their bedside. Each item was explained to the patients. Patients completed the questionnaire on their own. After the patient completed the questionnaire, the investigator reviewed the questionnaire and confirmed the item's answer with the patient. Because patients are prone to change their dietary habits after being diagnosed with gout, participants were informed to report their average intake frequency during the 1 year before the first gout flare. Questionnaires with a minimum of eight answered items were regarded as valid for statistical analysis.

For alcohol intake, patients were asked about the frequency, categories (beer, wine, or spirits), ethanol concentration, and volume consumed. A standard drink was used to evaluate alcohol intake. A standard drink contains approximately 14 g of alcohol, which is equivalent to 497 ml of 3.5% beer, 145 ml of 12% wine, or 44 ml of 40% spirits (25). Alcohol intake was calculated by multiplying the consumption of alcoholic drinks by the corresponding ethanol content. Alcohol intake in this study was divided into three categories: 0–28 g/day, 29–84 g/day, and >84 g/day. Daily consumption of red meat was categorized as less than 100 g/day, 101–200 g/day, and >200 g/day. Consumption of animal offal, seafood, hotpot, slow-cooking soup, SSB, tea, coffee, and milk and milk products was assessed by weekly frequency without portion sizes. Intake of these eight items was categorized as <once/week), 1–2 times/week, 3–4 times/week, and >4 times/week.

Statistical Analysis

Data were analyzed using SPSS Statistics for Windows 20.0 (IBM, Armonk, NY, United States). Categorical variables are presented as frequencies and percentages. Continuous variables are presented as means and standard deviations (SD) for normal distributed data or medians and interquartile ranges (IQRs) for data with a skewed distribution. The normal distribution of continuous variables was evaluated by Kolmogorov–Smirnov test. Differences in continuous variables between the two groups were tested using two-samples *t*-tests for the normally distributed variables and the Mann–Whitney test for non-normally distributed variables. A chi-square test was used for categorical variables. Missing data were addressed using pairwise deletion.

Correlations between dietary factors and obesity and sUA level of ≥600 μmol/L were evaluated using univariate logistic regression analyses. Further multivariate logistic regression analyses were applied to confirm the correlation between dietary factors and obesity after adjusting for potential confounding factors, which included duration of gout, family history, eGFR, comorbidities (i.e., hypertension, T2DM, dyslipidemia, metabolic syndrome, fatty liver disease, and coronary heart disease, and other dietary factors that were significant at $p < 0.05$ in the univariate logistic regression analysis. The correlation between dietary factors and sUA level ≥600 μmol/L was adjusted by the above confounding factors, obesity, and current medications (i.e., aspirin, diuretics, and lipid-lowering medication including atorvastatin and fenofibrate). The above correlations were performed both in male gout patients and early onset gout patients. All tests were conducted using a two-tailed 5% significance level.

RESULTS

Demographic Characteristics of Gout Patients

A total of 666 patients were recruited. Eleven patients were excluded, which included seven patients who answered fewer than eight items of the questionnaire and four patients who were re-diagnosed with monogenic gout (**Figure 1**). This resulted in 655 patients being valid for statistical analysis. Among these, 620 (94.6%) were males with a mean age of 42.7 ± 14.2 years and a mean age of gout onset of 37.5 ± 13.3 years. There were 35 (5.3%) females with gout, with a mean age of 63.2 ± 12.4 years and a mean age of gout onset of 58.5 ± 12.1 years. There were 161 (24.6%) patients who presented with tophi. The median gout duration (follow-up time) was 4 (2, 7) years. The mean sUA level was 537.1 ± 133.7 μmol/L, and 231 (35.3%) patients had an sUA level of over 600 μmol/L. Detailed demographic characteristics are shown in **Table 1**.

The highest proportion of patients had an onset age of 30–39 years (28.9%), followed by 20–29 years (28.1%) and 40–49 years (18.8%; **Figure 2**). There were 387 (59.1%) patients in the early onset group, with a mean onset age of 28.8 ± 6.0 years. Because all patients with early onset gout were males, subsequent statistical analyses included only the male patients.

Clinical Characteristics of Early Onset Gout Patients

Compared with later-onset male patients, early onset male gout patients presented with significantly higher sUA level (550.7 ± 135.2 μmol/L vs. 513.4 ± 129.0 μmol/L, $p < 0.05$), and had a higher proportion of patients who had an sUA level of ≥600 μmol/L (40.3 vs. 26.2%, $p < 0.05$). However, eGFR was significantly higher in the early onset group than

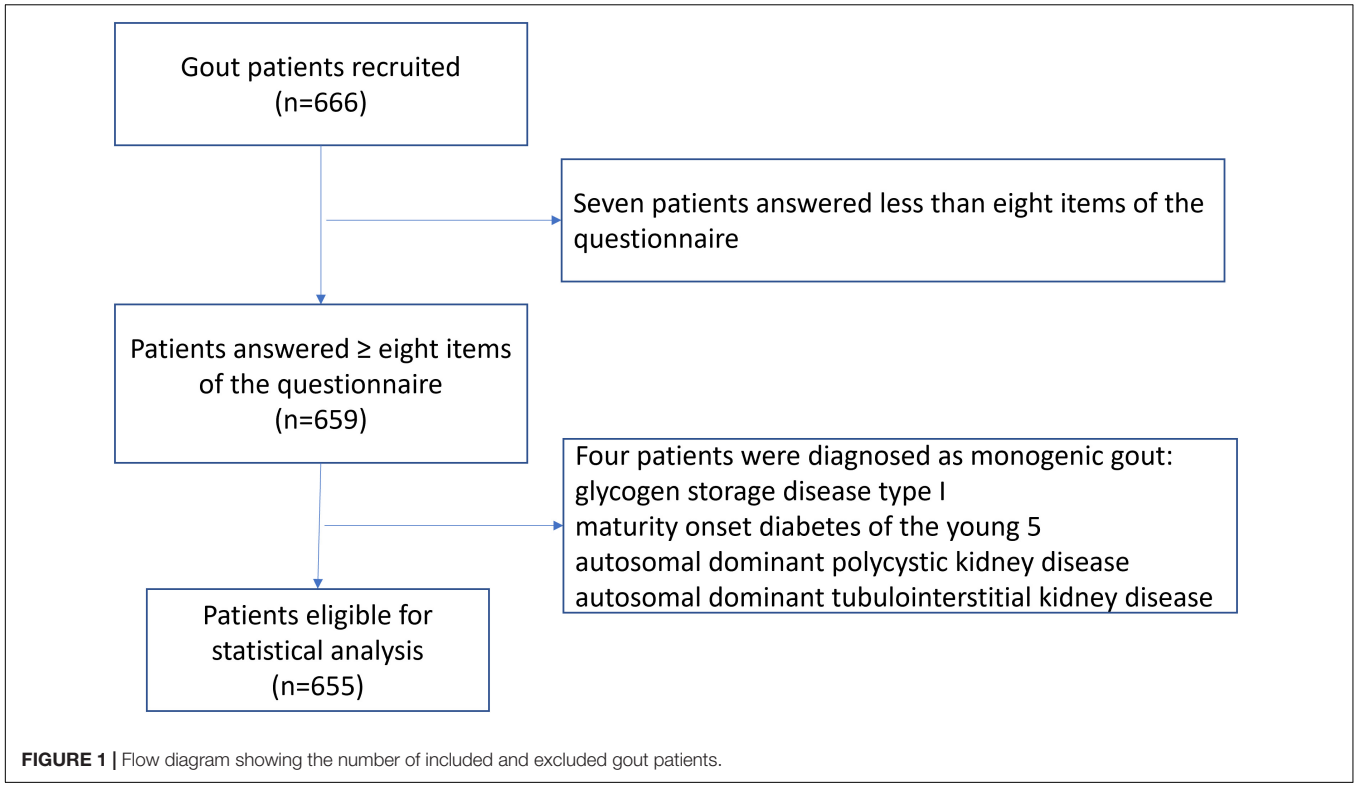

FIGURE 1 | Flow diagram showing the number of included and excluded gout patients.

that in the later-onset group (86.9 ± 16.6 ml/min/1.73 m^2 vs. 77.7 ± 32.3 ml/min/1.73 m^2, $p < 0.05$; **Table 2**). Compared with later-onset male patients, early onset male gout patients presented with a higher BMI (26.0 ± 3.9 kg/m^2 vs. 24.6 ± 2.8 kg/m^2; $p < 0.001$) and a higher prevalence of obesity (27.6 vs. 10.7%, $p < 0.001$; **Table 2**). Male gout patients with a later onset showed more hypertension (57.1 vs. 31.8%, $p < 0.05$), had higher rates of metabolic syndrome (51.5 vs. 41.9%, $p < 0.05$), T2DM (17.2 vs. 6.7%, $p < 0.05$), and coronary heart disease (5.6 vs. 0.3%, $p < 0.05$), and a higher count of ever-involved joints (4 vs. 3, $p < 0.05$) than those of early onset patients.

Food Intake Frequency in Early Onset Male Gout Patients

The frequency of consumption of each food is shown in **Table 3**. Among the male gout patients, 13.4% consumed >84 g/day of alcohol, 23.4% ate >200 g/day of red meat, 41.9% drank tea, 20.3% consumed SSB, and 10.2% consumed slow-cooking soup >4 times/week. Less than 10% of patients consumed animal offal, seafood, hotpot, coffee, or milk and milk products >4 times/week.

Compared with the later-onset gout patients, early onset male gout patients consumed more red meat (101–200 g/day: 43.6 vs. 26.0%, $p < 0.05$), SSB (>4 times/week: 27.9 vs. 7.7%, $p < 0.05$), and milk and milk products (1–2 times/week: 28.5 vs. 16.6%, $p < 0.05$), but less alcohol (>84 g/day: 8.5 vs. 21.5%, $p < 0.05$) and tea (>4 times/week: 35.7 vs. 52.4%, $p < 0.05$). There were no significant differences in intake of the other five foods between groups.

Correlation Between Food Intake Frequency and Serum Urate Level Over 600 μmol/L

For all male gout patients, the risk factors for sUA level over 600 μmol/L included red meat (compared with <100 g/day [reference], >200 g/day: odds ratio [OR] = 2.0, 95% confidence interval [CI]: 1.3, 3.0), animal offal (compared with <once/week [reference], >4 times/week: OR = 3.0, 95% CI: 1.3, 6.8), and SSB consumption (compared with <once/week [reference], >4 times/week: OR = 3.0, 95% CI: 2.0, 4.6; **Table 4**). For early onset gout patients, the risk factors for sUA level over 600 μmol/L were red meat (compared with <100 g/day [reference], >200 g/day: OR = 1.9, 95% CI: 1.1, 3.4) and SSB consumption (compared with <once/week [reference], >4 times/week: OR = 2.5, 95% CI: 1.5, 4.1). For later-onset gout patients, the correlation between food intake frequency and sUA level over 600 μmol/L weren't significant ($p > 0.05$). There was a marginal positive correlation between SSB consumption and sUA level over 600 μmol/L ($p = 0.056$).

After adjusting for potential confounding variables, the multivariate logistic regression analysis showed that the risk factors for sUA level over 600 μmol/L were animal offal (compared with <once/week [reference], >4 times/week: adjusted OR = 2.7, 95% CI: 1.1, 6.9) and SSB consumption (compared with <once/week [reference], >4 times/week: adjusted OR = 2.2, 95% CI: 1.4, 3.7). The correlation between SSB consumption and sUA level over 600 μmol/L was significant in early onset gout patients (compared with <once/week [reference], >4 times/week: adjusted OR = 2.1, 95% CI: 1.2, 3.7;

TABLE 1 | Demographic and clinical characteristics of 655 gout patients.

Characteristics	All patients (n = 655)	Male patients (n = 620)	Female patients (n = 35)	P
Age, years, mean ± SD	43.8 ± 14.8	42.7 ± 14.2	63.2 ± 12.4	**<0.001**
Onset age, years, mean ± SD	38.6 ± 14.0	37.5 ± 13.3	58.5 ± 12.1	**<0.001**
Gout duration, years, median (IQR)	4 (2, 7)	4 (2, 7)	3 (1, 7)	0.516
Count of ever involved joints, median (IQR)	4 (2, 7)	4 (2, 6)	4 (2, 8)	0.413
Flare times in the last year, median (IQR)	4 (2, 10)	4 (2, 10)	6 (2, 24)	0.212
Family history of gout, *n* (%)	235 (35.9)	219 (35.3)	16 (45.7)	0.277
Tophi, *n* (%)	161 (24.6)	151 (24.4)	10 (28.6)	0.687
sUA, μmol/L, mean ± SD	537.1 ± 133.7	536.7 ± 134.0	544.3 ± 129.9	0.745
sUA ≥ 600 μmol/L	231 (35.3)	214 (35.0)	14 (40.0)	0.587
SCr, μmol/L, mean ± SD	99.1 ± 23.9	99.3 ± 23.7	94.7 ± 27.9	0.341
eGFR, ml·min^{-1}·1.73 m^{-2}, mean ± SD	82.6 ± 24.4	83.4 ± 24.1	68.2 ± 25.0	**<0.001**
CKD, *n* (%)	65 (9.9)	50 (8.1)	15 (42.9)	**<0.001**
Urolithiasis, *n* (%)	167 (25.5)	160 (25.8)	7 (20.0)	0.552
BMI, kg/m^2, mean ± SD	25.4 ± 3.6	25.5 ± 3.6	25.3 ± 4.0	0.782
Obese, *n* (%)	141 (21.5)	132 (21.3)	9 (25.7)	0.171
Overweight, n (%)	283 (43.2)	269 (43.4)	14 (40.0)	
Normal BMI, *n* (%)	221 (33.7)	211 (34.0)	10 (28.6)	
Underweight, *n* (%)	10 (1.5)	8 (1.3)	2 (5.7)	
Waist circumstance, cm, mean ± SD	90.9 ± 9.7	91.0 ± 9.6	89.0 ± 10.2	0.241
Central obesity, *n* (%)	370 (56.5)	347 (56.0)	23 (65.7)	0.296
Dyslipidemia, *n* (%)	413 (63.12)	390 (62.9)	23 (65.7)	0.858
Hypercholesterolemia, *n* (%)	143 (21.8)	129 (20.8)	14 (40.0)	**0.011**
Hypertriglyceridemia, *n* (%)	197 (30.1)	185 (29.8)	12 (34.3)	0.705
LDL-C hyperlipidemia, *n* (%)	148 (22.6)	135 (21.8)	13 (37.1)	**0.040**
HDL-C hypolipidemia, *n* (%)	179 (27.3)	170 (27.4)	9 (25.7)	0.850
Metabolic syndrome, *n* (%)	302 (46.1)	282 (45.5)	20 (57.1)	0.220
Fatty liver disease, *n* (%)	316 (48.2)	301 (48.5)	15 (42.9)	0.603
T2DM, *n* (%)	80 (12.2)	66 (10.6)	14 (40.0)	**<0.001**
Hypertension, *n* (%)	278 (42.4)	256 (41.3)	22 (62.9)	**0.012**
Coronary heart disease, *n* (%)	19 (2.9)	14 (2.32)	5 (14.3)	**<0.001**

Bold values mean the difference is statistically significant.

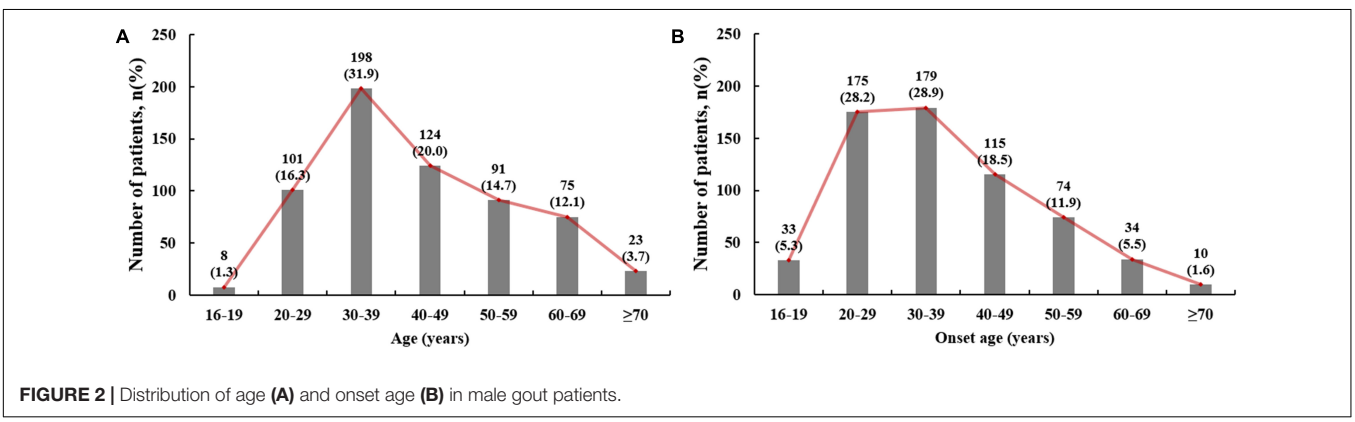

FIGURE 2 | Distribution of age **(A)** and onset age **(B)** in male gout patients.

Figure 3). The correlation between SSB consumption and sUA level over 600 μmol/L was marginal ($p = 0.062$, **Figure 3**).

Correlation Between Food Intake Frequency and Obesity

Similarly, the univariate logistic regression analysis for all male gout patients showed that red meat (compared with <100 g/day [reference], >200 g/day: OR = 2.0, 95% CI: 1.2, 3.3) and SSB consumption (compared with <once/week [reference], 1–2 times/week: OR = 2.5, 95% CI: 1.4, 4.4; 3–4 times/week: OR = 2.6, 95% CI: 1.4, 4.6; >4 times/week: OR = 2.9, 95% CI: 1.8, 4.7; **Table 5**) were positively correlated with obesity. In early onset gout patients, both red meat (compared with <100 g/day [reference], >200 g/day: OR = 2.0, 95% CI: 1.0, 3.5)

TABLE 2 | Comparison of demographic and clinical characteristics between male gout patients with early onset and later-onset.

Characteristics	Early onset group ($n = 387$)	Later-onset group ($n = 233$)	*P*
Age, years, mean ± SD	33.9 ± 7.8	57.3 ± 9.5	**<0.001**
Onset age, years, mean ± SD	28.8 ± 6.0	51.8 ± 9.0	**<0.001**
Gout duration, years, median (IQR)	3 (2, 7)	4 (2, 8)	0.070
Count of ever involved joints, median (IQR)	3 (2, 6)	4 (2, 8)	**0.002**
Flare times in the last year, median (IQR)	4 (2, 10)	4 (2, 12)	0.480
Family history of gout, *n* (%)	143 (37.0)	76 (32.6)	0.298
Tophi, *n* (%)	81 (20.9)	70 (30.0)	**0.012**
sUA, μmol/L, mean ± SD	550.7 ± 135.2	513.4 ± 129.0	**0.001**
sUA ≥ 600 μmol/L, *n* (%)	156 (40.3)	61 (26.2%)	**<0.001**
SCr, μmol/L, mean ± SD	97.6 ± 20.7	102.3 ± 27.8	**0.026**
eGFR, ml·min^{-1}·1.73 m^{-2}, mean ± SD	86.9 ± 16.6	77.7 ± 32.3	**<0.001**
CKD, *n* (%)	18 (4.7)	32 (13.7)	**<0.001**
Urolithiasis, *n* (%)	80 (20.7)	80 (34.3)	**<0.001**
BMI, kg/m^2, mean ± SD	26.0 ± 3.9	24.6 ± 2.8	**<0.001**
Obese, *n* (%)	107 (27.6)	25 (10.7)	**<0.001**
Overweight, *n* (%)	161 (41.6)	108 (46.4)	
Normal BMI, *n* (%)	113 (29.2)	98 (42.1)	
Underweight, *n* (%)	6 (1.5)	2 (0.9)	
Waist circumstance, cm, mean ± SD	91.2 ± 10.5	90.7 ± 8.1	0.450
Central obesity, *n* (%)	222 (57.4)	125 (53.6)	0.404
Dyslipidemia, *n* (%)	238 (61.5)	152 (65.2)	0.391
Hypercholesterolemia, *n* (%)	83 (21.4)	46 (19.7)	0.683
Hypertriglyceridemia, *n* (%)	123 (31.8)	62 (26.6)	0.176
LDL-C hyperlipidemia, *n* (%)	82 (21.2)	53 (22.7)	0.688
HDL-C hypolipidemia, *n* (%)	97 (25.1)	73 (31.3)	0.095
Metabolic syndrome, *n* (%)	162 (41.9)	120 (51.5)	**0.020**
Fatty liver disease, *n* (%)	198 (51.2)	103 (44.2)	0.098
T2DM, *n* (%)	26 (6.7)	40 (17.2)	**<0.001**
Hypertension, *n* (%)	123 (31.8)	133 (57.1)	**<0.001**
Coronary heart disease, *n* (%)	1 (0.3)	13 (5.6)	**<0.001**

sUA, serum uric acid; SCr, serum creatinine; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; T2DM, type 2 diabetes mellitus. Bold values mean the difference is statistically significant.

and SSB consumption (compared with <once/week [reference], 1–2 times/week: OR = 2.5, 95% CI: 1.3, 4.8; 3–4 times/week: OR = 2.2, 95% CI: 1.1, 4.2; >4 times/week: OR = 2.4, 95% CI: 1.3, 4.2; **Table 5**) were positively correlated with obesity. For later-onset gout patients, the correlation between food intake frequency and obesity weren't significant ($p > 0.05$).

After adjusting for potential confounding variables, the multivariate logistic regression analysis demonstrated that the risk factors for obesity were red meat (compared with <100 g/day [reference], >200 g/day: adjusted OR = 2.0, 95% CI: 1.1, 3.3) and SSB consumption (compared with <once/week [reference], 1–2 times/week: adjusted OR = 2.1, 95% CI: 1.1, 4.0; 3–4 times/week: adjusted OR = 1.8, 95% CI: 1.0, 3.5; >4 times/week: adjusted OR = 2.2, 95% CI: 1.2, 3.7). The correlation between SSB consumption and obesity was significant in early onset gout patients (compared with <once/week [reference], 1–2 times/week: adjusted OR = 2.4, 95% CI: 1.2, 4.9; 3–4 times/week: adjusted OR = 2.0, 95% CI: 1.0, 4.2; >4 times/week: adjusted OR = 2.5, 95% CI: 1.3, 4.7; **Figure 4**).

DISCUSSION

Our study showed that male gout patients with an early onset were characterized by a higher sUA level and a higher prevalence of obesity. For dietary habits before the onset of gout, early onset male gout patients consumed more SSB, red meat, and milk and milk product, but less alcohol and tea. In addition, a higher intake of SSB was associated with a higher sUA level and prevalence of obesity. Our results suggested that a higher sUA level and prevalence of obesity in male gout patients with an early onset is attributed to high SSB intake rather than other common gout-related dietary risk factors.

Both the prevalence of obesity and sUA level in the general population increase with age (26). Therefore, a higher prevalence of obesity and a higher sUA level in early onset than in later-onset male gout patients seems counter-intuitive. Obesity in youth has a detrimental effect on morbidity and mortality (26). Higher concentrations of sUA make it difficult to achieve a target sUA level. What's more, sUA is a risk factor for hypertension, coronary heart disease, and atrial stiffness (1, 27, 28). Thus, higher blood

TABLE 3 | Comparison of food intake frequency in male gout patients with early onset and later-onset.

Food intake frequency	All male gout patients ($n = 620$)	Early onset group ($n = 387$)	Later-onset group ($n = 233$)	*P*
Alcohol				
0–28 g/day, *n* (%)	466 (75.2)	312 (80.6)	154 (66.1)	**<0.001**
29–84 g/day, *n* (%)	71 (11.5)	42 (10.9)	29 (12.4)	
≥84 g/day, *n* (%)	83 (13.4)	33 (8.5)	50 (21.5)	
Red meat ($n = 592$)				
Less than 100 g/day, *n* (%)	232 (39.4)	130 (34.8)	102 (47.4)	**<0.001**
101–200 g/day, *n* (%)	219 (37.2)	163 (43.6)	56 (26.0)	
≥200 g/day, *n* (%)	138 (23.4)	81 (21.6)	57 (26.6)	
Animal offal				
Less than 1 time/week, *n* (%)	424 (68.4)	255 (65.9)	169 (72.5)	0.190
1–2 times/week, *n* (%)	128 (20.6)	83 (21.4)	45 (19.3)	
3–4 times/week, *n* (%)	42 (6.8)	32 (8.3)	10 (4.3)	
>4 times/week, *n* (%)	26 (4.2)	17 (4.4)	9 (3.9)	
Seafood				
Less than 1 time/week, *n* (%)	429 (69.2)	262 (67.7)	167 (71.7)	0.218
1–2 times/week, *n* (%)	108 (17.4)	76 (19.6)	32 (13.7)	
3–4 times/week, *n* (%)	44 (7.1)	24 (6.2)	20 (8.6)	
>4 times/week, *n* (%)	39 (6.3)	25 (6.5)	14 (6.0)	
Hotpot				
Less than 1 time/week, *n* (%)	546 (88.1)	341 (88.1)	205 (88.0)	0.993
1–2 times/week, *n* (%)	56 (9.0)	35 (9.0)	21 (9.0)	
3–4 times/week, *n* (%)	11 (1.8)	7 (1.8)	4 (1.7)	
>4 times/week, *n* (%)	7 (1.1)	4 (1.0)	3 (1.3)	
Slow-cooking soup				
Less than 1 time/week, *n* (%)	268 (43.2)	158 (40.8)	110 (47.2)	0.261
1–2 times/week, *n* (%)	196 (31.6)	126 (32.6)	70 (30.0)	
3–4 times/week, *n* (%)	93 (15.0)	65 (16.8)	28 (12.0)	
>4 times/week, *n* (%)	63 (10.2)	38 (9.8)	25 (10.7)	
Sugar-sweeten beverages				
Less than 1 time/week, *n* (%)	339 (54.7)	155 (40.1)	184 (79.0)	<0.001
1–2 times/week, *n* (%)	83 (13.4)	62 (16.0)	21 (9.0)	
3–4 times/week, *n* (%)	72 (11.6)	62 (16.0)	10 (4.3)	
>4 times/week, *n* (%)	126 (20.3)	108 (27.9)	18 (7.7)	
Tea				
Less than 1 time/week, *n* (%)	259 (41.8)	183 (47.3)	76 (32.6)	<0.001
1–2 times/week, *n* (%)	64 (10.3)	42 (10.9)	22 (9.4)	
3–4 times/week, *n* (%)	37 (6.0)	24 (6.2)	13 (5.6)	
>4 times/week, *n* (%)	260 (41.9)	138 (35.7)	122 (52.4)	
Coffee				
Less than 1 time/week, *n* (%)	562 (90.6)	346 (89.1)	217 (93.1)	0.346
1–2 times/week, *n* (%)	34 (5.5)	26 (6.7)	8 (3.4)	
3–4 times/week, *n* (%)	7 (1.1)	5 (1.3)	2 (0.9)	
>4 times/week, *n* (%)	17 (2.7)	11 (2.8)	6 (2.6)	
Milk and milk product ($n = 595$)				
Less than 1 time/week, *n* (%)	360 (60.8)	217 (57.9)	143 (65.9)	0.004
1–2 times/week, *n* (%)	143 (24.2)	107 (28.5)	36 (16.6)	
3–4 times/week, *n* (%)	40 (6.8)	26 (6.9)	14 (6.5)	
>4 times/week, *n* (%)	49 (8.3)	25 (6.7)	24 (11.1)	

Time/w: the frequency of dietary intake per week. Bold values mean the difference is statistically significant.

uric acid levels and a greater prevalence of obesity negatively impact young gout patients. Therefore, these issues must be addressed and managed appropriately during the management of these gout patients.

Worldwide, there are significant differences in eating habits between generations. The China National Nutrition and Health Survey showed a trend of increased SSB consumption and decreased alcohol and tea consumption in youth (29, 30). In

TABLE 4 | Univariate correlation between food intake frequency and sUA ≥600 μmol/L in male gout patients.

Food intake frequency	All male gout patients		Early onset gout patients		Later-onset gout patients	
	OR (95% CI)	*P*	OR (95% CI)	P	OR (95% CI)	*P*
Alcohol						
0–28 g/day	1	0.974	1	0.320	1	0.270
29–84 g/day	0.9 (0.6, 1.6)		1.4 (0.8, 2.8)		0.4 (0.1, 1.2)	
≥84 g/day	1.0 (0.6, 1.6)		1.5 (0.7, 3.1)		0.9 (0.4, 1.8)	
Red meat						
Less than 100 g/day	1	**0.008**	1	**0.043**	1	0.099
101–200 g/day	1.2 (0.8, 1.7)		1.0 (0.6, 1.7)		0.9 (0.4, 2.1)	
≥200 g/day	**2.0 (1.3, 3.0)**		**1.9 (1.1, 3.4)**		2.0 (0.9, 4.1)	
Animal offal						
Less than 1 time/week	1	**0.028**	1	0.202	1	0.094
1–2 times/week	0.8 (0.5, 1.2)		0.7 (0.4, 1.2)		1.0 (0.4, 2.1)	
3–4 times/week	1.2 (0.6, 2.2)		1.1 (0.5, 2.3)		0.8 (0.1, 3.7)	
>4 times/week	**3.0 (1.3, 6.8)**		2.0 (0.8, 5.5)		6.0 (1.4, 25.2)	
Seafood						
Less than 1 time/week	1	0.851	1	0.843	1	0.501
1–2 times/week	1.1 (0.7, 1.7)		0.8 (0.5, 1.5)		1.6 (0.7, 3.6)	
3–4 times/week	0.9 (0.5, 1.9)		1.2 (0.5, 2.9)		0.8 (0.2, 2.4)	
>4 times/week	1.3 (0.7, 2.6)		1.2 (0.5, 2.6)		1.7 (0.5, 5.4)	
Hotpot						
Less than 1 time/week	1	0.276	1	0.393	1	0.362
1–2 times/week	1.1 (0.6, 1.9)		1.1 (0.6, 2.3)		0.9 (0.3, 2.6)	
3–4 times/week	3.4 (1.0, 11.6)		3.8 (0.7, 19.9)		2.9 (0.4, 21.4)	
>4 times/week	1.4 (0.3, 6.5)		0.5 (0.0, 4.9)		5.9 (0.5, 66.2)	
Slow-cooking soup						
Less than 1 time/week	1	0.332	1	0.409	1	0.863
1–2 times/week	0.9 (0.6, 1.3)		0.9 (0.5, 1.4)		0.9 (0.4, 1.7)	
3–4 times/week	1.1 (0.7, 1.8)		1.1 (0.6, 2.0)		0.8 (0.3, 2.2)	
>4 times/week	0.6 (0.3, 1.1)		0.5 (0.3, 1.2)		0.6 (0.2, 1.8)	
Sugar-sweeten beverages						
Less than 1 time/week	1	**<0.001**	1	**0.002**	1	0.056
1–2 times/week	1.4 (0.8, 2.3)		1.3 (0.7, 2.4)		0.7 (0.2, 2.4)	
3–4 times/week	1.4 (0.8, 2.3)		0.9 (0.4, 1.6)		5.0 (1.0, 18.2)	
>4 times/week	**3.0 (2.0, 4.6)**		**2.5 (1.5, 4.1)**		2.6 (0.9, 7.1)	
Tea						
Less than 1 time/week	1	0.649	1	0.779	1	0.825
1–2 times/week	0.7 (0.4, 1.3)		0.8 (0.4, 1.5)		0.6 (0.2, 2.1)	
3–4 times/week	0.8 (0.4, 1.7)		0.7 (0.3, 1.7)		1.2 (0.3, 4.5)	
>4 times/week	0.9 (0.6, 1.2)		0.9 (0.6, 1.5)		1.0 (0.5, 2.0)	
Coffee						
Less than 1 time/week	1	0.429	1	0.310	1	0.193
1–2 times/week	0.7 (0.3, 1.4)		0.5 (0.2, 1.3)		1.0 (0.2, 5.1)	
3–4 times/week	2.5 (0.5, 11.1)		2.1 (0.4, 12.8)		3.0 (0.2, 49.1)	
>4 times/week	1.3 (0.5, 3.5)		0.5 (0.1, 2.0)		6.0 (1.0, 33.9)	
Milk and milk product						
Less than 1 time/week	1	0.895	1	0.812	1	0.918
1–2 times/week	1.1 (0.7, 1.7)		1.1 (0.7, 1.7)		0.9 (0.4, 2.0)	
3–4 times/week	1.2 (0.6, 2.4)		1.3 (0.6, 3.0)		1.1 (0.3, 3.6)	
>4 times/week	1.0 (0.5, 1.9)		1.4 (0.6, 3.2)		0.7 (0.2, 2.0)	

Bold values mean the difference is statistically significant.

Norway, Australia, Netherlands, and the United States, young people consume more SSB but less wine and tea than older people (31–33). Dietary factors that contribute to the development of gout have shifted from excessive alcohol consumption in later-onset gout patients to excessive intake of SSB and red meat in early onset gout patients.

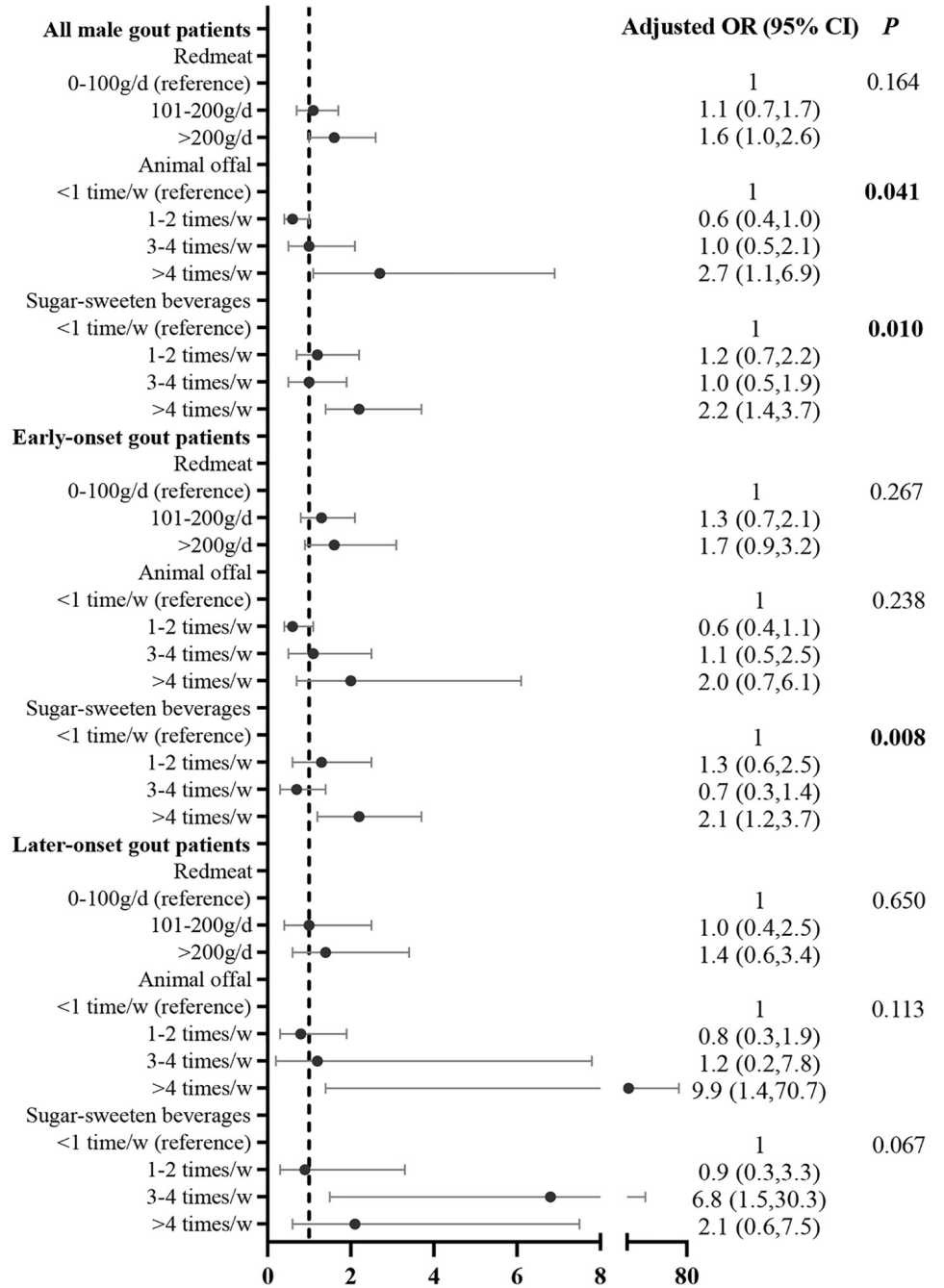

FIGURE 3 | Correlation between food intake frequency with sUA ≥600 μmol/L in male gout patients after adjusting for potential confounders. Confounders included age, duration of gout, family history, BMI, eGFR, tophi, comorbidities (i.e., hypertension, T2DM, dyslipidemia, and coronary heart disease, current medication) (i.e., aspirin, diuretics, and lipid-lowering medication), and other dietary factors that were significant in the univariate analysis.

SSB can increase the sUA level by depleting adenosine triphosphate during fructose metabolism, which, in turn, leads to the accumulation of adenosine monophosphate (AMP). The lack of free phosphate results in the conversion of AMP to the uric acid precursor inosine monophosphate (34). In prospective cohort studies, consumption of one serving per day of sugar-sweetened soft drinks led to a 45 and 74% higher gout risk in men (6) and women, respectively, than those who consumed less than one serving per month (5). In European ancestor gout patients, early onset gout patients consumed more SSB than later-onset gout patients (16). Similarly, our data revealed that early onset gout patients consumed more SSB and had a significantly higher sUA level than later-onset gout patients. Moreover, SSB consumption in male patients was positively correlated with sUA level over

TABLE 5 | Univariate correlation between food intake frequency and obesity in male gout patients.

Food intake frequency	All male gout patients		Early onset gout patients		Later-onset gout patients	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Alcohol						
0–28 g/day	1	0.137	1	0.136	1	0.606
29–84 g/day	0.8 (0.4, 1.5)		1.0 (0.5, 2.0)		0.6 (0.1, 2.9)	
≥84 g/day	**0.5 (0.3, 1.0)**		**0.3 (0.1, 1.0)**		1.4 (0.5, 3.6)	
Red meat						
Less than 100 g/day	1	**0.008**	1	**0.016**	1	0.117
101–200 g/day	1.1 (0.7, 1.7)		0.8 (0.5, 1.5)		0.9 (0.3, 3.1)	
≥200 g/day	**2.0 (1.2, 3.3)**		**2.0 (1.0, 3.5)**		2.5 (0.9, 6.7)	
Animal offal						
Less than 1 time/week	1	0.251	1	0.284	1	0.493
1–2 times/week	1.0 (0.6, 1.6)		1.2 (0.7, 2.1)		0.3 (0.7, 1.3)	
3–4 times/week	1.3 (0.6, 2.6)		1.2 (0.5, 2.7)		0.7 (0.1, 6.1)	
>4 times/week	0.1 (0.0, 1.1)		0.2 (0.0, 1.2)		0.0 (0.0, 0.0)	
Seafood						
Less than 1 time/week	1	0.315	1	0.370	1	0.944
1–2 times/week	1.6 (1.0, 2.5)		1.6 (0.9, 2.8)		0.8 (0.2, 2.9)	
3–4 times/week	1.2 (0.6, 2.5)		1.2 (0.5, 3.0)		1.3 (0.4, 5.1)	
>4 times/week	0.9 (0.4, 2.1)		1.1 (0.5, 2.9)		0.0 (0.0, 0.0)	
Hotpot						
Less than 1 time/week	1	0.900	0	1.0	1	1.0
1–2 times/week	0.8 (0.4, 1.6)		1.0 (0.5, 2.2)		0.0 (0.0, 0.0)	
3–4 times/week	0.8 (0.2, 3.7)		1.0 (0.2, 5.4)		0.0 (0.0, 0.0)	
>4 times/week	0.0 (0.0, 0.0)		0.0 (0.0, 0.0)		0.0 (0.0, 0.0)	
Slow-cooking soup						
Less than 1 time/week	1	0.759	1	0.512	1	0.972
1–2 times/week	1.0 (0.7, 1.6)		0.9 (0.6, 1.6)		1.0 (0.4, 2.7)	
3–4 times/week	0.7 (0.4, 1.4)		0.6 (0.3, 1.2)		1.0 (0.2, 3.7)	
>4 times/week	0.9 (0.5, 1.8)		1.0 (0.4, 2.1)		0.7 (0.1, 3.3)	
Sugar-sweeten beverages						
Less than 1 time/week	1	***P* < 0.001**	1	**0.009**	1	0.870
1–2 times/week	**2.5 (1.4, 4.4)**		**2.5 (1.3, 4.8)**		0.9 (0.2, 4.2)	
3–4 times/week	**2.6 (1.4, 4.6)**		**2.2 (1.1, 4.2)**		1.0 (0.1, 8.0)	
>4 times/week	**2.9 (1.8, 4.7)**		**2.4 (1.3, 4.2)**		1.7 (0.5, 6.5)	
Tea						
Less than 1 time/week	1	0.430	1	0.189	1	0.709
1–2 times/week	0.7 (0.3, 1.4)		0.8 (0.4, 1.9)		0.3 (0.0, 2.6)	
3–4 times/week	1.6 (0.7, 3.3)		2.2 (0.9, 5.3)		0.5 (0.1, 4.7)	
>4 times/week	1.0 (0.7, 1.5)		1.4 (0.8, 2.3)		0.8 (0.3, 1.9)	
Coffee						
Less than 1 time/week	1	0.489	1	0.260	1	0.547
1–2 times/week	0.8 (0.3, 2.0)		0.8 (0.3, 2.0)		0.0 (0.0, 0.0)	
3–4 times/week	1.5 (0.3, 7.8)		0.7 (0.1, 6.0)		8.0 (0.5, 132.7)	
>4 times/week	2.0 (0.7, 5.7)		3.2 (0.9, 10.7)		0.0 (0.0, 0.0)	
Milk and milk product						
Less than 1 time/week	1	0.800	1	0.917	1	0.647
1–2 times/week	1.1 (0.7, 1.8)		1.0 (0.6, 1.7)		0.7 (0.2, 2.6)	
3–4 times/week	1.1 (0.5, 2.3)		0.8 (0.3, 2.0)		2.2 (0.5, 8.6)	
>4 times/week	0.7 (0.3, 1.6)		1.2 (0.5, 2.9)		0.0 (0.0, 0.0)	

Bold values mean the difference is statistically significant.

600 μmol/L. Thus, higher SSB consumption may contribute to a paradoxically higher sUA level in younger gout patients with a higher eGFR.

A meta-analysis showed that intake of SSB increased the risk of being overweight or obese by 55% among groups with the highest intake compared with those with the lowest intake

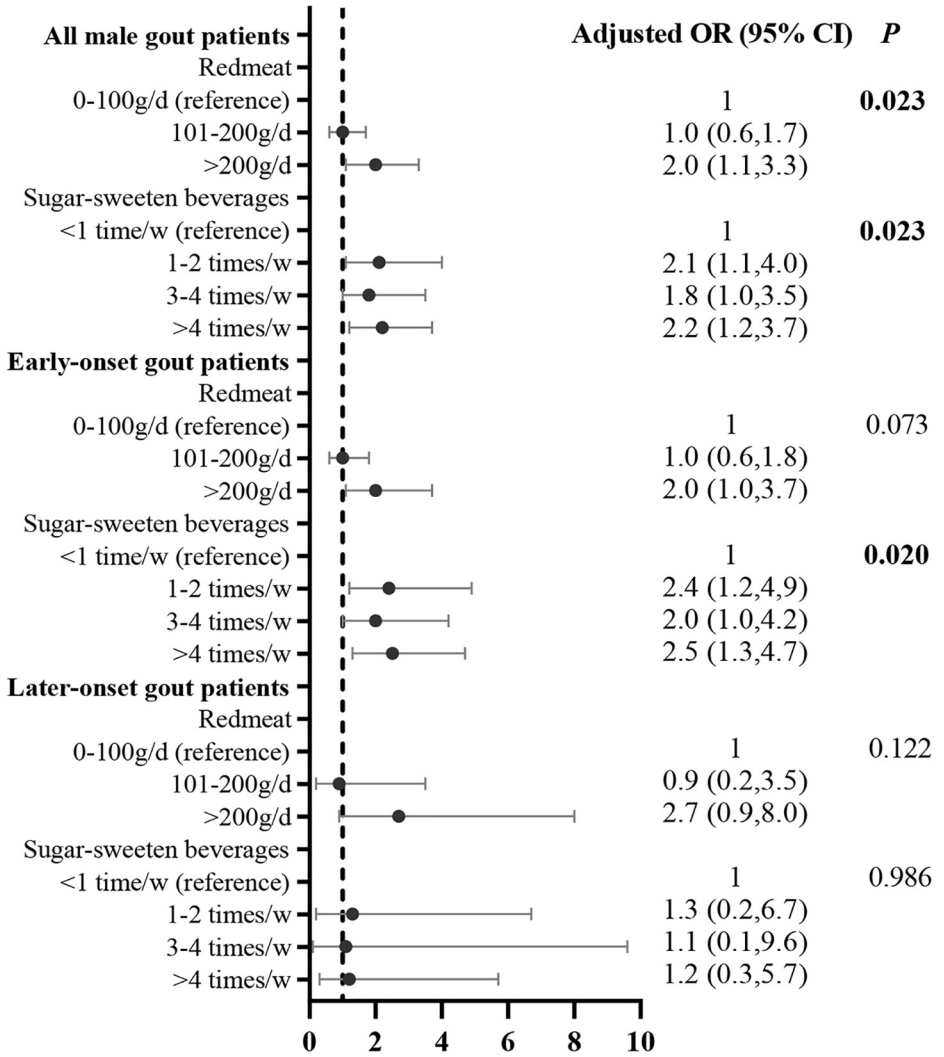

FIGURE 4 | Correlation between food intake frequency with obesity in male gout patients after adjusting for potential confounders. Confounders included age, duration of gout, tophi, eGFR, comorbidities (i.e., hypertension, T2DM, dyslipidemia, and coronary heart disease, and other dietary factors that were significant in the univariate analysis.

(35). Obesity increased sUA level and risk of developing gout. The United States National Health and Nutrition Examination Surveys conducted from 1988 to 1994 and 2007 to 2010 revealed that each unit increase in BMI was associated with a 5% higher prevalence of gout, even after adjustment for sUA level (36). Furthermore, patients with obesity are 2.24 times more likely to develop gout (37), and a prospective cohort study showed that obesity is associated with earlier onset of gout (38). Our study showed that SSB intake was positively correlated with obesity in male gout patients and early onset gout patients. Therefore, greater SSB intake likely contributes to a higher rate of obesity in early onset gout patients.

The correlation between intergenerational changes in dietary habits and intergenerational changes in clinical features of gout has been overlooked in previous studies (11–16). To our knowledge, the present study firstly demonstrated that of the many dietary factors, only SSB is associated with elevated sUA level and higher obesity rate in patients with early onset gout. In patients with early onset gout, attention should be paid to finding unfavorable dietary habits, with particular attention to SSB intake and advice to reduce added sugar intake. Dietary and exercise advice should be given to obese patients with early onset gout. However, reducing SSB intake cannot be left only to clinicians. The sugar industry has driven the growth of the SSB trend *via* pervasive marketing, which has influenced government policy, scientific research, and the diets of the general population (39). Thus, reducing the intake of SSB requires public health measures. The impact of introducing a sugar tax on the incidence of gout in the United States has been modeled, which showed that it could prevent almost 85,000 gout cases over 15 years, save more than 25,000 quality-adjusted life-years, and $3 billion (40).

There are several limitations to this study. First, this was a single-center study, which may have selection bias. Thus, our data from a university-affiliated hospital requires further confirmation using data from other centers. However, the patient characteristics of our study are similar to those of a study conducted at another university-affiliated hospital in China (13). Second, we investigated food intake frequency before the first gout attack. In the present study, patients had difficulty recalling the ingredients of the SSB and therefore could not calculate amount of fructose intake in detail. What's more, recall bias may be present in some patients, especially those with long disease duration. However, previous studies have suggested that past diets of up to 10 years prior are recalled with acceptable accuracy (41, 42). In this study, the median duration of gout was 3 years, and most patients had had gout for less than 10 years. Third, our study didn't include healthy population. Therefore, we couldn't explore the difference of gout related dietary habits between gout patients and healthy population. Fourth, total energy intake and physical activity of participants which were unavailable in the present study might be act as potential confounders. Differences in total energy intake and physical activity between early onset group and later-onset group is an interesting issue in future research. Lastly, our study assessed the relationship between dietary habits during the 1 year prior to an initial gout attack, current blood uric acid level, and obesity. A multicenter prospective study of a dynamic dietary survey on a representative community population should be conducted in the future which can assess whether dietary habits contribute to gout earlier onset, correlate with different gout clusters (43) and increase healthcare and economic burden of gout.

In conclusion, our study showed that the dietary factors that contribute to the development of gout have shifted from excessive alcohol consumption in later-onset gout patients to excessive SSB intake in early onset gout patients. This change was associated with a higher prevalence of obesity and higher sUA level in early onset gout patients. Thus, clinicians should provide specific dietary education for early onset and later-onset gout patients. The epidemic of excessive SSB consumption should be considered for the development of public health policies for the prevention of gout.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because disclosure of raw data requires regulatory approval. Requests to access the datasets should be directed to LD, dailie@mail.sysu.edu.cn.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Sun Yat-sen Memorial Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

Q-HL, Y-WZ, and LD: conception, design, and data analysis and interpretation. LD: administrative support. Q-HL, J-JL, Y-QM, and LD: provision of study materials or patients. S-YL, Y-FB, CD, and K-MY: collection and assembly of data. Q-HL and Y-WZ: writing the draft of the manuscript. All authors take part in draft revising and final approval of the manuscript.

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REFERENCES

1. Dalbeth N, Choi HK, Joosten LAB, Khanna PP, Matsuo H, Perez-Ruiz F, et al. Gout. *Nat Rev Dis Primers.* (2019) 5:69. doi: 10.1038/s41572-019-0115-y
2. Major TJ, Topless RK, Dalbeth N, Merriman TR. Evaluation of the diet wide contribution to serum urate levels: meta-analysis of population based cohorts. *BMJ.* (2018) 363:k3951. doi: 10.1136/bmj.k3951
3. Beyl RJ, Hughes L, Morgan S. Update on importance of diet in gout. *Am J Med.* (2016) 129:1153–8. doi: 10.1016/j.amjmed.2016.06.040
4. Cicero AFG, Fogacci F, Desideri G, Grandi E, Rizzoli E, D'Addato S, et al. Arterial stiffness, sugar-sweetened beverages and fruits intake in a rural population sample: data from the brisighella heart study. *Nutrients.* (2019) 11:2674. doi: 10.3390/nu11112674
5. Choi HK, Willett W, Curhan G. Fructose-rich beverages and risk of gout in women. *JAMA.* (2010) 304:2270–8. doi: 10.1001/jama.2010.1638
6. Choi HK, Curhan G. Soft drinks, fructose consumption, and the risk of gout in men: prospective cohort study. *BMJ.* (2008) 336:309–12. doi: 10.1136/bmj.39449.819271
7. Kuo CF, Grainge MJ, Zhang W, Doherty M. Global epidemiology of gout: prevalence, incidence and risk factors. *Nat Rev Rheumatol.* (2015) 11:649–62. doi: 10.1038/nrrheum.2015.91
8. Dehlin M, Jacobsson L, Roddy E. Global epidemiology of gout: prevalence, incidence, treatment patterns and risk factors. *Nat Rev Rheumatol.* (2020) 16:380–90. doi: 10.1038/s41584-020-0441-1
9. Zeng QY, Chen R, Darmawan J, Xiao ZY, Chen SB, Wigley R, et al. Rheumatic diseases in China. *Arthritis Res Ther.* (2008) 10:R17. doi: 10.1186/ar2368
10. Liu R, Han C, Wu D, Xia X, Gu J, Guan H, et al. Prevalence of hyperuricemia and gout in mainland China from 2000 to 2014: a systematic review and meta-analysis. *Biomed Res Int.* (2015) 2015:762820. doi: 10.1155/2015/762820
11. Xie LF, Zhao W, Zhong YC, Wang DL, Zhang XW. Investigation on the prevalence of hyperuricemia among young students. *Zhonghua Yi Xue Za Zhi.* (2018) 98:987. doi: 10.3760/cma.j.issn.0376-2491.2018.13.007
12. Pascart T, Norberciak L, Ea HK, Guggenbuhl P, Lioté F. GOSPEL 4 - Patients with early onset gout develop earlier severe joint involvement and metabolic comorbid conditions. *Arthritis Care Res.* (2019) 71:986–92. doi: 10.1002/acr.23706
13. Zhang B, Fang W, Zeng X, Zhang Y, Ma Y, Sheng F, et al. Clinical characteristics of early- and late-onset gout: a cross-sectional observational study from a Chinese gout clinic. *Medicine.* (2016) 95:e5425. doi: 10.1097/MD.0000000000005425
14. Lu CC, Wu SK, Chen HY, Chung WS, Lee MC, Yeh CJ. Clinical characteristics of and relationship between metabolic components and renal function among

patients with early-onset juvenile tophaceous gout. *J Rheumatol.* (2014) 41:1878–83. doi: 10.3899/jrheum.131240
15. Yu KH, Luo SF. Younger age of onset of gout in Taiwan. *Rheumatology.* (2003) 42:166–70. doi: 10.1093/rheumatology/keg035
16. Zaidi F, Narang RK, Phipps-Green A, Gamble GG, Tausche AK, So A, et al. Systematic genetic analysis of early-onset gout: ABCG2 is the only associated locus. *Rheumatology.* (2020) 59:2544–9. doi: 10.1093/rheumatology/kez685
17. Neogi T, Jansen TL, Dalbeth N, Fransen J, Schumacher HR, Berendsen D, et al. 2015 Gout classification criteria: an american college of rheumatology/european league against rheumatism collaborative initiative. *Arthritis Rheumatol.* (2015) 67:2557–68. doi: 10.1002/art.39254
18. Zhou BF, Cooperative Meta-Analysis Group of the Working Group on Obesity in China. Predictive values of body mass index and waist circumference for risk factors of certain related diseases in Chinese adults–study on optimal cut-off points of body mass index and waist circumference in Chinese adults. *Biomed Environ Sci.* (2002) 15:83–96.
19. Joint committee issued Chinese guideline for the management of dyslipidemia in adults. 2016 Chinese guideline for the management of dyslipidemia in adults. *Zhonghua Xin Xue Guan Bing Za Zhi.* (2016) 44:833–53. doi: 10.3760/cma.j.issn.0253-3758.2016.10.005
20. Chinese Diabetes Society. China guideline for type 2 diabetes (2013 edition). *Chin J Diabetes Mellit.* (2014) 6:447–98. doi: 10.3760/cma.j.issn.1000-6699.2014.10.020
21. Liu LS. Writing Group of 2010 Chinese Guidelines for the Management of Hypertension. 2010 Chinese guidelines for the management of hypertension. *Zhonghua Xin Xue Guan Bing Za Zhi.* (2011) 39:579–615.
22. Ma YC, Zuo L, Chen JH, Luo Q, Yu XQ, Li Y, et al. Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease. *J Am Soc Nephrol.* (2006) 17:2937–44. doi: 10.1681/ASN.2006040368
23. Zhao L, Ma G, Piao J, Zhang J, Yu D, He Y, et al. Scheme of the 2010-2012 Chinese nutrition and health surveillance. *Zhonghua Yu Fang Yi Xue Za Zhi.* (2016) 50:204–7. doi: 10.3760/cma.j.issn.0253-9624
24. Khanna D, Fitzgerald JD, Khanna PP, Bae S, Singh MK, Neogi T, et al. 2012 American College of Rheumatology guidelines for management of gout. Part 1: systematic nonpharmacologic and pharmacologic therapeutic approaches to hyperuricemia. *Arthritis Care Res.* (2012) 64:1431–46. doi: 10.1002/acr.21772
25. Welsh C, Earley K, Delahanty J, Wright KS, Berens T, Williams AA, et al. Residents' knowledge of standard drink equivalents: implications for screening and brief intervention for at-risk alcohol use. *Am J Addict.* (2014) 23:194–6. doi: 10.1111/j.1521-0391.2013.12080.x
26. Wang L, Zhou B, Zhao Z, Yang L, Zhang M, Jiang Y, et al. Body-mass index and obesity in urban and rural China: findings from consecutive nationally representative surveys during 2004-18. *Lancet.* (2021) 398:53–63. doi: 10.1016/S0140-6736(21)00798-4
27. Cicero A, Gitto S, Fogacci F, Rosticci M, Giovannini M, D'Addato S, et al. Fatty liver index is associated to pulse wave velocity in healthy subjects: data from the Brisighella Heart Study. *Eur J Intern Med.* (2018) 53:29–33. doi: 10.1016/j.ejim.2018.03.010
28. Cicero A, Fogacci F, Giovannini M, Grandi E, D'Addato S, Borghi C. Brisighella Heart Study group. Interaction between low-density lipoprotein-cholesterolaemia, serum uric level and incident hypertension: data from the Brisighella Heart Study. *J Hypertens.* (2019) 37:728–31. doi: 10.1097/HJH.0000000000001927
29. Gong W, Zhang Y, Yao Y, Chen Z, Liu A, Ding G. Beverage consumption among Chinese adults in 2010-2012. *Wei Sheng Yan Jiu.* (2018) 47:367–72.
30. Liu S, Yu D, Guo Q, Wang X, Xu X, Jia F, et al. Consumption status and trend of added sugar containing food among Chinese from 2002 to 2012. *Wei Sheng Yan Jiu.* (2016) 45:398–401.
31. Paulsen MM, Myhre JB, Andersen LF. Beverage consumption patterns among Norwegian adults. *Nutrients.* (2016) 8:561.
32. Sui Z, Zheng M, Zhang M, Rangan A. Water and beverage consumption: analysis of the Australian 2011-2012 national nutrition and physical activity survey. *Nutrients.* (2016) 8:678. doi: 10.3390/nu8090561
33. Özen AE, Bibiloni Mdel M, Pons A, Tur JA. Fluid intake from beverages across age groups: a systematic review. *J Hum Nutr Diet.* (2015) 28:417–42. doi: 10.1111/jhn.12250
34. Jamnik J, Rehman S, Blanco Mejia S, de Souza RJ, Khan TA, Leiter LA, et al. Fructose intake and risk of gout and hyperuricemia: a systematic review and meta-analysis of prospective cohort studies. *BMJ Open.* (2016) 6:e13191. doi: 10.1136/bmjopen-2016-013191
35. Te ML, Mallard S, Mann J. Dietary sugars and body weight: systematic review and meta-analyses of randomised controlled trials and cohort studies. *BMJ.* (2012) 346:e7492. doi: 10.1136/bmj.e7492
36. Teng GG, Tan CS, Santosa A, Saag KG, Yuan JM, Koh WP. Serum urate levels and consumption of common beverages and alcohol among Chinese in Singapore. *Arthritis Care Res.* (2013) 65:1432–40. doi: 10.1002/acr.21999
37. Evans PL, Prior JA, Belcher J, Mallen CD, Hay CA, Roddy E. Obesity, hypertension and diuretic use as risk factors for incident gout: a systematic review and meta-analysis of cohort studies. *Arthritis Res Ther.* (2018) 20:136. doi: 10.1002/acr.20479
38. DeMarco MA, Maynard JW, Huizinga MM, Baer AN, Köttgen A, Gelber AC, et al. Obesity and younger age at gout onset in a community-based cohort. *Arthritis Care Res.* (2011) 63:1108–14. doi: 10.1002/acr.20479
39. Singh GM, Micha R, Khatibzadeh S, Lim S, Ezzati M, Mozaffarian D, et al. Estimated global, regional, and national disease burdens related to sugar-sweetened beverage consumption in 2010. *Circulation.* (2015) 132:639–66. doi: 10.1161/CIRCULATIONAHA.114.010636
40. Yokose C, McCormick N, Choi HK. Dietary and lifestyle-centered approach in gout care and prevention. *Curr Rheumatol Rep.* (2021) 23:51. doi: 10.1007/s11926-021-01020-y
41. Eysteinsdottir T, Gunnarsdottir I, Thorsdottir I, Harris T, Launer LJ, Gudnason V, et al. Validity of retrospective diet history: assessing recall of midlife diet using food frequency questionnaire in later life. *J Nutr Health Aging.* (2011) 15:809–14. doi: 10.1007/s12603-011-0067-8
42. Takatsuka N, Kawakami N, Kawai K, Okamoto Y, Ishiwata K, Shimizu H. Validation of recalled food intake in the past in a Japanese population. *J Epidemiol.* (1996) 6:9–13. doi: 10.2188/jea.6.9
43. Richette P, Clerson P, Périssin L, Flipo RM, Bardin T. Revisiting comorbidities in gout: a cluster analysis. *Ann Rheum Dis.* (2015) 74:142–7. doi: 10.1136/annrheumdis-2013-203779

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Higher HEI-2015 scores are associated with lower risk of gout and hyperuricemia: Results from the national health and nutrition examination survey 2007–2016

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Gout, the most prevalent inflammatory arthritis, is becoming increasingly prevalent in the United States and across the world, and it adversely impacts people's quality of life and their health. Few studies have focused on the relationship between daily dietary quality and gout, so the topic requires further exploration. Data were derived from the National Health and Nutrition Examination Survey 2007–2016, and the inclusion criteria of the analytic sample were (1) adults, age ≥20 years, with complete information about HEI-2015, gout, and uric acid; (2) complete information of demographics, lifestyle (BMI, smoking, drinking), and disease history [hypertension, chronic kidney disease (CKD), diabetes]. The quality of the daily diet was reflected using the Healthy Eating Index 2015 (HEI-2015). The baseline features of different groups were examined using the Scott-Rao chi-square tests, and the association between the HEI-2015 score and the risk of gout/hyperuricemia (HUA) was investigated using weighted logistic regression models. The effects of different dietary components in the HEI-2015 on reducing the risk of gout/HUA were evaluated by weighted quantile sum (WQS) regression models. After adjusting for demographic characteristics, behavioral covariates, and disease history, higher HEI-2015 scores were associated with a significantly lower risk of gout (OR: 0.878, 95% CI: 0.876–0.880) and HUA (OR: 0.978, 95% CI: 0.976–0.979) in weighted logistic regression. Dairy, whole grains, plant proteins, and added sugar contributed greatly in HEI-2015 to reducing gout risk (weights of WQS index: 42, 17.18, 16.13, and 7.93%, respectively). Dairy, total fruits, greens and beans, and plant proteins contributed greatly in HEI-2015 to reducing HUA risk (weights of WQS

index: 28.9, 17.13, 16.84, and 11.39%, respectively). As the result, adherence to the American Dietary Guidelines may assist to decrease the risk of gout/HUA in American adults, and greater emphasis should be placed on dairy products, whole grains, fruits, legumes, and added sugars.

KEYWORDS

healthy eating index, gout, hyperuricemia, daily diet, weighted quantile sum (WQS) regression, NHANES

Introduction

Gout is inflammatory arthritis due to hyperuricemia (HUA), the most important pathological feature of which is the presence of sodium urate crystals in the joints. Gout episodes are frequently accompanied by excruciating joint pain and are closely linked to long-term conditions including obesity and hypertension (1–3), which impair the quality of life (4) and raise medical and care expenditures (5). There were 41.2 million prevalent cases of gout globally, with 7.4 million incident cases per year and almost 1.3 million years lived with disability (YLD) due to gout in 2017 (6, 7). Gout has become a global public health problem.

Recent research has revealed a strong link between diet and gout. According to a 26-year prospective cohort trial in the Health Professionals Follow-up Study (HPFS), the Dietary Approaches to Stop Hypertension (DASH) diet was beneficial in decreasing the incidence of gout and lowering serum uric acid concentrations in adult males, in comparison with a Western diet (8). Additionally, a clinical trial in Israel found that adherence to a Mediterranean diet can significantly lower serum uric acid and reduce the risk of gout in severely obese patients (9). Numerous studies have also evaluated the connection between particular foods or nutrients and gout, including dairy products (10), whole grains, fruits (8, 11, 12), added sugars (13, 14), and vitamins (12, 13). For instance, dairy products were shown to be beneficial in lowering serum uric acid and reducing the frequency of gout episodes in a randomized controlled study of gout patients (15). Moreover, a prospective cohort study showed that vegetables and fruits exhibited similar impacts to dairy products (11), and a cross-sectional analysis of the Brazilian Longitudinal Study of Adult Health (ELSA-Brazil) revealed that increased consumption of soft drinks and fructose was positively correlated with the risk of HUA (14).

The Healthy Eating Index (HEI) is a dietary quality indicator based on the Dietary Guidelines for Americans (DGA), and the HEI-2015 was the most recent version of the HEI. Multiple studies have discovered that the HEI score is related to health status, including physiological indicators (16), biochemical indicators (17), and disease risk (18, 19). The daily diet proposed by DGA is more adaptable in terms of food choices and is suited for a wider spectrum of people than the DASH diet and the Mediterranean diet (20). Furthermore, the adoption of the HEI-2015 also renders it possible to apply numerical values to represent how healthy a person's daily diet is, allowing for a comparison of diet quality.

To the best of our knowledge, no studies have investigated the relationship between HEI-2015 and gout or HUA. Therefore, this study intends to employ the HEI-2015 to estimate the overall health impact of various dietary components, in addition to analyzing the risk of developing gout or HUA in terms of adhering to DGA.

Materials and methods

Study sample

The National Health and Nutrition Examination Survey (NHANES) is a large open database developed to better understand the nutrition and diet of the American population. The database utilized a unique and complex multistage probabilistic design, so that sample weights could be used to reflect the non-institutionalized population of the United States. All subjects received a dietary survey and examination by a professional at mobile exam centers (MECs). The examination includes medical, dental, and physiological measurements, as well as laboratory tests, which were supervised by trained medical personnel. In addition, a variety of modern equipment enables NHANES to collect reliable, high-quality data.

We selected five consecutive survey cycles of NHANES (i.e., 2007–2008, 2009–2010, 2011–2012, 2013–2014, 2015–2016), and the overall sample size of adults (age $\geq$ 20) with no missing data for any variables is 23,109. The sample was weighted to represent a non-institutionalized adult population of 190 million Americans. Additional details of the study design, sampling, and exclusion criteria were illustrated in **Figure 1**. Only public data were used in the analysis, and ethical approval was not required for this study.

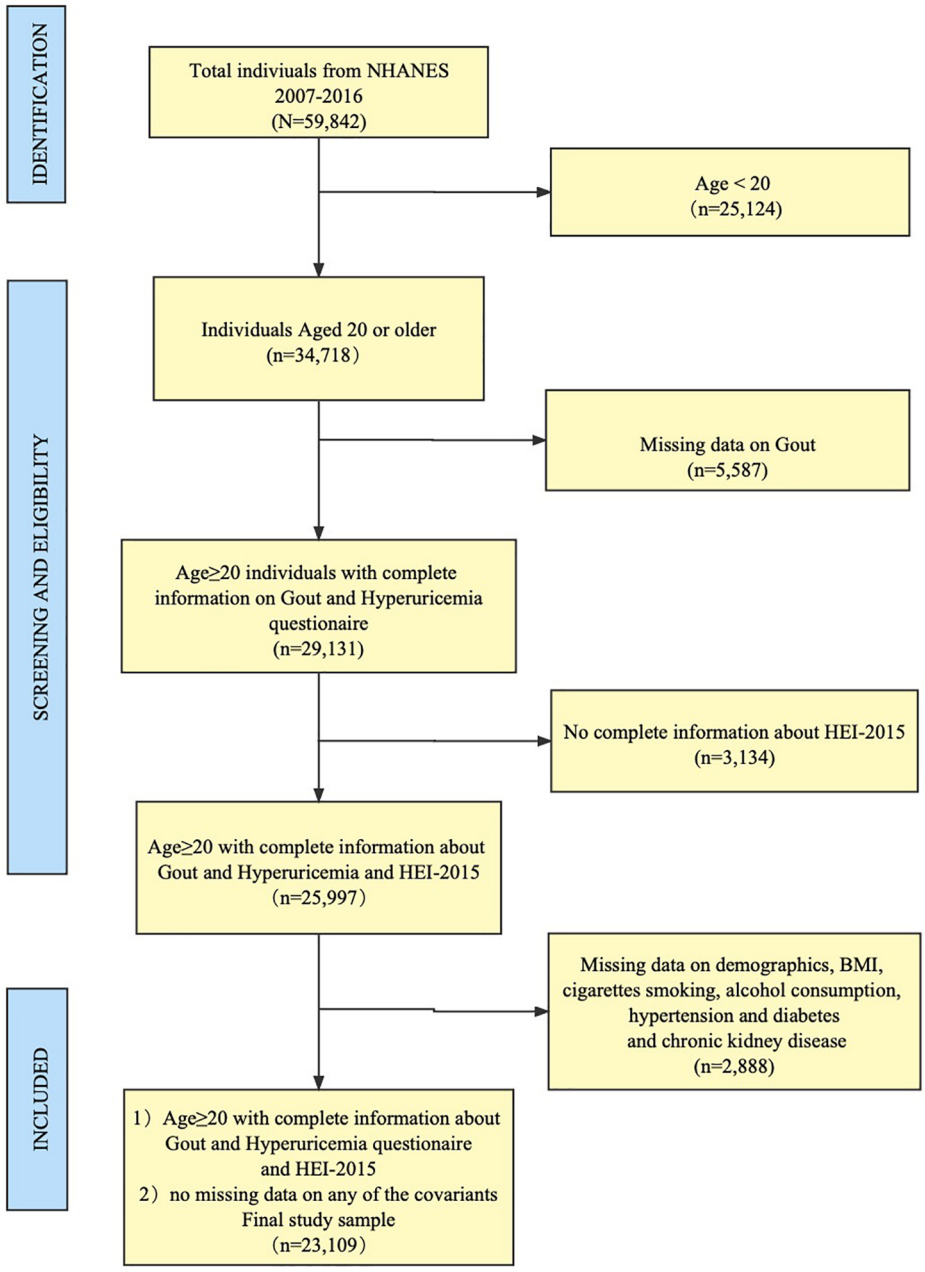

FIGURE 1
Flowchart of the study population.

Measurements and covariation assessment

Diet quality

HEI-2015 is utilized to assess the degree of adherence to DGA, which consists of 13 kinds of components (21). These 13 components were grouped into two forms, namely, the adequacy components (total vegetables, greens and beans, total fruits, whole fruits, whole grains, dairy, total protein foods, seafood and plant proteins, fatty acids) and the moderation components (sodium, refined grains, saturated fats, added sugars). Different maximum scores and weights were assigned to each component, and the overall 13 component scores (HEI-2015 scores) ranged from 0 to 100, with higher HEI scores reflecting better diet quality. Participants who had both two 24-h dietary recalls were included, and their dietary recall status was restricted to be reliable or had satisfied the minimum criteria for each day, which can reduce the bias of the HEI-2015. The

24-h dietary recall data were collected for 2 days, which were conducted by a trained interviewer face-to-face in the MEC on the first day and a follow-up interview 3–10 days later *via* phone. More details on dietary surveys and quality control can be found in the survey manual.[1] The HEI-2015 total and component scores were calculated by the simple HEI scoring algorithm using publicly available SAS macros. For weighted Scott–Rao chi-square tests and weighted logistic regressions, quartiles were used to categorize the HEI-2015 score into four groups, recorded as Q_1 (lowest diet quality, reference group), Q_2, Q_3, and Q_4 (highest diet quality), respectively (22).

Gout

All subjects were asked "Has a doctor or other health professional ever told you that you had gout?" and then categorized into non-gout subjects (reference group) and gout subjects according to the answers (23).

Hyperuricemia (HUA)

The level of uric acid in blood, plasma, and urine was determined using a timed endpoint approach. Uric acid is oxidized by uricase to produce allatoin and hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine (4-AAP) and 3,5-dichloro-2-hydroxybenzene sulfonate (DCHBS) in a reaction catalyzed by peroxidase to produce a colored product. The system monitors the change in absorbance at 520 nm at a fixed time interval. More quality control details can be found in the documentation (24). The uric acid values were recorded in mg/dl and then converted to μmol/L by multiplying 59.48. A serum urate level of >7.0 mg/dl in men and >5.7 mg/dl in women was considered HUA (23).

Covariates

Sex

Sex was classified as female (reference group) and male.

Age

Age was categorized into four groups, namely, 20–39 years group (reference group), 40–59 years group, 60–79 years group, and 80 years and above group (23).

Race

Race, according to NHANES classification, was categorized into non-Hispanic white (reference group), Mexican American group, non-Hispanic black, and other races (25).

Education

Education level was categorized as less than high school (reference group), high school graduate/GED, some college/AA degree, and college graduate or more (26).

Family income

Family income was categorized as ≤130% (reference group), >130–350%, and >350% by the ratio of family income to poverty (FPL) (27).

BMI status

Body mass index was calculated from measured height and weight as weight/height^2 (kg/m^2), then categorized into underweight (<18.5), normal (reference group, ≥18.5–24.9), overweight (≥25–29.9), and obese (≥30) (28).

Smoking status

Smoking behavior was measured by the "Smoking: Cigarette Use" questionnaire. The respondent was asked whether he/she had smoked at least 100 cigarettes in his/her life. If the respondent answered "no," he/she was classified as a never smoker (reference group). If the respondent had smoked at least 100 cigarettes in his/her life and still smokes when he/she answers the questionnaire, he/she is classified as a current smoker. The respondent was classified as a former smoker who has smoked 100 cigarettes in his life and had quit smoking when answering the questionnaire (29).

Drinking status

Drinking behavior was measured in the "alcohol use" questionnaire. In the "alcohol use" questionnaire, each respondent was asked how often he/she had drunk alcoholic drinks in the past 12 months and the average drinks on those days that he/she drank alcoholic beverages. According to these questions, the average number of alcoholic drinks consumed per week in the past 12 months could be calculated. A "drink" was defined as a 12-ounce beer, a 5-ounce glass of wine, or one-and-half ounces of liquor. Then, it was categorized into four strata (0, <1, 1–8, and ≥8 drinks per week) and defined as none (reference group), light, moderate, and heavy alcohol consumption, respectively (30).

Hypertension

All subjects were asked, "Ever been told by a doctor or other health professional that you had hypertension?," and then categorized into non-hypertension subjects (reference group) and hypertension subjects (31).

Chronic kidney disease

All subjects were asked, "Ever been told by a doctor or other health professional that you had weak or failing kidneys?," and then categorized into non-CKD subjects (reference group) and chronic kidney disease (CKD) subjects (32).

1 https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/manuals.aspx?BeginYear=2015

Diabetes

During the NHANES home interviews, all subjects were asked, "Ever been told by a doctor or other health professional that you had diabetes or sugar diabetes?," and then categorized into non-diabetic subjects (reference group) and diabetic subjects (33).

Statistical analysis

Analyses were conducted according to the Centers for Disease Control and Prevention (CDC) guidelines for analysis of NHANES data. A full sample 2-year mobile examination centers (MEC) weight was used to calculate the US non-institutionalized population.

Linear regression models were adopted to analyze the trends in the prevalence of gout and HUA in the five consecutive cycles. The baseline features of different groups were examined using the Scott-Rao chi-square tests, and the association between the HEI-2015 score and the risk of gout/HUA was investigated using weighted logistic regression models.

The weighted quartile sum (WQS) (34–37) regression model was used to assess the effects of mixed exposure to thirteen dietary components of HEI-2015. The WQS regression model calculates a weighted regression index that represents the overall dietary health effect for all thirteen dietary components of HEI-2015. The WQS model functions as follows:

$$g(\mu) = \beta_0 + \beta_1 \left(\sum_{i=0}^{c} \omega_i q_i \right) + z' \Phi$$

$$WQS = \sum_{j=1}^{c} \varpi_j q_j$$

where β_0 is the intercept; z' and Φ represent the matrix of covariates and the coefficients of the covariates. c is the number of dietary components considered in the analysis, and 13 dietary components were included in the current analysis. The whole sum of weighted indices (ω_i) is equal to 1, with the value of each component varying from 0 to $1\left(\sum_{i=0}^{c} \omega_i\right|_b = 1,\ 0 \geq \omega_i \geq 1\right).\beta_1$ is the regression coefficient of the WQS index. q_j represents the quartiles of a dietary component score (= 0, 1, 2, or 3 for the first, second, third, or fourth quartile, respectively). $g(\mu)$ is a logit link function, when the outcome of interest is binary (gout or not, HUA or not). The corresponding weight of each dietary component showed how much a specific dietary component contributed to the WQS index. The data were randomly split into two data sets (40% as training set and 60% as validation set).

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For all measures, we calculated 95% confidence intervals (CIs). The receiver operating characteristic curve (ROC) was used to validate the degree of WQS model fit.

All statistical tests were two-sided, and significance was considered at $P < 0.05$. WQS and ROC were performed with the R (version 4.1.0). RCS was implemented with the R package "rms" (version 6.3-0). WQS was implemented with the R package "gWQS" (version 3.0.4). Additional statistical analyses were performed using the SPSS statistical package (version 23.0; SPSS Inc., Chicago, IL, United States).

Results

Population characteristics between groups

Over the period of a total of five cycles from 2007 to 2016, the prevalence of gout (3.9% in 2007–2008 to 3.8% in 2015–2016, $P = 0.519$) and HUA (21.6% in 2007–2008 to 20.4% in 2015–2016, $P = 0.161$) among United States adults remained steady (**Table 1**).

The baseline characteristics of two groups (adults with/without gout) revealed that adults with gout were more likely to be male, over 60 years old, non-Hispanic black, have less than high school education, have low family income, obese, alcoholic, former smokers and suffer from CKD, hypertension, diabetes, HUA, and have lower HEI scores (**Table 2**).

Adults with HUA were more likely to be female, over 60 years old, non-Hispanic black, have a middle level of education, originate from low-income families, obese, alcoholics, or former smokers. They were also more likely to have CKD, hypertension, and diabetes, as well as lower HEI scores (**Supplementary Table 1**).

Higher healthy eating index score is associated with a lower risk of gout

After stepwise adjusting for covariates, a binary logistic regression model revealed that decreased gout risk was related to higher HEI-2015 scores (**Table 3**). In the model adjusted for age, sex, race/ethnicity, family income, and education, higher diet quality was linked to significantly decreased risks of gout [odds ratio (OR) 0.832 95% confidence intervals (CI): 0.830–0.834 for Q_4 compared with Q_1, $P < 0.001$]. Additional adjustments for BMI, smoking, and alcohol consumption did not significantly weaken this connection (OR: 0.886, 95%CI: 0.884–0.888 for Q_4 compared with Q_1, $P < 0.001$). The connection between HEI-2015 and gout risk remained significant (OR: 0.878, 95% CI: 0.876–0.880 for Q_4 compared with Q_1, $P < 0.001$) after further adjustments for chronic disease characteristics, including hypertension, diabetes, CKD, and HUA.

TABLE 1 The prevalence of gout and HUA among United States adults from 2007 to 2016.

	2007–2008 (n = 4831)	2009–2010 (n = 5006)	2011–2012 (n = 4150)	2013–2014 (n = 4620)	2015–2016 (n = 4502)	P-value
No. gout	224	223	173	186	216	
The prevalence of gout (Weighted%)	3.9%	3.8%	3.8%	4.1%	3.8%	0.519
No.HUA	1,115	1,084	892	964	928	
The prevalence of HUA (Weighted%)	21.60%	21.10%	20.10%	19.70%	20.40%	0.116

HUA, Hyperuricemia.

By applying the same analytical technique, we also discovered that higher HEI-2015 scores were related to a decreased risk of HUA (Q4 OR: 0.978, 95% CI: 0.976–0.979 for Q_4 compared with Q_1, $P < 0.001$, see **Supplementary Table 2**).

Figure 2 demonstrated the dose-response relationship between gout/HUA and HEI-2015 (as a continuous variable) by applying the restricted cubic splines (RCS) approach. Higher HEI-2015 scores did not demonstrate a protective effect of gout (OR: 0.988, 95% CI: 0.973–1.003, $P = 0.1223$) and instead showed a declining trend in OR (**Figure 2A**). Additionally, higher HEI-2015 scores revealed a declining OR trend (OR: 0.992, 95% CI: 0.985–0.999, $P = 0.0386$) and the preventive effects of HUA (**Figure 2B**).

Mixed effects of 13 dietary components on gout

The WQS regression was used to evaluate the health contributions of various dietary components to explore the health implications of dietary components in the total diet.

The WQS indices were statistically associated with gout. After adjusting for demographic variables, including age, sex, race, household income, and education level, the WQS index was significantly associated with progressively lower odds of gout (OR: 0.955, 95% CI, 0.930–0.982, $P = 0.0009$). The WQS index was significantly correlated with decreased risks of gout after further adjustments for BMI, smoking, and alcohol use (OR: 0.957, 95% CI, 0.933–0.983, $P = 0.0011$). These correlations were not significantly weakened by further adjustments for chronic disease characteristics (hypertension, diabetes, CKD, HUA) (OR: 0.963, 95% CI: 0.937–0.990, $P = 0.0067$).

Additionally, we discovered that the WQS indices significantly correlated with HUA (**Supplementary Table 3**). The WQS index was significantly linked with progressively decreased odds of HUA after multiple adjustments (OR: 0.934, 95% CI: 0.919–0.950, $P < 0.0001$).

The weight of each dietary component in the WQS regression model represented the contributions of the overall dietary health effects. Dairy, whole grains, and plant proteins were the highest weighted dietary components in the model of gout (42, 17.18, and 16.13%, respectively). Added sugar as a moderation component was also the highest weighted dietary component in the model of gout (7.93%) (**Figure 3A**). Similarly, dairy, total fruits, greens, beans, and plant proteins were the highest weighted dietary components in the model of HUA (**Figure 4A**) (28.9, 17.13, 16.84, and 11.39%, respectively). The evaluation of the WQS models showed that the area under the ROC curves (AUCs) was 0.834 in gout (**Figure 3B**) and 0.710 in HUA (**Figure 4B**).

Discussion

In agreement with the American College of Rheumatology report (23), this study discovered that the prevalence of both gout and HUA remained steady from 2007 to 2016. Our stepwise logistic regression models demonstrated that higher HEI-2015 scores were independently related to a decreased risk of gout/HUA. The findings supported the concept of HEI-2015's recommendations for a healthy diet (20, 21), which noted that a high-quality diet improved the quality of life.

Patients with HUA who followed a Mediterranean diet showed a decrease in blood uric acid concentration of 119 μmol/L over 6 months in a randomized controlled study (P for within-group comparison < 0.001)[9]. The quality of the Mediterranean diet, however, was not measured in this study. After quantifying the quality of the Mediterranean diet, MD Kontogianni et al. found a 70% reduction in the risk of HUA in those scoring in the Q_4 of the Mediterranean diet compared with those scoring in the Q_1 (OR: 0.30, 95%CI: 0.11–0.82) (38). According to a prospective cohort study of adult men conducted by Rai et al., higher DASH dietary scores were linked to a decreased incidence of gout (OR: 0.68, 95% CI: 0.57–0.80, $P < 0.001$, Q_5 vs. Q_1) (8). However, certain studies, such as those on the Mediterranean diet (39, 40) and the DASH diet (41, 42), estimated dietary quality scores based on the frequency of meals. In our study, we determined the consumption of each dietary component and then estimated the HEI-2015 score in the form of nutritional density (43). The HEI-2015 has 13 dietary components, compared with the 11 components of the Mediterranean diet score and the 8 components of the DASH diet, which improves the validity of our findings.

The HEI-2015 score calculation was based on the DGA, which included daily dietary recommendations for the US population and sought to improve dietary quality (20, 44). We

TABLE 2 Characteristics among adults aged 20 years or older by gout.

Characteristics	Adults without gout (n, %)	Adults with gout (n, %)	*P*-value
Sex no. (Weighted%)			<0.001
Female	11,369 (51.77)	302 (30.6)	
Male	10,698 (48.23)	740 (69.4)	
Age group no. (Weighted%)			<0.001
20–39 year	7,296 (35.24)	54 (6.39)	
40–59 year	7,416 (37.74)	264 (34.17)	
60–79 year	5,966 (22.52)	557 (47.3)	
80+ year	1,389 (4.51)	167 (12.13)	
Race no. (Weighted%)			<0.001
Non-Hispanic white	9,615 (68.56)	560 (77.16)	
Mexican American	3,528 (8.62)	73 (3.04)	
Non-Hispanic black	4,367 (10.34)	265 (11.83)	
Other	4,557 (12.48)	144 (7.97)	
Education no. (Weighted%)			<0.001
<High school	5,417 (16.12)	269 (17.13)	
High school/GED	4,997 (22.09)	269 (24.27)	
College/AA degree	6,491 (31.86)	296 (32.01)	
College or above	5,162 (29.94)	208 (26.6)	
Family income no. (Weighted%)			<0.001
0∼130 FPL	6,496 (19.97)	309 (20.88)	
>130∼350 FPL	7,550 (33.22)	368 (32.41)	
>350 FPL	8,021 (46.82)	365 (46.71)	
BMI no. (Weighted%)			<0.001
Normal weight	5,861 (27.78)	143 (11.59)	
Underweight	330 (1.47)	6 (0.35)	
Overweight	7,352 (33.63)	307 (30.87)	
Obese	8,524 (37.11)	586 (57.19)	
Drink level no. (Weighted%)			<0.001
None	7,133 (26.02)	407 (34.53)	
Light	6,786 (30.65)	268 (25.86)	
Moderate	7,548 (40.63)	337 (36.39)	
Heavy	600 (2.69)	30 (3.22)	
Smoke status no. (Weighted%)			<0.001
Never smoker	12,282 (55.65)	429 (42.83)	
Former smoker	5,214 (24.4)	446 (42.16)	
Current smoker	4,571 (19.95)	167 (15.01)	
CKD no. (Weighted%)			<0.001
No	21,419 (97.69)	919 (90.71)	
Yes	648 (2.31)	123 (9.29)	
Diabetes no. (Weighted%)			
No	19,411 (91.1)	726 (75.52)	<0.001
Yes	2,656 (8.9)	316 (24.48)	
Hypertension no. (Weighted%)			<0.001
No	14,432 (69.22)	261 (30.13)	
Yes	7,635 (30.78)	781 (69.87)	
HUA no. (Weighted%)			<0.001
No	17,577 (80.47)	549 (53.71)	
Yes	4,490 (19.53)	493 (46.29)	
HEI category no. (Weighted%)			<0.001
Q_1	5,492 (25.04)	233 (23.75)	
Q_2	5,491 (25.02)	259 (24.7)	
Q_3	5,590 (24.92)	294 (27.06)	
Q_4	5 494 (25.02)	256 (24.49)	

Values are survey-weighted percentages. FPL, family income to poverty; CKD, chronic kidney disease; HUA, Hyperuricemia; HEI, healthy eating index.

concentrated on the contributions of different components to identify the component that contributed the most to offering better dietary guidance to individuals with gout/HUA and at risk of gout/HUA. The results indicated that added sugars, dairy products, whole grains, seafood, and plant proteins contributed the most to lowering the risk of gout in the HEI-2015, implying that increasing dietary intake of dairy products, whole grains, vegetables, and legumes, as well as lowering added sugar intake within the recommended range, may decrease the risk of gout. Additionally, the diet's ability to protect against HUA was most strongly influenced by dairy products, total fruits, greens and beans, seafood and vegetable protein, whole grains, and whole fruits. It was shown that consuming more dairy products, whole grains, fruits, vegetables, and legumes while consuming added sugars less frequently and within the recommended range can lower the risk of HUA.

Dairy products provide a lot of high-quality protein, and it has been shown that casein and whey protein from milk can lower blood uric acid levels in healthy people (45). Dairy products are also low in purines and contribute less to the purine load associated with other high-quality protein sources, such as meat and seafood (10). Proteins and lipids from dairy products were also found to inhibit inflammatory responses associated with monosodium urate monohydrate (MSU) crystals *in vivo*/*in vitro* experiments (46). A study based on NHANES data revealed that a higher ratio of refined grains to whole grains was associated with a greater risk of CKD, and a high intake of whole grains was associated with low serum uric acid levels (47). Whole grains are a rich source of fiber, minerals, vitamins, phenolic compounds, phytoestrogens, and related antioxidants (48). These ingredients are beneficial for disease prevention and management (49–52). The association between fruit and uric acid/gout is disputed, since fructose metabolism generated urates, and fresh fruit is high in fructose (53, 54). Some studies have also found that fruit intake reduces the risk of gout because fruits contribute to urine alkalization and promote uric acid excretion (55). Fruits are rich in various nutrients, such as vitamin C (56, 57), potassium (58, 59), fiber (60), epicatechin, and flavonols (61, 62), which may alter the effects of fructose and urate. Studies on added sugars and gout

TABLE 3 Relationship between HEI-2015 and gout among adults aged 20 years or older.

Variable	OR (95% CI)			*P*-value
	Model 1	Model 2	Model 3	
Sex (reference, female)				<0.001
Male	2.743 (2.739, 2.748)	2.643 (2.639, 2.648)	3.027 (3.022, 3.033)	
Age group (reference, 20–39 year)				<0.001
40–59 year	5.204 (5.188, 5.220)	4.522 (4.508, 4.537)	3.68 (3.668, 3.692)	
60–79 year	12.372 (12.334, 12.411)	9.993 (9.961, 10.025)	6.234 (6.213, 6.254)	
80+ year	17.066 (17.002, 17.129)	15.729 (15.669, 15.790)	8.465 (8.431, 8.499)	
Race (reference, non-Hispanic white)				<0.001
Mexican American	0.430 (0.428, 0.432)	0.398 (0.396, 0.400)	0.452 (0.450, 0.454)	
Non-Hispanic black	1.245 (1.242, 1.248)	1.231 (1.228, 1.234)	1.022 (1.019, 1.024)	
Other	0.756 (0.753, 0.758)	0.828 (0.826, 0.830)	0.809 (0.807, 0.812)	
Education (reference, <high school)				<0.001
High school/GED	1.046 (1.044, 1.049)	1.056 (1.054, 1.059)	1.098 (1.095, 1.101)	
College/AA degree	1.122 (1.120, 1.125)	1.113 (1.110, 1.115)	1.121 (1.118, 1.124)	
College or above	0.932 (0.929, 0.934)	1.061 (1.059, 1.064)	1.152 (1.148, 1.155)	
Family income (reference,0∼130% FPL)				<0.001
>130∼350% FPL	0.703 (0.702, 0.705)	0.676 (0.674, 0.677)	0.722 (0.721, 0.724)	
>350% FPL	0.700 (0.699, 0.702)	0.681 (0.679, 0.682)	0.774 (0.772, 0.776)	
HEI category (reference, Q_1)				<0.001
Q_2	0.910 (0.908, 0.912)	0.903 (0.901, 0.905)	0.888 (0.886, 0.890)	
Q_3	0.974 (0.972, 0.976)	1.01 (1.007, 1.012)	0.991 (0.989, 0.993)	
Q_4	0.832 (0.830, 0.834)	0.886 (0.884, 0.888)	0.878 (0.876, 0.880)	
BMI (reference, normal weight)				<0.001
Underweight		0.619 (0.611, 0.627)	0.689 (0.680, 0.698)	
Overweight		1.678 (1.674, 1.683)	1.361 (1.358, 1.365)	
Obese		3.062 (3.054, 3.069)	1.907 (1.902, 1.912)	
Drink level (reference, none)				<0.001
Light		0.822 (0.820, 0.823)	0.871 (0.869, 0.872)	
Moderate		0.878 (0.876, 0.879)	0.913 (0.912, 0.915)	
Heavy		1.314 (1.308, 1.320)	1.203 (1.197, 1.209)	
Smoke status (reference, never)				<0.001
Former		1.335 (1.332, 1.337)	1.266 (1.264, 1.268)	
Current		1.019 (1.017, 1.022)	1.05 (1.047, 1.052)	
CKD (reference, no)				<0.001
Yes			2.051 (2.045, 2.057)	
Diabetes (reference, no)				<0.001
Yes			1.33 (1.327, 1.332)	
Hypertension (reference, no)				<0.001
Yes			2.304 (2.300, 2.308)	
HUA (reference, no)				<0.001
Yes			2.484 (2.480, 2.488)	

FPL, family income to poverty; CI, confidence interval; OR, odds ratio; CKD, chronic kidney disease; Model 1, adjusted for demographic characteristics (sex, age group, race, education, family income); Model 2, adjusted for demographic characteristics (sex, age group, race, education, family income); BMI, smoking, and drinking status; Model 3, adjusted for demographic characteristics (sex, age group, race, education, family income); BMI, smoking, drinking status, hypertension, CKD, diabetes, and hyperuricemia.

have also indicated that reducing added sugar intake helps to decrease uric acid and prevent gout.

According to the Dietary Guidelines for Americans 2020–2025 (20), adults in the United States should consume 8 ounces of seafood per week; however, in our study, 79% of participants did not reach this recommended standard. However, we found that the seafood and plant protein components have a healthy contribution to gout/HUA. We assumed that the protective

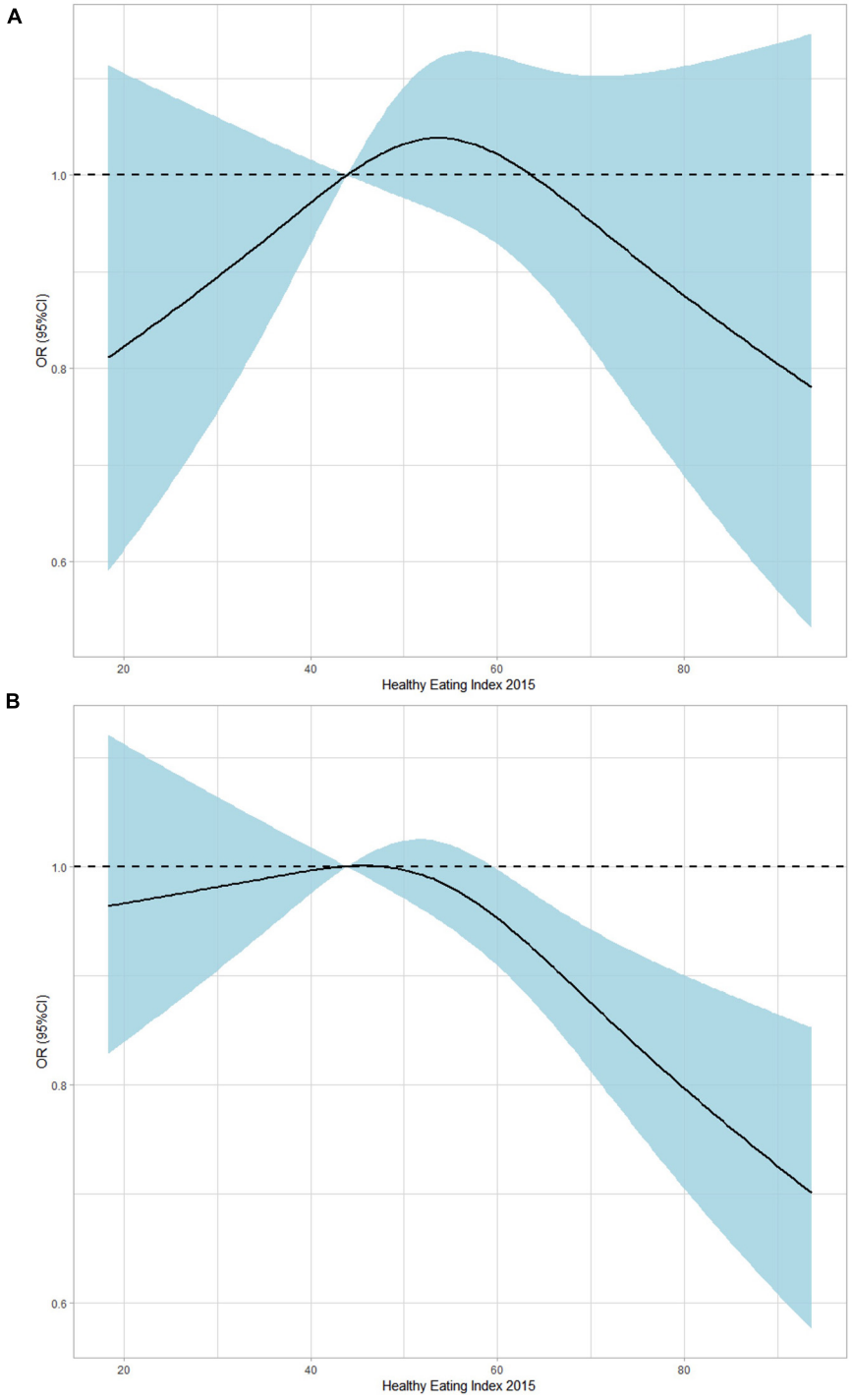

FIGURE 2
Dose-response association between HEI (it continues) and gout/HUA using restricted cubic splines (RCS). The models of gout **(A)** were adjusted for sex, age group, race, education, family income, BMI, smoking, drinking status, hypertension, CKD, diabetes, and hyperuricemia. The models of HUA **(B)** were adjusted for sex, age group, race, education, family income, BMI, smoking, drinking status, hypertension, CKD, and diabetes.

effects exhibited by the seafood and plant protein were due to seafood intake within reasonable limits (63) and the protective effects of plant protein (64, 65). The impact of low-dose seafood consumption and uric acid in healthy individuals required more exploration. Encouragement of greater consumption of dairy products, whole grains, low-fructose fruits, and high-quality

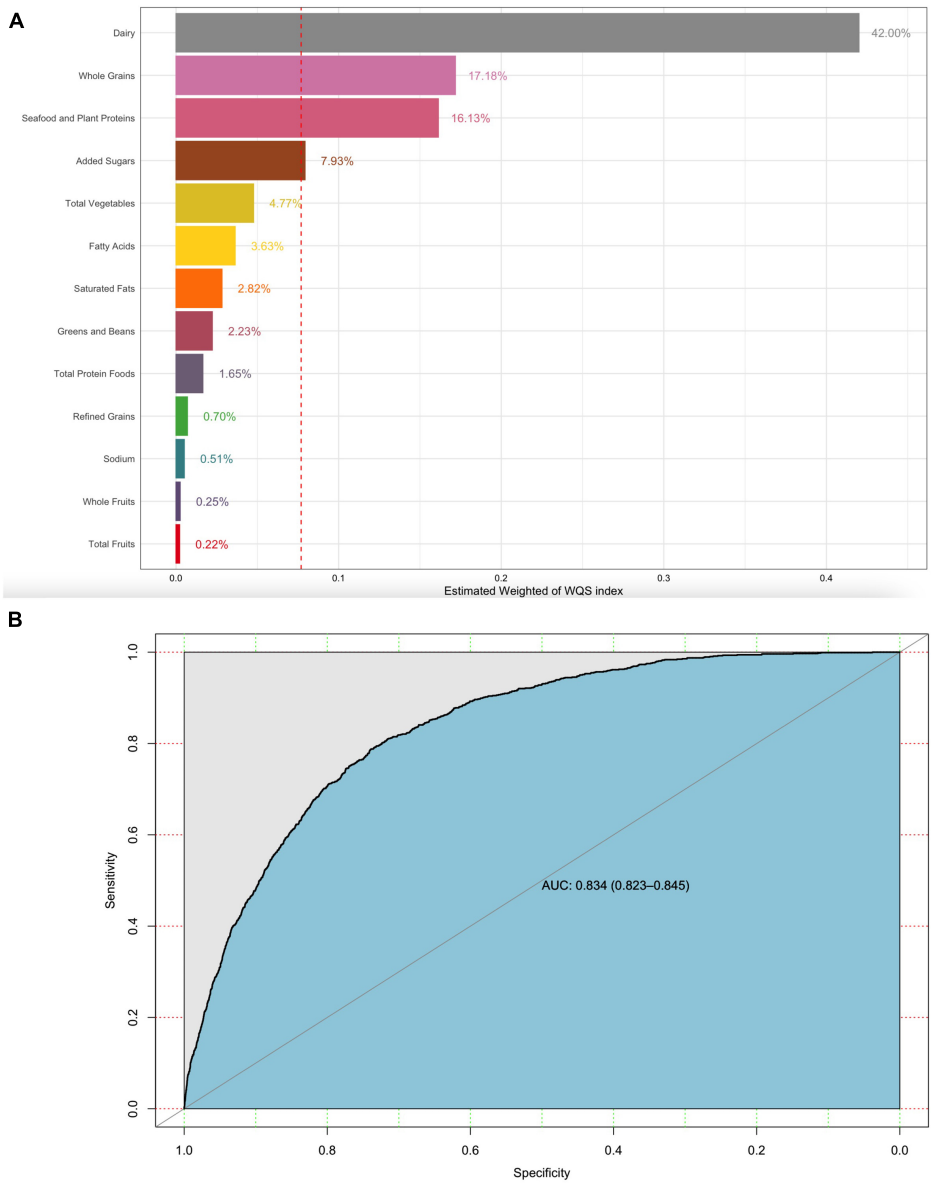

FIGURE 3
WQS model regression index weights for gout **(A)** and the AUCs of the WQS models **(B)**. Models were adjusted for sex, age group, race, education, family income, BMI, smoking, drinking status, hypertension, CKD, diabetes, and hyperuricemia.

protein from plant sources, particularly legumes, is crucial for preventing gout and decreasing blood uric acid.

We discovered additional risk variables for gout/HUA, including male gender, advanced age, non-Hispanic black race, middle education level, low income, obesity, alcohol intake, hypertension, and CKD. Due to estrogen's stimulation of uric acid excretion, women have always had low rates of HUA and gout (3). Poor education and income levels are risk factors for gout because they are directly associated with socioeconomic position, which affects access to healthcare and treatment compliance (66). Drinking promotes uric acid metabolism, raises blood uric acid levels, and increases the risk of gout/HUA, which was consistent with our results (67). Aging, obesity, hypertension, and CKD are all common gout risk factors, raising the incidence of gout by affecting uric acid metabolism or excretion (3). Interestingly, we found that smoking was a protective factor for HUA, as smoking may lower blood uric acid levels by metabolically interacting with superoxide (68). Another rationale is that since there was only one blood uric acid test implemented in this study, it cannot accurately represent long-term uric acid levels. However, the damage of long-term smoking to overall health also increases the risk of gout (69).

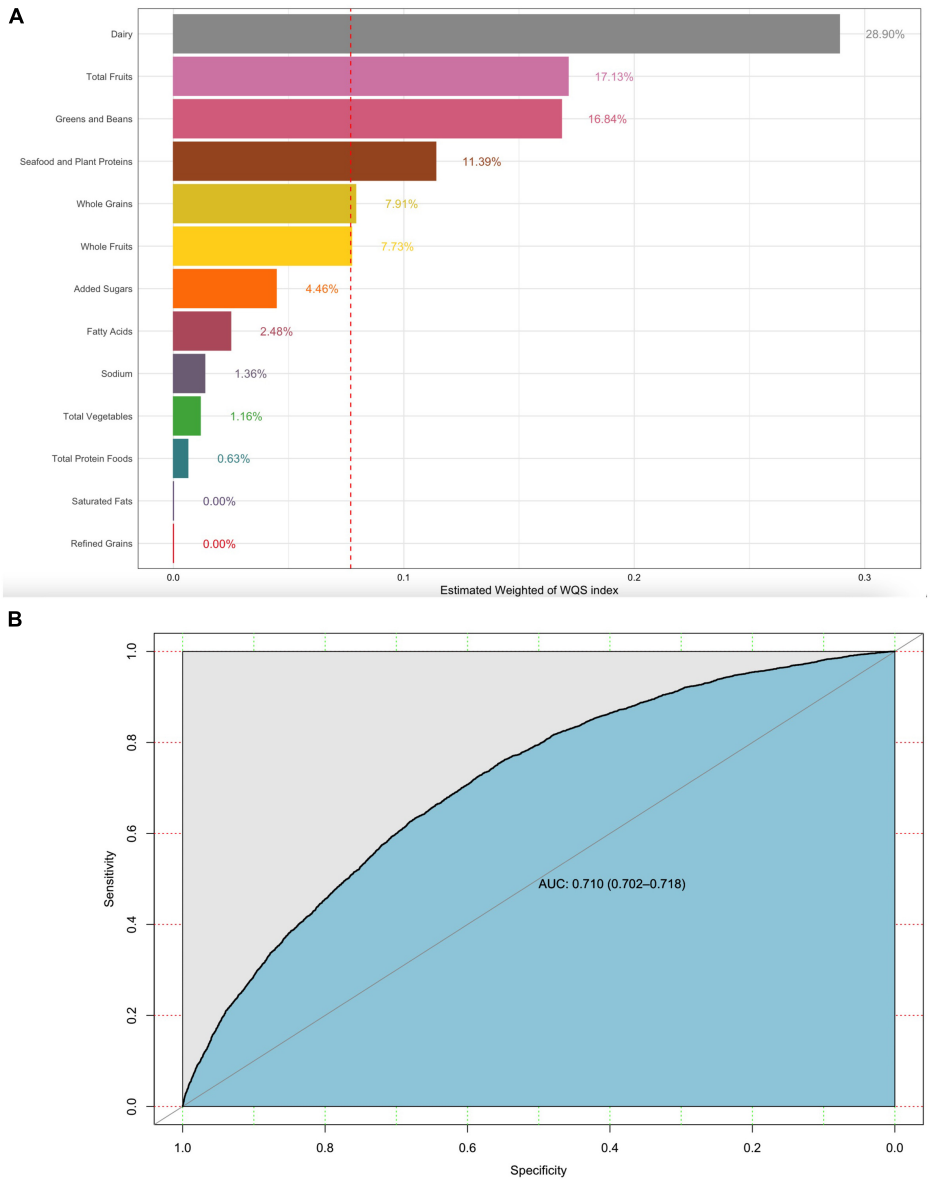

FIGURE 4
WQS model regression index weights for HUA **(A)** and the AUCs of the WQS models **(B)**. Models were adjusted for sex, age group, race, education, family income, BMI, smoking, drinking status, hypertension, CKD, and diabetes.

Diabetes was found to be a protective factor for HUA in this investigation, according to the logistic regression results of the HUA model. Diabetes, as a complication of HUA, has a very close relationship with HUA. Our study discovered that the added sugar scores in diabetes patients were substantially higher than those in non-diabetic subjects, indicating a decreased intake of added sugar in diabetic people. Research indicated that added sugar intake may raise serum uric acid levels (70, 71) and raise the risk of developing gout (72). In the liver, the metabolism of fructose, a major source of added sugar in the daily diet, stimulates adenosine monophosphate deaminase which promotes the degradation of adenosine monophosphate to inosine monophosphate. This process could induce insulin resistance (73–75) and promote the production of uric acid in the liver (43), thereby increasing the risk of HUA. Therefore, we assumed that diabetes showed as a protective factor for HUA in the results due to the significantly lower intake of added sugars in diabetic subjects than in non-diabetic subjects. Then, we proposed that restricting added sugar consumption might significantly lower the risk of HUA, including the diabetic population.

The article had several limitations due to the nature of the study. As with any observational study, our estimates were still subject to residual and unmeasured confounders, and no causal relationship can be inferred. Second, self-reported data about disease were subject to recall bias, and lack of adjustment for medication history for disease would affect results. Finally, only one serum uric acid test did not reflect the long-term uric acid level of the subject and thus caused possible erroneous results.

Nonetheless, our study also has some strengths. A major strength is the use of a large, nationally representative database. All dietary interviewers were required to complete professional courses and training and regularly reinforce training annually. Data from two 24-h dietary survey were used to reduce recall bias from the food frequency questionnaire. The HEI was developed and validated using HNANES dietary data, and dietary data were collected in the same manner as the HEI-2015 score. Effective and reasonable quantification of dietary quality is also a strength of this article. Second, the WQS model had been used in fewer nutrition-related studies. We used the WQS model to identify the highest contributing dietary components, and this study was a new application of the WQS model. Through this article, we hope to make the public aware of the benefits of a healthier daily diet for gout and HUA.

Conclusion

We found that people with the highest HEI-2015 score had a reduced risk of gout and HUA by 12.2 and 2.2% compared to people with the lowest HEI-2015 score. Dairy products, whole grains, fruits, and plant protein-related foods contributed the most to the health effects of the daily diet represented by HEI-2015. Therefore, people at high risk of gout or gout sufferers should pay more attention to maintaining a healthy diet and follow the DGA, especially increasing the intake of dairy products, whole grains, fruits, and legumes and reducing the intake of added sugars.

Data availability statement

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

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.921550/full#supplementary-material

References

1. Richette P, Bardin T. Gout. *Lancet (Lond Engl).* (2010) 375:318–28. doi: 10.1016/S0140-6736(09)60883-7

2. Dalbeth N, Merriman TR, Stamp LK. Gout. *Lancet (Lond Engl).* (2016) 388:2039–52. doi: 10.1016/S0140-6736(16)00346-9

3. Dalbeth N, Merriman TR, Stamp LK. Gout. *Lancet (Lond Engl).* (2021) 397:1843–55. doi: 10.1016/S0140-6736(21)00569-9

4. Smith E, Hoy D, Cross M, Merriman TR, Vos T, Buchbinder R, et al. The global burden of gout: estimates from the Global Burden of Disease 2010 study. *Ann Rheum Dis.* (2014) 73:1470–6. doi: 10.1136/annrheumdis-2013-204647

5. Danve A, Neogi T. , Rising global burden of gout: time to act. *Arthr Rheumatol (Hoboken N J).* (2020) 72:1786–8. doi: 10.1002/art.41453

6. Safiri S, Kolahi AA, Hill C, Smith E, Carson-Chahhoud K, Mansournia MA, et al. Prevalence, deaths, and disability-adjusted life years due to musculoskeletal disorders for 195 countries and territories 1990-2017. *Arthr Rheumatol (Hoboken N J).* (2021) 73:702–14. doi: 10.1002/art.41571

7. Dehlin M, Jacobsson L, Roddy E. Global epidemiology of gout: prevalence, incidence, treatment patterns and risk factors. *Nat Rev Rheumatol.* (2020) 16:380–90. doi: 10.1038/s41584-020-0441-1

8. Rai SK, Fung TT, Lu N, Keller SF, Curhan GC, Choi HK, et al. The dietary approaches to Stop Hypertension (DASH) diet, Western diet, and risk of gout in men: prospective cohort study. *BMJ (Clin Res ed).* (2017) 357:j1794. doi: 10.1136/bmj.j1794

9. Yokose C, McCormick N, Rai SK, Lu N, Curhan G, Schwarzfuchs D, et al. Effects of low-fat, mediterranean, or low-carbohydrate weight loss diets on serum urate and cardiometabolic risk factors: a secondary analysis of the dietary intervention randomized controlled trial (DIRECT). *Diab Care.* (2020) 43:2812–20. doi: 10.2337/dc20-1002

10. Choi HK, Atkinson K, Karlson EW, Willett W, Curhan G. Purine-rich foods, dairy and protein intake, and the risk of gout in men. *N Engl J Med.* (2004) 350:1093–103. doi: 10.1056/NEJMoa035700

11. Pan W-H, Wu HJ, Yeh CJ, Chuang SY, Chang HY, Yeh NH, et al. Diet and health trends in Taiwan: comparison of two nutrition and health surveys from 1993-1996 and 2005-2008. *Asia Pac J Clin Nutr.* (2011) 20:238–50.

12. Towiwat P, Li Z-G. The association of vitamin C, alcohol, coffee, tea, milk and yogurt with uric acid and gout. *Int J Rheum Dis.* (2015) 18:495–501. doi: 10.1111/1756-185X.12622

13. Zheng Z, Harman JL, Coresh J, Köttgen A, McAdams-DeMarco MA, Correa A, et al. The dietary fructose:vitamin c intake ratio is associated with hyperuricemia in african-american adults. *J Nutr.* (2018) 148:419–26. doi: 10.1093/jn/nxx054

14. Siqueira JH, Mill JG, Velasquez-Melendez G, Barreto SM, Benseñor IM, et al. Sugar-sweetened soft drinks and fructose consumption are associated with hyperuricemia: cross-sectional analysis from the brazilian longitudinal study of adult health (ELSA-Brasil). *Nutrients.* (2018) 10:981. doi: 10.3390/nu10080981

15. Dalbeth N, Wong S, Gamble GD, Mason B, Pool B, et al. Acute effect of milk on serum urate concentrations: a randomised controlled crossover trial. *Ann Rheum Dis.* (2010) 69:1677–82. doi: 10.1136/ard.2009.124230

16. Xu B, Houston DK, Locher JL, Ellison KJ, Gropper S, Buys DR, et al. Higher Healthy Eating Index-2005 scores are associated with better physical performance. *J Gerontol Ser A Biol Sci Med Sci.* (2012) 67:93–9. doi: 10.1093/gerona/glr159

17. Liu Y, Ajami NJ, El-Serag HB, Hair C, Graham DY, White DL, et al. Dietary quality and the colonic mucosa-associated gut microbiome in humans. *Am J Clin Nutr.* (2019) 110:701–12. doi: 10.1093/ajcn/nqz139

18. Xu B, Houston D, Locher JL, Zizza C. The association between Healthy Eating Index-2005 scores and disability among older Americans. *Age Ageing.* (2012) 41:365–71. doi: 10.1093/ageing/afr158

19. de Koning L, Chiuve SE, Fung TT, Willett WC, Rimm EB, Hu FB, et al. Diet-quality scores and the risk of type 2 diabetes in men. *Diabetes Care.* (2011) 34:1150–6. doi: 10.2337/dc10-2352

20. Phillips JA. Dietary guidelines for americans, 2020-2025. *Workplace Health Safety.* (2021) 69:395. doi: 10.1177/21650799211026980

21. 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. doi: 10.1016/j.jand.2018.05.021

22. Yoo ER, Kim D, Vazquez-Montesino LM, Escober JA, Li AA, Tighe SP, et al. Diet quality and its association with nonalcoholic fatty liver disease and all-cause and cause-specific mortality. *Liver Int Off J Int Associat Study Liver.* (2020) 40:815–24. doi: 10.1111/liv.14374

23. Chen-Xu M, Yokose C, Rai SK, Pillinger MH, Choi HK. Contemporary prevalence of gout and hyperuricemia in the united states and decadal trends: the national health and nutrition examination survey, 2007-2016. *Arthr Rheumatol (Hoboken NJ).* (2019) 71:991–9. doi: 10.1002/art.40807

24. National Health and Nutrition Examination Survey. *Contract for Difference (CFD).* (2016). Available online at: https://wwwn.cdc.gov/nchs/data/nhanes/2015-2016/manuals/2016_MEC_Laboratory_Procedures_Manual.pdf (accessed January 30, 2022).

25. Tsai J, Homa DM, Gentzke AS, Mahoney M, Sharapova SR, Sosnoff CS, et al. Exposure to secondhand smoke among nonsmokers - United States, 1988-2014. *MMWR Morbid Mortal Weekly Report.* (2018) 67:1342–6. doi: 10.15585/mmwr.mm6748a3

26. Scholes S, Bann D. Education-related disparities in reported physical activity during leisure-time, active transportation, and work among US adults: repeated cross-sectional analysis from the National Health and Nutrition Examination Surveys, 2007 to 2016. *BMC Public Health* (2018) 18:926. doi: 10.1186/s12889-018-5857-z

27. Montgomery J, Lu J, Ratliff S, Mezuk B. Food insecurity and depression among adults with diabetes: results from the national health and nutrition examination survey (NHANES). *Diabetes Educat.* (2017) 43:260–71. doi: 10.1177/0145721717699890

28. Lu N, Dubreuil M, Zhang Y, Neogi T, Rai SK, Ascherio A, et al. Gout and the risk of Alzheimer's disease: a population-based BMI-matched cohort study. *Ann Rheum Dis.* (2016) 75:547–51. doi: 10.1136/annrheumdis-2014-206917

29. Alharthi SSY, Dubreuil M, Zhang Y, Neogi T, Rai SK, Ascherio A, et al. Association between time since quitting smoking and periodontitis in former smokers in the National Health and Nutrition Examination Surveys (NHANES) 2009 to 2012. *J Periodontol.* (2019) 90:16–25. doi: 10.1002/JPER.18-0183

30. Breslow RA, Chen CM, Graubard BI, Jacobovits T, Kant AK. Diets of drinkers on drinking and nondrinking days: NHANES 2003-2008. *Am J Clin Nutr.* (2013) 97:1068–75. doi: 10.3945/ajcn.112.050161

31. Santos, D, and Dhamoon, MS. Trends in antihypertensive medication use among individuals with a history of stroke and hypertension, 2005 to 2016. *JAMA Neurol.* (2020) 77:1382–89. doi: 10.1001/jamaneurol.2020.2499

32. Florea, A, Jacobs, ET, Harris, RB, Klimentidis, YC, Thajudeen, B, Kohler, LN, et al. Chronic kidney disease unawareness and determinants using 1999-2014 National Health and Nutrition Examination Survey Data. *J Public Health.* (2021) 9:fdab112. doi: 10.1093/pubmed/fdab112

33. Saydah SH, Siegel KR, Imperatore G, Mercado C, Gregg EW, et al. The cardiometabolic risk profile of young adults with diabetes in the U.S. *Diabetes Care.* (2019) 42:1895–902. doi: 10.2337/dc19-0707

34. Carrico C, Gennings C, Wheeler DC, Factor-Litvak P. Characterization of weighted quantile sum regression for highly correlated data in a risk analysis setting. *J Agricult Biol Environ Statist.* (2015) 20:100–20. doi: 10.1007/s13253-014-0180-3

35. Czarnota J, Gennings C, Colt JS, De Roos AJ, Cerhan JR, Severson RK, et al. Analysis of environmental chemical mixtures and non-hodgkin lymphoma risk in the NCI-SEER NHL Study. *Environ Health Perspect.* (2015) 123:965–70. doi: 10.1289/ehp.1408630

36. Czarnota J, Gennings C, Wheeler DC. Assessment of weighted quantile sum regression for modeling chemical mixtures and cancer risk. *Cancer Informatics.* (2015) 14(Suppl. 2):159–71. doi: 10.4137/CIN.S17295

37. Zhang Y, Dong T, Hu W, Wang X, Xu B, Lin Z, et al. Association between exposure to a mixture of phenols, pesticides, and phthalates and obesity: comparison of three statistical models. *Environ Int.* (2019) 123:325–36. doi: 10.1016/j.envint.2018.11.076

38. Kontogianni MD, Panagiotakos DB, Tsetsekou E, Zeimbekis A, Pitsavos C, et al. Adherence to the Mediterranean diet and serum uric acid: the ATTICA study. *Scand J Rheumatol.* (2012) 41:442–9. doi: 10.3109/03009742.2012.679964

39. Panagiotakos DB, Pitsavos C, Stefanadis C. Dietary patterns: a Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. Nutrition, metabolism, and cardiovascular diseases. *NMCD.* (2006) 16:559–68. doi: 10.1016/j.numecd.2005.08.006

40. Panagiotakos DB, Pitsavos C, Arvaniti F, Stefanadis C. Adherence to the mediterranean food pattern predicts the prevalence of hypertension, hypercholesterolemia, diabetes and obesity, among healthy adults; the accuracy of the MedDietScore. *Preventive Med.* (2007) 44:335–40. doi: 10.1016/j.ypmed.2006.12.009

41. Fung TT, Chiuve SE, McCullough ML, Rexrode KM, Logroscino G, Hu FB. Adherence to a DASH-style diet and risk of coronary heart disease and stroke in women. *Arch Int Med.* (2008) 168:713–20. doi: 10.1001/archinte.168.7.713

42. Taylor EN, Fung TT, Curhan GC. DASH-style diet associates with reduced risk for kidney stones. *J Am Soc Nephrol.* (2009) 20:2253–9. doi: 10.1681/ASN.2009030276

43. Lerman JL, Krebs-Smith SM, Kirkpatrick SI, Pannucci TE, Wilson MM, et al. Evaluation of the healthy eating index-2015. *J Acad Nutr Dietet.* (2018) 118:1622–33. doi: 10.1016/j.jand.2018.05.019

44. Snetselaar LG, de Jesus JM, DeSilva DM, Stoody EE. Dietary guidelines for Americans, 2020-2025: understanding the scientific process guidelines, and key recommendations. *Nutrit Today.* (2021) 56:287–95. doi: 10.1097/NT.0000000000000512

45. Garrel DR, Verdy M, PetitClerc C, Martin C, Brulé D, Hamet P, et al. Milk- and soy-protein ingestion: acute effect on serum uric acid concentration. *Am J Clin Nutr.* (1991) 53:665–9. doi: 10.1093/ajcn/53.3.665

46. Dalbeth N, Gracey E, Pool B, Callon K, McQueen FM, Cornish J, et al. Identification of dairy fractions with anti-inflammatory properties in models of acute gout. *Ann Rheum Dis.* (2010) 69:766–9. doi: 10.1136/ard.2009.113290

47. Mazidi M, Katsiki N, Mikhailidis DP, Banach M. A higher ratio of refined grain to whole grain is associated with a greater likelihood of chronic kidney disease: a population-based study. *Br J Nutr.* (2019) 121:1294–302. doi: 10.1017/S0007114518003124

48. Aune D, Keum N, Giovannucci E, Fadnes LT, Boffetta P, Greenwood DC, et al. Whole grain consumption and risk of cardiovascular disease, cancer, and all cause and cause specific mortality: systematic review and dose-response meta-analysis of prospective studies. *BMJ (Clin Res ed.).* (2016) 353:i2716. doi: 10.1136/bmj.i2716

49. Liese AD, Roach AK, Sparks KC, Marquart L, D'Agostino RB Jr., Mayer-Davis EJ, et al. Whole-grain intake and insulin sensitivity: the Insulin Resistance Atherosclerosis Study. *Am J Clin Nutr.* (2003) 78:965–71. doi: 10.1093/ajcn/78.5.965

50. Steffen LM, Jacobs DR Jr., Murtaugh MA, Moran A, Steinberger J, Hong CP, et al. Whole grain intake is associated with lower body mass and greater insulin sensitivity among adolescents. *Am J Epidemiol.* (2003) 158:243–50. doi: 10.1093/aje/kwg146

51. Ajani UA, Ford ES, Mokdad AH. Dietary fiber and C-reactive protein: findings from national health and nutrition examination survey data. *J Nutr.* (2004) 134:1181–5. doi: 10.1093/jn/134.5.1181

52. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol.* (2015) 11:577–91. doi: 10.1038/nrendo.2015.128

53. Nakagawa T, Lanaspa MA, Johnson RJ. The effects of fruit consumption in patients with hyperuricaemia or gout. *Rheumatol (Oxf Engl).* (2019) 58:1133–41. doi: 10.1093/rheumatology/kez128

54. Zou F, Zhao X, Wang F. A review on the fruit components affecting uric acid level and their underlying mechanisms. *J Food Biochem.* (2021) 45:e13911. doi: 10.1111/jfbc.13911

55. Kanbara A, Hakoda M, Seyama I. Urine alkalization facilitates uric acid excretion. *Nutr J.* (2010) 9:45. doi: 10.1186/1475-2891-9-45

56. Berger L, Gerson CD, Yü TF. The effect of ascorbic acid on uric acid excretion with a commentary on the renal handling of ascorbic acid. *Am J Med.* (1977) 62:71–6. doi: 10.1016/0002-9343(77)90351-5

57. Wu T-K, Wei CW, Pan YR, Cherng SH, Chang WJ, Wang HF, et al. Vitamin C attenuates the toxic effect of aristolochic acid on renal tubular cells via decreasing oxidative stress-mediated cell death pathways. *Mol Med Rep.* (2015) 12:6086–92. doi: 10.3892/mmr.2015.4167

58. Cappuccio FP, MacGregor GA. Does potassium supplementation lower blood pressure? A meta-analysis of published trials. *J Hypertens.* (1991) 9:465–73. doi: 10.1097/00004872-199105000-00011

59. Reungjui S, Roncal CA, Mu W, Srinivas TR, Sirivongs D, Johnson RJ, et al. Thiazide diuretics exacerbate fructose-induced metabolic syndrome. *J Am Soc Nephrol.* (2007) 18:2724–31. doi: 10.1681/ASN.2007040416

60. Cronin P, Joyce SA, O'Toole PW, O'Connor EM. Dietary fibre modulates the gut microbiota. *Nutrients.* (2021) 13:1655. doi: 10.3390/nu13051655

61. Wang H, Nair MG, Strasburg GM, Chang YC, Booren AM, Gray JI, et al. Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. *J Nat Products.* (1999) 62:802. doi: 10.1021/np990184z

62. Nijveldt RJ, van Nood E, Boelens PG, van Norren K, van Leeuwen PA. Flavonoids: a review of probable mechanisms of action and potential applications. *Am J Clin Nutr.* (2001) 74:418–25. doi: 10.1093/ajcn/74.4.418

63. Teng GG, Pan A, Yuan JM, Koh WP. Food sources of protein and risk of incident gout in the singapore chinese health study. *Arthr Rheumatol (Hoboken N J).* (2015) 67:1933–42. doi: 10.1002/art.39115

64. Sucher S, Markova M, Hornemann S, Pivovarova O, Rudovich N, Thomann R, et al. Comparison of the effects of diets high in animal or plant protein on metabolic and cardiovascular markers in type 2 diabetes: a randomized clinical trial. *Diab Obes Metab.* (2017) 19:944–52. doi: 10.1111/dom.12901

65. Belanger MJ, Wee CC, Sacks FM, Miller ER, Appel LJ, Mukamal KJ, et al. Effects of dietary macronutrients on serum urate: results from the OmniHeart trial. *Am J Clin Nutr.* (2021) 113:1593–9. doi: 10.1093/ajcn/nqaa424

66. Bowen-Davies Z, Muller S, Mallen CD, Hayward RA, Roddy E. Gout severity. *Arthr Care Res.* (2018) 70:1822–8. doi: 10.1002/acr.23562

67. Nieradko-Iwanicka B. The role of alcohol consumption in pathogenesis of gout. *Crit Rev Food Sci Nutr.* (2021) 19:1–9. doi: 10.1080/10408398.2021.1911928

68. Tomita M, Mizuno S, Yokota K. Increased levels of serum uric acid among ex-smokers. *J Epidemiol.* (2008) 18:132–4. doi: 10.2188/jea.JE2006332

69. Burke BT, Köttgen A, Law A, Grams M, Baer AN, Coresh J, et al. Gout in older adults: the atherosclerosis risk in communities study. *J Gerontol Ser A Biol Sci Med Sci.* (2016) 71:536–42. doi: 10.1093/gerona/glv120

70. Gao X, Qi L, Qiao N, Choi HK, Curhan G, Tucker KL, et al. Intake of added sugar and sugar-sweetened drink and serum uric acid concentration in US men and women. *Hypertension (Dallas Tex 1979).* (2007) 50:306–12. doi: 10.1161/HYPERTENSIONAHA.107.091041

71. Johnson RJ, Nakagawa TM, Sanchez-Lozada LG, Shafiu M, Sundaram S, Le M, et al. Sugar, uric acid, and the etiology of diabetes and obesity. *Diabetes.* (2013) 62:3307–15. doi: 10.2337/db12-1814

72. Choi HK, Curhan G. Soft drinks, fructose consumption, and the risk of gout in men: prospective cohort study. *BMJ (Clin Res ed).* (2008) 336:309–12. doi: 10.1136/bmj.39449.819271.BE

73. DiNicolantonio JJ, O'Keefe JH, Lucan SC. Added fructose: a principal driver of type 2 diabetes mellitus and its consequences. *Mayo Clinic Proc.* (2015) 90:372–81. doi: 10.1016/j.mayocp.2014.12.019

74. Bray GA. Energy and fructose from beverages sweetened with sugar or high-fructose corn syrup pose a health risk for some people. *Adv Nutr (Bethesda Md).* (2013) 4:220–5. doi: 10.3945/an.112.002816

75. Burner TW, Rosenthal AK. Diabetes and rheumatic diseases. *Curr Opini Rheumatol.* (2009) 21:50–4. doi: 10.1097/BOR.0b013e32831bc0c4

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The association of healthy eating index with periodontitis in NHANES 2013–2014

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Background: Periodontal disease is very common worldwide and is one of the main causes of tooth loss in adults. Periodontal disease is characterized by chronic inflammation that can destroy adjacent alveolar bone and lead to a loss of periodontal ligaments. Although previous studies have found that a daily diet can influence the development of periodontal disease (e.g., a diet low in carbohydrates and rich in vitamins C and D and fiber can have a protective effect). Periodontal disease may present as gingivitis or periodontitis. However, studies on the role of healthy eating index in periodontitis are lacking. The purpose of this study was to assess the association between healthy eating index and periodontitis.

Methods: We analyzed data collected from participants in the National Health and Nutrition Examination Survey (NHANES), a nationally representative survey conducted in 2-year cycles from 2013 to 2014. As part of our analysis, we developed multivariate logistic regression models to examine the independent association between the healthy eating index and periodontitis. We evaluated the significance of association using odds ratios (OR) with 95% confidence intervals (95%CI).

Results: Individuals with a lower total healthy eating index had a higher prevalence of periodontitis. Adjusted multivariate regression models showed that a higher healthy diet index was associated with a lower prevalence of periodontitis (OR = 0.69, 95% CI: 0.55–0.86, $P < 0.05$).

Conclusion: The results of the study showed that dietary structure was associated with the prevalence of periodontitis. Patients with a higher healthy eating index had a lower prevalence of periodontitis. These findings will need to be confirmed by longitudinal, prospective studies in the future.

KEYWORDS

periodontal disease, healthy eating index, periodontitis, dietary structure, HEI

Introduction

Periodontal disease is a complex inflammatory disease caused by pathogenic plaque biofilm that affects not only the gums and bone but also the alveolar bone, resulting in bleeding gums, periodontal pocket formation, and loss of attachment, which can lead to loosening and loss of teeth in the long run (1). According to statistics from the United States, 47% of adults older than 30 years have chronic periodontitis, 30% have

moderate periodontal disease and 8.5% have severe periodontal disease (2, 3). Previous studies have suggested that several factors such as lifestyle, obesity and metabolic syndrome and genetic factors increase the risk of periodontitis (4). Healthy eating habits promote health, while unhealthy eating habits are associated with a variety of chronic diseases (5). Based on dietary guidelines for Americans (DGA) recommendations, Healthy Eating Index (HEI) indicates how closely a diet adheres to the DGA, with a higher score indicating better compliance (6, 7). The HEI-2015 consists of 13 dietary components. Nine adequate components: including total fruit, all fruit, all vegetables, greens and legumes, whole grains, dairy products, all protein foods, seafood and vegetable proteins, and fatty acids. Four moderate components: including refined grains, sodium, added sugars and saturated fats. Each element has a maximum score of 5 or 10 points. The total score is 100, and the higher the score means the closer to the recommended range or number (8). HEI is a valid and reliable measure of dietary quality for different population subgroups and interventions related to nutrition (8). However, there are limited studies on the role of HEI in periodontitis. The purpose of this study was to assess the prevalence of periodontitis between those participants with a lower HEI and those with a higher HEI.

Methods

Study population

Data were drawn from the 2013–2014 NHANES. All adults aged 30 or older who had a permanent tooth were eligible for full-mouth periodontal examinations (9). Participants in NHANES completed a questionnaire at home, followed by a physical examination and interviews at a mobile exam center (MEC). Data collected at the clinical examinations were standardized with minimal site-specific bias. Because only a subset of NHANES participants underwent MEC examinations, we included only those who reported a complete dental examination. Dietary quality was obtained from 24 h dietary recalls and was assessed by HEI (2015) scores. We also included other demographic variables (including age, gender, race, educational attainment, smoking status, and alcohol use status) and BMI (Body Mass Index). Ultimately, we included 3.001 participants for the next step of analysis.

Study variables

Socio-demographic characteristics

Socio-demographic characteristics were set as age, gender (male/female), race (Mexican American; white; black and other), education level (less than high school; high school and college or above), smoking status (former smoker; never smoker and now smoker), diabetes (DM; no), drinking status (never; former; mild; moderate and heavy) and poverty income ratio (PIR).

The classification of smoking status is based on the following criteria (10): A never smoker is defined as an adult who has never smoked or smoked <100 cigarettes in their lifetime; Smokers who reported smoking ≥100 cigarettes in their lifetime and were currently non-smokers were identified as former smokers; whereas current smokers were defined as those who smoked ≥100 cigarettes on some days or every day in their lifetime. Never drinkers were defined as those who reported drinking <12 drinks; ever drinkers were defined as those who had more than 12 drinks in their lifetime but not in the past year; current drinkers were further classified as light, moderate and heavy current drinkers. Heavy current drinkers were defined as ≥3 drinks per day for women and ≥4 drinks per day for men, with five or more binge drinking days per month; moderate drinkers were defined as ≥2 drinks per day for women and ≥3 drinks per day for men, with ≥2 binge drinking days per month. Light drinkers: did not meet the above criteria (11, 12). In the US, PIR is defined as the ratio between a household's self-reported income and the poverty line. According to the PIR, we categorize family income into three levels: low (PIR <1.35), medium (1.35 ≤ PIR <3.0) and high (PIR ≥3.0) (13). BMI was divided into four categories (14): underweight (BMI <18.5), normal weight (BMI = 18.5–24.9), overweight (BMI = 25–29.9) and obese (BMI >30.0). An individual who reports themselves to have diabetes mellitus or who uses antidiabetic medication is considered to have diabetes.

Periodontitis

The full-mouth periodontal examination was performed by a calibrated dentist who assessed the periodontal status of the participants. Periodontal examinations comprised probing depths (PD) and clinical attachment levels (AL) at the MEC. Supplementary Table 1 shows the criteria for classification according to periodontal status (15).

HEI

Using an automated multiple-pass method, the NHANES dietary data include 24-h dietary recalls collected by computer-assisted dietary interview software. According to the United States Department of Agriculture (USDA) Food and Nutrient Database for Dietary Studies, the nutrient values for each food were assigned (16). Food components, with the exception of fatty acids, are scored on a density basis (per 1,000 kcal or as a percentage of energy). Fatty acids are expressed as a ratio of unsaturated to saturated fatty acids (17). Dietary components and standards for scoring are shown in Supplementary Table 2. For the adequacy components, better quality is reflected in higher scores. For the moderation components, a lower intake will result in a higher score.

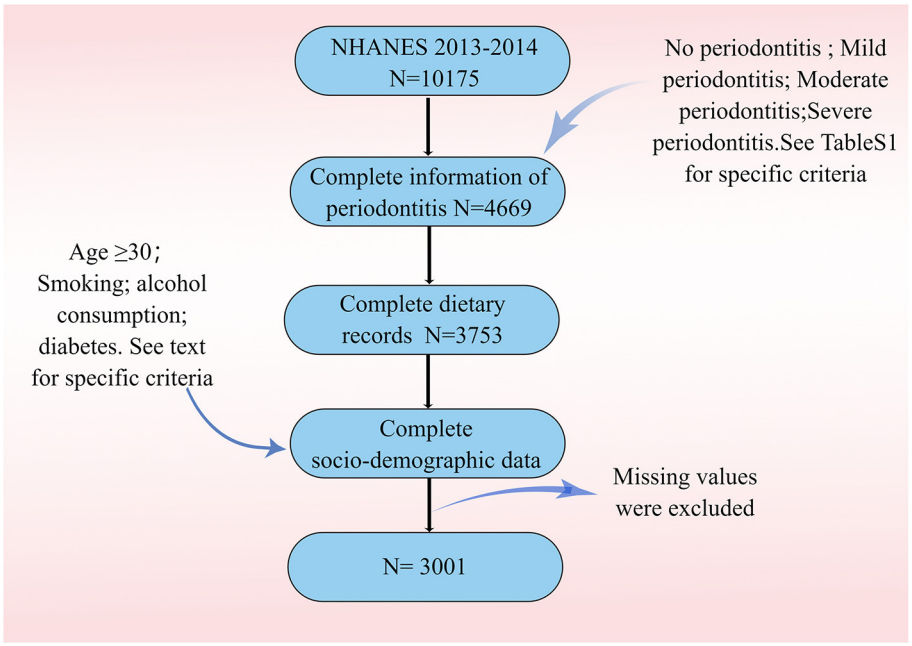

FIGURE 1
Flowchart of the study population. (ID:PORYR171ff).

Statistical analysis

For descriptive analyses, continuous variables were described by means and standard deviations; categorical variables were described by frequencies and percentages. HEI-2015 classification according to quartiles (quartile 1: <25th percentile, quartile 2: ≥25 to 50th percentile, quartile 3: ≥50 to 75th percentile, quartile 4: ≥75th percentile) (18). We used Fisher's exact test to assess the difference in distributions of categorical variables (19). We then presented descriptive statistics comparing the prevalence of periodontitis by HEI. We examined the association between HEI and periodontitis using invariable and multivariable logistic regression model while adjusting for age, sex, race, smoking, drinking, education level and BMI.

Results

Population characteristics

Figure 1 describes study recruitment and inclusion/exclusion criteria. Multiple socio-demographic groups had statistically significant higher rates of periodontitis (Table 1). The average age of participants with periodontitis was 55 years, higher than those who did not have periodontitis (49 years). In the four HEI groups, the prevalence of severe periodontitis was 32.7, 25.9, 25.5, and 15.9% in that order. The prevalence of periodontitis is highest in non-Hispanic white, followed by non-Hispanic black. At the same time, the prevalence of periodontitis was significantly higher among participants with low PIR and among those with high school or less education. Periodontitis is most prevalent in light drinkers and least prevalent in never drinkers. Finally, the incidence of periodontitis is higher in those with a history of smoking compared to non-smokers. The prevalence of periodontitis was higher in participants with DM than in those without DM (58.6 vs. 40.2%). In terms of BMI, obese participants had the highest number of people with periodontitis, but the prevalence was not statistically different.

Outcome and exposure factors

According to the total scores, participants were divided into four quartiles and periodontitis ever accounted for 27.1% in Q1, 26.4% in Q2, 25.5% in Q3, and 21% in Q4. Participants with HEI scores in the highest quartile had a lower prevalence of periodontitis than those in the lowest quartile (21.0 vs. 27.1%). At the same time, our results suggest a gradual decrease in the prevalence of periodontitis as the HEI rises. Also, in terms of the severity of periodontitis, participants in the top quartile of HEI scores had a lower proportion (15.9 %) of severe periodontitis than participants in the other groups. Supplementary Table 3

TABLE 1 Characteristics of the overall target population according to periodontitis.

Variables	Total ($n = 3,001$)	No ($n = 1,715$)	Mild ($n = 37$)	Moderate ($n = 998$)	Severe ($n = 251$)	*p*
HEI, *n* (%)						<0.001
Q1	750 (25.0)	402 (23.4)	12 (32.4)	254 (25.5)	82 (32.7)	
Q2	750 (25.0)	410 (23.9)	7 (18.9)	268 (26.9)	65 (25.9)	
Q3	750 (25.0)	422 (24.6)	12 (32.4)	252 (25.3)	64 (25.5)	
Q4	751 (25.0)	481 (28)	6 (16.2)	224 (22.4)	40 (15.9)	
Age, Mean ± SD	52.1 ± 14.3	49.4 ± 13.9	43.4 ± 12.6	55.9 ± 14.4	55.9 ± 11.9	<0.001
Sex, *n* (%)						<0.001
Female	1523 (50.7)	985 (57.4)	17 (45.9)	441 (44.2)	80 (31.9)	
Male	1478 (49.3)	730 (42.6)	20 (54.1)	557 (55.8)	171 (68.1)	
DM, *n* (%)						<0.001
DM	435 (14.6)	180 (10.6)	6 (16.2)	211 (21.2)	38 (15.1)	
No	2551 (85.4)	1525 (89.4)	31 (83.8)	782 (78.8)	213 (84.9)	
Race, *n* (%)						<0.001
Black	570 (19.0)	259 (15.1)	9 (24.3)	219 (21.9)	83 (33.1)	
Mexican American	382 (12.7)	168 (9.8)	5 (13.5)	168 (16.8)	41 (16.3)	
Other Race	667 (22.2)	401 (23.4)	11 (29.7)	212 (21.2)	43 (17.1)	
White	1382 (46.1)	887 (51.7)	12 (32.4)	399 (40)	84 (33.5)	
PIR, *n* (%)						<0.001
High	1071 (35.7)	782 (45.6)	10 (27)	237 (23.7)	42 (16.7)	
Low	883 (29.4)	378 (22)	10 (27)	386 (38.7)	109 (43.4)	
Medium	1047 (34.9)	555 (32.4)	17 (45.9)	375 (37.6)	100 (39.8)	
Education, *n* (%)						<0.001
College or above	1819 (60.6)	1230 (71.7)	21 (56.8)	482 (48.3)	86 (34.3)	
High school	655 (21.8)	297 (17.3)	11 (29.7)	267 (26.8)	80 (31.9)	
Less high school	527 (17.6)	188 (11)	5 (13.5)	249 (24.9)	85 (33.9)	
Smoke, *n* (%)						<0.001
Former	776 (25.9)	400 (23.3)	5 (13.5)	305 (30.6)	66 (26.3)	
Never	1686 (56.2)	1103 (64.3)	26 (70.3)	463 (46.4)	94 (37.5)	
Now	539 (18.0)	212 (12.4)	6 (16.2)	230 (23)	91 (36.3)	
Alcohol, *n* (%)						<0.001
Former	511 (17.0)	235 (13.7)	4 (10.8)	221 (22.1)	51 (20.3)	
Heavy	515 (17.2)	274 (16)	9 (24.3)	174 (17.4)	58 (23.1)	
Mild	1115 (37.2)	683 (39.8)	15 (40.5)	334 (33.5)	83 (33.1)	
Moderate	458 (15.3)	294 (17.1)	4 (10.8)	127 (12.7)	33 (13.1)	
Never	402 (13.4)	229 (13.4)	5 (13.5)	142 (14.2)	26 (10.4)	
BMI, *n* (%)						0.348
Normal	756 (25.2)	457 (26.6)	9 (24.3)	235 (23.5)	55 (21.9)	
Obese	1188 (39.6)	653 (38.1)	18 (48.6)	413 (41.4)	104 (41.4)	
Overweight	1028 (34.3)	592 (34.5)	10 (27)	338 (33.9)	88 (35.1)	
Underweight	29 (1.0)	13 (0.8)	0 (0)	12 (1.2)	4 (1.6)	

TABLE 2 Association of HEI-2015 with periodontitis.

Exposure	Unadjusted model	Model 1	Model 2	Model 3
HEI_Q1	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
HEI_Q2	0.96 (0.78–1.17); 0.678	0.89 (0.72–1.1); 0.293	0.99 (0.79–1.24); 0.935	0.99 (0.8–1.23); 0.938
HEI_Q3	0.9 (0.73–1.1); 0.299	0.78 (0.63–0.96); 0.02	0.91 (0.72–1.14); 0.414	0.91 (0.73–1.14); 0.399
HEI_Q4	0.65 (0.53–0.8); <0.001	0.53 (0.43–0.66); <0.001	0.75 (0.59–0.95); 0.016	0.69 (0.55–0.86); 0.001
P-value for trend	<0.001	<0.001	0.045	0.036

Model 1: adjusted for gender, age, race.
Model 2: Model 1+ adjusted for education, PIR.
Model 3: Model 2+smoke, DM, alcohol, and BMI.

TABLE 3 Association of HEI-2015 components with periodontitis.

HEI-2015 components	Unadjusted model	Model 1	Model 2	Model 3
Adequacy				
Total vegetables	0.93 (0.89–0.97); 0.001	0.91 (0.86–0.95); <0.001	0.94 (0.9–0.99); 0.016	0.93 (0.89–0.97); 0.031
Greens and beans	0.95 (0.92–0.98); 0.001	0.94 (0.91–0.97); 0.001	0.98 (0.95–1.02); 0.342	0.94 (0.91–1.03); 0.414
Total fruit	0.96 (0.93–0.99); 0.02	0.93 (0.9–0.96); <0.001	0.92 (0.88–0.95); <0.001	0.96 (0.91–0.99); 0.031
Whole fruits	0.96 (0.93–0.99); 0.013	0.94 (0.91–0.97); <0.001	0.93 (0.9–0.97); <0.001	0.98 (0.92–1.02); 0.054
Whole grains	0.98 (0.96–1); 0.027	0.96 (0.94–0.98); <0.001	0.97 (0.95–1); 0.021	0.96 (0.94–0.98);0.02
Dairy	0.98 (0.96–1);0.033	0.98 (0.96–1); 0.102	1 (0.97–1.02); 0.821	1 (0.98–1.03); 0.733
Total protein foods	0.97 (0.92–1.03); 0.359	0.95 (0.89–1); 0.067	0.93 (0.87–0.99); 0.018	0.97 (0.91–1.21); 0.144
Seafood and plant proteins	0.93 (0.9–0.96); <0.001	0.92 (0.89–0.95); <0.001	0.93 (0.9–0.97); <0.001	0.93 (0.90–0.96); 0.001
Fatty acids	0.97 (0.96–0.99); 0.01	0.97 (0.95–0.99); 0.006	0.96 (0.94–0.98); <0.001	0.96 (0.94–0.98); 0.005
Moderation				
Sodium	1 (0.98–1.02);0.782	1.01 (0.98–1.03); 0.614	1 (0.98–1.02); 0.852	0.97 (0.95–1.02); 0.536
Refined grains	0.99 (0.97–1.01); 0.265	0.98 (0.96–1); 0.022	0.99 (0.97–1.02); 0.635	0.97 (0.95–1.02); 0.123
Saturated fats	1.01 (0.99–1.03); 0.328	1.01 (0.99–1.04); 0.234	1.01 (0.99–1.03); 0.497	1.21 (0.99–1.33); 0.213
Added sugars	0.98 (0.96–1.01); 0.137	0.96 (0.94–0.98); <0.001	0.98 (0.95–1); 0.043	0.99 (0.95–1); 0.122

Model 1: adjusted for gender, age, race.
Model 2: Model 1+ adjusted for education, PIR.
Model 3: Model 2+smoke, DM, alcohol, and BMI.

showed the distributions of HEI-2015 components by categories of HEI.

Multivariate regression analysis

All four models showed a negative correlation between HEI and the prevalence of periodontitis in 2015, based on the quartiles of HEI (Table 2). That is, logistic regression analysis demonstrated that higher quartiles of 2015 HEI were associated with a lower prevalence of periodontitis. Compared to the lowest quartile of the HEI-2015 population, the fourth quartile had a lower prevalence of periodontitis in model 1 (OR = 0.53; 95%CI 0.43–0.66), model 2 (OR = 0.75; 95%CI 0.59–0.95) and model 3 (OR = 0.69; 95%CI 0.55–0.86). *P* for trend was < 0.05 in all models. The results of the multivariate regression analysis of the 2015 HEI components showed that that scores of fatty acids, seafood and plant proteins, whole grains; total fruit and total vegetables were all significantly associated with periodontitis (Table 3). Table 4 shows the actual effect of smoking, age and gender, with the results suggesting that the absence of a smoking history has a protective effect against periodontitis.

Spline smoothing

After adjusting for potential confounders, smooth curve fitting indicated a non-linear relationship between 2015 HEI and periodontitis. It is evident from this curve that there is linear relationship between the 2015 HEI and the prevalence of periodontitis (Figure 2). HEI is negatively correlated with periodontitis, i.e., higher HEI is associated with less periodontitis. The middle of the line has a greater slope than the ends of the line.

TABLE 4 The actual effect of smoking, age and gender.

Variable	adj. OR_95CI	adj. *P*_value
Smoke		
Former	1(Ref)	
Now	2.53 (1.98–3.24)	<0.001
Never	0.73 (0.6–0.88)	0.001
Age		
≤60	1(Ref)	
>60	1.26 (0.94–1.68)	0.121
Sex		
Female	1 (Ref)	
male	1.16 (0.92–1.31)	0.311

Subgroup analyses

To identify potential effect modifiers, we also performed a subgroup analysis (Supplementary Table 4). The results showed that the effect sizes differed significantly across drinking status and DM. For mild drinkers, HEI above 50 (HEI_Q3, HEI_Q4) is protective against periodontitis. Similarly, for participants without diabetes, higher HEI helped to reduce the prevalence of periodontitis. In contrast, among participants with diabetes, the difference was not statistically significant. It is suggested that the promotional effect of diabetes on periodontitis is greater than the protective effect of HEI.

Discussion

In the cohort we analyzed, 42.9% of the participants had periodontitis, with 8.4% of them having severe periodontitis. Our analysis showed that a higher HEI score was significantly associated with a lower prevalence of periodontitis. People with a better diet quality intake had a lower risk of periodontitis. Of the 13 components of the HEI, fatty acids, seafood and plant proteins, whole grains; total fruit and total vegetables were most associated with periodontitis.

There is an association between periodontitis and diet (20). Previous studies have found that eating naturally fibrous foods reduces plaque build-up and that eating soft foods over a long period of time increases plaque build-up, leading to periodontal disease. In addition, the consumption of a highly inflammatory diet by patients with periodontitis can not only exacerbate clinical symptoms but also increase the risk of other associated systemic diseases (20).

The HEI is a valid and rapid method of assessing the quality of the diet and is made up of the following components: adequacy components (total fruits, whole fruits, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant proteins, fatty acids) and moderation components (sodium, refined grains, added sugars, saturated fats) (18). Our findings suggest that fatty acids, seafood and plant proteins, whole grains; total fruit and total vegetables are all associated with a lower prevalence of periodontitis. Consumption of potassium-rich foods, such as green vegetables and fruits, has been reported to be effective in reducing the incidence of periodontitis (21). Free fatty acids play a role in the development of periodontitis and omega-3 fatty acids appear to have a positive effect on periodontal wound healing and may reduce periodontitis (22, 23). There are fewer studies on the link between seafood, plant proteins and periodontitis, but previous studies have concluded that a daily intake of ∼1 gram of calcium is beneficial for periodontal health and that there is a strong relationship between calcium intake and the development of periodontitis, with lower dietary calcium intake contributing to the incidence of periodontitis (24). Thus, this indirect evidence also suggests that better diet quality is associated with a lower prevalence of periodontitis, which is consistent with our results.

Previous clinical studies have shown that smokers have a higher prevalence of chronic periodontitis, more severe alveolar bone resorption and deeper periodontal pockets, and that smoking is an important contributing factor to chronic periodontitis (25). The analysis showed that the prevalence of periodontitis was higher in participants who were current and former smokers. Non-smoking had a protective effect on periodontitis. The mechanism by which smoking contributes to the development of periodontitis is not yet clear. It has been suggested that smoking inhibits the antimicrobial action of the periodontal gingival sulcus fluid. Smoking can cause changes in inflammatory factors in the gingival sulcus fluid, resulting in an inflammatory response and structural damage to periodontal tissues (26, 27). The negative association between HEI and the risk of periodontitis was more pronounced in those who did not have DM and in those who consumed alcohol lightly. The results suggest that for participants with light alcohol consumption and no DM, obtaining a higher HEI by adjusting their diet may reduce the prevalence of periodontitis.

Previous studies have analyzed the relationship between single nutrients and periodontitis, but the present study is a large population-based cross-sectional study exploring HEI and periodontitis. HEI is a more comprehensive method of nutritional assessment and is more representative of an individual's comprehensive nutritional intake. Therefore, analyzing the relationship between HEI and periodontitis can provide a more comprehensive picture of the relationship between dietary intake and periodontitis. Furthermore, we conclude that the prevalence of periodontitis can be reduced by adjusting dietary intake. Based on a multiple regression analysis of the 13 components of the HEI, we were able to further

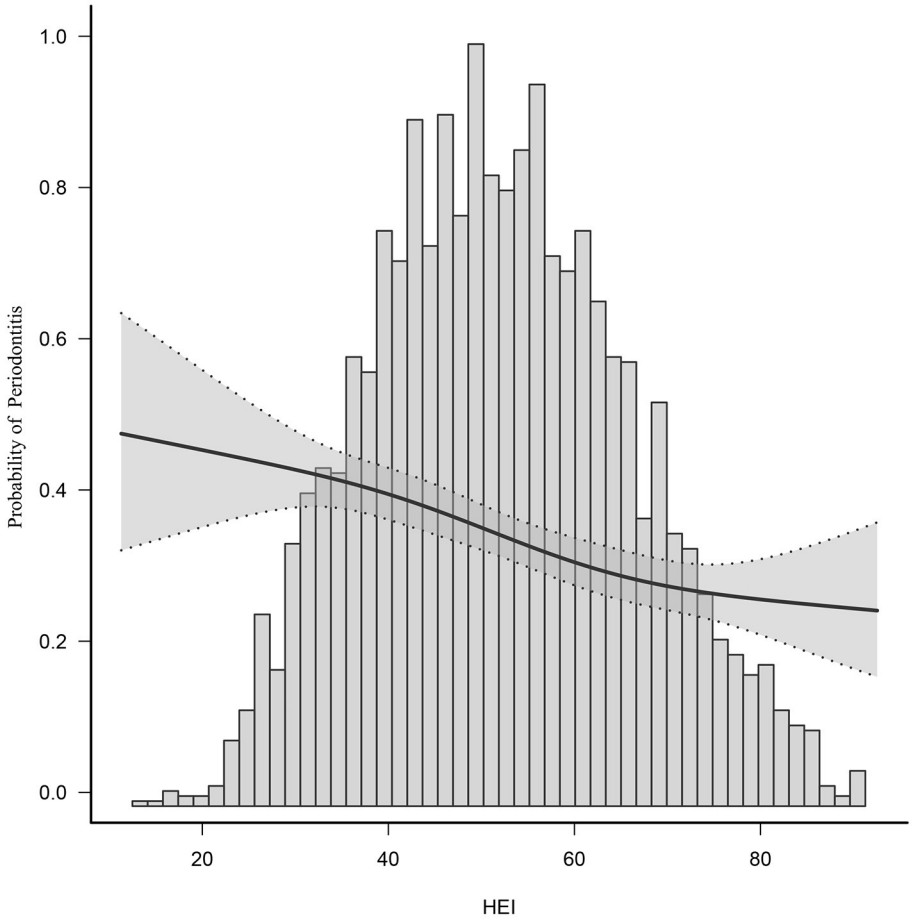

FIGURE 2
Smooth curve fitting of periodontitis and HEI-2015.

investigate the relationship between different types of diet and periodontitis.

However, there remain several limitations. Because cross-sectional observational studies cannot indicate causation and directionality, our findings should be interpreted with care. Although confounding has been extensively adjusted for, residual confounding still cannot be fully ruled out. Because of the retrospective nature of the questionnaire, there was a possibility of recall bias on the part of patients.

Conclusion

Our study found that a lower HEI was associated with a higher incidence of periodontitis, suggesting that a comprehensive health promotion including dietary modification is needed to reduce the burden of periodontal disease in society. Because the findings from this study were cross-sectional, prospective studies are needed to clarify the causal relationship between HEI and periodontitis.

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

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data, took part in drafting the article or revising it critically for important intellectual content, agreed to submit to the current journal, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.968073/full#supplementary-material

References

1. Khajuria DK, Zahra SF, Razdan R. Effect of locally administered novel biodegradable chitosan based risedronate/zinc-hydroxyapatite intra-pocket dental film on alveolar bone density in rat model of periodontitis. *J Biomat Sci Pol Ed.* (2018) 29:74–91. doi: 10.1080/09205063.2017.1400145

2. Kaschwich M, Behrendt C, Heydecke G, Bayer A, Debus ES, Seedorf U, et al. The association of periodontitis and peripheral arterial occlusive disease-a systematic review. *Int J Mol Sci.* (2019) 20:2936. doi: 10.3390/ijms20122936

3. Kugaji MS, Kumbar VM, Peram MR, Patil S, Bhat KG, Diwan PV. Effect of Resveratrol on biofilm formation and virulence factor gene expression of Porphyromonas gingivalis in periodontal disease. *Apmis: Acta Pathologica, Microbiologica, Et Immunologica Scandinavica.* (2019) 127:187–95. doi: 10.1111/apm.12930

4. Genco RJ, Borgnakke WS. Risk factors for periodontal disease. *Periodontology.* (2000) 62:59–94.. doi: 10.1111/j.1600-0757.2012.00457.x

5. Noratto GD, Lage NN, Chew BP, Mertens-Talcott SU, Talcott ST, Pedrosa ML. Non-anthocyanin phenolics in cherry (Prunus avium L) modulate IL-6, liver lipids and expression of PPARδ and LXRs in obese diabetic (db/db) mice. *Food Chem.* (2018) 266:405–14. doi: 10.1016/j.foodchem.2018.06.020

6. Chin EL, Huang L, Bouzid YY, Kirschke CP, Durbin-Johnson B, Baldiviez LM, et al. Association of lactase persistence genotypes (rs4988235) and ethnicity with dairy intake in a healthy U.S. *Populat Nutr.* (2019) 11:1860. doi: 10.3390/nu11081860

7. Mahmoudi-Nezhad M, Farhangi MA, Kahroba H. Cocaine and amphetamine-regulated transcript pre-propeptide gene (CARTPT) polymorphism interacts with Diet Quality Index-International (DQI-I) and Healthy Eating Index (HEI) to affect hypothalamic hormones and cardio-metabolic risk factors among obese individuals. *J Transl Med.* (2020) 18:16. doi: 10.1186/s12967-020-02208-z

8. Reedy J, Lerman JL, Krebs-Smith SM, Kirkpatrick SI, Pannucci TE, Wilson MM, et al. Evaluation of the healthy eating index-2015. *J Acad Nutr Diet.* (2018) 118:1622–33. doi: 10.1016/j.jand.2018.05.019

9. Dye BA, Afful J, Thornton-Evans G, Iafolla T. Overview and quality assurance for the oral health component of the national health and nutrition examination survey (NHANES), 2011-2014. *BMC Oral Health.* (2019) 19:95. doi: 10.1186/s12903-019-0777-6

10. Kim K, Song Y, Oh TJ, Choi SH, Jang HC. Association between iron intake and diabetic peripheral neuropathy in Type 2 diabetes: significance of iron intake and the ratio between iron intake and polyunsaturated fatty acids intake. *Nutrients.* (2020) 12:3365. doi: 10.3390/nu12113365

11. Tsai J, Ford ES Li C, Zhao G. Past and current alcohol consumption patterns and elevations in serum hepatic enzymes among US adults. *Addict Behav.* (2012) 37:78–84. doi: 10.1016/j.addbeh.2011.09.002

12. Rattan P, Penrice DD, Ahn JC, Ferrer A, Patnaik M, Shah VH, et al. Inverse association of telomere length with liver disease and mortality in the US population. *Hepatol Commun.* (2022) 6:399–410. doi: 10.1002/hep4.1803

13. Xu F, Lu B. Prospective association of periodontal disease with cardiovascular and all-cause mortality: NHANES III follow-up study. *Atherosclerosis.* (2011) 218:536–42. doi: 10.1016/j.atherosclerosis.2011.07.091

14. Hastie CE, Padmanabhan S, Slack R, Pell ACH, Oldroyd KG, Flapan AD, et al. Obesity paradox in a cohort of 4880 consecutive patients undergoing percutaneous coronary intervention. *Eur Heart J.* (2010) 31:222–6. doi: 10.1093/eurheartj/ehp317

15. Eke PI, Page RC, Wei L, Thornton-Evans G, Genco RJ. Update of the case definitions for population-based surveillance of periodontitis. *J Periodontol.* (2012) 83:1449–54. doi: 10.1902/jop.2012.110664

16. Cogswell ME, Loria CM, Terry AL, Zhao L, Wang C, Chen T, et al. Estimated 24-h urinary sodium and potassium excretion in US adults. *Jama.* (2018) 319:1209–20. doi: 10.1001/jama.2018.1156

17. Haring B, Crandall CJ, Wu C, LeBlanc ES, Shikany JM, Carbone L, et al. Dietary patterns and fractures in postmenopausal women: results from the women's health initiative. *JAMA Intern Med.* (2016) 176:645–52. doi: 10.1001/jamainternmed.2016.0482

18. Fan Y, Zhang Y, Li J, Liu Y, Chang H, Jiang Y, et al. Association between healthy eating index-2015 and various cognitive domains in US adults aged 60 years or older: the national health and nutrition examination survey (NHANES) 2011–2014. *BMC Public Health.* (2021) 21:1862. doi: 10.1186/s12889-021-11914-2

19. Yamaguchi S, Hamano T, Doi Y, Oka T, Kajimoto S, Kubota K, et al. Hidden hypocalcemia as a risk factor for cardiovascular events and all-cause mortality among patients undergoing incident hemodialysis. *Sci Rep.* (2020) 10:4418. doi: 10.1038/s41598-020-61459-4

20. Machado V, Botelho J, Viana J, Pereira P, Lopes LB, Proença L, et al. Association between dietary inflammatory index and periodontitis: a cross-sectional and mediation analysis. *Nutrients.* (2021) 13:1194. doi: 10.3390/nu13041194

21. Yamori M, Njelekela M, Mtabaji J, Yamori Y, Bessho K. Hypertension, periodontal disease, and potassium intake in nonsmoking, nondrinker African women on no medication. *Int J Hypertens.* (2011) 2011:695719. doi: 10.4061/2011/695719

22. Kruse AB, Kowalski CD, Leuthold S, Vach K, Ratka-Krüger P, Woelber JP. What is the impact of the adjunctive use of omega-3

fatty acids in the treatment of periodontitis? A systematic review and meta-analysis. *Lip Health Dis.* (2020) 19:100. doi: 10.1186/s12944-020-01267-x

23. Shikama Y, Kudo Y, Ishimaru N, Funaki M. Potential role of free fatty acids in the pathogenesis of periodontitis and primary Sjögren's syndrome. *Int J Mol Sci.* (2017) 18:836. doi: 10.3390/ijms18040836

24. Amarasena N, Yoshihara A, Hirotomi T, Takano N, Miyazaki H. Association between serum calcium and periodontal disease progression in non-institutionalized elderly. *Gerodontology.* (2008) 25:245–50. doi: 10.1111/j.1741-2358.2007.00211.x

25. Leite FRM, Nascimento GG, Scheutz F, López R. Effect of smoking on periodontitis: a systematic review and meta-regression. *Am J Prev Med.* (2018) 54:831–41. doi: 10.1016/j.amepre.2018.02.014

26. Khan S, Khalid T, Awan KH. Chronic periodontitis and smoking. Prevalence and dose-response relationship. *Saudi Med J.* (2016) 37:889–94. doi: 10.15537/smj.2016.8.14223

27. Choi S, Kim K, Chang J, Kim SM, Kim SJ, Cho H, et al. Association of chronic periodontitis on Alzheimer's disease or vascular dementia. *J Am Geriat Soc.* (2019) 67:1234–9. doi: 10.1111/jgs.15828

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Association of dietary and nutrient patterns with systemic inflammation in community dwelling adults

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Purpose: Evidence investigating associations between dietary and nutrient patterns and inflammatory biomarkers is inconsistent and scarce. Therefore, we aimed to determine the association of dietary and nutrient patterns with inflammation.

Methods: Overall, 1,792 participants from the North-West Adelaide Health Study were included in this cross-sectional study. We derived dietary and nutrient patterns from food frequency questionnaire data using principal component analysis. Multivariable ordinal logistic regression determined the association between dietary and nutrient patterns and the grade of inflammation (normal, moderate, and severe) based on C-reactive protein (CRP) values. Subgroup analyses were stratified by gender, obesity and metabolic health status.

Results: In the fully adjusted model, a plant-sourced nutrient pattern (NP) was strongly associated with a lower grade of inflammation in men ($OR_{Q5vsQ1} = 0.59$, 95% CI: 0.38–0.93, p-trend = 0.08), obesity ($OR_{Q5vsQ1} = 0.43$; 95% CI: 0.24–0.77, p-trend = 0.03) and metabolically unhealthy obesity ($OR_{Q5vsQ1} = 0.24$; 95% CI: 0.11–0.52, p-trend = 0.01). A mixed NP was positively associated with higher grade of inflammation ($OR_{Q5vsQ1} = 1.35$; 95% CI: 0.99–1.84, p-trend = 0.03) in all participants. A prudent dietary pattern was inversely associated with a lower grade of inflammation (OR_{Q5vsQ1} = 0.72, 95% CI: 0.52–1.01, p-trend = 0.14). In contrast, a western dietary pattern and animal-sourced NP were associated with a higher grade of inflammation in the all participants although BMI attenuated the magnitude of association (OR_{Q5vsQ1} = 0.83, 95% CI: 0.55–1.25; and $OR_{Q5vsQ1} = 0.94$, 95% CI: 0.63–1.39, respectively) in the fully adjusted model.

Conclusion: A plant-sourced NP was independently associated with lower inflammation. The association was stronger in men, and those classified as obese and metabolically unhealthy obese. Increasing consumption of plant-based foods may mitigate obesity-induced inflammation and its consequences.

KEYWORDS

nutrient pattern, dietary pattern, inflammation, C-reactive protein, obesity

Introduction

Low grade systemic inflammation is a risk factor for many chronic illnesses, including cardiovascular diseases, diabetes, non-alcoholic fatty liver diseases, depression, and cancers, which all contribute to global morbidity and mortality (1–4). Inflammation is also known as a hallmark criterion in obesity, a precursor to metabolic syndromes and related diseases (5). Many risk factors can influence systemic inflammation, such as genetics, environmental and behavioral conditions (6), as well as diet; a key modifiable factor in prevention and treatment strategies for obesity and chronic diseases.

Adherence to a healthy diet is associated with a reduced risk of developing chronic diseases (7). A possible mechanism underlying this protective effect is through reducing inflammation. Previous studies examining the association between diet and systemic inflammation were focused on specific food items or nutrient components rather than diet as a whole (8) and do not take into account the overall interactions between different dietary components, given foods are generally consumed in combination.

Studies, then, have shifted to using a dietary pattern approach to capture the diet-inflammation relationship. According to a recent systematic review, many have explored the association between food group-based dietary patterns (DP) and systemic inflammation in European countries and the United States but the results are inconsistent, particularly for data-driven DP (9). Evidence in the Australian context are also scarce (9). Another method, a nutrient group-based dietary patterns (NP), has also been used to determine the diet-inflammation relationship. However, the association remains unclear. Only one study has examined the association between NPs and systemic inflammation markers to date, suggesting an inverse association between plant-sourced NP and systemic inflammation in men (10). DP and NP are different, given the former is constructed based on food groups and the latter is based on nutrient groups of the dietary data. The use of food groups and DP reflect dietary habits of the population. On the other hand, NP and the nutrient groups can portray the physiological roles of dietary components in the association and provide an easier comparison between populations as they are less diverse compared to food groups (11). Nevertheless, no studies have compared DP and NP to examine diet-inflammation relationship.

Therefore, in this study, we aimed (1) to explore the association between DPs and NPs with a clinical marker of systemic inflammation, namely C-reactive protein (CRP) (12), in the Australian population; and (2) to determine whether the association affected by gender, obesity and metabolic health status.

Methods

Study design and population

We used data from the North-West Adelaide Health Study (NWAHS) cohort whose characteristics and recruitment have been described in detail previously (13, 14). In brief, the NWAHS represented, at the time of recruitment, ∼1 third of the South Australian population and half of the metropolitan area. Participants (age ≥18 years old) were randomly selected from the electronic White Page® from the northern and western suburbs of Adelaide, South Australia. The recruitment was conducted by telephone. Data collection was conducted three times using a computer-assisted telephone interview (CATI), self-administered questionnaire and clinic examination in 1999–2003 (stage 1), 2004–2006 (stage 2) and 2008–2010 (stage 3). A follow up study using a self-completed online or postal survey was conducted in 2015. The dietary intake data was only collected at stage 3. Data for ethnicity were not available for this cohort.

For this study, we used data from Stage 3 (2008–2010). We included 1,792 participants with complete dietary intake and CRP data (Figure 1). We excluded participants with (1) implausible energy intake value (i.e., <800 kcal for men, <600 kcal for women and >4,000 kcal for both men and women); (2) CRP value >10 mg/L which indicates acute inflammation (15); (3) participants who had been diagnosed with cancer after 2010; and (4) participants with a missing value for covariates (Figure 1). Ethics approval was obtained from The Human Ethics Research Committee, Queen Elizabeth Hospital, South Australia. All participants provided written informed consent.

Dietary intake assessment and analysis

Dietary intake data were obtained at Stage 3 using a validated Cancer Council Victoria Dietary Questionnaire for Epidemiological Studies (DQES-V3.1) (16). This is a 167-item food frequency questionnaire (FFQ) that assessed dietary habit of participants in the previous 12 months. Nutrient intake was computed from the dietary data based on the NUTTAB95 database (17).

The construction of DPs and NPs using principal component analysis (PCA) have been described previously (18, 19). In brief, "healthy" (prudent) and "unhealthy" (Western) DP were identified. Thirty-nine DPs were derived from the food groups. Two retained factors were determined using a scree plot, an eigenvalue (>1) and interpretability. We applied varimax rotation to increase factor interpretability. Daily intake of individual food items was used to standardize the sum of factor loading coefficients. Factor scores for each participant per the retained factors were calculated using the standardized coefficients. Sample adequacy was checked using

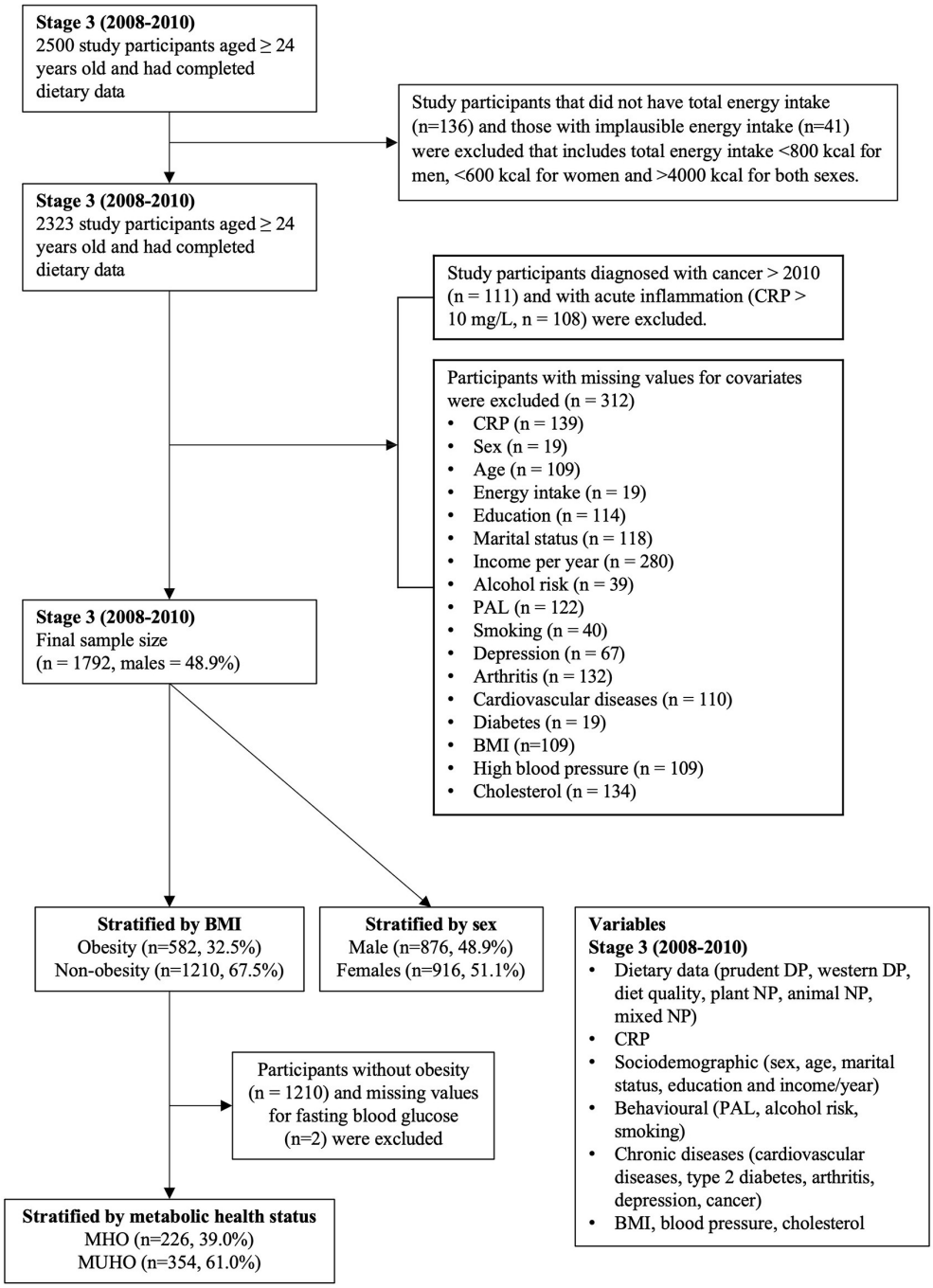

FIGURE 1
Flowchart of participants included in the study design. *CRP* C-reactive protein, *PAL* physical activity level, *BMI* body mass index *MHO* metabolically healthy obesity, *MUHO* metabolically unhealthy obesity, *DP* dietary patterns, *NP* nutrient patterns.

a Kaiser-Meyer-Olkin test. A measure of "diet quality" was developed by subtracting western from prudent DP scores (20). The same steps were performed to identify the NPs. Plant-sourced, animal-sourced, and mixed-sourced NPs were identified based on thirty-one nutrient groups from the overall measured nutrients. Additionally, the Pearson's correlation coefficient between each NP and thirty-nine food groups was determined.

CRP measurement and categories

A fasting blood sample was obtained, and high-sensitivity CRP levels was measured using an automated chemistry analyzer Olympus AU5400 (Beckman Coulter, USA). The grade of inflammation were determined based on CRP values (low, <1 mg/L; moderate, 1–3 mg/L; and high, >3 mg/L) (15).

Measurement of covariates

We included potential confounders including anthropometric, behavioral, sociodemographic and chronic conditions that are associated with diet and inflammation in this study. Measurement of these covariates has been described in a previous study (13).

Anthropometric measurements (i.e., height, weight, waist circumference and blood pressure) were obtained following standard protocols in the clinic examination. For each participant, height and weight were used to compute BMI [weight (kg)/height $(m)^2$] (21). Waist circumference was measured three times and the calculated mean value was used. Blood pressure was measured twice and the average of two recorded measures was used. Measurement of total cholesterol, high density lipoprotein, low density lipoprotein and fasting blood glucose were obtained from a fasting blood sample from each participant taken during the clinic examination.

For smoking status, participants were grouped into non-smoker, ex-smoker and current smoker. The 1989 National Heart Foundation Risk Factor Prevalence study classification formula was used to classify alcohol risk into non-drinkers and no-risk, low risk, intermediate risk, and high to very high risk drinkers (22). The Active Australia Survey was utilized to assess physical activity levels (PAL) with results classified into no activity, insufficient activity and sufficient activity (23).

Information regarding sex and age were obtained using the CATI. Participant socioeconomic status (i.e., income per year, education and marital status) and chronic conditions [i.e., cardiovascular diseases (CVD), diabetes, and arthritis] were obtained from a self-administered questionnaire. Depression was measured using the Center for Epidemiologic Studies Depression scale (CES-D) (24). Linked data from the South Australia Cancer Registry (SA Health), organized through the South Australia and Northern Territory datalink, was used to obtain participants cancer information until 2015. Participants in Stage 3 provided consent to access data from the administrative data sets.

Statistical analyses

DPs and NPs [scores] were categorized into quintiles. Descriptive analysis of other covariates was conducted across quintiles. Mean and standard deviation were calculated for continuous and normally distributed variables while proportions were used for categorical variables. ANOVA and Chi-square test were used to determine significant differences across DP and NP quintiles.

Multivariable ordinal logistic regression was used to estimate the odds ratio to determine the association of DP and NP with the grade of inflammation. In addition, we performed linear regression analysis to examine the association between dietary and nutrient patterns with CRP as a continuous variable. The CRP data was log transformed prior to the analysis due to skewed distribution. We developed four models. Model one was adjusted for energy intake and sociodemographic factor (i.e., sex, age, income per year, education and marital status). Model two was additionally adjusted for behavioral factor (i.e., smoking status, PAL and alcohol risk). Model three was additionally adjusted for chronic conditions (CVD, diabetes, depression, arthritis, and cancer) and related markers (blood pressure and total cholesterol). Model four was additionally adjusted for BMI. We did not include medications (e.g., for glucose-, lipid-, and blood pressure-lowering) as confounders given that they do not affect diet as an exposure variable.

Subgroup analysis by gender, obesity status and metabolic health status were performed using the same statistical analysis method as the overall participants. Stratification for obesity status was based on the WHO definition of obesity by BMI. Participants with a BMI <30 kg/m^2 were categorized as non-obese while participants with a BMI ≥30 kg/m^2 were categorized as obese. Stratification based on metabolic health phenotype of the obese participants were determined based on the National Cholesterol Education Program adult treatment program III (NCEP-ATP III) criteria of metabolic syndromes: (1) abdominal obesity, waist circumference >102 cm for men and >88 cm for women, (2) triglycerides ≥150 mg/dL, (3) HDL cholesterol <40 mg/dL for men and <50 mg/dL for women, (4) blood pressure ≥130/≥85 mmHg, and (5) fasting glucose ≥100 mg/dL (25). Participants were categorized into metabolically unhealthy obesity (MUHO) if having ≥2 criteria (waist circumference was excluded) (26).

Sensitivity analyses to account for waist circumference (WC) and waist-to-hip ratio (WHR) were also performed separately to assess the potential effect of fat distribution in the association (27). For the analysis of the overall participants and subgroup analysis by gender, WC and WHR were accounted as a continuous variable. For subgroup analysis by obesity and metabolic health status, WC and WHR were used as determinants of obesity based on the WHO cut-off (28). Participants with WC >102 cm or WHR ≥0.90 for men and WC >88 cm or WHR ≥0.85 for women were categorized to have abdominal obesity.

The *p*-value for trend was determined using quintiles as continuous variable. All analyses were performed using

TABLE 1 Characteristics of all participants by extreme quintiles of DPs and NPs (Stage 3, 2008–2010; $n = 1,792$).

Characteristics	Overall	Prudent DP		*P*-trend	Western DP		*P*-trend	Plant NP		*P*-trend	Animal NP		*P*-trend
		Q1	Q5		Q1	Q5		Q1	Q5		Q1	Q5	
Sex[b] (*n* %)													
Male	876 (48.9%)	217 (60.4%)	150 (41.9%)	**<0.001**	96 (26.7%)	269 (75.1%)	**<0.001**	203 (56.5%)	169 (47.2%)	**0.01**	150 (41.8%)	169 (47.2%)	**<0.001**
Female	916 (51.1%)	142 (39.6%)	208 (58.1%)		263 (73.3%)	89 (24.9%)		156 (43.5%)	189 (52.8%)		209 (58.2%)	189 (52.8%)	
Agea (mean, SD)	56.6 (13.6)	54.3 (14.1)	59.8 (12.6)	**<0.001**	57.7 (12.9)	54.1 (14.0)	**0.003**	55.6 (14.6)	58.3 (12.1)	**0.03**	57.8 (13.1)	58.3 (12.1)	0.08
BMI[a] (mean, SD)	28.5 (5.2)	28.8 (5.3)	27.8 (4.9)	0.053	27.4 (4.7)	29.4 (5.8)	**<0.001**	28.8 (5.6)	28.0 (4.9)	0.28	27.7 (4.7)	28.6 (5.2)	**0.03**
Obesity[b] (*n* %)													
Non-obese	1,210 (67.5%)	233 (64.9%)	257 (71.8%)	0.06	273 (76.0%)	218 (60.9%)	**<0.001**	242 (67.4%)	259 (72.3%)	0.14	269 (74.9%)	259 (72.3%)	**0.004**
Obese	582 (32.5%)	126 (35.1%)	101 (28.2%)		86 (24.0%)	140 (39.1%)		117 (32.6%)	99 (27.7%)		90 (25.1%)	99 (27.7%)	
CRP[b] (*n* %)													
<1.0 mg/L	518 (28.9%)	85 (23.7%)	117 (32.7%)	0.08	117 (32.6%)	99 (27.7%)	0.83	73 (20.3%)	120 (33.5%)	**0.003**	115 (32.0%)	99 (27.7%)	0.21
1.0–3.0 mg/L	748 (41.7%)	156 (43.5%)	143 (39.9%)		139 (38.7%)	153 (42.7%)		164 (45.7%)	149 (41.6%)		132 (36.8%)	153 (42.7%)	
>3.0 mg/L	526 (29.4%)	118 (32.9%)	98 (27.4%)		103 (28.7%)	106 (29.6%)		122 (34.0%)	89 (24.9%)		112 (31.2%)	106 (29.6%)	
Energy (kcal/day)[a] (mean, SD)	2,056.5 (579.6)	1,761.9 (562.4)	2,460.4 (562.9)	**<0.001**	1,548.6 (422.9)	2,675.0 (506.7)	**<0.001**	1,795.4 (550.3)	2,423.4 (583.8)	**<0.001**	1,516.4 (378.9)	2,423.4 (583.8)	**<0.001**
Educational status[b] (*n* %)													
Did not complete school/high school level	891 (49.7%)	214 (59.6%)	153 (42.7%)	**<0.001**	184 (51.3%)	191 (53.4%)	**0.001**	203 (56.5%)	147 (41.1%)	**<0.001**	182 (50.7%)	147 (41.1%)	0.11
Trade/certificate/diploid	575 (32.1%)	122 (34.0%)	114 (31.8%)		95 (26.5%)	126 (35.2%)		124 (34.5%)	122 (34.1%)		110 (30.6%)	122 (34.1%)	
Degree or higher	326 (18.2%)	23 (6.4%)	91 (25.4%)		80 (22.3%)	41 (11.5%)		32 (8.9%)	89 (24.9%)		67 (18.7%)	89 (24.9%)	
Marital status[b] (*n* %)													
Married/living with partner	1,252 (69.9%)	210 (58.5%)	242 (67.6%)	**<0.001**	224 (62.4%)	248 (69.3%)	**<0.001**	218 (60.7%)	258 (72.1%)	**<0.001**	233 (64.9%)	258 (72.1%)	0.11
Separated/divorced	245 (13.7%)	72 (20.1%)	54 (15.1%)		71 (19.8%)	49 (13.7%)		68 (18.9%)	47 (13.1%)		66 (18.4%)	47 (13.1%)	
Widowed	155 (8.6%)	32 (8.9%)	40 (11.2%)		41 (11.4%)	20 (5.6%)		28 (7.8%)	32 (8.9%)		30 (8.4%)	32 (8.9%)	
Never married	140 (7.8%)	45 (12.5%)	22 (6.1%)		23 (6.4%)	41 (11.5%)		45 (12.5%)	21 (5.9%)		30 (8.4%)	21 (5.9%)	
Income per year[b] (*n* %)													
Up to $20,000	249 (13.9%)	66 (18.4%)	65 (18.2%)	**0.02**	64 (17.8%)	47 (13.1%)	**0.01**	61 (17.0%)	61 (17.0%)	**0.04**	54 (15.0%)	61 (17.0%)	0.57
$20,001–$40,000	462 (25.8%)	93 (25.9%)	84 (23.5%)		92 (25.6%)	89 (24.9%)		101 (28.1%)	75 (20.9%)		97 (27.0%)	75 (20.9%)	
$40,001–$60,000	305 (17.0%)	53 (14.8%)	67 (18.7%)		50 (13.9%)	56 (15.6%)		55 (15.3%)	63 (17.6%)		56 (15.6%)	63 (17.6%)	
$60,001–$80,000	258 (14.4%)	49 (13.6%)	43 (12.0%)		42 (11.7%)	68 (19.0%)		55 (15.3%)	48 (13.4%)		41 (11.4%)	48 (13.4%)	
More than $80,000	518 (28.9%)	98 (27.3%)	99 (27.7%)		111 (30.9%)	98 (27.4%)		87 (24.2%)	111 (31.0%)		111 (30.9%)	111 (31.0%)	
Alcohol risk[b] (*n* %)													
Non-drinkers and no risk	889 (49.6%)	187 (52.1%)	177 (49.4%)	**0.01**	145 (%)	220 (%)	**<0.001**	182 (50.7%)	183 (51.1%)	0.08	172 (47.9%)	183 (51.1%)	**0.01**

(Continued)

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TABLE 1 Continued

Characteristics	Overall	Prudent DP		*P*-trend	Western DP		*P*-trend	Plant NP		*P*-trend	Animal NP		*P*-trend
		Q1	Q5		Q1	Q5		Q1	Q5		Q1	Q5	
Low risk	680 (37.9%)	111 (30.9%)	147 (41.1%)		161 (%)	85 (%)		123 (34.3%)	139 (38.8%)		130 (36.2%)	139 (38.8%)	
Intermediate risk	71 (4.0%)	26 (7.2%)	10 (2.8%)		14 (%)	25 (%)		21 (5.8%)	12 (3.4%)		14 (3.9%)	12 (3.4%)	
High to very high risk	14 (0.8%)	4 (1.1%)	1 (0.3%)		6 (%)	3 (%)		2 (0.6%)	1 (0.3%)		6 (1.7%)	1 (0.3%)	
Incomplete information	138 (7.7%)	31 (8.6%)	23 (6.4%)		33 (%)	25 (%)		31 (8.6%)	23 (6.4%)		37 (10.3%)	23 (6.4%)	
PAL[b] (*n* %)													
No activity	302 (16.9%)	86 (24.0%)	30 (8.4%)	**<0.001**	48 (13.4%)	76 (21.2%)	**0.042**	90 (25.1%)	40 (11.2%)	**<0.001**	63 (17.5%)	40 (11.2%)	0.49
Activity but not sufficient	795 (44.4%)	166 (46.2%)	144 (40.2%)		148 (41.2%)	156 (43.6%)		164 (45.7%)	133 (37.2%)		150 (41.8%)	133 (37.2%)	
Sufficient activity	695 (38.8%)	107 (29.8%)	184 (51.4%)		163 (45.4%)	126 (35.2%)		105 (29.2%)	185 (51.7%)		146 (40.7%)	185 (51.7%)	
Smoking status[b] (*n* %)													
Non smoker	824 (46.0%)	131 (36.5%)	173 (48.3%)	**<0.001**	170 (47.4%)	138 (38.5%)	**<0.001**	133 (37.0%)	171 (47.8%)	**<0.001**	164 (45.7%)	171 (47.8%)	0.18
Ex-smoker	714 (39.8%)	144 (40.1%)	151 (42.2%)		151 (42.1%)	136 (38.0%)		147 (40.9%)	153 (42.7%)		145 (40.4%)	153 (42.7%)	
Current smoker	254 (14.2%)	84 (23.4%)	34 (9.5%)		38 (10.6%)	84 (23.5%)		79 (22.0%)	34 (9.5%)		50 (13.9%)	34 (9.5%)	
Cardiovascular diseases[b] (*n* %)													
No CVD	1,641 (91.6%)	322 (89.7%)	324 (90.5%)	0.16	323 (90.0%)	327 (91.3%)	0.36	320 (89.1%)	330 (92.2%)	0.41	324 (90.3%)	330 (92.2%)	0.12
CVD (inc TIA)	151 (8.4%)	37 (10.3%)	34 (9.5%)		36 (10.0%)	31 (8.7%)		39 (10.9%)	28 (7.8%)		35 (9.7%)	28 (7.8%)	
Arthritis[b,c] (*n* %)													
No arthritis	1,153 (64.3%)	226 (63.0%)	216 (60.3%)	0.52	216 (60.2%)	251 (70.1%)	0.15	231 (64.3%)	235 (65.6%)	0.72	230 (64.1%)	235 (65.6%)	0.78
Arthritis	562 (31.4%)	116 (32.3%)	128 (35.8%)		122 (34.0%)	92 (25.7%)		116 (32.3%)	112 (31.3%)		110 (30.6%)	112 (31.3%)	
Diabetes[b] (*n* %)													
No diabetes	1,622 (90.5%)	320 (89.1%)	320 (89.4%)	0.40	330 (91.9%)	315 (88.0%)	0.45	326 (90.8%)	327 (91.3%)	0.96	329 (91.6%)	327 (91.3%)	**0.04**
Diabetes	170 (9.5%)	39 (10.9%)	38 (10.6%)		29 (8.1%)	43 (12.0%)		33 (9.2%)	31 (8.7%)		30 (8.4%)	31 (8.7%)	
Depression[b] (*n* %)													
No depressive symptoms	1,491 (83.2%)	285 (79.4%)	305 (85.2%)	**0.03**	311 (86.6%)	274 (76.5%)	**0.01**	283 (78.8%)	306 (85.5%)	0.06	301 (83.8%)	306 (85.5%)	0.70
Mild depression	193 (10.8%)	44 (12.3%)	35 (9.8%)		34 (9.5%)	52 (14.5%)		42 (11.7%)	33 (9.2%)		38 (10.6%)	33 (9.2%)	
Moderate to severe depression	108 (6.0%)	30 (8.4%)	18 (5.0%)		14 (3.9%)	32 (8.9%)		34 (9.5%)	19 (5.3%)		20 (5.6%)	19 (5.3%)	
Cancer[b] (*n* %)													
No	1,740 (97.1%)	347 (96.7%)	344 (96.1%)	0.64	346 (96.4%)	354 (98.9%)	**0.04**	345 (96.1%)	345 (96.4%)	0.48	345 (96.1%)	345 (96.4%)	0.40
Yes	52 (2.9%)	12 (3.3%)	14 (3.9%)		13 (3.6%)	4 (1.1%)		14 (3.9%)	13 (3.6%)		14 (3.9%)	13 (3.6%)	
High blood pressuse[b] (*n* %)													
No	848 (47.3%)	174 (48.5%)	165 (46.1%)	0.92	180 (50.1%)	170 (47.5%)	0.55	169 (47.1%)	165 (46.1%)	0.57	165 (46.0%)	165 (46.1%)	0.63
Yes	944 (52.7%)	185 (51.5%)	193 (53.9%)		179 (49.9%)	188 (52.5%)		190 (52.9%)	193 (53.9%)		194 (54.0%)	193 (53.9%)	

(Continued)

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TABLE 1 Continued

Characteristics	Overall	Prudent DP		*P*-trend	Western DP		*P*-trend	Plant NP		*P*-trend	Animal NP		*P*-trend
		Q1	Q5		Q1	Q5		Q1	Q5		Q1	Q5	
High cholesterol[b] **(*n* %)**													
No	1,050 (58.6%)	207 (57.7%)	213 (59.5%)	0.87	207 (57.7%)	219 (61.2%)	0.23	211 (58.8%)	191 (53.4%)	0.23	203 (56.5%)	191 (53.4%)	0.30
Yes	742 (41.4%)	152 (42.3%)	145 (40.5%)		152 (42.3%)	139 (38.8%)		148 (41.2%)	167 (46.6%)		156 (43.5%)	167 (46.6%)	
Prudent DP[a] (mean, SD)	0.0 (1.0)				0.0 (1.0)	−0.0 (1.1)	0.56	−1.1 (0.5)	1.3 (0.9)	**<0.001**	−0.2 (1.0)	1.3 (0.9)	**<0.001**
Western DP [a] (mean, SD)	0.0 (1.0)	0.1 (1.1)	0.0 (1.0)	0.12				0.1 (1.1)	0.1 (1.0)	0.57	−0.8 (0.7)	0.1 (1.0)	**<0.001**
Diet quality[a] (mean, SD)	−0.0 (1.4)	−1.4 (1.2)	1.5 (1.2)	**<0.001**	1.2 (1.1)	−1.6 (1.4)	**<0.001**	−1.1 (1.3)	1.3 (1.4)	**<0.001**	0.6 (1.3)	1.3 (1.4)	**<0.001**
Plant NP[a] (mean, SD)	0.0 (1.0)	−1.0 (0.5)	1.3 (0.9)	**<0.001**	0.0 (1.0)	0.0 (1.1)	0.24				−0.0 (0.9)	0.1 (1.0)	**0.02**
Animal NP[a] (mean, SD)	0.0 (1.0)	−0.1 (1.0)	0.3 (1.1)	**<0.001**	−0.8 (0.7)	1.0 (0.9)	**<0.001**	0.1 (1.0)	0.1 (1.0)	0.29			
Mixed NP[a] (mean, SD)	−0.0 (1.0)	−0.1 (1.0)	0.2 (1.1)	**<0.001**	−0.4 (0.9)	0.5 (1.1)	**<0.001**	−0.0 (0.9)	−0.0 (1.2)	0.87	−0.0 (1.0)	−0.0 (1.2)	0.31

BMI, body mass index; PAL, physical activity level; CVD, cardiovascular disease; TIA, transient ischaemic attack; DP, DPs; NP, nutrient patterns; [a]ANOVA [b]Pearson's Chi-squared test [c]Participants who refused to state/don't know their arthritis data were not reported. Bold indicates significant in *p*-value.

STATA/SE version 16 (Stata, StataCorp LP, College Station, TX, USA).

Results

Characteristics of sample population based on quintiles of DPs and NPs are presented in Table 1 and Supplementary Tables S1, S2. We observed no difference in CRP categories across quintiles of identified DPs and NPs, except for the plant-sourced NP. Participants in higher quintiles of the prudent pattern, diet quality and plant-sourced NP were older, married or living with a partner, had a higher proportion of women, higher levels of education, more physically active and non-/ex-smoker. In contrast, participants with a high score of western pattern were likely to be men, younger, and had BMI $\geq$30 kg/m^2.

The odds ratio for the association of quintiles of DPs and NPs with grade of inflammation in all study participants are presented in Table 2. In the fully adjusted model, the highest quintile of plant-sourced NP was associated with a 43% odds reduction of inflammation compared to the first quintile (OR_{Q5vsQ1} = 0.57, 95% CI = 0.42–0.78, *p*-trend = 0.01). Likewise, non-significant, inverse association was observed for prudent DP and overall diet quality with inflammation, and the effect was moderate for diet quality (7% reduction). In contrast, mixed-sourced NP was associated with a 35% increase in inflammation (OR_{Q5vsQ1} = 1.35, 95% CI = 0.99–1.84, *p*-trend = 0.03). Non-significant, positive association was observed for both western DP and animal-sourced NP with grade of inflammation after adjusting for sociodemographic, behavioral factors, blood pressure, total cholesterol, and chronic diseases (Model 1–3). However, the association was reversed when BMI was included in the model (Model 4).

Subgroup analysis by gender and obesity status showed a similar pattern of association (Figure 2; Supplementary Tables S3–S5). The prudent pattern was inversely associated with inflammation in all subgroups. For the western pattern, inclusion of BMI into the model attenuated the association in both males and females (Supplementary Table S3). There was no association between western DP and CRP in participants with a BMI <30 kg/m^2. However, the association was positive in the obesity group (OR_{Q5vsQ1} = 1.62, 95% CI: 0.78–3.38). We also observed a greater association between diet quality and grade of inflammation in obesity compared to the overall population, where the highest quintile was associated with a 30% reduction in inflammation (OR_{Q5vsQ1} = 0.70, 95% CI: 0.39–1.24, p = 0.24) (Supplementary Table S4).

Furthermore, the inverse association between plant-sourced NP and the grade of inflammation remained strong in subgroup analysis (Figure 3; Supplementary Tables S3–S5). The effect size in the highest quintile of plant-sourced NP was greater in males compared to females (41 vs. 37%

TABLE 2 Association of DPs and NPs with grade of inflammation in all participants in the NWAHS (Stage 3, 2008–2010; $n = 1,792$).

	Odds ratio (95% confidence interval)					*P*-trend
	Q1	Q2	Q3	Q4	Q5	
Prudent DP						
Model 1	1.00	0.77 (0.59–1.02)	0.70 (0.53–0.94)	0.88 (0.66–1.18)	0.64 (0.47–0.88)	0.05
Model 2	1.00	0.80 (0.60–1.05)	0.74 (0.56–0.99)	0.95 (0.70–1.27)	0.72 (0.52–0.99)	0.23
Model 3	1.00	0.83 (0.63–1.10)	0.77 (0.57–1.03)	0.94 (0.70–1.27)	0.74 (0.54–1.03)	0.24
Model 4	1.00	0.81 (0.61–1.09)	0.70 (0.52–0.95)	0.86 (0.63–1.17)	0.72 (0.52–1.01)	0.14
Western DP						
Model 1	1.00	1.30 (0.98–1.72)	1.28 (0.96–1.72)	1.38 (1.00–1.89)	1.57 (1.07–2.29)	**0.03**
Model 2	1.00	1.25 (0.95–1.67)	1.21 (0.90–1.63)	1.28 (0.93–1.76)	1.36 (0.92–2.00)	0.16
Model 3	1.00	1.19 (0.89–1.58)	1.15 (0.86–1.55)	1.24 (0.90–1.72)	1.26 (0.85–1.86)	0.26
Model 4	1.00	1.05 (0.78–1.41)	0.99 (0.73–1.35)	0.95 (0.68–1.34)	0.83 (0.55–1.25)	0.37
Diet quality						
Model 1	1.00	0.85 (0.65–1.13)	0.87 (0.66–1.16)	0.73 (0.54–0.97)	0.71 (0.53–0.96)	**0.02**
Model 2	1.00	0.90 (0.68–1.20)	0.95 (0.71–1.27)	0.81 (0.60–1.10)	0.82 (0.60–1.11)	0.16
Model 3	1.00	0.92 (0.69–1.22)	0.96 (0.72–1.29)	0.81 (0.60–1.09)	0.84 (0.62–1.14)	0.19
Model 4	1.00	0.93 (0.69–1.25)	1.00 (0.74–1.35)	0.80 (0.59–1.10)	0.93 (0.67–1.28)	0.44
Plant NP						
Model 1	1.00	0.63 (0.48–0.83)	0.69 (0.52–0.92)	0.76 (0.57–1.01)	0.55 (0.41–0.75)	**0.01**
Model 2	1.00	0.65 (0.50–0.86)	0.73 (0.55–0.96)	0.81 (0.61–1.07)	0.60 (0.45–0.82)	**0.03**
Model 3	1.00	0.67 (0.51–0.88)	0.72 (0.54–0.96)	0.82 (0.62–1.10)	0.61 (0.45–0.83)	**0.03**
Model 4	1.00	0.64 (0.48–0.86)	0.69 (0.51–0.92)	0.74 (0.55–1.00)	0.57 (0.42–0.78)	**0.01**
Animal NP						
Model 1	1.00	1.12 (0.85–1.49)	0.95 (0.71–1.28)	1.31 (0.95–1.79)	1.24 (0.85–1.82)	0.18
Model 2	1.00	1.13 (0.85–1.50)	0.94 (0.70–1.26)	1.28 (0.94–1.76)	1.20 (0.82–1.75)	0.26
Model 3	1.00	1.09 (0.82–1.45)	0.91 (0.67–1.22)	1.27 (0.92–1.75)	1.13 (0.77–1.66)	0.34
Model 4	1.00	0.91 (0.68–1.22)	0.78 (0.57–1.06)	1.18 (0.85–1.64)	0.94 (0.63–1.39)	0.69
Mixed NP						
Model 1	1.00	0.95 (0.73–1.25)	1.03 (0.78–1.36)	1.19 (0.90–1.57)	1.28 (0.96–1.72)	**0.04**
Model 2	1.00	0.93 (0.70–1.22)	1.00 (0.76–1.32)	1.17 (0.89–1.55)	1.29 (0.96–1.73)	**0.03**
Model 3	1.00	0.91 (0.69–1.20)	1.00 (0.76–1.32)	1.15 (0.87–1.53)	1.31 (0.97–1.77)	**0.03**
Model 4	1.00	0.94 (0.71–1.25)	0.98 (0.73–1.31)	1.16 (0.87–1.55)	1.35 (0.99–1.84)	**0.03**

DP, dietary patterns; NP, nutrient patterns. Model 1: adjusted for sociodemographic factor (sex, age, education, marital status, income per year) and total energy intake (including fiber). Model 2: additionally adjusted for behavioral factor (alcohol risk, PAL, smoking status). Model 3: additionally adjusted for blood pressure, total cholesterol, and chronic diseases (cardiovascular diseases, arthritis, diabetes, depression, cancer). Model 4: additionally adjusted for BMI. Bold indicates significant in *p*-value.

reduction), greater for participants with obesity compared to participants without obesity (57 vs. 40% reduction). The plant-sourced NP was also associated with a 76% reduction in inflammation ($OR_{Q5vsQ1} = 0.24$, 95% CI: 0.11–0.52; p-trend = 0.01) in MUHO. We found an interaction between the highest quintile of plant-sourced nutrient pattern and MUHO (p-interaction = 0.023). No interactions were observed for the other dietary or nutrient patterns with sex, obesity status and metabolic health status (data not shown).

For the mixed-sourced NP, the magnitude of association was 2-fold greater with the grade of inflammation in the obesity group and the trends were significant ($OR_{Q5vsQ1} = 2.36$, 95% CI: 1.32–4.23; p-trend = 0.002). In this study, we also performed subgroup analyses for MHO and MUHO (Supplementary Table 5). BMI remained to attenuate the association of western DP or animal-sourced NP with the grade of inflammation in males and females (Supplementary Table S3).

In the sensitivity analysis adjusted for WC or WHR, the inverse association between plant-sourced NP and systemic inflammation remained strong in overall participants and subgroup analyses (Supplementary Tables S6, S7). Minimal differences in the estimates were observed in the association between other dietary and nutrient patterns with systemic inflammation compared to the models adjusted for BMI.

When we examined the association with CRP as a continuous variable, we found that plant-sourced NP showed

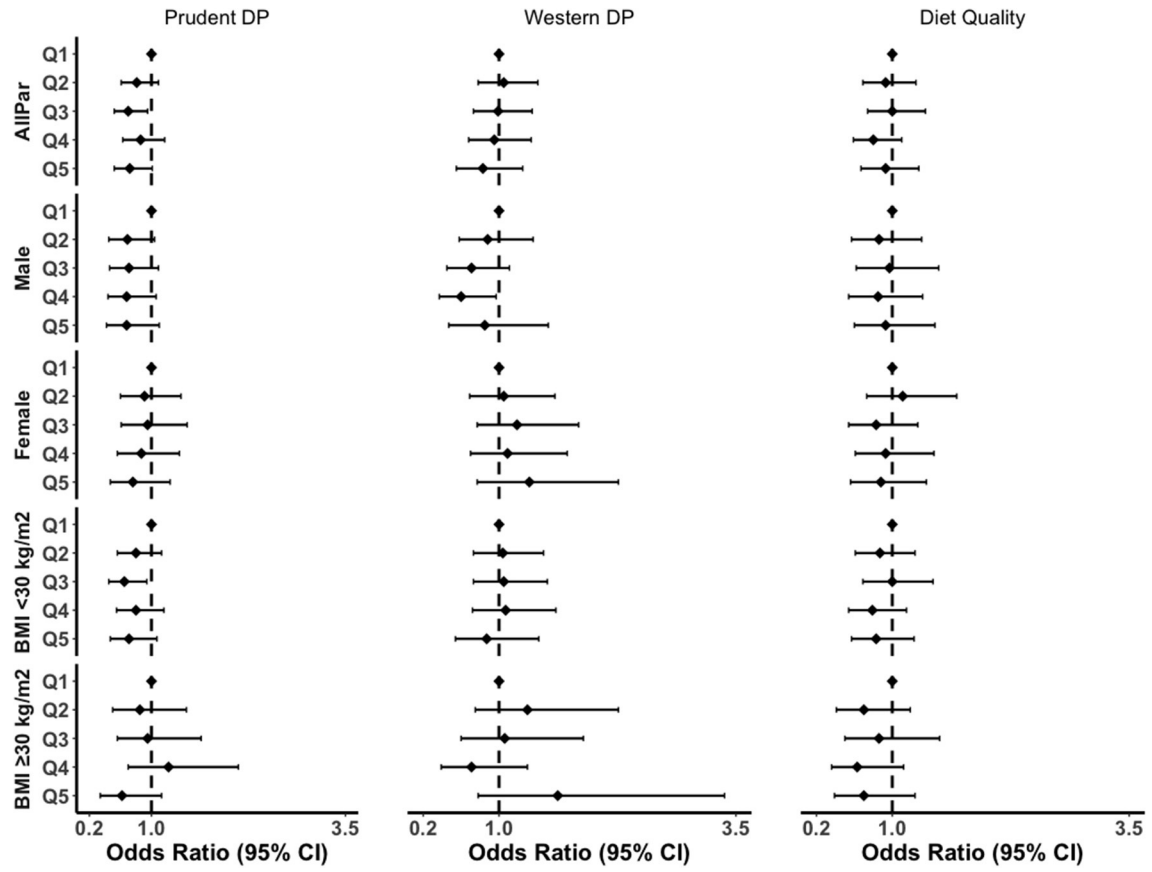

FIGURE 2
Subgroup analyses of the association between DPs and grade of inflammation in the fully adjusted model. BMI, body mass index; CI, confidence interval; AllPar, All Participants. Q1 (quintile 1) was a reference.

significant negative association with log-transformed CRP levels in the overall participants ($\beta_{Q5vsQ1} = -0.21$, 95% CI: −0.34 to −0.07) (Supplementary Table S8). The highest quintile of plant-sourced NP was also associated with a significant decrease of log-transformed CRP values in subgroup analysis for male ($\beta_{Q5vsQ1} = -0.20$, 95% CI: −0.39 to −0.02), non-obese ($\beta_{Q5vsQ1} = -0.19$, 95% CI: −0.37 to −0.02), obese ($\beta_{Q5vsQ1} = -0.34$, 95% CI: −0.56 to −0.11) and MUHO ($\beta_{Q5vsQ1} = -0.51$, 95% CI: −0.81 to −0.21).

Discussion

We found an independent association of dietary and nutrient patterns with inflammation. Prudent and plant-sourced NPs were associated with lower levels of inflammation. Furthermore, a plant-sourced NP was strongly associated with lower inflammation, particularly among males, people with obesity and MUHO. In contrast, mixed-sourced NPs were associated with higher levels of inflammation. BMI attenuated the association between western DP and animal-sourced NP with higher inflammation.

Comparison with other studies

We found that adherence to a prudent/healthy diet was inversely associated with inflammation. This is consistent with earlier studies reporting the association between empirically derived healthy DPs and CRP levels in different populations (29, 30). In contrast, the western DP was associated with increased inflammation. However, BMI strongly attenuated the effect size in the overall population and subgroup analysis by gender. Some evidence reported that the association between western/unhealthy DPs and CRP levels remained positive after adjustment for BMI (29, 31). However, consistent with the current study, other studies (32, 33) have shown that the association between unhealthy DPs and CRP levels was inversed after adjustment for BMI. As an indicator of increased adiposity (34), BMI may confound or mediate the association between

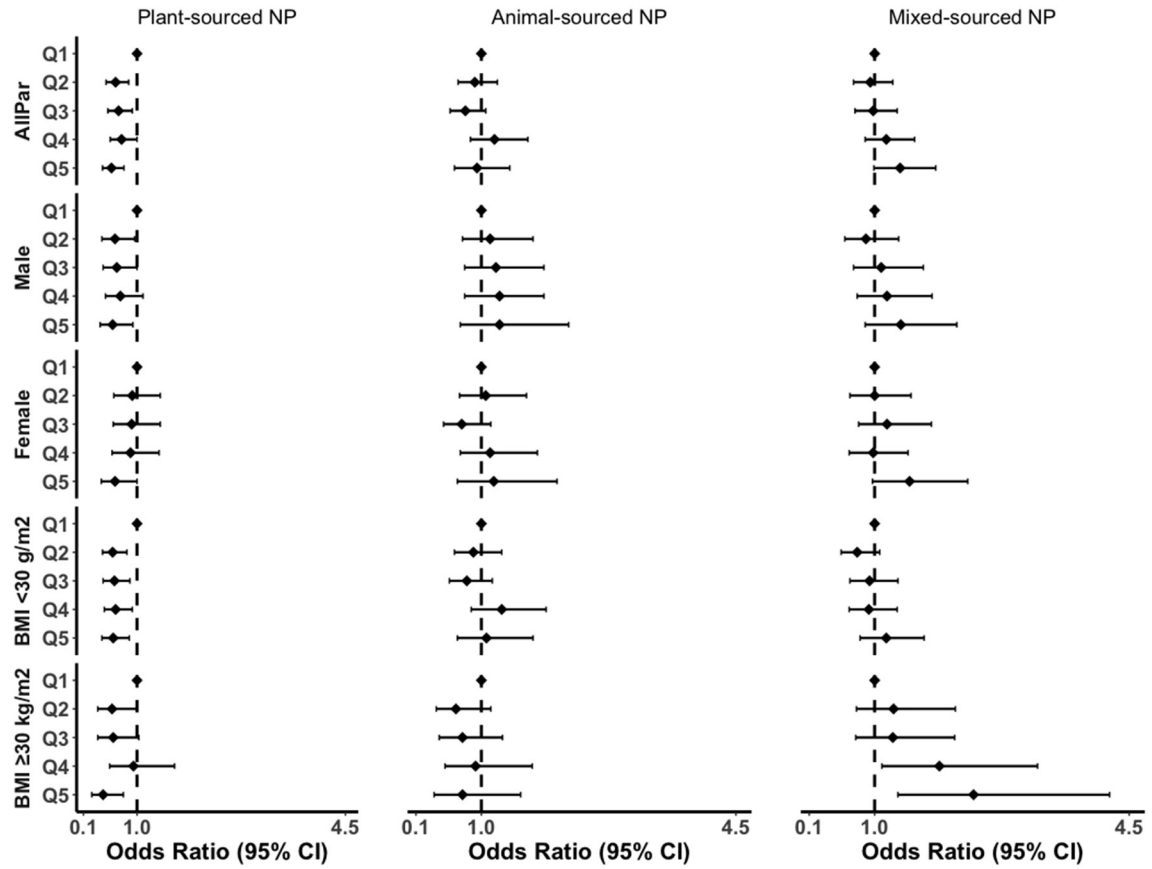

FIGURE 3
Subgroup analyses of the association between NPs and grade of inflammation in the fully adjusted model. BMI, body mass index; CI, confidence interval; AllPar, All Participants. Q1 (quintile 1) was a reference.

diet, inflammation and obesity (35). It has been suggested that high adiposity influences the effect magnitude of diet on CRP (36). This may explain the effect of BMI in the association between western DP and CRP in this population.

Furthermore, we observed that adherence to a higher diet quality was associated with lower inflammation in the overall population and people with obesity. This is consistent with results from studies using index-based diet quality measures [e.g., the Healthy Eating Index (37) and the Dietary Approaches to Stop Hypertension (38)] and supports the notion that adherence to a higher diet quality, or healthier dietary habits, may reduce inflammation.

Studies investigating the association between NP and systemic inflammation are scant. A previous study in Men Androgen Inflammation Lifestyle Environment and Stress (MAILES) cohort in Australia reported an inverse association between plant-sourced NP and CRP levels and a positive association between animal-sourced NP and CRP levels (10). Our findings are consistent with this study, and our plant- and animal-sourced NP also shared similarities in characteristics of nutrient groups that compose those two nutrient patterns, namely beta-carotene, lutein and zeaxanthin for plant-sourced NP, and cholesterol and omega-6 for animal-sourced NP. Nevertheless, evidence examining the association between NP and diseases associated with systemic inflammation suggests that plant-sourced nutrients may lower systemic inflammation. A study in the same population has shown an inverse association between plant-sourced NP and depressive symptoms, a condition associated with systemic inflammation (19, 39). A few cross-sectional studies have also reported that NP, generally characterized by high loadings of plant-based nutrients, including vitamins, micronutrients and MUFA, was associated with reduced risk of weight gain (16), metabolic syndromes (40), non-alcoholic fatty liver disease (41), colorectal cancer (42) and hypertension (43); conditions associated with elevated inflammation. On the other hand, NP characterized by animal-sourced nutrients, has been associated with increased odds of metabolic syndromes (40, 44). A prospective study in the EPIC cohort also showed that NP with similar characteristics to our mixed-sourced

NP (i.e., protein, vitamin B2, phosphorus and calcium) was associated with greater risk of weight gain (16). This evidence supports our findings on the association between NP and systemic inflammation.

In addition, other studies have used the Dietary Inflammatory Index (DII®) to examine the association between diet and systemic inflammation, inflammatory biomarkers (e.g., CRP) (45, 46), or chronic conditions. Higher scores of DII, indicating a more pro-inflammatory diet, have been associated with detrimental conditions related to pro-inflammatory state in the body, such as increased inflammatory biomarkers (47), risks of all-cause and CVD mortality (48), lower muscle mass and strength (49) and increased adiposity (50). The DII scores are based on 45 dietary components that include foods and nutrients which were associated with modulation of six inflammatory biomarkers (IL-1β, IL-4, IL-6, IL-10, TNF-α and CRP) (51). The majority of our identified nutrient groups in the NPs are in accordance with the components of DII. Using *a posteriori* method, the derived DP and NP in this study can depict dietary habit and interaction between food and nutrient groups to elicit the inflammatory effect in the population. This interaction may not be reflected, in *a priori* index-based measure, such as the DII.

Potential mechanisms

Modulation of inflammation by diet is related to oxidative stress levels. It is partly facilitated by anti-inflammatory components through their antioxidant capacity, reducing oxidative stress and preventing oxidative damage (52, 53) and pro-inflammatory components that can induce oxidative stress and promote release of inflammatory cytokines triggering inflammation (54). In this study, the identified prudent DP is characterized by a high intake of fruit and vegetables, nuts, fish and legumes (18), which have been associated with lower CRP concentrations in observational and clinical studies (55–58). We found adherence to the plant-sourced NP was associated with lower inflammation. Characterized by a high intake of –β-carotene, vitamin C, potassium, lutein, zeaxanthin and dietary fiber (19)– commonly found as anti-inflammatory components in fruit and vegetables (e.g., leafy greens) (59, 60), this suggests these antioxidant components may mediate the effect of a healthy DP to reduce inflammation in this population.

The plant-sourced NP also showed the most consistent association compared to other identified dietary and NPs in the subgroup analyses, even after the adjustment for BMI, WC and WHR. In support of Cao et al. (10), we observed that a plant-sourced NP was associated with reduced inflammation in men, and the effect was greater compared to women. Men are more prone to weight gain and chronic diseases. This could be due to their: (1) tendency to consume a unhealthy diet (61); and (2) differences in response to oestradiol on body weight regulation (62), compared to women. Furthermore, a marked reduction of inflammation was observed in people with obesity and MUHO who adhered to the plant-sourced NP. Intervention using antioxidants, such as vitamin C, has been demonstrated to reduce CRP, interleukin-6 and fasting blood glucose levels in hypertensive or diabetic obese participants (63). Altogether, this suggests adherence to an antioxidant rich diet may be beneficial to reduce inflammation, particularly for men and people with obesity and metabolic syndromes.

Conversely, adherence to an animal-sourced NP was associated with a very moderate increase in inflammation in the overall population. This finding is not unexpected, given the animal-sourced NP in this study is characterized by a combination of anti- and pro-inflammatory components, with polyunsaturated fatty acids or PUFA (omega-3 and omega-6), monounsaturated fats (MUFA), vitamin E, saturated fats (SFA) and cholesterol scored among the highest in loading factors (19). Omega-3 and MUFA (8), as well as vitamin E have antioxidant properties and have been associated with a reduction in inflammatory biomarker levels, including CRP. On the other hand, omega-6 (64), SFA and cholesterol, predominantly found in oils and processed foods, have pro-inflammatory properties. In combination, it is possible they may cancel each other effect, resulting in a small magnitude of effect size in the association. In addition, the effect of PUFA metabolism by desaturase activity may modify the availability of PUFA and its bioactive derivatives in the tissue (65). Omega-3 and omega-6 are both substrates of desaturase enzyme which conversion results in eicosapentanoic acid and arachidonic acid, respectively (66). The former and its derivates are generally more anti-inflammatory compared to the latter (65). Subsequently, competing amount of omega-3 and omega-6 in the diet may alter the ratio of pro- and anti-inflammatory derivatives from PUFA metabolism and their inflammatory effect. This may also explain a moderate increase observed in the association between animal-sourced NP and systemic inflammation in this study. Interestingly, the animal-sourced NP was inversely associated with the grade of inflammation in obesity but not the non-obese group. This indicates the anti-inflammatory components of an animal-sourced NP may provide a beneficial effect by reducing inflammation in people with obesity. However, further studies are required to confirm this hypothesis.

This study also revealed a significant association between the higher adherence to a mixed-sourced NP and the higher inflammation that was stronger in obese participants. A mixed-sourced NP was characterized by phosphorus, protein, vitamin B2, B3 and B12, iodine, zinc, saturated fats, calcium, sodium, retinol, iron, and cholesterol (19). These nutrients are primarily found in meat, dairy products and processed foods, and typical to a western DP. Consistent with our findings, studies have reported an association between protein (67), iron (68), saturated fats (69) and cholesterols (70) with increased inflammatory biomarkers. Interestingly, similar to the plant-sourced NP, the association between mixed-sourced NP and CRP

was not affected by BMI. The NP approach may provide improved precision in predicting the association between diet and inflammation, further studies are needed to confirm these findings.

Strength and limitations

We included a large sample size and provided comprehensive analysis on the interaction between DP and NPs with the grade of CRP in the general adult population, stratified by gender, obesity and metabolic health. The results of this study should be considered in the context of several limitations. As this is a cross sectional study, we cannot infer the causal relationship between diet and inflammation. Given the nature of observational studies, there are likely to be residual confounders which have not been included in the analysis that may affect the association. There is also potential misreporting of the dietary intake data collected using a self-reported FFQ. However, the FFQ has been widely used to generate DP (18) and NP (10) data in cohort studies, validating the reliability of FFQ to assess overall dietary intake. Furthermore, we determined inflammation based on a single inflammatory biomarker, CRP. Nonetheless, CRP is a widely used clinical marker and a strong predictor of many inflammatory-related diseases (12). In addition, consideration should be taken in interpreting results given the wide confidence intervals, which could be due to a sample size limitation.

Conclusion

This study is the first to combine DP and NP to explore the association between dietary patterns and systemic inflammation. The study revealed independent associations of DP and NPs with inflammation. A plant-sourced NP, characterized by antioxidants and fibers, was inversely associated with inflammation; an association stronger in men, obesity and MUHO. In combination with prudent DP, this suggests a possible biological pathway underlying the protective effect of a healthy diet against chronic diseases by reducing inflammation through the anti-inflammatory properties derived from fruit and vegetables. This finding supports current dietary recommendations to increase intake of fruit and vegetables and highlights the need to improve the clinical and public health message, particularly for men and people with obesity. Future studies are required to confirm the association of DP and NPs with inflammation in the longitudinal setting, and to include other inflammatory biomarkers, health outcomes and different populations.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: Not applicable. Requests to access these datasets should be directed to tiffany.gill@adelaide.edu.au.

Ethics statement

The studies involving human participants were reviewed and approved by the Human Ethics Research Committee, Queen Elizabeth Hospital, South Australia. The patients/participants provided their written informed consent to participate in this study.

Author contributions

YW, YAM, AP, and TG conceived the study. YW wrote the manuscript, analyzed, and interpreted the data. YAM constructed the dietary and nutrient patterns as well as analyzed the data. YAM, AP, and TG provided expert opinion, gave comment on the manuscript, and approved the final version. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.977029/full#supplementary-material

References

1. Buckley DI, Fu R, Freeman M, Rogers K, Helfand M. C-Reactive protein as a risk factor for coronary heart disease: a systematic review and meta-analyses for the U.S. Preventive services task force. *Ann Int Med.* (2009) 151:483–95. doi: 10.7326/0003-4819-151-7-200910060-00009

2. Miller AA, Spencer SJ. Obesity and neuroinflammation: a pathway to cognitive impairment. *Brain Behav Immun.* (2014) 42:10–21. doi: 10.1016/j.bbi.2014.04.001

3. Netea MG, Balkwill F, Chonchol M, Cominelli F, Donath MY, Giamarellos-Bourboulis EJ, et al. A guiding map for inflammation. *Nat Immunol.* (2017) 18:826–31. doi: 10.1038/ni.3790

4. Stefan N, Cusi K. A global view of the interplay between non-alcoholic fatty liver disease and diabetes. *Lancet Diab Endocrinol.* (2022) 10:284–96. doi: 10.1016/S2213-8587(22)00003-1

5. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: the linking mechanism and the complications. *Arch Med Sci.* (2017) 13:851–63. doi: 10.5114/aoms.2016.58928

6. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of disease across the life span. *Nat Med.* (2019) 25:1822–32. doi: 10.1038/s41591-019-0675-0

7. Willett WC, Stampfer MJ. Current evidence on healthy eating. *Annu Rev Public Health.* (2013) 34:77–95. doi: 10.1146/annurev-publhealth-031811-124646

8. Galland L. Diet and inflammation. *Nutr Clin Pract.* (2010) 25:634–40. doi: 10.1177/0884533610385703

9. Hart MJ, Torres SJ, McNaughton SA, Milte CM. Dietary patterns and associations with biomarkers of inflammation in adults: a systematic review of observational studies. *Nutr J.* (2021) 20:24. doi: 10.1186/s12937-021-00674-9

10. Cao Y, Wittert G, Taylor AW, Adams R, Appleton S, Shi Z. Nutrient patterns and chronic inflammation in a cohort of community dwelling middle-aged men. *Clin Nutr.* (2017) 36:1040–7. doi: 10.1016/j.clnu.2016.06.018

11. Freisling H, Fahey MT, Moskal A, Ocké MC, Ferrari P, Jenab M, et al. Region-specific nutrient intake patterns exhibit a geographical gradient within and between European countries. *J Nutr.* (2010) 140:1280–6. doi: 10.3945/jn.110.121152

12. Luan Y-Y, Yao Y-M. The clinical significance and potential role of c-reactive protein in chronic inflammatory and neurodegenerative diseases. *Front Immunol.* (2018) 9:1302. doi: 10.3389/fimmu.2018.01302

13. Grant JF, Chittleborough CR, Taylor AW, Dal Grande E, Wilson DH, Phillips PJ, et al. The north west adelaide health study: detailed methods and baseline segmentation of a cohort for selected chronic diseases. *Epidemiol Perspect Innov.* (2006) 3:4. doi: 10.1186/1742-5573-3-4

14. Grant JF, Taylor AW, Ruffin RE, Wilson DH, Phillips PJ, Adams RJ, et al. Cohort profile: the north west adelaide health study (Nwahs). *Int J Epidemiol.* (2009) 38:1479–86. doi: 10.1093/ije/dyn262

15. Pearson TA, Mensah GA, Hong Y, Smith Jr SC. Cdc/Aha workshop on markers of inflammation and cardiovascular disease: application to clinical and public health practice: overview. *Circulation.* (2004) 110:e543–4. doi: 10.1161/01.CIR.0000148979.11121.6B

16. Freisling H, Pisa P, Ferrari P, Byrnes G, Moskal A, Dahm C, et al. Main nutrient patterns are associated with prospective weight change in adults from 10 European countries. *Eur J Nutr.* (2016) 55:2093–104. doi: 10.1007/s00394-015-1023-x

17. *Food Standards Australian New Zealand (Nuttab) 2010 Online Searchable Database*. Available online at: https://www.foodstandards.gov.au/science/monitoringnutrients/afcd/Pages/default.aspx (accessed May 10, 2021).

18. Shakya PR, Melaku YA, Page A, Gill TK. Association between dietary patterns and adult depression symptoms based on principal component analysis, reduced-rank regression and partial least-squares. *Clin Nutr.* (2020) 2811–23. doi: 10.1016/j.clnu.2019.12.011

19. Shakya PR, Melaku YA, Page AJ, Gill TK. Nutrient patterns and depressive symptoms among australian adults. *Eur J Nutr.* (2021) 60:329–43. doi: 10.1007/s00394-020-02243-y

20. Wang YB, Shivappa N, Hébert JR, Page AJ, Gill TK, Melaku YA. Association between dietary inflammatory index, dietary patterns, plant-based dietary index and the risk of obesity. *Nutrients.* (2021) 13:1536. doi: 10.3390/nu13051536

21. NLBI Obesity Education Initiative Expert Panel on the Identification EaToOaOiA. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults–the evidence report. *Nat Inst Health Obes Res.* (1998) 6(Suppl. 2):51s—209s.

22. AIHW. *Risk Factor Prevalence Study: Survey No. 3 1989*. Canberra: AIHW (1990).

23. Armstrong T, Bauman AE, Davies J. *Physical Activity Patterns of Australian Adults: Results of the 1999 National Physical Activity Survey*. Canberra, ACT: Australian Institute of Health and Welfare (2000).

24. Radloff LS. The Ces-D scale:a self-report depression scale for research in the general population. *Appl Psychol Meas.* (1977) 1:385–401. doi: 10.1177/014662167700100306

25. Peeters PJ, Aerssens J, de Hoogt R, Stanisz A, Gohlmann HW, Hillsley K, et al. Molecular profiling of murine sensory neurons in the nodose and dorsal root ganglia labeled from the peritoneal cavity. *Physiol Genomics.* (2006) 24:252–63. doi: 10.1152/physiolgenomics.00169.2005

26. Schulze MB. Metabolic health in normal-weight and obese individuals. *Diabetologia.* (2019) 62:558–66. doi: 10.1007/s00125-018-4787-8

27. Stefan N. Causes, consequences, and treatment of metabolically unhealthy fat distribution. *Lancet Diab Endocrinol.* (2020) 8:616–27. doi: 10.1016/S2213-8587(20)30110-8

28. WHO. *Waist Circumference and Waist-Hip Ratio : Report of a Who Expert Consultation, Geneva, 8-11 December 2008*. Geneva: World Health Organization (2011).

29. Centritto F, Iacoviello L, di Giuseppe R, De Curtis A, Costanzo S, Zito F, et al. Dietary patterns, cardiovascular risk factors and c-reactive protein in a healthy italian population. *Nutr Metabol Cardiov Dis.* (2009) 19:697–706. doi: 10.1016/j.numecd.2008.11.009

30. Nanri A, Yoshida D, Yamaji T, Mizoue T, Takayanagi R, Kono S. Dietary patterns and c-reactive protein in japanese men and women. *Am J Clin Nutr.* (2008) 87:1488–96. doi: 10.1093/ajcn/87.5.1488

31. Nettleton JA, Steffen LM, Mayer-Davis EJ, Jenny NS, Jiang R, Herrington DM, et al. Dietary patterns are associated with biochemical markers of inflammation and endothelial activation in the multi-ethnic study of atherosclerosis (Mesa). *Am J Clin Nutr.* (2006) 83:1369–79. doi: 10.1093/ajcn/83.6.1369

32. Ko BJ, Park KH, Shin S, Zaichenko L, Davis CR, Crowell JA, et al. Diet quality and diet patterns in relation to circulating cardiometabolic biomarkers. *Clin Nutr.* (2016) 35:484–90. doi: 10.1016/j.clnu.2015.03.022

33. Fung TT, Rimm EB, Spiegelman D, Rifai N, Tofler GH, Willett WC, et al. Association between dietary patterns and plasma biomarkers of obesity and cardiovascular disease risk. *Am J Clin Nutr.* (2001) 73:61–7. doi: 10.1093/ajcn/73.1.61

34. Taylor AE, Ebrahim S, Ben-Shlomo Y, Martin RM, Whincup PH, Yarnell JW, et al. Comparison of the associations of body mass index and measures of central adiposity and fat mass with coronary heart disease, diabetes, and all-cause mortality: a study using data from 4 Uk cohorts. *Am J Clin Nutr.* (2010) 91:547–56. doi: 10.3945/ajcn.2009.28757

35. Schlesinger S, Neuenschwander M, Schwedhelm C, Hoffmann G, Bechthold A, Boeing H, et al. Food groups and risk of overweight, obesity, and weight gain: a systematic review and dose-response meta-analysis of prospective studies. *Adv Nutr.* (2019) 10:205–18. doi: 10.1093/advances/nmy092

36. Papier K, Hartman L, Tong TYN, Key TJ, Knuppel A. Higher meat intake is associated with higher inflammatory markers, mostly due to adiposity: results from Uk biobank. *J Nutr.* (2022) 152:183–9. doi: 10.1093/jn/nxab314

37. Millar SR, Navarro P, Harrington JM, Perry IJ, Phillips CM. Dietary quality determined by the healthy eating index-2015 and biomarkers of chronic low-grade inflammation: a cross-sectional analysis in middle-to-older aged adults. *Nutrients.* (2021) 13:222. doi: 10.3390/nu13010222

38. Soltani S, Chitsazi MJ, Salehi-Abargouei A. The effect of dietary approaches to stop hypertension (Dash) on serum inflammatory markers: a systematic review and meta-analysis of randomized trials. *Clin Nutr.* (2018) 37:542–50. doi: 10.1016/j.clnu.2017.02.018

39. Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, et al. So depression is an inflammatory disease, but where does the inflammation come from? *BMC Med.* (2013) 11:200. doi: 10.1186/1741-7015-11-200

40. Vajdi M, Farhangi MA, and Nikniaz L. Diet-derived nutrient patterns and components of metabolic syndrome: a cross-sectional community-based study. *BMC Endocrine Disord.* (2020) 20:69. doi: 10.1186/s12902-020-0547-0

41. Tien NV, Arisawa K, Uemura H, Imaeda N, Goto C, Katsuura-Kamano S. Association between nutrient patterns and fatty liver index: baseline survey of the japan multi-institutional collaborative cohort study in tokushima, Japan. *J Epidemiol.* (2021) 32:376–83. doi: 10.2188/jea.JE20200447

42. Moskal A, Freisling H, Byrnes G, Assi N, Fahey MT, Jenab M, et al. Main nutrient patterns and colorectal cancer risk in the european prospective investigation into cancer and nutrition study. *Bri J Cancer.* (2016) 115:1430–40. doi: 10.1038/bjc.2016.334

43. Mazidi M, Ofori-Asenso R, George ES, Vatanparast H. Association between nutrient patterns and hypertension among adults in the United States: a population-based survey. *High Blood Press Cardiovasc Prev.* (2020) 27:133–8. doi: 10.1007/s40292-020-00364-3

44. Sadeghi O, Sadeghi A, Mozaffari-Khosravi H, Shokri A. The association between nutrient patterns and metabolic syndrome among iranian adults: cross-sectional analysis of shahedieh cohort study. *Public Health Nutr.* (2021) 24:3379–88. doi: 10.1017/S1368980020001639

45. Shivappa N, Blair CK, Prizment AE, Jacobs DR, Hébert JR. Prospective study of the dietary inflammatory index and risk of breast cancer in postmenopausal women. *Mol Nutr Food Res.* (2017) 61:1600592. doi: 10.1002/mnfr.201600592

46. Shivappa N, Wirth MD, Murphy EA, Hurley TG, Hébert JR. Association between the dietary inflammatory index (Dii) and urinary enterolignans and c-reactive protein from the national health and nutrition examination survey-2003-2008. *Eur J Nutr.* (2019) 58:797–805. doi: 10.1007/s00394-018-1690-5

47. Shivappa N, Wirth MD, Hurley TG, Hébert JR. Association between the dietary inflammatory index (Dii) and telomere length and c-reactive protein from the national health and nutrition examination survey-1999-2002. *Mol Nutr Food Res.* (2017) 61:1600630. doi: 10.1002/mnfr.201600630

48. Yuan S, Song C, Zhang R, He J, Dou K. Dietary inflammation index and its association with long-term all-cause and cardiovascular mortality in the general us population by baseline glycemic status. *Nutrients.* (2022) 14:2556. doi: 10.3390/nu14132556

49. Chen L, Ming J, Chen T, Hébert JR, Sun P, Zhang L, et al. Association between dietary inflammatory index score and muscle mass and strength in older adults: a study from national health and nutrition examination survey (Nhanes) 1999–2002. *Eur J Nutr.* (2022). doi: 10.1007/s00394-022-02941-9. [Epub ahead of print].

50. Lozano CP, Wilkens LR, Shvetsov YB, Maskarinec G, Park S-Y, Shepherd JA, et al. Associations of the dietary inflammatory index with total adiposity and ectopic fat through the gut microbiota, lps, and c-reactive protein in the multiethnic cohort–adiposity phenotype study. *Am J Clin Nutr.* (2021) 115:1344–56. doi: 10.1093/ajcn/nqab398

51. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hebert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. *Public Health Nutr.* (2014) 17:1689–96. doi: 10.1017/S1368980013002115

52. Fiedor J, Burda K. Potential role of carotenoids as antioxidants in human health and disease. *Nutrients.* (2014) 6:466–88. doi: 10.3390/nu6020466

53. Righi NC, Schuch FB, De Nardi AT, Pippi CM, de Almeida Righi G, Puntel GO, et al. Effects of vitamin c on oxidative stress, inflammation, muscle soreness, and strength following acute exercise: meta-analyses of randomized clinical trials. *Eur J Nutr.* (2020) 59:2827–39. doi: 10.1007/s00394-020-02215-2

54. Maurizi G, Della Guardia L, Maurizi A, Poloni A. Adipocytes properties and crosstalk with immune system in obesity-related inflammation. *J Cell Physiol.* (2018) 233:88–97. doi: 10.1002/jcp.25855

55. Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB, Willett WC. Fruit and vegetable intakes, c-reactive protein, and the metabolic syndrome. *Am J Clin Nutr.* (2006) 84:1489–97. doi: 10.1093/ajcn/84.6.1489

56. Yu Z, Malik VS, Keum N, Hu FB, Giovannucci EL, Stampfer MJ, et al. Associations between Nut consumption and inflammatory biomarkers. *Am J Clin Nutr.* (2016) 104:722–8. doi: 10.3945/ajcn.116.134205

57. Pot GK, Geelen A, Majsak-Newman G, Harvey LJ, Nagengast FM, Witteman BJM, et al. Increased consumption of fatty and lean fish reduces serum c-reactive protein concentrations but not inflammation markers in feces and in colonic biopsies. *J Nutr.* (2009) 140:371–6. doi: 10.3945/jn.109.113472

58. Esmaillzadeh A, Azadbakht L. Legume consumption is inversely associated with serum concentrations of adhesion molecules and inflammatory biomarkers among iranian women. *J Nutr.* (2011) 142:334–9. doi: 10.3945/jn.111.146167

59. Brighenti F, Valtueña S, Pellegrini N, Ardigò D, Del Rio D, Salvatore S, et al. Total antioxidant capacity of the diet is inversely and independently related to plasma concentration of high-sensitivity c-reactive protein in adult italian subjects. *Br J Nutr.* (2005) 93:619–25. doi: 10.1079/BJN20051400

60. Ma Y, Griffith JA, Chasan-Taber L, Olendzki BC, Jackson E, Stanek EJ, et al. Association between dietary fiber and serum c-reactive protein. *Am J Clin Nutr.* (2006) 83:760–6. doi: 10.1093/ajcn/83.4.760

61. Kiefer I, Rathmanner T, Kunze M. Eating and dieting differences in men and women. *J Mens Health Gend.* (2005) 2:194–201. doi: 10.1016/j.jmhg.2005.04.010

62. Rubinow KB. Estrogens and body weight regulation in men. *Adv Exp Med Biol.* (2017) 1043:285–313. doi: 10.1007/978-3-319-70178-3_14

63. Ellulu MS, Rahmat A, Patimah I, Khaza'ai H, Abed Y. Effect of vitamin c on inflammation and metabolic markers in hypertensive and/or diabetic obese adults: a randomized controlled trial. *Drug Des Devel Ther.* (2015) 9:3405–12. doi: 10.2147/DDDT.S83144

64. Innes JK, Calder PC. Omega-6 fatty acids and inflammation. *Prostagl Leukot Essent Fatty Acids.* (2018) 132:41–8. doi: 10.1016/j.plefa.2018.03.004

65. Schulze MB, Minihane AM, Saleh RNM, Risérus U. Intake and metabolism of omega-3 and omega-6 polyunsaturated fatty acids: nutritional implications for cardiometabolic diseases. *Lancet Diab Endocrinol.* (2020) 8:915–30. doi: 10.1016/S2213-8587(20)30148-0

66. Layé S, Madore C, St-Amour I, Delpech J-C, Joffre C, Nadjar A, et al. N-3 polyunsaturated fatty acid and neuroinflammation in aging and Alzheimer's disease. *Nutr Aging.* (2015) 3:33–47. doi: 10.3233/NUA-150049

67. Gögebakan O, Kohl A, Osterhoff MA, van Baak MA, Jebb SA, Papadaki A, et al. Effects of weight loss and long-term weight maintenance with diets varying in protein and glycemic index on cardiovascular risk factors: the diet, obesity, and genes (diogenes) study: a randomized, controlled trial. *Circulation.* (2011) 124:2829–38. doi: 10.1161/CIRCULATIONAHA.111.033274

68. de Oliveira Otto MC, Alonso A, Lee DH, Delclos GL, Jenny NS, Jiang R, et al. Dietary micronutrient intakes are associated with markers of inflammation but not with markers of subclinical atherosclerosis. *J Nutr.* (2011) 141:1508–15. doi: 10.3945/jn.111.138115

69. Clarke R, Shipley M, Armitage J, Collins R, Harris W. Plasma phospholipid fatty acids and chd in older men: whitehall study of london civil servants. *Br J Nutr.* (2009) 102:279–84. doi: 10.1017/S0007114508143562

70. Tannock LR, O'Brien KD, Knopp RH, Retzlaff B, Fish B, Wener MH, et al. Cholesterol feeding increases c-reactive protein and serum amyloid a levels in lean insulin-sensitive subjects. *Circulation.* (2005) 111:3058–62. doi: 10.1161/CIRCULATIONAHA.104.506188

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Adherence to mediterranean diet and the risk of differentiated thyroid cancer in a European cohort: The EPIC study

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Background: The Mediterranean diet (MD) has been proposed as a healthy diet with a potential to lower the incidence of several types of cancer, but there is no data regarding thyroid cancer (TC). We investigated the association between MD adherence, and its components, and the differentiated TC risk within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort.

Methods: Over 450,000 men and women from nine European countries were followed up for a mean of 14.1 years, during which 712 differentiated TC cases were identified. Adherence to MD was estimated using the relative MD (rMED) score, an 18-point scale including alcohol, and the adapted rMED (arMED) score, a 16-point scale excluding alcohol. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox regression models adjusted for potential confounding factors.

Results: Adherence to the arMED score was not associated with the risk of differentiated TC ($HR_{high\ vs.\ low\ adherence} = 0.94$, 95% CI: 0.70–1.25; p-trend 0.27), while a suggestive, but non-statistically significant inverse relationship was observed with rMED ($HR_{high\ vs.\ low\ adherence} = 0.88$, 95% CI: 0.68–1.14; p-trend 0.17). Low meat ($HR_{low\ vs.\ high\ meat\ intake} = 0.81$, 95% CI: 0.67–0.99; p-trend = 0.04) and moderate alcohol ($HR_{moderate\ vs.\ non-moderate\ intake} = 0.88$, 95% CI: 0.75–1.03) intake were related with lower differentiated TC risk.

Conclusions: Our study shows that a high adherence to MD is not strongly related to differentiated TC risk, although further research is required to confirm the impact of MD and, especially, meat intake in TC risk.

KEYWORDS

thyroid cancer (TC), Mediterranean diet (MD), meat, intake, EPIC study, cohort

Introduction

Thyroid cancer (TC) represents the most common endocrine malignancy worldwide (1). Lastly, the TC incidence has gradually increased, in part driven by overdiagnosis due to the use of ultrasound examinations and increased medical surveillance, leading to higher TC prevalence in high-income countries (2). The transformation of thyroid follicular cells may result in differentiated or undifferentiated TC. Differentiated TC, including papillary and follicular carcinoma, represents more than 90% of all TC cases (3). Poorly differentiated and anaplastic thyroid carcinomas are rare but more aggressive tumor types (3). Exposure to ionizing radiation, particularly during childhood (4), previous history of benign thyroid hyperplasia (5), and overweight/obesity (6, 7) are the mostwell-established risk factors for TC.

The Mediterranean diet (MD) is characterized by a high consumption of fruits, vegetables, complex carbohydrates and fish, a low amount of meat and dairy products and a daily glass of wine (8). In this dietary pattern, olive oil is the main source of fats (9). There is evidence that relates high adherence to MD with lower risk of cancer incidence and mortality (e.g., breast, colorectal, head and neck, respiratory, gastric, liver and bladder) (10, 11), obesity (12), and type 2 diabetes (13). Convincing evidence is consistently showing a positive moderate association for overweight and obesity (6, 7), and type 2 diabetes (14, 15) with TC incidence. MD is rich in polyphenols, fibers, phytosterols, monounsaturated and polyunsaturated fatty acids, which are probably the main drivers of the protection of MD against cancer (16). The potential underlying mechanisms of action involve anti-oxidative and anti-inflammatory effects, reduction of tumor cell growth, increase of chemoprotective effects, and inhibition of tumor development (16). Several dietary factors of MD have been suggested to play a role in TC etiology, but the results are inconclusive (17, 18). Previous

Abbreviations: arMED, adapted relative Mediterranean diet score; BMI, body mass index; EPIC, European Prospective Investigation into Cancer and Nutrition; MD, Mediterranean diet; rMED, relative Mediterranean diet score; TC thyroid cancer; T3 triiodothyronine; T4 thyroxine.

studies investigating TC have mainly focused on separate food items and only few on dietary patterns (17, 18). Dietary pattern analysis examines the overall effects of diet and could be a better approach to investigate the role of diet in chronic diseases (19).

To our knowledge, there are no studies on the relationship between MD adherence and TC risk. Therefore, in the current study we aimed to investigate the association between MD adherence and the risk of differentiated TC within the European Prospective Investigation into Cancer and Nutrition (EPIC) study.

Materials and methods

Subjects and study design

EPIC is a large prospective cohort study designed to investigate the relationship between diet, lifestyle, environmental factors, and cancer. The full methods and study design have been described previously (20). In brief, 521,324 participants, mostly aged between 35 and 70 years, were recruited between 1992 and 2000 in 23 centers from 10 Western European countries. All participants provided written informed consent, and the study was approved by the local ethics committees in the participating countries and the ethical review board of the International Agency for Research on Cancer (IARC). We excluded participants with prevalent cancer other than non-melanoma skin cancer at baseline or with missing information on date of diagnosis or censoring data, missing dietary and lifestyle information (did not complete the questionnaires), had extreme energy intake and/or expenditure (participants in the top or bottom 1% of the distribution of the ratio of total energy intake to energy requirement) and participants from Greece (data not provided for the current study) (Supplementary Figure 1).

Dietary and lifestyle ascertainment

Dietary information was collected at enrollment, using country-specific dietary questionnaires (20). Total energy intake was estimated by using the standardized EPIC Nutrient Database (21). At baseline, information on socio-demographic characteristics, tobacco consumption, physical activity, reproductive history, use of oral contraceptives and hormone replacement therapy, and medical history were self-reported using standardized lifestyle questionnaires (20). Anthropometry (weight and height) was measured at recruitment by trained personnel, except for Oxford (United Kingdom), Norway, and France, where measurements were self-reported.

The adherence to MD was measured using the adapted relative MD score (arMED), a version of the relative MD (rMED) (22) based on the original MD score by Trichopoulou et al. (23), excluding alcohol. The arMED incorporates 8 selected components of MD and is a 16-point scale. Each component was calculated as a function of energy density (g/2,000 kcal per day) and then divided into cohort-wide tertiles of intakes (except for olive oil). For five of the six components that positively reflect MD: fruits (including nuts and seeds), vegetables (excluding potatoes), legumes, fish (including seafood), and cereal products, a score of 0-1-2 was assigned to the lowest to highest intake tertiles, respectively. The score was inverted (2-1-0 assigned to the intake tertiles) for the two components that negatively reflect MD: total meat (red meat, processed meat, poultry, game, and offal) and dairy products. The score for olive oil was adapted for non-Mediterranean countries owing to their low consumption, by assigning 0 to non-consumers, 1 for subjects below the median intake and 2 for subjects equal to or above this median. The arMED score was further classified into low (0–5 points), medium (6–9 points) or high (10–16 points) adherence levels, as previously categorized in the EPIC study (10).

In a previous EPIC study, moderate alcohol intake was inversely associated with differentiated TC risk (24). Therefore, the rMED score (22), including alcohol, was also computed. The rMED incorporates the same previous 8 components plus alcohol and is an 18-point scale. Alcohol in the rMED score was scored dichotomously assigning 2 points for moderate consumption (sex-specific cut off points: 5–25 g per day for women and 10–50 g per day for men) and 0 points for intakes outside this range. The rMED score was further classified into low (0–6 points), medium (7–10 points) or high (11–18 points) adherence levels, as previously categorized in the EPIC study (22, 25).

Follow-up and case assessment

Incident cancer cases were identified through population cancer registries in all countries except France Germany, and Naples (Italy) where cases were identified through active follow-up, directly from the participants and confirmed by a combination of methods, including health insurance records, and cancer and pathology registries. Vital status was obtained from mortality registries at the regional or national level. Complete follow-up censoring dates ranged from December 2010 to December 2014, depending on the study center. In this TC study (code C73 according to the International Classification of Diseases, 10th Revision), only first primary differentiated TC cases were included, and therefore, 52 undifferentiated TC (such as medullary, anaplastic, lymphoma, and other morphologies) were excluded (Supplementary Figure 1). Finally, 712 first primary incident differentiated TC cases were considered: 573 papillary TCs, 108 follicular TCs, and 31 not otherwise specified (NOS) TCs, most likely to be papillary TCs. Data on the stage of differentiated TC at diagnosis were collected from each center where possible. A total of 468 cases (65.7%) had tumor-node-metastasis staging score information, of which 371 were

TABLE 1 Description of the EPIC study by country and by adapted relative Mediterranean diet score (arMED).

			Differentiated thyroid cancer cases (*n*)				arMED score		arMED score (%)		
Country	***N***	**Women (%)**	**Overall**	**Papillary**	**Follicular**	**NOS**	**Mean**	**SD**	**Low (0–5)**	**Medium (6–9)**	**High (10–16)**
Denmark	55,005	52.2	39	28	11	0	6.0	2.5	44.5	46.4	9.1
France	67,391	100	248	227	19	2	8.9	2.4	7.5	51.7	40.8
Germany	48,551	56.4	82	58	21	3	6.6	2.2	31.6	58.2	10.2
Italy	44,543	68.5	127	97	19	11	10.4	2.1	0.9	33.9	65.2
Norway	33,972	100	36	31	4	1	7.8	2.1	13.6	65.8	20.6
Spain	39,984	62.1	80	66	13	1	10.7	2.2	1.3	26.2	72.5
Sweden	48,666	54.2	39	25	7	7	4.6	2.1	69.0	29.4	1.5
Netherlands	36,537	73.7	17	12	4	1	5.8	2.1	46.9	48.3	4.9
UK	75,415	69.7	44	29	10	5	8.7	2.4	10.1	51.6	38.2
Total	450,064	70.8	712	573	108	31	7.8	3.0	24.2	46.1	29.7

NOS: not otherwise specified; SD standard deviation.

classified as low-risk tumors (T1–T2) and 97 were classified as high-risk tumors (T3–T4).

Statistical analysis

Cox proportional hazard models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) between adherence to the MD measured by arMED and rMED score, its individual components and differentiated TC risk. Age was the primary time variable in all models. Entry time was age at recruitment and exit time was age at first diagnosis (cases), death, or censoring date (loss or end of follow-up), whichever occurred first. Both arMED and rMED scores were computed as categorical variables (low, medium, and high) and as continuous variables (per 1-unit). Trend tests were obtained by scoring the arMED or rMED categories in a continuous scale from 1 to 3. The basic model for the association between arMED and differentiated TC was stratified by sex, age at recruitment (1-year interval), study center, and adjusted for total energy intake (kcal/day). Variables associated with TC in previous EPIC studies (24, 26–28) were a-priori selected as potential confounders. Thus, the most-adjusted model was additionally adjusted for body mass index (BMI, kg/m^2), smoking status, alcohol (g/day), education level and physical activity (according to the Cambridge Physical Activity Index) (29). In women the model was also adjusted for menopausal status and type, ever use of oral contraceptives, and history of infertility problems. Results from the two models were almost identical, therefore, the most-adjusted model was selected for presentation. Similar models were applied for the rMED score, without alcohol consumption (g/day) as adjustment variable. All models met the proportional hazard assumption, tested using the Schoenfeld goodness-of-fit test. In addition, we estimated the associations between individual components of MD and TC risk. Each component was evaluated as a categorical variable (tertile points assigned for the arMED/rMED score calculation), except for alcohol (moderate vs. non-moderate consumption). Interactions on the multiplicative scale with sex, smoking status (never, former, and current smokers), alcohol (low, moderate, and high), BMI (<25 and ≥ 25 kg/m^2), were examined using the likelihood ratio test.

Separate analyses were performed for differentiated TC subtypes: follicular and papillary tumors, and disease stage: low-risk (T1–T2) and high-risk (T3–T4) tumors. Heterogeneity of risk between TC subtypes was assessed with the Wald test. Separate models were also computed to check the variability between countries with a high and low TC incidence. EPIC countries with TC incidence rates of >5/10,000 in women (i.e., France, Germany, Italy, and Spain) were considered to have a high TC incidence. Moreover, separate models were conducted only in women, because of the small proportion of men with TC (10.4%). Finally, we conducted separate analyses by geographical regions: South (Spain, Italy, France), Central (UK, Germany, and the Netherlands) and North Europe (Denmark, Norway, and Sweden) because dietary habits can differ between European regions (30). Sensitivity analyses were performed by repeating the models after the exclusion of differentiated TC cases diagnosed during the first 2 years of follow-up, since participants may have changed their diets in the pre-diagnostic period. For all analyses, p-values < 0.05 were considered statistically significant. Statistical analyses were conducted using R 3.2.1 software.

Results

In the current analysis of 450,064 EPIC participants (70.8% women), 712 were diagnosed with differentiated TC (89.6% women) (Supplementary Figure 1). The mean arMED score

TABLE 2 Baseline characteristics of included participants from the EPIC study according to the adapted relative Mediterranean diet score (arMED).

Characteristics	All	arMED score		
		Low (0-5)	Medium (6–9)	High (10–16)
N	450,064	108,791	207,470	133,803
Sex, %				
Women	70.8	53.6	74.7	78.9
Men	29.2	46.4	25.3	21.3
Age, years [mean (SD)]	51.1 (9.8)	51.9 (10.0)	51.5 (9.5)	49.9 (9.7)
Total energy, kcal/day [mean (SD)]	2,077 (619)	2,176 (654)	2,039 (601)	2,054 (607)
Alcohol, g/day [median (IQR)]	5.5 (0.9-15.2)	6.0 (1.3-16.8)	5.7 (1.1-15.2)	4.9 (0.5-13.5)
BMI, %				
<25 kg/m^2	53.3	48.3	55.1	54.4
25 to <30 kg/m^2	34.4	38.6	33.4	32.6
≥ 30 kg/m^2	12.4	13.1	11.5	13.0
Smoking status (%)				
Never	48.7	41.1	48.8	54.8
Former	27.3	27.5	28.1	25.7
Current	22.2	30.1	20.9	17.6
Unknown	1.9	1.2	0.2	1.9
Physical activity (%)				
Inactive or moderately inactive	52.9	49.3	51.2	58.3
Active or moderately active	45.2	47.6	46.6	41.0
Unknown	2.0	3.2	2.2	0.7
Education level (%)				
Primary or lower	28.1	31.0	24.3	31.7
Secondary or higher	68.1	67.3	71.3	63.8
Unknown	3.8	1.7	4.4	4.5
Menopausal status and type[a] (%)				
Premenopausal	34.7	29.5	33.2	39.9
Perimenopausal	19.8	20.6	20.6	17.9
Postmenopausal	42.8	47.6	43.6	38.9
Surgical menopause	2.8	2.3	2.6	3.3
Ever use of contraceptive pill[a] (%)				
No	37.9	33.4	36.5	42.5
Yes	59.4	58.6	61.5	56.9
Unknown	2.6	7.9	2.0	0.6
Infertility problems[a] (%)				
No	62.3	37.1	60.9	78.4
Yes	3.1	1.3	2.9	4.2
Unknown	34.6	61.6	36.2	17.4

arMED, adapted relative Mediterranean diet; BMI, body mass index; IQR, interquartile range; SD, standard deviation. [a]Only in women ($n = 318,647$).

was 7.8 (3.0) ranging between 4.6 (in Sweden) and 10.7 points (in Spain) (Table 1). Participants with high arMED score were more likely to be women, younger, never smoker, physically inactive/moderate inactive, and to consume less alcohol and slightly less total energy at recruitment, compared to those with a lower arMED score (Table 2). Women with high arMED score compared to those with low, tended to be premenopausal.

We found no association between arMED score (excluding the alcohol component) and the risk of overall differentiated TC in the fully-adjusted model ($HR_{\text{high vs. low adherence}} = 0.94$, 95% CI: 0.70–1.25; p-trend = 0.27) (Table 3). No differences

TABLE 3 Hazard ratios (95% Confidence Intervals) for the associations between relative Mediterranean diet score (rMED), adapted rMED (arMED) and differentiated thyroid cancer (TC) risk in the EPIC study.

	N	Overall differentiated TC	Papillary TC	Follicular TC	p for heterogeneity
Cases (*n*)		712	573	108	
arMED score (adjusted for alcohol)					0.82
Low (0–5)	108,791	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Medium (6–9)	207,470	1.13 (0.88–1.45)	1.14 (0.85–1.52)	1.26 (0.70–2.27)	
High (10–16)	133,803	0.94 (0.70–1.25)	0.96 (0.70–1.33)	0.99 (0.48–2.03)	
P-trend		0.27	0.38	0.83	
Continuous (per unit)	450,064	0.98 (0.95–1.02)	0.99 (0.95–1.03)	0.99 (0.91–1.08)	
rMED score (not adjusted for alcohol)					0.58
Low (0–6)	121,208	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Medium (7–10)	201,933	1.05 (0.84–1.32)	1.10 (0.85–1.42)	0.88 (0.51–1.51)	
High (11–18)	126,923	0.88 (0.68–1.14)	0.87 (0.65–1.17)	0.97 (0.51–1.84)	
P-trend		0.17	0.14	0.97	
Continuous (per unit)	450,064	0.98 (0.95–1.01)	0.98 (0.94–1.01)	0.98 (0.91–1.07)	

Cox models were stratified by sex, age at recruitment, study center, and adjusted for total energy intake (kcal/day, continuous), body mass index (kg/m^2, continuous), smoking status, alcohol (g/day, continuous, when applied), education level, and physical activity. In addition, in women, they were further adjusted for menopausal status and type, ever use of oral contraceptives, and history of infertility problems.

were observed in associations by TC subtype (p-value for heterogeneity = 0.82). No interactions were found for sex, smoking status, BMI, and alcohol intake. No statistically significant differences were observed in the associations between arMED score and differentiated TC risk by tumor stage, country-incidence rate, and European region (Supplementary Table 1). Similar non-statistically significant results were observed in women only and in the sensitivity analysis excluding the TC cases diagnosed within the first 2-years of follow-up (Supplementary Table 1).

A non-statistically significant inverse relationship between rMED score (including the alcohol component) and overall differentiated TC ($HR_{high\ vs.\ low\ adherence}$ = 0.88, 95% CI: 0.68–1.14; p-trend = 0.17), especially against papillary TC risk ($HR_{high\ vs.\ low\ adherence}$ = 0.87, 95% CI: 0.65–1.17; p-trend = 0.14) was observed (Table 3). In the analysis of each component of MD, we found an inverse association between low meat intake and differentiated TC risk ($HR_{low\ vs.\ high\ adherence}$ = 0.81, 95% CI: 0.67–0.99) (Table 4). The HR for moderate vs. non-moderate alcohol intake was 0.88 (95% CI 0.72–1.03). The other components were not related to differentiated TC risk.

Discussion

Adherence to MD, measured by arMED score (without the alcohol component) was not associated with the risk of differentiated TC in this large European prospective cohort study (n = 450,064) with a long follow-up (mean = 14.1 years), and a relatively high number of cases (n = 712). The results were also non-statistically significant in all sub-analyses. However, there was a statistically non-significant inverse relationship with rMED (including the alcohol component), probably driven by the inverse trend with alcohol intake and the positive association with meat intake.

In our longitudinal study, we did not find a clear association of differentiated TC risk with MD adherence. Whereas, in an US population-based case-control study, a tendency for an inverse association between a dietary pattern high in fruits and vegetables and risks of both overall and papillary TC were observed (31). Similarly, a traditional Polynesian dietary pattern characterized by a high consumption of fish, seafood and fruits, and low consumption of meat was inversely related, but was not statistically significant, with overall and papillary TC risk (32). In a Greek case-control study, inverse associations were found between the risk of overall and papillary TC and three dietary patterns rich in fresh fruit, raw vegetables, and mixed raw vegetables and fruits. Contrarily, a dietary pattern rich in fish and cooked vegetables, which is a dietary habit of Mediterranean populations, showed a higher risk of follicular TC (33). In a cross-sectional study, a high adherence to MD correlated with lower circulating levels of triiodothyronine (T3) and thyroxine (T4), but not with thyroid-stimulating hormone (TSH) (34). However, associations of TC with hypo- or hyperthyroidism and thyrotoxicosis are weaker and less consistent. High concentrations of free T4, TSH and the T4/T3 ratio were related to a higher differentiated TC risk in a small Canadian case-control study (35). Nevertheless, in a previous EPIC analysis, only low levels of TSH and high levels of thyroglobulin were associated with a higher differentiated TC risk, but not plasma concentrations of either T3 or T4 (36).

Except meat and a suggestive association for alcohol, none of the other components presumed to fit MD were related to differentiated TC risk in our analysis. In previous EPIC analyses,

TABLE 4 Hazard ratios (95% Confidence Intervals) of the association between each component of Mediterranean diet (MD) and differentiated thyroid cancer risk in the EPIC study.

rMED components (g/day per 2,000 kcal)	Median (33–67th percentiles)	0 point at rMED	1 point at rMED	2 points at rMED	*P*-trend
Vegetables	167.7 (125.9–221.1)	1.00 (ref)	0.99 (0.81–1.21)	0.89 (0.71–1.11)	0.26
Fruits	193.1 (135.3–265.0)	1.00 (ref)	1.08 (0.88–1.32)	1.07 (0.86–1.31	0.60
Legumes	4.8 (0.8–11.3)	1.00 (ref)	1.01 (0.82–1.26)	0.94 (0.74–1.19)	0.57
Cereals	170.4 (138.8–204.3)	1.00 (ref)	0.98 (0.81–1.18)	0.92 (0.75–1.12)	0.39
Olive oil	0.0 (0.0–0.8)	1.00 (ref)	1.08 (0.85–1.39)	1.19 (0.94–1.50)	0.14
Fish	17.9 (10.2–27.7)	1.00 (ref)	1.10 (0.89–1.36)	1.20 (0.95–1.51)	0.13
Meat	94.1 (74.5–114.5)	1.00 (ref)	0.95 (0.79–1.13)	0.81 (0.67–0.99)	0.04
Dairy	286.0 (205.9–386.0)	1.00 (ref)	0.93 (0.77–1.12)	0.86 (0.71–1.05)	0.15
Alcohol (categorical)	5.6 (2.1–10.9)	1.00 (ref)		0.88 (0.75–1.03)	

rMED, relative Mediterranean diet score. Each component was calculated as a function of energy density (g/2,000 kcal per day) and then divided into tertiles of intakes (except for olive oil and alcohol). In the rMED score, for five of the six components that positively reflect Mediterranean diet: fruits (including nuts and seeds), vegetables (excluding potatoes), legumes, fish (including seafood), and cereals, points of 0-1-2 were assigned to the intake tertiles. For olive oil, 0 was assigned to non-consumers, 1 for subjects below the median intake and 2 for subjects equal or above this median. For meat and dairy products, which negatively reflect Mediterranean diet, 2-1-0 points were assigned to the first, second and third intake tertiles, respectively. Alcohol was scored dichotomously assigning 2 points for moderate consumption (sex-specific cut off points: 5–25 g per day for women and 10–50 g per day for men) and 0 points for intakes outside this range. Cox models were stratified by sex, age at recruitment, study center, and adjusted for total energy intake (kcal/day, continuous), body mass index (kg/m^2, continuous), smoking status, alcohol (except for the alcohol model), education level, physical activity. In addition, in women they were further adjusted for menopausal status and type, ever use of oral contraceptives, and history of infertility problems.

similar null results were observed with the consumption of fruit and vegetables (37). Likewise, a meta-analysis using 19 case-control studies found no association with the intake of fruit and vegetables including cruciferous vegetables, which have been studied in more detail due to their content of goitrogens (38). Fish is a rich natural source of iodine which is essential for thyroid function. A meta-analysis of six case-control studies suggested that consumption of fish may decrease the risk of TC in iodine deficient areas, but not in iodine-rich areas (38). No association with fish intake was reported in a previous EPIC analysis, where very low or very high iodine intakes are rare (39). Intake of grains was not related to TC risk in a meta-analysis of three case-control studies (38). Although anti-cancer effects of olive oil and its compounds are proposed (11), neither olive oil or its compounds were associated with differentiated TC risk in previous EPIC analyses (28, 40). Similar to our findings, the incidence of TC was not related to either dairy products or calcium intake in the NIH-AARP Diet and Health Study, a large US cohort (41). Finally, our results on alcohol are broadly in agreement with a previous EPIC analysis (24) and a meta-analysis of observational studies (42), where moderate alcohol intake was associated with lower risk of TC.

In the current study, low consumption of meat was associated with a 19% lower risk of differentiated TC compared with high consumption. Only a few studies have assessed the direct role of meat intake in TC risk (17). Some classes of meat such as poultry, lamb and pork were positively associated with TC risk in case-control studies conducted in Kuwait (43), Greece (33), and the US (44), but not in Sweden and Norway (45). Potential underlying mechanisms may be related to the concentrations of haem iron and the formation of N-nitroso compounds in meats, especially in red and processed meats (46). Indeed, nitrate can inhibit iodine uptake by the thyroid (17), dysregulate thyroid hormone production and result in thyroid tumor onset (47). Another potential mechanism of action could be the formation of heterocyclic amines and polycyclic aromatic hydrocarbons, which are well-known human mutagens/carcinogens (48). Therefore, the role of meat consumption in thyroid carcinogenesis merits further investigation.

Several limitations of this study should be considered. Dietary data derived from self-reported information relying on participants' memory is prone to measurement error. Dietary data were measured only at recruitment and do not reflect longitudinal changes in dietary intake. Nevertheless, an influence of dietary changes during the pre-diagnostic period of TC is unlikely, since sensitivity analyses excluding incident cases diagnosed within the first 2 years of follow-up were similar to the entire follow-up. Both arMED and rMED scores also have limitations, as a similar weight is given to each component and the foods within them, but not all may have equivalent effects on health or TC risk. Our risk estimates were adjusted for several confounding factors; however, we cannot rule out the possibility of residual confounding by other unmeasured factors. For instance, medical history of benign thyroid diseases, a well-established risk factor for TC was not available in EPIC. The strengths of our study are its prospective design, the relatively large number of TC cases (except for follicular TC subtype), and the wide variation in MD adherence, allowing sufficient statistical power for subgroup analyses. We also minimized any

potential bias due to overdiagnosis by stratifying the analysis into countries with high or low incidence rates and into associations with low- or high-risk TC at diagnosis.

Conclusions

In summary, our study showed no association between adherence to arMED score and differentiated TC risk. However, a potential inverse trend with rMED was suggested, potentially driven by the consumption of a low amount of meat and a moderate amount of alcohol. Future research is required to confirm this potential association with meat intake and to evaluate which type of meat (i.e., red meat, processed meat, poultry, etc.) is responsible for these suggested harmful effects. Lastly, replication and meta-analysis of our findings with other prospective studies is required to further elucidate a possible association with MD adherence.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. For information on how to submit an application for gaining access to the EPIC data and/or biospecimens, please follow the instructions at http://epic.iarc.fr/access/index.php.

Ethics statement

The studies involving human participants were reviewed and approved by Ethical review board of the International Agency for Research on Cancer (IARC). The patients/participants provided their written informed consent to participate in this study.

Author contributions

RZ-R: conceptualization. AA, MSa, AE, AT, M-CB-R, NL, TT, CLe, VKa, MSc, DP, VKr, SS, RT, FR, GS, TJ, SC, CLa, MR-B, PA, JHu, MG, MA, LN, JH, KP, AH, EW, and SR: data resources. VC: statistical analysis. RZ-R: funding acquisition. FL and MF: writing—original draft preparation. SR and RZ-R: writing—review and editing. All authors have read and agreed to the final version of the manuscript.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.982369/full#supplementary-material

References

1. Pizzato M, Li M, Vignat J, Laversanne M, Singh D, La Vecchia C, et al. The epidemiological landscape of thyroid cancer worldwide: GLOBOCAN estimates for incidence and mortality rates in 2020. *Lancet Diabetes Endocrinol.* (2022) 10:264–72. doi: 10.1016/S2213-8587(22)00035-3

2. Vaccarella S, Franceschi S, Bray F, Wild CP, Plummer M, Dal Maso L. Worldwide thyroid-cancer epidemic? The increasing impact of overdiagnosis. *N Engl J Med.* (2016) 375:614–7. doi: 10.1056/NEJMp1604412

3. Nabhan F, Dedhia PH, Ringel MD. Thyroid cancer, recent advances in diagnosis and therapy. *Int J Cancer.* (2021) 149:984–92. doi: 10.1002/ijc.33690

4. D'Avanzo B, La Vecchia C, Franceschi S, Negri E, Talamini R. History of thyroid diseases and subsequent thyroid cancer risk. *Cancer Epidemiol Prev Biomarkers.* (1995) 4:193–9.

5. Cardis E, Kesminiene A, Ivanov V, Malakhova I, Shibata Y, Khrouch V, et al. Risk of thyroid cancer after exposure to 131I in childhood. *J Natl Cancer Inst.* (2005) 97:724–32. doi: 10.1093/jnci/dji129

6. Kitahara CM, McCullough ML, Franceschi S, Rinaldi S, Wolk A, Neta G, et al. Anthropometric factors and thyroid cancer risk by histological subtype: pooled analysis of 22 prospective studies. *Thyroid.* (2016) 26:306–18. doi: 10.1089/thy.2015.0319

7. Kitahara CM, Pfeiffer RM, Sosa JA, Shiels MS. Impact of overweight and obesity on US papillary thyroid cancer incidence trends (1995–2015). *JNCI J Natl Cancer Inst.* (2020) 112:810. doi: 10.1093/jnci/djz202

8. Willett WC, Sacks F, Trichopoulou A, Drescher G, Ferro-Luzzi A, Helsing E T, et al. Mediterranean diet pyramid: a cultural model for healthy eating. *Am J Clin Nutr.* (1995) 61 (6 Suppl):1402S—6S. doi: 10.1093/ajcn/61.6.1402S

9. Bach A, Serra-Majem L, Carrasco JL, Roman B, Ngo J, Bertomeu I, et al. The use of indexes evaluating the adherence to the mediterranean diet in epidemiological studies: a review. *Public Health Nutr.* (2006) 9:132–46. doi: 10.1079/PHN2005936

10. Buckland G, Travier N, Cottet V, González CA, Luján-Barroso L, Agudo A, et al. Adherence to the editerranean diet and risk of breast cancer in the European prospective investigation into cancer and nutrition cohort study. *Int J Cancer.* (2013) 132:2918–27. doi: 10.1002/ijc.27958

11. Morze J, Danielewicz A, Przybyłowicz K, Zeng H, Hoffmann G, Schwingshackl L. An updated systematic review and meta-analysis on adherence to Mediterranean diet and risk of cancer. *Eur J Nutr.* (2021) 60:1561–86. doi: 10.1007/s00394-020-02346-6

12. Esposito K, Kastorini CM, Panagiotakos DB, Giugliano D. Mediterranean diet and weight loss: meta-analysis of randomized controlled trials. *Metab Syndr Relat Disord.* (2011) 9:1–12. doi: 10.1089/met.2010.0031

13. Esposito K, Maiorino MI, Bellastella G, Chiodini P, Panagiotakos D, Giugliano D. A journey into a Mediterranean diet and type 2 diabetes: a systematic review with meta-analyses. *BMJ Open.* (2015) 5:e008222. doi: 10.1136/bmjopen-2015-008222

14. Li H, Qian J. Association of diabetes mellitus with thyroid cancer risk: a meta-analysis of cohort studies. *Medicine.* (2017) 96:e8230. doi: 10.1097/MD.0000000000008230

15. Yeo Y, Ma SH, Hwang Y, Horn-Ross PL, Hsing A, Lee KE, et al. Diabetes mellitus and risk of thyroid cancer: a meta-analysis. *PLoS ONE.* (2014) 9:e98135. doi: 10.1371/journal.pone.0098135

16. Mentella MC, Scaldaferri F, Ricci C, Gasbarrini A, Miggiano GAD. Cancer and mediterranean diet: a review. *Nutrients.* (2019) 11:2059. doi: 10.3390/nu11092059

17. Choi WJ, Kim J. Dietary factors and the risk of thyroid cancer: a review. *Clin Nutr Res.* (2014) 3:75. doi: 10.7762/cnr.2014.3.2.75

18. Barrea L, Gallo M, Ruggeri RM, Giacinto PD, Sesti F, Prinzi N, et al. Nutritional status and follicular-derived thyroid cancer: an update. *Crit Rev Food Sci Nutr.* (2021) 61:25–59. doi: 10.1080/10408398.2020.1714542

19. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol.* (2002) 13:3–9. doi: 10.1097/00041433-200202000-00002

20. Riboli E, Hunt K, Slimani N, Ferrari P, Norat T, Fahey M, et al. European prospective investigation into cancer and nutrition (EPIC): study populations and data collection. *Public Health Nutr.* (2002) 5:1113–24. doi: 10.1079/PHN2002394

21. Slimani N, Deharveng G, Unwin I, Southgate DA, Vignat J, Skeie G, et al. The EPIC nutrient database project (ENDB): a first attempt to standardize nutrient databases across the 10 European countries participating in the EPIC study. *Eur J Clin Nutr.* (2007) 61:1037–56. doi: 10.1038/sj.ejcn.1602679

22. Buckland G, Agudo A, Luján L, Jakszyn P, Bueno-de-Mesquita HB, Palli D, et al. Adherence to a Mediterranean diet and risk of gastric adenocarcinoma within the European prospective investigation into cancer and nutrition (EPIC) cohort study. *Am J Clin Nutr.* (2010) 91:381–90. doi: 10.3945/ajcn.2009.28209

23. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a greek population. *N Engl J Med.* (2003) 348:2599–608. doi: 10.1056/NEJMoa025039

24. Sen A, Tsilidis KK, Allen NE, Rinaldi S, Appleby PN, Almquist M, et al. Baseline and lifetime alcohol consumption and risk of differentiated thyroid carcinoma in the EPIC study. *Br J Cancer.* (2015) 113:840–7. doi: 10.1038/bjc.2015.280

25. Romaguera D, Guevara M, Norat T, Langenberg C, Forouhi NG, Sharp S, et al. Mediterranean diet and type 2 diabetes risk in the European prospective investigation into cancer and nutrition (EPIC) study: the InterAct project. *Diabetes Care.* (2011) 34:1913–8. doi: 10.2337/dc11-0891

26. Rinaldi S, Lise M, Clavel-Chapelon F, Boutron-Ruault MC, Guillas G, Overvad K, et al. Body size and risk of differentiated thyroid carcinomas: findings from the EPIC study. *Int J Cancer.* (2012) 131:E1004–14. doi: 10.1002/ijc.27601

27. Zamora-Ros R, Rinaldi S, Biessy C, Tjønneland A, Halkjaer J, Fournier A, et al. Reproductive and menstrual factors and risk of differentiated thyroid carcinoma: the EPIC study. *Int J Cancer.* (2015) 5:1218–27. doi: 10.1002/ijc.29067

28. Zamora-Ros R, Rinaldi S, Tsilidis KK, Weiderpass E, Boutron-Ruault MC, Rostgaard-Hansen AL, et al. Energy and macronutrient intake and risk of differentiated thyroid carcinoma in the European prospective investigation into cancer and nutrition study. *Int J Cancer.* (2016) 138:65–73. doi: 10.1002/ijc.29693

29. Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European prospective investigation into cancer and nutrition (EPIC) study. *Public Health Nutr.* (2003) 6:407–13. doi: 10.1079/PHN2002439

30. Agudo A, Slimani N, Ocké MC, Naska A, Miller AB, Kroke A, et al. Consumption of vegetables, fruit and other plant foods in the European prospective investigation into cancer and nutrition (EPIC) cohorts from 10 European countries. *Public Health Nutr.* (2002) 5:1179–96. doi: 10.1079/PHN2002398

31. Liang J, Zhao N, Zhu C, Ni X, Ko J, Huang H, et al. Dietary patterns and thyroid cancer risk: a population-based case-control study. *Am J Transl Res.* (2020) 12:180–90.

32. Cléro É, Doyon F, Chungue V, Rachédi F, Boissin JL, Sebbag J, et al. Dietary patterns, goitrogenic food, and thyroid cancer: a case-control study in French polynesia. *Nutr Cancer.* (2012) 64:929–36. doi: 10.1080/01635581.2012.713538

33. Markaki I, Linos D, Linos A. The influence of dietary patterns on the development of thyroid cancer. *Eur J Cancer.* (2003) 39:1912–9. doi: 10.1016/s0959-8049(03)00432-5

34. Zupo R, Castellana F, Panza F, Lampignano L, Murro I, Di Noia C, et al. Adherence to a mediterranean diet and thyroid function in obesity: a cross-sectional apulian survey. *Nutrients.* (2020) 12:1–10. doi: 10.3390/nu12103173

35. Sasson M, Kay-Rivest E, Shoukrun R, Florea A, Hier M, Forest VI, et al. The T4/T3 quotient as a risk factor for differentiated thyroid cancer: a case control study. *J Otolaryngol Head Neck Surg.* (2017) 46:28. doi: 10.1186/s40463-017-0208-0

36. Rinaldi S, Plummer M, Biessy C, Tsilidis KK, Østergaard JN, Overvad K, et al. Thyroid-stimulating hormone, thyroglobulin, and thyroid hormones and risk of differentiated thyroid carcinoma: the EPIC study. *J Natl Cancer Inst.* (2014) 106:dju097. doi: 10.1093/jnci/dju097

37. Zamora-Ros R, Béraud V, Franceschi S, Cayssials V, Tsilidis KK, Boutron-Ruault MC, et al. Consumption of fruits, vegetables and fruit juices and differentiated thyroid carcinoma risk in the European prospective investigation into cancer and nutrition (EPIC) study. *Int J Cancer.* (2018) 142:449–59. doi: 10.1002/ijc.30880

38. Liu ZT, Lin AH. Dietary factors and thyroid cancer risk: a meta-analysis of observational studies. *Nutr Cancer.* (2014) 66:1165–78. doi: 10.1080/01635581.2014.951734

39. Zamora-Ros R, Castañeda J, Rinaldi S, Cayssials V, Slimani N, Weiderpass E, et al. Consumption of fish is not associated with risk of differentiated thyroid carcinoma in the European prospective investigation into cancer and nutrition (EPIC) study. *J Nutr.* (2017) 147:1366–73. doi: 10.3945/jn.117.247874

40. Zamora-Ros R, Cayssials V, Franceschi S, Kyrø C, Weiderpass E, Hennings J, et al. Polyphenol intake and differentiated thyroid cancer risk in the European prospective investigation into cancer and nutrition (EPIC) cohort. *Int J Cancer.* (2020) 146:1841–50. doi: 10.1002/ijc.32589

41. Park Y, Leitzmann MF, Subar AF, Hollenbeck A, Schatzkin A. Dairy food, calcium, and risk of cancer in the NIH-AARP diet and health study. *Arch Intern Med.* (2009) 169:391–401. doi: 10.1001/archinternmed.2008.578

42. Hong SH, Myung SK, Kim HS. Alcohol intake and risk of thyroid cancer: a meta-analysis of observational studies. *Cancer Res Treat.* (2017) 49:534–47. doi: 10.4143/crt.2016.161

43. Memon A, Varghese A, Suresh A. Benign thyroid disease and dietary factors in thyroid cancer: a case-control study in Kuwait. *Br J Cancer.* (2002) 86:1745–50. doi: 10.1038/sj.bjc.6600303

44. Daniel CR, Cross AJ, Graubard BI, Hollenbeck AR, Park Y, Sinha R. Prospective investigation of poultry and fish intake in relation to cancer risk. *Cancer Prev Res.* (2011) 4:1903–11. doi: 10.1158/1940-6207.CAPR-11-0241

45. Galanti MR, Hansson L, Bergström R, Wolk A, Hjartåker A, Lund E, et al. Diet and the risk of papillary and follicular thyroid carcinoma: a population-based case-control study in Sweden and Norway. *Cancer Causes Control.* (1997) 8:205–14. doi: 10.1023/a:1018424430711

46. Cross AJ, Pollock JRA, Bingham SA. Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. *Cancer Res.* (2003) 63:2358–60.

47. Aschebrook-Kilfoy B, Shu X-O, Gao Y-T, Ji BT, Yang G, Li HL, et al. Thyroid cancer risk and dietary nitrate and nitrite intake in the Shanghai Women's health study. *Int J Cancer.* (2013) 132:897–904. doi: 10.1002/ijc.27659

48. Cross AJ, Sinha R. Meat-related mutagens/carcinogens in the etiology of colorectal cancer. *Environ Mol Mutagen.* (2004) 44:44–55. doi: 10.1002/em.20030

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Disease specific symptoms indices in patients with celiac disease—A hardly recognised entity

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Background: Celiac disease (CD) was considered a rare disease before and was perceivably only limited to children but now affects almost 1–2% of the global population. This abrupt increase in prevalence is due to advancements in diagnostic criteria and medical facilities but still many countries lack the basic data that can assess the severity of this health issue. The present study was conducted with the aim to assess the common but rarely diagnosed condition with the identification of its underlying secondary ailments.

Materials and methods: Patients visiting public sector hospitals were recruited and tested for clinical symptoms secondary to gluten-containing foods (wheat and barley, etc.), followed by serological testing for immunoglobulin A, tissue transglutaminase A, and anti-endomysial antibodies. Only seropositive candidates were included in the endoscopic and biopsy examination for the features of villous atrophy and intestinal cell damage. The secondary ailments including anemia, growth retardation, and gastrointestinal symptoms were also documented for the tested positive patients. The modified European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) criterion was followed throughout the study.

Results: From 647 suspected cases from March 2018 to July 2019, 113 were confirmed with CD while 58% were female children and 42% were male children. The majority of them were from a lower class (75%) and 26% of them had a positive family history of CD. A total of 67% of patients with CD were underweight while wasting was observed in 38%, and 80% were stunted as well. Of the positively tested patients with CD, 49% had moderate anemia with 15% having severe anemia. Approximately 33% had hypoalbuminemia as well. The majority of them had a mild to severe range of gastrointestinal symptoms, such as abdominal pain, diarrhea, flatus, eructation, diarrhea, and steatorrhea.

Conclusion: The study finding indicates an increased number of patients diagnosed with CD with an excessive sum of secondary ailments, such as anemia, growth failure, growth retardation, malnutrition, and gastrointestinal symptoms.

KEYWORDS

celiac disease, anemia, wasting, hypoalbuminemia, gastrointestinal discomforts

Introduction

Celiac disease (CD) is an autoimmune systemic enteropathy that can be triggered by the ingestion of gluten proteins (mainly from wheat, rye, and barley) with manifestation in the small intestine and extra interstitial organs prevalent in almost all age groups (1, 2). Earlier CD was considered a rare disease that only occurred in children. According to John Walker-Smith and Gee, CD was some kind of chronic indigestion affecting children between the ages of 1–5 with symptoms of foul-smelling feces, pale in color, and bulk in nature. These concepts were altered by the findings of Dicke, reporting gluten as the causative agent for CD, and it can be caused by all cereals, especially wheat flour. Later on, mucosal lesions, i.e., villous atrophy and crypt hyperplasia were identified as diagnostic features in CD (3). The onset of the disease was perceived to be gradual with loss of muscle and fat (cachexia), failure to thrive, and mal-absorption syndrome (4). The advances in diagnostic criteria and true understanding of the nature of disease made the scientists realize and calculate the true prevalence of CD (5). For more than 2 decades, CD has emerged as a major public health concern with an initial prevalence of 1% reported by various European countries (6).

Patients with CD may also present with adjacent underlying complications, i.e., failure to thrive, short stature, delayed puberty, tiredness, loss of weight, muscle mass, and fat mass but 10% of the patients with CD can be obese, and therefore they should not be overlooked. CD may also present with various gastrointestinal complaints, i.e., diarrhea, cramping, bloating, flatulence, nausea, and electrolyte imbalance (7, 8). There are certain non-classical symptoms of CD, such as iron deficiency anemia, increased transaminases, constipation, ataxia, lethargy, osteoporosis, and dyspepsia (9). Currently, the diagnostic criterion of CD is based on the guidelines described in the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN). The criterion includes documentation of history, serology, and histology. Marsh measures are used in the ESPGHAN for histology interpretation and CD confirmation including attenuation of duodenal folds with crypt hyperplasia and excessive aggregation of intraepithelial lymphocytes (10).

The prevalence of CD has been increasing with the passage of time (0.6% from 1991 to 2000 and 0.8% from 2000 to 2016) but still missing data from developing countries, such as Pakistan. A systemic review and meta-analysis conducted to assess the global prevalence of CD suggested various readings including a 1.4% seroprevalence of CD based on 275,818 individuals (95% *CI*; confidence interval, 1.1–1.7%) but Asia was among the highest CD prevalent region (1.8%) from the whole. The seroprevalence was based on the quantification of anti-tissue transglutaminase and anti-endomysial antibodies. Based on 138,792 individuals, it was reduced up to 0.7% (95% *CI*, 0.5–0.9%) based on the biopsy confirmation having clear indications of villous atrophy and crypts hyperplasia. European countries were found to have the highest CD prevalence with 0.8% along with Asia (0.6%). CD prevalence was 0.5% in North America and Africa. The least prevalence of 0.4% was found in South America. The overall proportion of CD was more among female children compared with male children (female children 0.6%, male children 0.4%, $p < 0.01$) with a ratio of 1:3 (male:female) (11) and children were the most affected age group compared with adults (children 0.9%, adults 0.5%, $p < 0.01$) (12). A province (Asia) based study reported 1.6% of seroprevalence of CD and 0.5% based on biopsy parameters (13). The Human

Leukocyte Antigen on alleles DQ2 and DQ8 (heterodimeric; surface receptors) have great significance in the CD diagnosis. The prevalence also varies based on these high-risk populations (1.2–55% CD) and low-risk populations (0.14–5.7%).

Due to the multisystem nature of the disease with a lot of expensive and invasive diagnostic procedures, the majority of cases go undiagnosed, and the chances of having a false positive also increase because of the high level of doubt (9). Among the top 10 most populated countries around the globe, only 4 of them (US, India, Brazil, and Russia) had population-based data on the prevalence of CD. Pakistan, China, Indonesia, Nigeria, Bangladesh, and Japan lack the data, although CD has been reported in all these countries except Nigeria (12). Various studies conducted to assess the prevalence of CD along with existing medical conditions, such as anemia and short stature, are available in the literature. A longitudinal study conducted to assess the CD among the children with the study duration of years reported a 60.03% prevalence. Approximately 26% of them were anemic, 90.7% were underweight, 83.9% had short stature, and 40–50% reported diarrhea, abdominal pain, and distension (14). A cross-sectional study conducted in the public sector hospital in Lahore reported a 12.1% prevalence of CD with 80.3% having iron deficiency anemia (IDA) (15) in another study prevalence was 28.2% with 53.8% having macrocytic anemia and 38.46% having microcytic anemia (16, 17).

All these studies either had a low sample size or were carried out with reference to an existing underlying clinical manifestation. In some of the studies, only seroprevalence was measured and ESPGHAN criteria were not followed. The prevalence of all typical and atypical symptoms was not considered in these studies also. Therefore, the present study was conducted to assess the prevailing gastrointestinal symptoms, signs of growth failure, anemia, and cachexia in patients presenting in a public and private sector hospital in Lahore city-Pakistan.

Materials and methods

The present study was a descriptive cross-sectional survey conducted to identify the patients with CD or person along with prevailing gastrointestinal complaints, signs of growth failure, and malnutrition.

Consent and ethical considerations

The study protocol was approved by the Ethical board for biomedical research, the University of Veterinary and Animal Sciences, Lahore, Pakistan. (No.029/IRC/BMR) Written informed consent was obtained from the parents of children or the legal guardian before enrolling any participant in the study.

Study participants

Patients with celiac disease/gluten sensitivity were recruited according to the modified ESPGHAN criteria (Figure 1) (18). A total of 647 participants suspected of CD were enrolled for further assessment. A gluten-free diet was recommended for the confirmed CD cases immediately.

Nutritional and clinical assessment

Screening of the patients was done through symptoms ranging from classic signs of a mal-absorption syndrome, such as diarrhea (increase in liquidity and frequency than normal for >2 weeks), weight loss (weight for age below 5th percentile), growth failure (linear height below the 5th percentile for age), and anemia (pallor) to non-specific symptoms, such as chronic constipation or abdominal pain.

Anthropometry

Body weight was measured using a weighing scale with standardization done after every 20 readings. Height was measured (without shoes) in the position, back and hips touching the wall by using wall-mounted stedio-meter. The mid-upper arm circumference (MUAC) was measured by MUAC measuring tape from the midpoint between the acromion process of the scapula and the tip of the elbow. The body mass index (BMI) was calculated as weight in kilograms divided by height in meter square (19). The WHO standard growth charts, designed separately for boys and girls were used as a research tool to assess their nutritional status. A patient adherent to a gluten-free diet and its duration without positive serologic test results for CD or diagnosed by the physician were identified by age and sex also.

Serology

Celiac disease was defined as having either double-positive serologic test results on IgA, tissue trans-glutaminase through ELISA, or a reported diagnosis by a healthcare professional coupled with gluten-free diet consumption. On the basis of symptoms, in the case of suspected CD, serum levels of IgA autoantibodies to tissue trans-glutaminase IgA (tTG-IgA) were determined (20).

Endoscopy

Those individuals who were found to be positive for ELISA were contacted for further tests as per

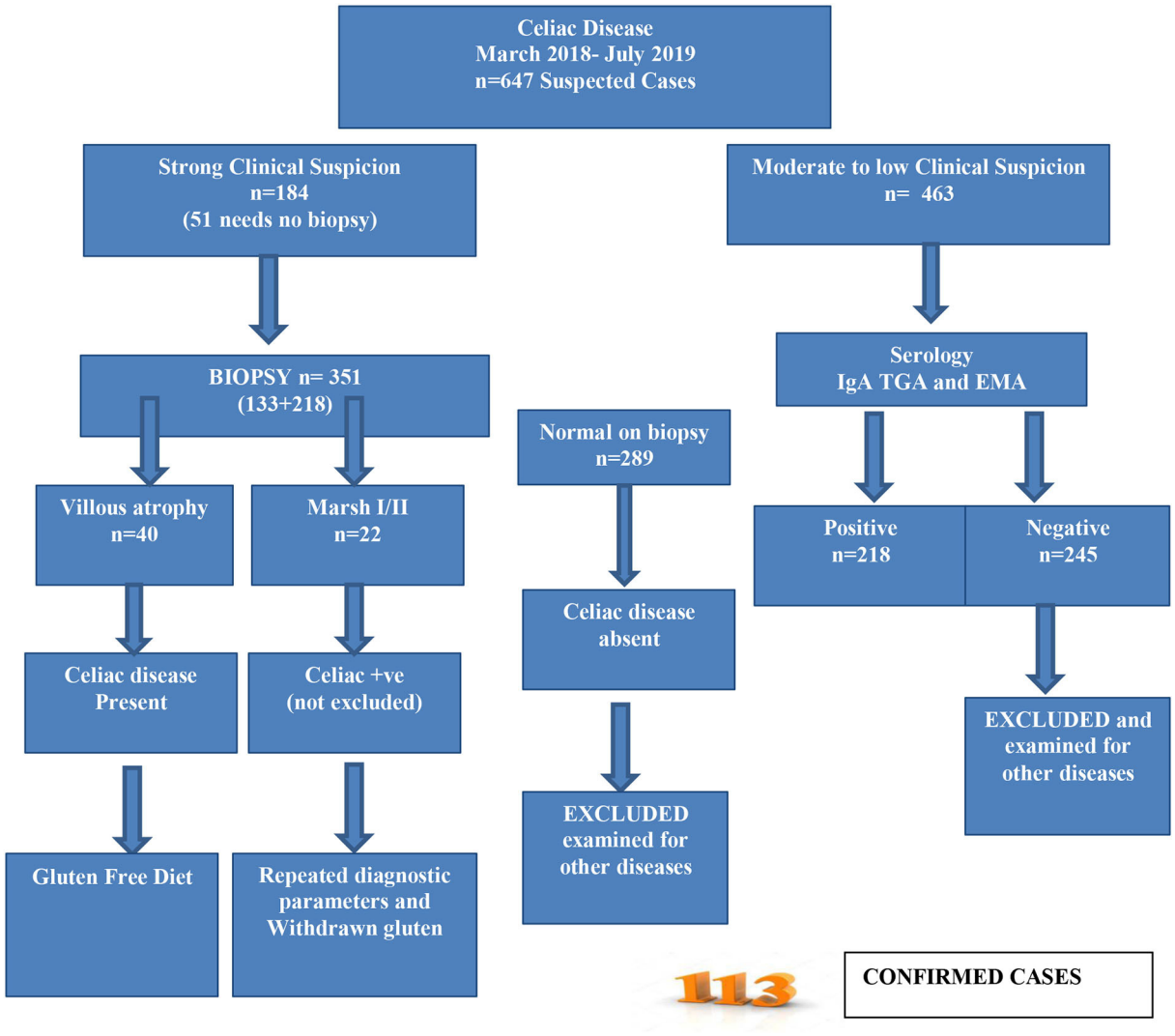

FIGURE 1
Diagnosis and screening of celiac patients according to ESPGHAN criteria.

protocol. A detailed clinical and hematological evaluation, complete blood count (CBC), and biochemical tests of liver enzymes were conducted. Upper gastrointestinal and duodenal endoscopic examinations were done to check villous atrophy by using a video endoscope (21).

Statistical analysis

Data obtained from the study were analyzed using a statistical package for social sciences (SPSS) and expressed as mean ± standard deviation (SD), frequencies, and percentages. A descriptive analysis was performed to obtain the before-mentioned data.

Results

The present study was conducted to find the symptoms of gluten insensitivity/CD in both genders. Data related to demographics, anthropometry, diagnostic tests related to blood, and endoscopy were performed alongside the frequency of gastrointestinal complaints.

From the collected data, 58% were female children and 42% were male children. The majority of the participants were from the age group of 2–5 years and 6–9 years and only 2% were from the age group of >15 years with a mean age of 7.27 years. Almost three-fourths of the study participants had their own houses and 27% were living in rental houses. The majority of the study participant's fathers were either laborers (39%) or had private jobs (34%), and 11% were government servants. Almost half of

TABLE 1 Demographic profile of patients with celiac disease (CD).

Gender	Frequency (*n*)	Percent (%)
Male	47	41.6
Female	66	58.4
Age (Years)		
2-5	49	43.3
6-9	36	31.8
10-15	26	23
>15	2	1.8
Age (Years); Mean ± SD	7.27 ± 3.68	
Residence		
Own house	83	73.4
Rental	30	26.6
Father's occupation		
Laborer	44	39
Farmer	17	15
Govt. job	13	11.5
Private job	39	34.5
Family's income/month (PKR:USD)		
<20,000 PKR/<100USD	46	40.8
20,000–50,000PKR/100–250 USD	48	42.4
>50,000PKR/>250–USD	19	16.8
Socioeconomic Status		
Lower class	85	75.2
Middle class	28	24.8

TABLE 2 Anthropometric measurements of patients with celiac disease.

Weight (Kg); **Mean ± SD**	17.29 ± 9.18
Height (cm); **Mean ± SD**	103.5 ± 23.04
Body mass index (BMI); **Mean ± SD**	15.43 ± 4.35

Weight for age (Percentile)	**Frequency (*n*)**	**Percent (%)**
<1st	43	38.1
3rd	33	29.2
>5th	24	21.2
>15th	12	10.6
>75th	1	0.9
Height for age (Percentile)		
<1st	9	8
3rd	80	70.8
>5th	13	11.5
>15th	11	9.7
Short stature		
Yes	89	78.7
No	24	21.3

the participant's family income was between 20,000 and 50,000 PKR and only 17% had a family income of more than 50,000 PKR in the collected data. All the participants either belonged to the lower or middle socioeconomic class with 75% from the lower socioeconomic class (Table 1).

The majority of the study participants were undernourished, as their weight for age (percentile) was from <1st and 3rd percentiles, indicating under-nutrition, from which 38% were severely undernourished (<1st percentile) and 29% were from 3rd percentile. The same trend was observed in the height for age parameter, with 70% of the participants having <5th percentile in height for age and 8% were severely stunted (<1st percentile). Approximately 79% of the participants were of short stature (Table 2).

Of the total study population, 95% had positive levels of IgG and all of them had positive IgA levels for CD. Almost 15% of the participants had severe anemia and 50% had moderate level anemia while 70% of the participants had lower hematocrit levels. Approximately, 33% of the participants had hypoalbuminemia. Liver enzymes were increased in almost 34–36% of the participants (Table 3).

Almost 46% of the participants had moderate abdominal pain, and 22% had severe abdominal pain. About 36% were having moderate heartburn and 5.5% had severe heartburn. Almost 33% had moderate regurgitation and 4% had severe regurgitation. About 38% were facing moderate nausea and 46% had no nausea. Approximately, 24% of the participants had moderate borborygmus and 63% of them had severe episodes. From the collected data, 67 and 53% of the participants had no Eructation and Flatus, respectively. Hard stool, urgency, and incomplete evacuation were absent in approximately 93–99% of the participants. Approximately 12% had severe episodes of steatorrhea (Table 4).

The majority of the participants were diagnosed with CD for more than 1 year presenting with clinical symptoms (39.4%) and approximately 16% were diagnosed for 1 month while 44% were newly diagnosed patients (Figure 2). Only 26% had a positive family history of CD. From the collected data, 20% of the participants showed Marsh I/II and 35% had Marsh III on endoscopic findings, which are clear indications of CD (Table 5). A total of 45% of participants did not undergo endoscopic examination due to strong clinical suspicion and responsiveness to a gluten-free diet.

Discussion

The present study was conducted with the objective to assess the prevailing symptoms of CD with a special focus on the underlying conditions, such as anemia, malnutrition, and gastrointestinal symptoms. CD, also known as heterogeneous

TABLE 3 Blood chemistry of patients with celiac disease.

Parameters	Frequency (*n*)	Percent (%)	Mean ± S.D
Immunoglobulin A (tTG-IgA)			142 ± 46
Positive	113	100	
Hemoglobin			9.5 ± 1.4
Normal >11 mg/dL	40	35.4	
Moderate anemia 8–10 mg/dL	56	49.5	
Severe anemia <8 mg/dL	17	15.1	
Hematocrit (HCT)			-
Normal >32–37%	34	30.1	
Low <32–37%	79	69.9	
Albumin			2.94±0.99
Normal >3.3–3.8 g/dL	76	67.2	
Low	37	32.8	
Serum glutamic pyruvic transaminase (SGPT)			28.3 ± 6.4
Normal	69	61.1	
Low	4	3.5	
High	40	35.4	
Serum glutamic-oxaloacetic transaminase (SGOT)			35.2 ± 4.9
Normal	72	63.7	
Low	2	1.8	
High	39	34.5	

TABLE 4 Gastrointestinal complaints of patients with celiac disease.

Gastrointestinal Complaints		Mild	Moderate	Severe	Absent
Abdominal pain	Frequency (*n*)	18	50	24	17
	Percent (%)	16.5	45.9	22	15.6
Heartburn	Frequency (*n*)	29	39	6	35
	Percent (%)	26.6	35.8	5.5	32.1
Regurgitation	Frequency (*n*)	26	36	4	43
	Percent (%)	23.9	33	3.7	39.4
Nausea	Frequency (*n*)	8	41	10	49
	Percent (%)	7.3	37.6	9.2	45.9
Borborygmus	Frequency (*n*)	12	26	2	69
	Percent (%)	11	23.9	1.8	63.3
Eructation	Frequency (*n*)	9	24	3	73
	Percent (%)	8.3	22	2.8	67
Flatus	Frequency (*n*)	10	27	14	58
	Percent (%)	9.2	24.8	12.8	53.2
Diarrhea	Frequency (*n*)	7	49	25	28
	Percent (%)	6.4	45	22.9	25.7
Loose Stool	Frequency (*n*)	6	52	22	29
	Percent (%)	5.5	47.7	20.2	26.6
Hard Stool	Frequency (*n*)	1	0	0	108
	Percent (%)	0.9	0	0	99.1
Urgency	Frequency (*n*)	2	5	0	102
	Percent (%)	1.8	4.6	0	93.6
Incomplete Evacuation	Frequency (*n*)	3	6	2	98
	Percent (%)	2.8	5.5	1.8	89.9
Steatorrhea	Frequency (*n*)	7	39	13	50
	Percent (%)	6.4	35.8	11.9	45.9
Fever	Frequency (*n*)	0	73	36	0
	Percent (%)	0	67	33	0
Cough	Frequency (*n*)	0	34	75	0
	Percent (%)	0	31.2	68.8	0

autoimmune disease, Is triggered when gluten is ingested. It was considered rare in early times, but now it is one of the common public health concerns. The global prevalence of gluten allergy is increasing rapidly now. Studies have shown the prevalence is higher in infants and children. Some studies highlighted that it may be due to the early introduction of gluten-related products (22) but some denied this fact. One Norwegian study concludes that gluten must be introduced to infants over 6 months of age (23). In Italy, one study is done with school-going children having CD and the results showed that prevalence in Italy is increased by 1.5% in the last 25 years (24). The present study showed that majorly children, 113 out of 647, affected due to this autoimmune disease belonged to low-income status. Of all of the participants, 113 had 10 times greater tTG-IgA levels (> 7 U/ml). Male children were 41.6% and female children were 58.4% with an M:F ratio of 1:1.4. Similar trend was observed in the study in which out of 350, 126 patients fulfilled the criteria including 54 male children and 71 female children with an M:F ratio of 1:1.3 (25).

This disease is responsible for a broad spectrum of symptoms and problems. Starting from malnutrition to failure to thrive, CD children are prone to many other underlying issues, such as gastrointestinal problems, anemia, liver problems, and many others (7). Most studies done in this context are proof of malnutrition in relation to CD. Mal-absorption contributes the most important role in causing malnutrition and growth problems in patients with CD (26). As a result of malabsorption, a lesser amount of substrate is available due to which the "energy compensation" mechanism in the body is activated. In this mechanism, the body stores fats (in adipose tissues), and proteins (in muscles) start to deplete. This whole process results in severe weight loss and retardation in the growth process. If it continues for 3–4 months, the weight loss will result in stunting (27, 28). All the infants and children are considered short-statured when compared with growth charts (24). Delayed puberty in CD adolescent girls is also of primary concern. Delayed bone aging and amenorrhea accompanied by infertility can be caused if the patient is not treated well-according to the condition (29). In our study, this fact was proved as more than 79% of participants were short stature and 88% were severely

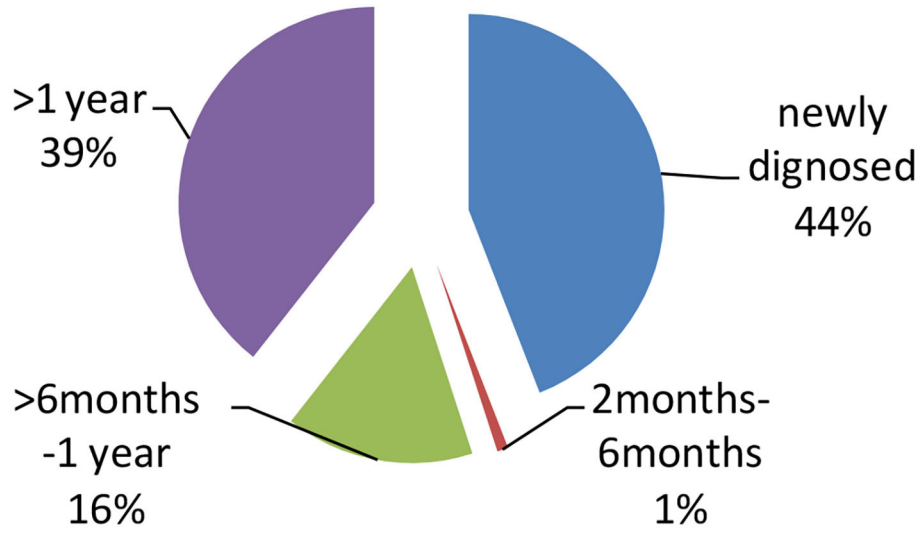

FIGURE 2
Distribution of patients with celiac disease (CD) according to the duration of diagnosis.

TABLE 5 Disease history and endoscopic findings of patients with celiac disease.

Family History	Frequency (*n*)	Percent (%)
Positive	29	25.7
Negative	84	74.3
Endoscopic findings		
Not done	51	45.1
Marsh 1	20	17.7
Marsh 2	2	1.8
Marsh 3	40	35.4

wasted as <5th percentile when growth charts were plotted. The mean BMI of the study participants was 15.43 ± 4.35.

Some common manifestations of the gastro-intestinal tract in celiac patients are abdominal distension accompanied by diarrhea, abdominal pain, anorexia, vomiting, and in some cases ulcers and binge eating (30). Overweight or children with excessive appetite get usually masked due to the presence of some other diseases with CD. The current study reported severe abdominal pain in 22% of cases while nausea was present in 9.2 and 70% of patients with CD were suffering from different degrees of diarrhea (mild, moderate, and severe). When reviewed in the literature, it is noted that abdominal distension is one of the most common symptoms faced by patients with CD (31, 32). Similarly, in (33) study, diarrhea is observed in more than 70% of patients with CD. Comparing facts with our present study, it was clear that such symptoms were also present in our targeted population in clear significant percentages.

The blood chemistry of patients with CD is also altered in a visible manner. Most of the patients with CD (65%) suffer from anemia as the main issue as well. Along with anemia, some other problems, such as major changes in the endocrine system of the patients are also seen (28). A significant inverse relationship between the exposure to gluten and insulin-like growth hormone 1 (IGF-1) has been studied. It is noticed that the secretion of this particular hormone is only affected when the gluten is exposed for a very long time. These changes lead to a decrease in the growth velocity. When the condition is worsened, it is studied that the growth hormone released from the hypothalamus is also affected (28, 34, 35). Due to malabsorption, anemia is very prevalent in patients with CD. Our study showed clear results of anemia in patients with CD. Some were highly anemic and a major population was moderately anemic. Along with lower hemoglobin levels, hematocrit levels were also lesser in patients with CD when the values were compared with normal people. With low hemoglobin levels, albumin levels are also affected in patients with CD. Due

to malabsorption, despite of high protein diet, the patients do not respond to a protein diet hence leading to hypo-albuminemia in patients who are not treated properly. In addition, our study showed hypo-albuminemia in patients with CD.

For screening, some very reliable and sensitive tests are performed. These tests are specific screening tests; IgA anti-tissue Transglutaminase (IgA tTG antibodies) are prescribed for checking and confirming CD (36). These antibodies are generated when gluten is exposed to the small intestine and the auto-immune system gets triggered. The mucosal lining of the intestine is damaged due to immune-mediated response as the result of gluten intolerance (37). Our study showed that all the targeted patients were positive for this test showing the occurrence of CD. IgG immunoglobulin tests were also done and the majority of them showed positive results for these immune globulins. The liver is also affected by CD. Many researchers have found a relationship between CD and autoimmune liver injuries (38). More than 30% of participants in our study showed increased liver enzymes level. For diagnosis, endoscopic findings are considered. About 18–36% of the study participants had moderate to severe villous atrophy upon endoscopic and biopsy examination. The endoscopic examination is considered the gold standard invasive method for the diagnosis of CD (39). Compared with our findings, all 75 of the patients found positive with serological screening had prominent histological and endoscopic changes in the intestines in terms of villous atrophy (40).

Conclusion

Population-based data on CD are missing for various underdeveloped/developing countries including Pakistan. It is not uncommon in our population. The present study found that a large number of celiac children aged between 2 and 9 years presented with anemia, diarrhea, growth failure, mal-digestion, and malabsorption. A total of 28% cases were reported with non-diarrheal CD. Disturbed serological titers and biopsy findings are hallmarks of the disease.

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 Institutional Review Committee For Biomedical Research Uvas, Lahore, Pakistan. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

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

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References

1. Lauret E, Rodrigo L. Celiac disease and autoimmune-associated conditions. *BioMed Res Int.* (2013) 2013:7589. doi: 10.1155/2013/127589

2. Nardecchia S, Auricchio R, Discepolo V, Troncone R. Extra-intestinal manifestations of coeliac disease in children: clinical features and mechanisms. *Front Pediat.* (2019) 7:56. doi: 10.3389/fped.2019.00056

3. Ascher H, Holm K, Kristiansson B, Mäki M. Different features of coeliac disease in two neighbouring countries. *Arch Dis Child.* (1993) 69:375–80. doi: 10.1136/adc.69.3.375

4. Dowd B, Walker-Smith J. Samuel Gee, Aretaeus, and the coeliac affection. *Br Med J.* (1974) 2:45. doi: 10.1136/bmj.2.5909.45

5. Lionetti E, Catassi C. New clues in celiac disease epidemiology, pathogenesis, clinical manifestations, and treatment. *Int Rev Immunol.* (2011) 30:219–31. doi: 10.3109/08830185.2011.602443

6. Johnston S, Watson R, McMillan S, Sloan J, Love A. Coeliac disease detected by screening is not silent–simply unrecognized. *QJM: J Assoc Physic.* (1998) 91:853–60. doi: 10.1093/qjmed/91.12.853

7. Jamma S. Rubio–Tapia A, Kelly CP, Murray J, Najarian R, Sheth S, et al. Celiac crisis is a rare but serious complication of celiac disease in adults. *Clin Gastroenterol Hepatol.* (2010) 8:587–90. doi: 10.1016/j.cgh.2010.04.009

8. Murray JA, Watson T, Clearman B, Mitros F. Effect of a gluten-free diet on gastrointestinal symptoms in celiac disease. *Am J Clin Nutr.* (2004) 79:669–73. doi: 10.1093/ajcn/79.4.669

9. Oxentenko AS, Rubio-Tapia A, editors. *Celiac Disease. Mayo Clinic Proceedings*. Rochester: Elsevier (2019). doi: 10.1016/j.mayocp.2019.02.019

10. Freeman HJ. Adult celiac disease in the elderly. *World Journal of Gastroenterology: WJG.* (2008) 14:6911. doi: 10.3748/wjg.14.6911

11. Tortora R, Zingone F, Rispo A, Bucci C, Capone P, Imperatore N, et al. Coeliac disease in the elderly in a tertiary centre. *Scand J Gastroenterol.* (2016) 51:1179–83. doi: 10.1080/00365521.2016.1186222

12. Singh P, Arora A, Strand TA, Leffler DA, Catassi C, Green PH, et al. Global prevalence of celiac disease: systematic review and meta-analysis. *Clin Gastroenterol Hepatol.* (2018) 16:823–36. doi: 10.1016/j.cgh.2017.06.037

13. Singh P, Arora S, Singh A, Strand TA, Makharia GK. Prevalence of celiac disease in Asia: A systematic review and meta-analysis. *J Gastroenterol Hepatol.* (2016) 31:1095–101. doi: 10.1111/jgh.13270

14. Javed F, Sattar F, Gill AA, Lodhi Y, Iqbal K, Nazir A. Clinical spectrum of celiac disease in children: experience from a public hospital in Pakistan. *Annals of Punjab Medical College (APMC).* (2019) 13:192–6. doi: 10.29054/apmc/2019.145

15. Asghar A, Zafar MH, Munir S. Frequency of celiac disease in patients presenting with nutritional anemia. *Annals Punjab Med College (APMC).* (2018) 12:151–3. doi: 10.29054/apmc/2018.135

16. Qureshi SH, Qureshi SH, Shah FU, Shah SF, Qureshi AH. Frequency of Celiac Disease in anemic patients presenting at Federal Government Poly Clinic Hospital, Islamabad, Pakistan. *Rawal Med J.* (2020) 45:51–3.

17. Talpur MTH, Rehman S, Haider S, Sheikh QH, Jafri SM, Maheshwary N. Prevalence of celiac disease among patients presenting with iron deficiency anemia. *Med Channel.* (2017) 23:45.

18. Husby S, Koletzko S, Korponay-Szabó I, Mearin M, Phillips A, Shamir R, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr.* (2012) 54:136–60. doi: 10.1097/MPG.0b013e31821a23d0

19. Brambilla P, Picca M, Dilillo D, Meneghin F, Cravidi C, Tischer M, et al. Changes of body mass index in celiac children on a gluten-free diet. *Nutrition Metabol Cardiovasc Dis.* (2013) 23:177–82. doi: 10.1016/j.numecd.2011.10.002

20. Smarrazzo A, Misak Z, Costa S, Mičetić-Turk D, Abu-Zekry M, Kansu A, et al. Diagnosis of celiac disease and applicability of ESPGHAN guidelines in Mediterranean countries: a real life prospective study. *BMC Gastroenterol.* (2017) 17:1–8. doi: 10.1186/s12876-017-0577-x

21. Makharia GK, Verma AK, Amarchand R, Bhatnagar S, Das P, Goswami A, et al. Prevalence of celiac disease in the northern part of India: a community based study. *J Gastroenterol Hepatol.* (2011) 26:894–900. doi: 10.1111/j.1440-1746.2010.06606.x

22. Welander A, Tjernberg AR, Montgomery SM, Ludvigsson J, Ludvigsson JF. Infectious disease and risk of later celiac disease in childhood. *Pediatrics.* (2010) 125:e530–e6. doi: 10.1542/peds.2009-1200

23. Størdal K, White RA, Eggesbø M. Early feeding and risk of celiac disease in a prospective birth cohort. *Pediatrics.* (2013) 132:e1202–9. doi: 10.1542/peds.2013-1752

24. Gatti S, Lionetti E, Balanzoni L, Verma AK, Galeazzi T, Gesuita R, et al. Increased prevalence of celiac disease in school-age children in Italy. *Clin Gastroenterol Hepatol.* (2020) 18:596–603. doi: 10.1016/j.cgh.2019.06.013

25. Waheed N, Cheema HA, Suleman H, Fayyaz Z, Mushtaq I, Hashmi MA. Celiac crisis: a rare or rarely recognized disease. *J Ayub Medical Coll Abbottabad.* (2016) 28:672–5.

26. Catassi C, Fasano A. Celiac disease as a cause of growth retardation in childhood. *Curr Opin Pediatr.* (2004) 16:445–9. doi: 10.1097/01.mop.0000133637.64414.20

27. Boersma B, Houwen R, Blum W, Van Doorn J, Wit J. Catch-up growth and endocrine changes in childhood celiac disease. *Hormone Res Paediat.* (2002) 58(Suppl. 1):57–65. doi: 10.1159/000064771

28. Cornean RE, Gheban D, Simionescu B, Margescu M. Celiac disease among adolescents. Poor growth and delayed puberty. *Int J Celiac Dis.* (2018) 2:52–7. doi: 10.12691/ijcd-6-2-1

29. Bona G, Marinello D, Oderda G. Mechanisms of abnormal puberty in coeliac disease. *Hormone Res Pædiat.* (2002) 57(Suppl. 2):63–5. doi: 10.1159/000058103

30. Beniwal N, Ameta G, Chahar CK. Celiac disease in children with severe acute malnutrition (SAM): a hospital based study. *Indian J Pediat.* (2017) 84:339–43. doi: 10.1007/s12098-017-2300-x

31. Mohindra S, Yachha S, Srivastava A, Krishnani N, Aggarwal R, Ghoshal U, et al. Coeliac disease in Indian children: assessment of clinical, nutritional and pathologic characteristics. *J Health Popul Nutri.* (2001) 204:8. Available online at: https://www.jstor.org/stable/23498861

32. Kumar P, Mishra K, Singh P, Rai K. Should we screen children with severe acute malnutrition for celiac disease. *Indian Pediatr.* (2012) 49:330–1.

33. Thapa B. Celiac disease: Indian experience. In: *Nutrition in Children in Developing Countries*. New Delhi: B.I Publications Pvt Ltd (2004). p. 355.

34. Argente J, Pozo J, Hernández M. The growth hormone axis in malnutrition. *An Esp Pediatr.* (1993) 39:173–80.

35. Maes M, Underwood L. Growth failure in chronic disease: pathophysiology and treatment. *Int Semin Paediatr Gastroent Nutr.* (1997) 6:3–7.

36. Fasano A, Catassi C. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. *Gastroenterology.* (2001) 120:636–51. doi: 10.1053/gast.2001.22123

37. Newton KP, Singer SA. Celiac disease in children and adolescents: Special considerations. In: *Seminars in Immunopathology*. Berlin; Heidelberg: Springer (2012). doi: 10.1007/s00281-012-0313-0

38. Anania C, De Luca E, De Castro G, Chiesa C, Pacifico L. Liver involvement in pediatric celiac disease. *World J Gastroenterol: WJG.* (2015) 21:5813. doi: 10.3748/wjg.v21.i19.5813

39. Buchanan R, Dennis S, Gendel S, Acheson D, Assimon SA, Beru N, et al. Approaches to establish thresholds for major food allergens and for gluten in food. *J Food Prot.* (2008) 71:1043–88. doi: 10.4315/0362-028X-71.5.1043

40. Paul SP, Sandhu BK, Spray CH, Basude D, Ramani P. Evidence supporting serology-based pathway for diagnosing celiac disease in asymptomatic children from high-risk groups. *J Pediatr Gastroenterol Nutr.* (2018) 66:641–4. doi: 10.1097/MPG.0000000000001757

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Triglyceride glucose index and its combination with the Get with the Guidelines-Heart Failure score in predicting the prognosis in patients with heart failure

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Background: Heart failure (HF) is associated with generalized insulin resistance (IR). Recent studies demonstrated that triglyceride glucose (TyG) is an effective alternative index of IR. However, the relationship between the TyG index and in-hospital mortality in patients with HF is unclear. In the present study, we aimed to clarify the association between the TyG index and in-hospital mortality in patients with HF.

Methods: A retrospective study consisting of 4,411 patients diagnosed with HF from 2015 to 2018 was conducted. All-cause mortality during hospitalization was the primary endpoint. The association between the TyG index and in-hospital mortality was assessed using the logistic regression analysis.

Results: The risk of in-hospital mortality was significantly associated with increased TyG index (OR: 1.886, 95% CI: 1.421–2.501, $p < 0.001$) under logistic regression with multivariable adjustment. When divided into three groups based on the TyG index, Tertile 3 demonstrated significantly higher in-hospital mortality than the other two Tertiles (OR: 2.076, 95% CI: 1.284–3.354, $p = 0.001$). Moreover, the TyG index improved the prediction efficiency of the Get with the Guidelines-Heart Failure (GWTG-HF) score (absolute integrated discrimination improvement = 0.006, $p < 0.001$; category-free net reclassification improvement = 0.075, $p = 0.005$). In subgroup analysis, the TyG index exhibited similar predictive

performance of in-hospital mortality when groups were stratified based on type 2 diabetes mellitus (T2DM) and coronary artery disease (CAD).

Conclusion: TyG is a potential index for predicting in-hospital mortality in patients with HF, independent of T2DM or CAD status. The TyG index may be combined with the GWTG-HF score to further improve its predictive efficacy.

KEYWORDS

heart failure, triglyceride glucose index, type 2 diabetes, prognosis, GWTG-HF risk score

Introduction

Heart failure (HF) is the end stage of many cardiovascular diseases, with high morbidity and mortality (1, 2). Due to the continuous development of oral drugs, the poor prognosis of HF has improved to some extent in recent years. However, poor prognosis remains the main cause of mortality in cardiovascular disease (3). Therefore, early risk stratification and accurate identification of high-risk patients with HF are essential for their effective management and treatment.

Insulin resistance (IR) is common in cardiovascular disease and has been established as a predictor of outcomes independent of type 2 diabetes mellitus (T2DM) status (4–7). As research has progressed, IR has been found to play a crucial role in the development of HF because of associated reductions in cardiac insulin metabolic signaling (8–10). To date, the closest approach to the gold diagnostic standard in IR is hyperinsulinemic–euglycemic clamp (11). However, the complexity of the test process limits its clinical applications. Based on the features of IR, hyperglycemia, and dyslipidemia, the triglyceride glucose (TyG) index was developed. TyG is calculated using fasting plasma glucose (FPG) and triglycerides, which are easily obtained by blood tests (12, 13). Numerous clinical studies indicated that TyG is significantly associated with IR obtained by the hyperinsulinemic–euglycemic clamp and even shows a better performance than the homeostasis model assessment of IR (HOMA-IR) in patients with and without diabetes (12, 13).

Previous studies primarily focused on the correlation between the TyG index and coronary artery disease (CAD; 6, 7). However, no study analyzed the predictive value of TyG for adverse events in HF patients with or without T2DM. Therefore, in this study, we aimed to explore the relationship between TyG levels and in-hospital mortality in patients with HF. Furthermore, we evaluated whether TyG could enhance the prediction efficiency of the Get with the Guidelines-Heart Failure (GWTG-HF) score.

Materials and methods

Study population and design

The current study population was based on a retrospective observational cohort study, which enrolled 5,126 consecutive patients with HF at the Shengjing Hospital of China Medical University (from January 2015 to December 2018). The diagnosis of HF was confirmed by symptoms (such as breathlessness, ankle swelling, and fatigue), signs (such as elevated jugular venous pressure, pulmonary crackles, and peripheral edema), echocardiography, and the results of laboratory tests as recommended by the modified Framingham criteria (14), including *de novo* HF or decompensation of chronic HF. HF with reduced ejection fraction was defined as left ventricular ejection fraction (LVEF) $\leq$ 40%, HF with mildly reduced ejection fraction as 41% $\leq$ LVEF $\leq$ 49%, and HF with preserved ejection fraction (HFpEF) as LVEF $\geq$ 50% (15). Patient data were recorded in a database set up specifically for this study. The TyG index was calculated according to the formula:

$$TyG = In\left[fasting\ triglycerides\ \left(\frac{mg}{dL}\right) \times fasting\ plasma\ glucose\ \left(\frac{mg}{dL}\right) \div 2\right] (12, 13)$$

The GWTG-HF risk score was calculated based on seven previously reported indicators: systolic blood pressure, heart rate, age, sodium, blood urea nitrogen (BUN), history of chronic obstructive pulmonary disease, and race (16). Within 24 h of admission, fasting venous blood samples were collected from all the patients. FPG and triglyceride levels were measured by an enhanced immunonephelometric assay using an automated analyzer (AU5800; Beckman Coulter, Inc., Carlsbad, CA, United States) in the Shengjing Hospital core laboratory. All-cause in-hospital mortality was the primary endpoint.

The exclusion criteria were as follows: no availability of fasting triglyceride or FPG data, cardiogenic shock, chronic kidney failure with dialysis on admission and/or diagnosed liver disease, and hormone therapy before admission. The present study included 4411 patients with HF.

Patient selection is shown in the flow diagram in Figure 1. The participants were divided into two groups according to their in-hospital mortality. This study complied with the Helsinki Declaration and was approved by the Research Ethics Committee of Shengjing Hospital of China Medical University (IRB number: 2019PS594K).

Statistical analysis

All patients were divided into two groups according to in-hospital mortality. Continuous variables are expressed as mean ± standard deviation, or median and interquartile range, depending on whether or not they were normally

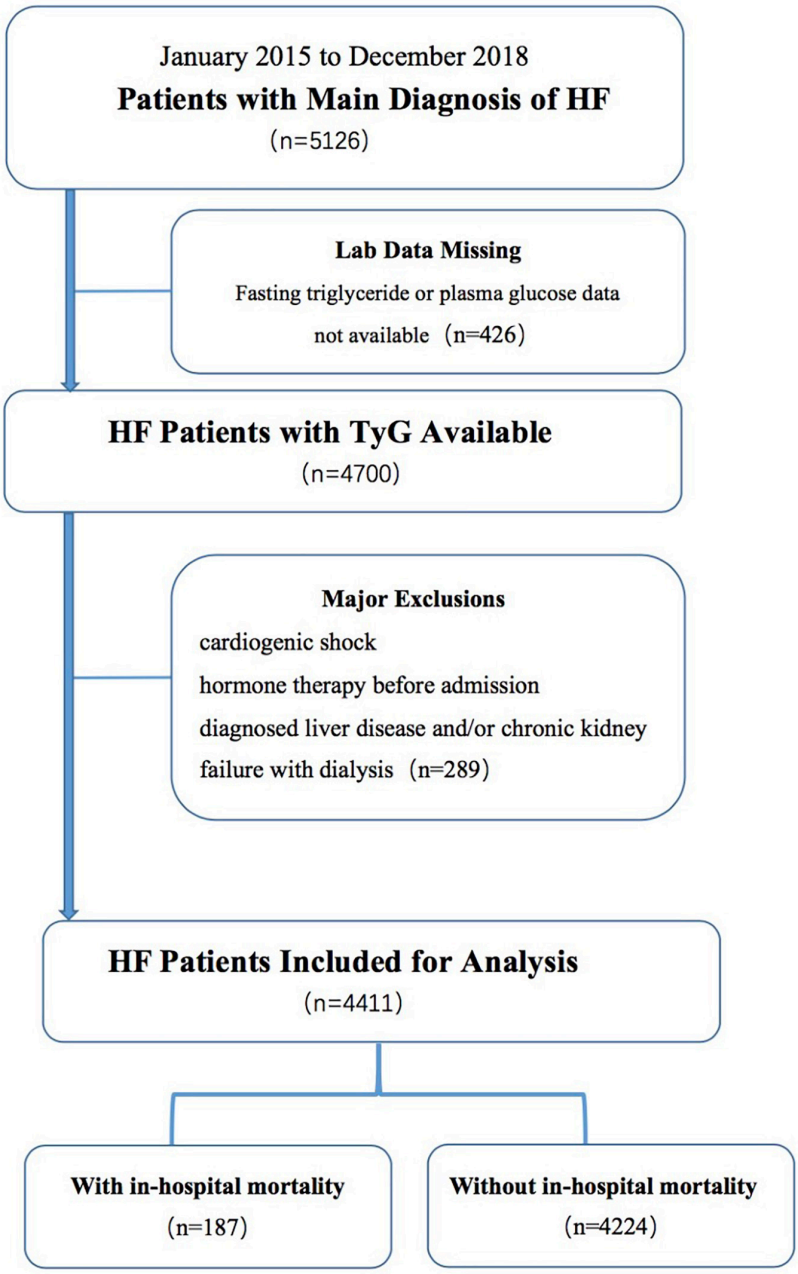

FIGURE 1
Flowchart of participant selection.

distributed. The differences in continuous variables between groups were analyzed using Student's *t*-test or the Kruskal–Wallis *H* test. Categorical variables are expressed as numbers and percentages (%). The differences in categorical variables between the groups were analyzed using the chi-square test or Fisher's exact test. Predictors associated with the primary endpoint were analyzed using univariate and multivariate logistic regression analyses. TyG levels were considered to be a continuous variable. To further explore the association between TyG and in-hospital mortality, TyG was also analyzed as a categorical variable, with Tertile 1 as the reference group. The relationship between the TyG index and primary endpoint was given as an odds ratio (OR) with associated 95% confidence intervals (CIs). The predictive potential of the TyG index and TyG + GWTG-HF score was analyzed. In brief, the area under the curve associated with the primary endpoint through the receiver operating characteristic curve was calculated by the statistical software MedCalc (version 18.1.1; MedCalc Software, Ostend, Belgium; 17). Absolute integrated discrimination improvement (IDI) and category-free net reclassification improvement (NRI) were used to evaluate the improvement in predictive efficiency of the TyG + GWTG-HF score compared to the GWTG-HF score (18). The interaction was tested with a likelihood ratio test, and the ORs with associated 95% CIs were represented by a forest plot. Statistical analyses were performed using SAS (version 9.4; Statistical Analysis Software Institute, Inc., Cary, NC, United States), and statistical significance was set at a p-value < 0.05.

Results

General characteristics

A total of 4,411 patients with HF (mean age: 70.6 ± 12.6) were enrolled in our study, and 48.4% of patients were men. The mean length of hospitalization was 10.0 ± 6.2 days, and 187 (4.2%) patients died during hospitalization. The proportions of patients with HF with reduced ejection fraction, HF with mildly reduced ejection fraction, and HFpEF were 18.9, 40.1, and 41%, respectively. The baseline characteristics of the patients are summarized in **Table 1**. Patients with in-hospital mortality were likely to have a poor New York Heart Association (NYHA) grade, older age, high systolic blood pressure on admission, higher TyG index, higher GWTG-HF score, and a history of CAD. The patients who met in-hospital mortality also had higher creatinine, BUN, uric acid, N-terminal brain natriuretic peptide and cardiac troponin I levels, and lower hemoglobin, serum sodium, and albumin levels on admission than surviving patients. In terms of medications, the in-hospital mortality group had lower treatment rates with angiotensin-converting enzyme inhibitors (ACEI)/angiotensin receptor blockers (ARB)/angiotensin receptor-neprilysin inhibitor (ARNI), diuretics, and aldosterone antagonists (**Table 1**).

Ability of triglyceride glucose index to predict prognosis

Indicators of the primary endpoint identified by univariate analysis were also analyzed using multivariate analysis. **Supplementary File 1** shows the univariate logistic analysis and the indicators related to in-hospital mortality: TyG index, age, sex, NYHA grade, systolic blood pressure, albumin, total bilirubin, BUN, creatinine, uric acid, hemoglobin, serum sodium, N-terminal brain natriuretic peptide, cardiac troponin I, LVEF, history of CAD, atrial fibrillation, smoking, and the use of ACEI/ARB/ARNI, beta-blockers, diuretics, or aldosterone antagonists.

Moreover, the logistic univariate analysis demonstrated that TyG, as a continuous variable, was associated with in-hospital mortality (OR: 1.266, 95% CI: 1.031–1.551, $p = 0.024$). Multiple adjustments for confounders did not attenuate this prediction (OR: 1.886, 95% CI: 1.421–2.501, $p < 0.001$) (**Table 2**). When patients were divided into three groups based on their TyG index, TyG remained a strong predictor of the primary endpoint. Using Tertile 1 (TyG index < 8.25) as a reference, multivariate analysis revealed that the TyG index in Tertiles 2 ($8.25 \leq$ TyG index < 8.78) and 3 (TyG index ≥ 8.78) increased the OR for in-hospital mortality in patients with HF (Tertile 2: OR 1.536, 95% CI 1.002–2.354, $p = 0.049$; Tertile 3: OR 2.076, 95% CI 1.284–3.354, $p = 0.003$) (**Table 2**) (The details of the multivariate analysis are shown in **Supplementary Files 2, 3**).

Prognostic accuracy of triglyceride glucose and comparison of different parameters

Combining the TyG index and GWTG-HF score enhanced the predictive efficiency of the new model compared to that with the GWTG-HF score only (IDI = 0.006, $p < 0.001$; NRI = 0.075, $p = 0.005$) (**Table 3**).

Relevant clinical indicators for in-hospital mortality such as age (< 65 vs. ≥ 65 years), ejection fraction ($< 50\%$ vs. $\geq 50\%$), sex (men vs. women), CAD (yes vs. no), and T2DM (yes vs. no) were evaluated using *post hoc* subgroup analysis. In subgroup analysis, we observed that a higher TyG index was significantly associated with an increased risk of in-hospital mortality after stratification by age, sex, CAD, and T2DM. However, the TyG index was less effective for predicting the endpoint in patients with HFpEF (**Figure 2**).

TABLE 1 Characteristics of subjects divided by in-hospital mortality, median (IQR), or N (%), or means ± SD.

Variable	Overall (n = 4,411)	Patients with in-hospital mortality (n = 187)	Patients without in-hospital mortality (n = 4,224)	P-value
Age (years)	70.6 ± 12.6	77.1 ± 10.4	70.3 ± 12.6	<0.001
Men [n (%)]	2,927 (48.4)	83 (44.4)	2,205 (52.2)	0.036
NYHA grading [n (%)]				<0.001
II	897 (20.3)	6 (3.2)	891 (21.1)	
III	1,712 (38.8)	40 (21.4)	1,672 (39.6)	
IV	1,802 (40.9)	141 (75.4)	1,661 (39.3)	
Heart rate on admission, bpm	88.2 ± 22.4	89.1 ± 21.1	88.1 ± 22.5	0.552
SBP on admission, mmHg	137.3 ± 23.4	132.5 ± 27.2	137.5 ± 23.2	0.004
GWTG-HF score	57.2 ± 8.8	63.3 ± 7.5	57.9 ± 8.7	<0.001
TyG index	8.58 ± 0.68	8.69 ± 0.74	8.57 ± 0.68	0.024
Triglyceride, mg/dl	111.4 ± 84.2	109.1 ± 75.6	111.6 ± 84.5	0.701
FPG, mg/dl	118.2 ± 44.0	138.3 ± 66.8	117.3 ± 44.7	<0.001
Albumin, g/L	37.1 ± 4.3	34.3 ± 4.6	37.2 ± 4.3	<0.001
TBIL, μmol/L	16.2 ± 11.2	17.4 ± 13.3	16.2 ± 11.1	0.145
LDL, mmol/L	2.58 ± 0.97	2.53 ± 1.06	2.58 ± 0.96	0.438
BUN, mmol/L	8.7 ± 5.3	15.5 ± 9.7	8.5 ± 4.8	<0.001
Creatinine, mg/dl	1.15 ± 0.87	1.84 ± 1.29	1.12 ± 0.83	<0.001
eGFR, ml/min/1.73 m^2	74.6 ± 30.1	49.4 ± 29.3	75.7 ± 29.7	<0.001
Uric Acid, μmol/L	444.1 ± 144.5	539.0 ± 216.7	436.8 ± 138.9	<0.001
Hemoglobin, g/L	126.6 ± 23.0	112.4 ± 28.5	127.2 ± 22.5	<0.001
HbA1c, %	6.47 ± 1.36	6.61 ± 1.47	6.46 ± 1.35	0.156
Serum sodium, mmol/L	139.1 ± 3.9	137.4 ± 5.9	139.2 ± 3.8	<0.001
cTNI, ng/ml	0.04 (0.01, 0.21)	0.19 (0.05, 2.6)	0.04 (0.01, 0.19)	<0.001
NT-proBNP, pg/ml	5,036 (1,592, 5,328)	5,327 (4,978, 11,833)	4,718 (1,494, 5,329)	<0.001
LVEF, %	49.1 ± 10.4	47.9 ± 8.2	49.2 ± 10.5	0.105
Comorbidities, *n (%)*				
CAD	3,133 (71.0)	147 (78.6)	2,986 (70.7)	0.020
Hypertension	2,778 (63.0)	109 (58.3)	2,669 (63.2)	0.175
AF	1,375 (31.2)	43 (23.0)	1,332 (31.5)	0.014
T2DM	1,414 (32.1)	70 (37.4)	1,344 (31.8)	0.107
COPD	1,051 (23.8)	37 (19.8)	1,014 (24.0)	0.185
Smoking, *n (%)*	1,222 (27.7)	35 (18.7)	1,187 (28.1)	0.005
Medications, *n (%)*				
ACE-I/ARB/ARNI	3,581 (81.2)	113 (60.4)	3,468 (82.1)	<0.001
Beta blockers	3,592 (81.4)	159 (85.0)	3,433 (81.3)	0.196
Diuretic	3,724 (84.4)	141 (75.4)	3,583 (84.8)	0.001
Aldosterone antagonists	1,284 (29.1)	41 (21.9)	1,243 (29.4)	0.027

ACEI, angiotensin-converting enzyme inhibitors; AF, atrial fibrillation; ARB, angiotensin II receptor blockers; ARNI, angiotensin receptor blocker-neprilysin inhibitors; BUN, blood urea nitrogen; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; cTNI, cardiac troponin I; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GWTG-HF, Get With the Guidelines-Heart Failure; HbA1c, glycated hemoglobin; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal brain natriuretic peptide; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; TBIL, total bilirubin; TyG, triglyceride glucose.

Discussion

The TyG level was found to be a potential independent index for predicting in-hospital mortality. Furthermore, when combined with the GWTG-HF score, the TyG index produced better prognostic efficiency than the GWTG-HF score alone. Additionally, a similar predictive performance was found when patients were stratified by CAD and T2DM, which indicates that the TyG index plays an important role in prediction, independent of T2DM or CAD status. Therefore, our study reveals a relationship between TyG and HF in the general population for the first time.

Insulin resistance is classically defined as the failure of insulin to promote its metabolic activity in organs and tissues, such as the liver, the skeletal muscle, and the adipose tissue (8, 9). In 2005, Ingelsson et al. reported that IR could predict HF incidence even when adjusted for established risk factors for HF (19). In their study, insulin sensitivity was measured using the euglycemic insulin clamp (20). Despite the improvements in accuracy from euglycemic insulin and hyperinsulinemic–euglycemic clamps, these complex procedures remain difficult to use in the clinic. In the ARIC study, Vardeny et al. utilized HOMA-IR, which was calculated using FPG and fasting insulin, to explore the relationship between IR and HF. They observed that lower levels of HOMA-IR (1.0–2.0) than previously considered were associated with an increased risk of HF (21). Other than FPG and insulin, the symptoms of IR may manifest as dyslipidemia due to its influence on the

TABLE 2 Effects of multiple variables on clinical outcomes in univariate and multivariate analyses.

	Univariate analysis			*Multivariate analysis*		
	OR	***95% CI***	***P***	***OR***	***95% CI***	***P***
GWTG-HF, per 1 score increase	1.099	1.078–1.121	<0.001	–	–	–
TyG as a continuous variable						
TyG, per 1 score increase	1.266	1.031–1.554	0.024	1.886	1.421–2.501	<0.001*
TyG as a categories variable						
Tertile 1	Reference			Reference		
Tertile 2	1.054	0.726–1.531	0.781	1.536	1.002–2.354	0.049*
Tertile 3	1.301	0.911–1.860	0.148	2.076	1.284–3.354	0.003*

*Adjusted for age, sex, NYHA grading, heart rate on admission, SBP on admission, albumin, TBIL, LDL, BUN, creatinine, uric acid, hemoglobin, serum sodium, cTNI, NT-proBNP, LVEF, and the history of CAD, hypertension, AF, DM, COPD, smoking, ACEI/ARB/ARNI, beta-blockers, diuretic, aldosterone antagonists.

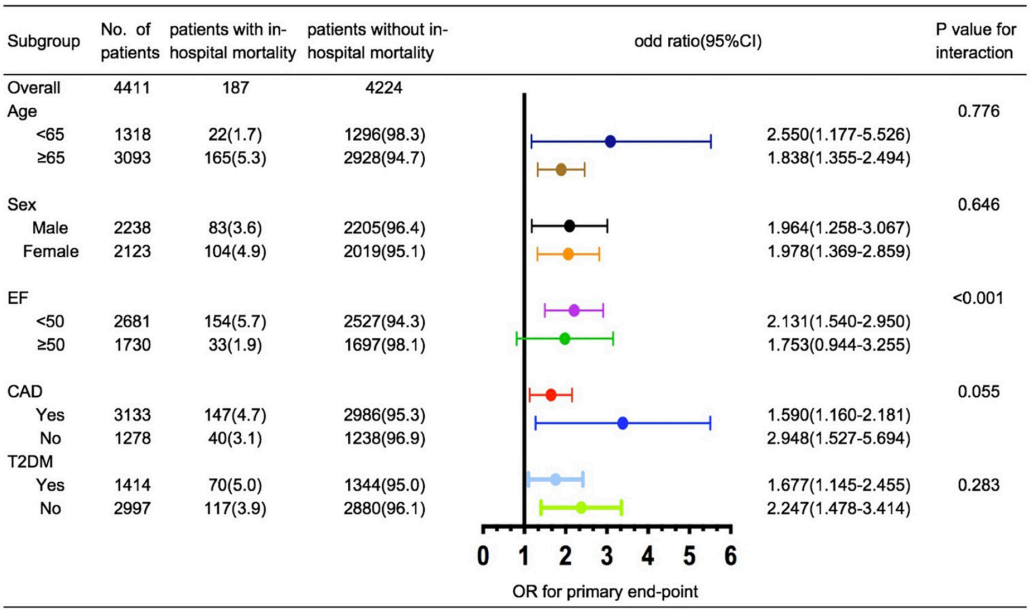

FIGURE 2
Post hoc subgroup analysis of TyG index for the primary endpoint.

TABLE 3 Comparisons of the predictive performance of GWTG-HF and TYG+GWTG-HF for the prognosis prediction.

	AUC (95%CI)	z for C-statistic	P for C-statistic	NRI	P for NRI	IDI	P for IDI
GWTG-HF score	0.712 (0.698–0.725)	–	–	–	–	–	–
TyG+GWTG-HF vs. GWTG-HF	0.720 (0.706–0.733)	1.195	0.232	0.075	0.005	0.006	<0.001

AUC, area under the curve; GWTG-HF, Get with the Guidelines-Heart Failure score; TyG, triglyceride glucose.

liver (5, 22). Based on glucotoxicity and lipotoxicity, TyG was developed as a potential index to represent IR. In previous studies, TyG has shown a good correlation with IR, better than HOMA-IR (12, 13). Wenqin et al. studied 546 HF patients with T2DM and demonstrated a positive correlation between the TyG index and prognosis (23). However, the predictive effect of the TyG index in patients with HF who are non-diabetic remains unclear. To the best of our knowledge, our study is the first to explore the prognostic value of the TyG index in patients with HF, independent of T2DM status. We observed that TyG is a potential independent index for predicting in-hospital mortality in patients with HF. Moreover, the TyG index showed good performance in patients who are non-diabetic with HF (interaction p = 0.283, Figure 2). This observation suggests that IR affects the prognosis of patients with HF independent of diabetes. To further explore the clinical

conditions of patients, we performed a subgroup analysis. A similar predictive performance was found for the TyG index under CAD stratification (interaction $p = 0.055$, **Figure 2**). This result was supported by the ARIC study, indicating that the relationship between IR and HF is not mediated by an increased risk of CAD (21). Notably, the results of our subgroup analysis of LVEF differ from those of previous studies (24, 25). In a prospective study of HF patients with LVEF > 40%, Tamariz et al. reported that patients with metabolic syndrome had a worse prognosis at 2.6 years of follow-up than those without metabolic syndrome (26). In contrast, Zhou et al. studied 1,548 patients with HFpEF and found no differences in cardiovascular and all-cause mortality between groups with and without metabolic syndrome. This is consistent with the results of our subgroup analyses. The predictive efficiency of the TyG index was impaired in patients with HFpEF (**Figure 2**). This result may be related to the heterogeneous nature of HFpEF (27). Age, female sex, diabetes, hypertension, obesity, metabolic syndrome, and atrial fibrillation have been identified as classical risk factors for the development of HFpEF (28). Thus, interactions between these comorbidities may make it challenging to evaluate specific indicators of in-hospital mortality. In addition, the 95% CI for the TyG index in HFpEF was wide and included a potentially important effect size; therefore, further understanding of the pathophysiology of HFpEF and long-term follow-up studies may be required to assess the TyG index for patients with HFpEF.

In 2010, Peterson et al. set up the GWTG-HF score to predict adverse events in hospitals, and the points-scoring system has been widely utilized since the publishing of studies by Peterson et al., Suzuki et al., and Shiraishi et al. (16, 29, 30). We further explored whether TyG can enhance the predictive efficiency of the GWTG-HF score. Because the receiver operating characteristic analysis could not provide pertinent information about whether a combination of GWTG-HF score with a new index would enhance the accuracy of the model for predicting the risk of in-hospital mortality, we also conducted IDI and NRI to measure the incremental value (31, 32). In our study, both IDI and NRI demonstrated a significant improvement in the reclassification of in-hospital mortality by TyG + GWTG-HF vs. GWTG-HF alone (IDI = 0.006, $p < 0.001$; NRI = 0.075, $p = 0.005$) (**Table 3**).

Although the pathophysiology of IR in HF requires further research, the effect of the TyG index on patient prognosis may be related to known mechanisms. In conditions of IR, the myocardium utilizes free fatty acids and decreases glucose uptake and oxidation (33). Because glucose is a more efficient substrate than fatty acids, converting cardiac metabolism from glucose metabolism to fatty acid oxidation reduces cardiac efficiency. This metabolic disorder increases the predisposition to stress overload and ischemia. In addition to glucotoxicity and lipotoxicity, the dysregulation of neurohumoral factors, cytokines, and oxidative stress is the main cause of cardiac IR and impaired cardiac function (34). Furthermore, IR can lead to hyperinsulinemia, which is recognized as a factor that accelerates cardiac remodeling (35, 36). Hyperinsulinemia can also lead to the retention of sodium and the activation of the sympathetic nervous system, which contributes to HF (37, 38). Consequently, IR influences glucose and lipid metabolism, leading to a mismatch in energy needs, affecting neurohumoral factors, and causing HF progression.

The TyG index is simple to calculate and easy to obtain at the bedside. Therefore, it improves upon other IR prediction indicators and must be popularized. Monitoring the TyG index can better identify patients with a high risk of in-hospital mortality from HF, independent of T2DM and CAD status. Additionally, a new model consisting of the TyG index and GWTG-HF score can further enhance the predictive efficacy of GWTG-HF, potentially benefiting clinical practice.

The present study has some limitations. First, this was a retrospective study, and the causal relationship of the association between the TyG index and in-hospital mortality requires further confirmation through prospective studies. Second, because the TyG index was assessed only at admission without dynamic monitoring, we could not assess whether lowering the TyG index might improve patient prognosis. Finally, although we included as many clinically relevant variables as possible in the multivariate analysis, potential confounders likely remained.

In summary, TyG was a potential independent index for predicting in-hospital mortality in patients with HF, independent of T2DM or CAD status. The TyG index can further improve the predictive efficacy of the GWTG-HF score.

Data availability statement

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

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

All authors were involved in the conception and design of the study, as well as in the collection, analysis and interpretation of data, reviewed, and approved the final manuscript.

Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.950338/full#supplementary-material

References

1. Chandra A, Vaduganathan M, Lewis E, Claggett BL, Rizkala AR, Wang W, et al. Health-related quality of life in heart failure with preserved ejection fraction: The PARAGON-HF trial. *JACC Heart Fail.* (2019) 7:862–74. doi: 10.1016/j.jchf.2019.05.015

2. Ma L, Chen W, Gao R, Liu LS, Zhu ML, Wang YJ, et al. China cardiovascular diseases report 2018: An updated summary. *J Geriatr Cardiol.* (2020) 17:1–8. doi: 10.11909/j.issn.1671-5411.2020.01.001

3. Gager G, Gelbenegger G, Jilma B, von Lewinski D, Sourij H, Eyileten C, et al. Cardiovascular outcome in patients treated with SGLT2 inhibitors for heart failure: A meta-analysis. *Front Cardiovasc Med.* (2021) 8:691907. doi: 10.3389/fcvm.2021.691907

4. Rask-Madsen C, Kahn CR. Tissue-specific insulin signaling, metabolic syndrome, and cardiovascular disease. *Arterioscler Thromb Vasc Biol.* (2012) 32:2052–9.

5. Ormazabal V, Nair S, Elfeky O, Aguayo C, Salomon C, Zu?iga FA, et al. Association between insulin resistance and the development of cardiovascular disease. *Cardiovasc Diabetol.* (2018) 17:122.

6. Ma X, Dong L, Shao Q, Cheng Y, Lv S, Sun Y, et al. Triglyceride glucose index for predicting cardiovascular outcomes after percutaneous coronary intervention in patients with type 2 diabetes mellitus and acute coronary syndrome. *Cardiovasc Diabetol.* (2020) 19:31. doi: 10.1186/s12933-020-01006-7

7. Luo E, Wang D, Yan G, Qiao Y, Liu B, Hou J, et al. High triglyceride-glucose index is associated with poor prognosis in patients with acute ST-elevation myocardial infarction after percutaneous coronary intervention. *Cardiovasc Diabetol.* (2019) 18:150. doi: 10.1186/s12933-019-0957-3

8. Aroor A, Mandavia C, Sowers J. Insulin resistance and heart failure: Molecular mechanisms. *Heart Fail Clin.* (2012) 8:609–17.

9. Riehle C, Abel E. Insulin signaling and heart failure. *Circ Res.* (2016) 118:1151–69.

10. Velez M, Kohli S, Sabbah HN. Animal models of insulin resistance and heart failure. *Heart Fail Rev.* (2014) 19:1–13.

11. American Diabetes Association [ADA]. Consensus development conference on insulin resistance. 5–6 November 1997. American Diabetes Association. *Diabetes Care.* (1998) 21:310–4. doi: 10.2337/diacare.21.2.310

12. Guerrero-Romero F, Simental-Mendia LE, Gonzalez-Ortiz M, Martínez-Abundis E, Ramos-Zavala MG, Hernández-González SO, et al. The product of triglycerides and glucose, a simple measure of insulin sensitivity comparison with the euglycemic–hyperinsulinemic clamp. *J Clin Endocrinol Metab.* (2010) 95:3347–51. doi: 10.1210/jc.2010-0288

13. Vasques AC, Novaes FS, de Oliveira MDS, Souza JRM, Yamanaka A, Pareja JC, et al. TyG index performs better than HOMA in a Brazilian population: A hyperglycemic clamp validated study. *Diabetes Res Clin Pract.* (2011) 93:e98–100. doi: 10.1016/j.diabres.2011.05.030

14. McKee P, Castelli W, McNamara P, Kannel WB. The natural history of congestive heart failure: The Framingham study. *New Engl J Med.* (1971) 285:1441–6.

15. McDonagh T, Metra M, Adamo M, Gardner RS, Baumbach A, Bohm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: Developed by the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). With the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail.* (2022) 24:4–131.

16. Peterson P, Rumsfeld J, Liang L, Albert NM, Hernandez AF, Peterson ED, et al. A validated risk score for in-hospital mortality in patients with heart failure from the American Heart Association Get With the Guidelines program. *Circ Cardiovasc Qual.* (2010) 3:25–32.

17. DeLong, E, DeLong D, Clarke-Pearson D. Comparing the areas under two or more correlated receiver operating characteristic curves: A nonparametric approach. *Biometrics.* (1988) 44:837–45.

18. Redelmeier D, Bloch D, Hickam D. Assessing predictive accuracy: How to compare Brier scores. *J Clin Epidemiol.* (1991) 44:1141–6.

19. Ingelsson E, Sundström J, Arnlöv J. Insulin resistance and congestive heart failure. *JAMA.* (2005) 294:334–41.

20. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: A method for quantifying insulin secretion and resistance. *Am J Physiol.* (1979) 237:E214–23.

21. Vardeny O, Gupta D, Claggett B, Burke S, Shah A, Loehr L, et al. Insulin resistance and incident heart failure the ARIC study (Atherosclerosis Risk in Communities). *JACC Heart Fail.* (2013) 1:531–6.

22. Klop B, Elte J, Cabezas M. Dyslipidemia in obesity: Mechanisms and potential targets. *Nutrients.* (2013) 5:1218–40.

23. Guo W, Zhao L, Mo F, Peng C, Li L, Xu Y, et al. The prognostic value of the triglyceride glucose index in patients with chronic heart failure and type 2 diabetes: A retrospective cohort study. *Diabetes Res Clin Pract.* (2021) 177:108786. doi: 10.1016/j.diabres.2021.108786

24. Arcopinto M, Schiavo A, Salzano A, Bossone E, D'Assante R, Marsico F, et al. Metabolic syndrome in heart failure: Friend or foe? *Heart Fail Clin.* (2019) 15:349–58.

25. Tamariz L, Hassan B, Palacio A, Arcement L, Horswell R, Hebert K, et al. Metabolic syndrome increases mortality in heart failure. *Clin Cardiol.* (2009) 32:327–31.

26. Zhou Y, Fu L, Sun J, Zhu Z, Xing Z, Zhou S, et al. Association between metabolic syndrome and an increased risk of hospitalization for heart failure in population of HFpEF. *Front Cardiovasc Med.* (2021) 8:698117. doi: 10.3389/fcvm.2021.698117

27. Zheng S, Chan F, Nabeebaccus A, Shah AM, McDonagh T, Okonko DO, et al. Drug treatment effects on outcomes in heart failure with preserved ejection fraction: A systematic review and meta-analysis. *Heart.* (2018) 104:407–15.

28. Ferrari R, Bohm M, Cleland JG, Paulus WJS, Pieske B, Rapezzi C, et al. Heart failure with preserved ejection fraction: Uncertainties and dilemmas. *Eur J Heart Fail.* (2015) 17:665–71.

29. Suzuki S, Yoshihisa A, Sato Y, Kanno Y, Watanabe S, Abe S, et al. Clinical significance of get with the guidelines-heart failure risk score in patients with chronic heart failure after hospitalization. *J Am Heart Assoc.* (2018) 7:e008316. doi: 10.1161/JAHA.117.008316

30. Shiraishi Y, Kohsaka S, Abe T, Mizuno A, Goda A, Izumi Y, et al. Validation of the Get With The Guideline-Heart Failure risk score in Japanese patients and the potential improvement of its discrimination ability by the inclusion of B-type

natriuretic peptide level. *Am Heart J.* (2016) 171:33–9. doi: 10.1016/j.ahj.2015.10.008

31. Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. *Circulation.* (2007) 115:928–35.

32. Pickering JW, Endre ZH. New metrics for assessing diagnostic potential of candidate biomarkers. *Clin J Am Soc Nephrol.* (2012) 7:1355–64.

33. Witteles RM, Fowler MB. Insulin-resistant cardiomyopathy clinical evidence, mechanisms, and treatment options. *J Am Coll Cardiol.* (2008) 51:93–102.

34. Hattori Y. Insulin resistance and heart failure during treatment with sodium glucose cotransporter 2 inhibitors: Proposed role of ketone utilization. *Heart Fail Rev.* (2020) 25:403–8. doi: 10.1007/s10741-020-09921-3

35. Rutter MK, Parise H, Benjamin EJ, Levy D, Larson MG, Meigs JB, et al. Impact of glucose intolerance and insulin resistance on cardiac structure and function: Sex-related differences in the Framingham Heart Study. *Circulation.* (2003) 107:448–54. doi: 10.1161/01.cir.0000045671.62860.98

36. Sundstrom J, Lind L, Nystrom N, Zethelius B, Andrén B, Hales CN, et al. Left ventricular concentric remodeling rather than left ventricular hypertrophy is related to the insulin resistance syndrome in elderly men. *Circulation.* (2000) 101:2595–600. doi: 10.1161/01.cir.101.22.2595

37. DeFronzo RA, Cooke CR, Andres R, Faloona GR, Davis PJ. The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. *J Clin Invest.* (1975) 55:845–55. doi: 10.1172/JCI107996

38. Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL. Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *J Clin Invest.* (1991) 87:2246–52. doi: 10.1172/JCI115260

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Association between the empirical dietary inflammatory index and cardiorespiratory fitness in Tehranian adults in 2017–2018

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Background: Inflammatory-related chronic diseases are increasing in Iran with high consumption of a diet containing pro-inflammatory potential and a sedentary lifestyle. The empirical dietary inflammatory index (EDII) was developed as a tool to assess dietary effects on systemic inflammation. We examined the hypothesis that specific dietary patterns reflecting systemic inflammation are associated with cardiorespiratory fitness (CRF) in Tehranian adults.

Methods: This cross-sectional study was carried out on 270 adults who are residents of Tehran. Dietary intake was assessed using a 168-item valid and reliable food frequency questionnaire. The EDII score was developed according to participant dietary intakes of 21-item pre-defined food groups. CRF was assessed by using a graded exercise treadmill test. Anthropometric measurements were assessed using standard methods. To discover the association between CRF and EDII, we used multivariable logistic regression analysis.

Results: Those who were in the third tertile of the EDII had 57% lower odds of having better VO_{2Max} (ml/kg/min) than those in the first tertile (OR: 0.43; 95% CI: 0.16, 1.12, $p = 0.01$). There were no significant differences between tertiles of the EDII score in terms of VO2 (L·min) and VO2 (LBM) before and after adjusting for confounders. There was a significant decrease in VO_{2Max} (ml/kg/min) across tertiles of the EDII after controlling for covariates (p-value = 0.04). There was a significant inverse association between the EDII score and VO_{2Max} (ml/kg/min) ($\beta = -0.35$, $p = 0.001$).

Conclusions: Our finding demonstrated that a higher EDII might be associated with lower CRF in Tehranian adults. Prospective studies are needed to shed light on the causal link between the EDII and CRF.

KEYWORDS

empirically dietary inflammatory index, inflammation, cardiorespiratory fitness, $VO2_{max}$, food-based dietary inflammatory index

Introduction

Chronic inflammation is a process in which inflammation develops slowly and lasts for a long period, ranging from months to years (1). An increase in inflammatory mediators such as C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), and interleukin 6 (IL6) is supposed to be associated with inflammation (2–4). Inflammation is a risk factor for chronic diseases such as cardiovascular diseases (CVDs), diabetes, and various cancers (5–7). Some research shows that cardiovascular diseases are one of the leading causes of mortality in adults older than 45 years (8). Also, there are numerous clinical investigations indicating that a strong association exists between low cardiorespiratory fitness (CRF) and the risk of mortality from cardiovascular disease (8–10).

CRF refers to the maximum capacity of the cardiovascular and respiratory systems to supply oxygen to skeletal muscles during activities such as exercise (10, 11). There are several studies available today which have examined the association between exercise and physical activity with inflammation, and their reports suggested that exercise has an anti-inflammatory role (12–14). Also, biological plausibility in the general population suggests that exercise has the potential to develop an acute and long-term anti-inflammatory phenotype (12, 14). The results of the study by Church et al. indicated that the level of CRF was inversely related to CRP levels in men (13).

According to studies, diet is one of the main determinants of inflammation (4, 15). Because of the interactions between food and micronutrients, the assessment of food intake is comprehensive when different food groups are examined together in the form of dietary patterns or food indexes. Dietary patterns or dietary indexes provide a more comprehensive assessment of an individual's diet than the approach of using single foods or nutrients to determine diet–disease associations (16, 17). It seems that the use of two different indicators (EDII and DII) to assess the inflammatory potential of the diet can cause differences in the results of different studies.

The two main indicators to describe the inflammatory potential of the diet include a literature-derived dietary inflammatory index (DII) and an empirical dietary inflammatory index (EDII) (15–18). The DII is essentially an *a priori* index focusing on the inflammatory potential of dietary nutrients (2), while the EDII is an *a posteriori* index focusing on the inflammatory potential of food groups, and this index appears to provide a new dimension of the inflammatory potential of the whole diet of individuals (15–17). According to the results of a meta-analysis of 17 related studies published in 2018, a higher DII increased the risk of CVD by about 35% and also showed a higher risk for all-cause CVD and cancer death (19). Recently, data from three large prospective cohort studies in the United States using the EDII showed that a higher incidence of CVD, CHD, and stroke was significantly associated with dietary patterns with higher inflammatory potential (20). On the other hand, Tabung et al. indicated that in contrast to the EDII, which is based on the food groups, the DII is a nutrient-based indicator and is strongly influenced by the consumption of nutritional supplements by individuals (15).

Given that Iran is a country with an increasing rate of several inflammatory diseases, we designed this cross-sectional study to investigate whether the inflammatory potential of diet (using the EDII to achieve a new dimension of results in the inflammation of food groups) is related to cardiorespiratory readiness in Tehranian adults.

Methods

Study population

This is a cross-sectional study in which 270 apparently healthy (118 males and 152 female) adults aged between 18 and 45 years living in Tehran from February 2017 to December 2018 with a mean age of 36.77 ± 13.19 years and an average BMI of 62.25 Kg/m^2 were recruited by using the convenience sampling method. Based on the previously calculated correlation coefficient between the dietary pattern and cardiorespiratory fitness (21), our target number of subjects was 256 [$(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta} \times \sqrt{1-r^2}\ /r) = 256$, where $\alpha = 0.05$, $1\text{–}\beta = 0.95$, and $r = 0.34$]. To compensate for the potential exclusion of participants due to under- and overreporting of total energy intake, or attrition due to other reasons, the final sample size of 270 participants was selected for inclusion. The inclusion criteria were age range of 18–45 years, being healthy, desire to participate in the study, and being residents of Tehran. Some people were excluded from the study who had a dietary intake of less than 800 kcal/d or more than 4200 kcal/d,

infectious conditions, and active inflammatory diseases; who were pregnant and lactating; or who used drugs such as hormonal drugs or sedatives, as well as people who used any supplements for weight loss such as caffeine and green tea supplements and conjugated linoleic acid (CLA).

Ethical considerations

In this study, written consent was obtained from all participants to participate in the study. Also, all methods were based on the Helsinki guideline and ethical standards of the Tehran University of Medical Sciences (TUMS.VCR.REC.1396.4085), which approved the protocol and the form of informed consent.

Data collection

Participants' lifestyle information including age, gender, cardiovascular disease (yes or no), respiratory disease (yes or no), and smoking (yes or no) was collected through a self-administered questionnaire. Participants' physical activity was also evaluated using the Iranian version of the International Physical Activity Questionnaire (IPAQ) (22). Then, the participants were grouped into three categories: very low (< 600 MET-minute/week), low (600–3000 MET-minute/week), and moderate and high (> 3000 MET-minute/week) (23).

Anthropometric measures and body composition

Body weight was measured in the participants while wearing light clothes to the nearest 0.1 kg by using a Seca scale. Participants' height was first measured by using a stadiometer (Seca, Germany), and their waist circumference (WC) was measured by placing a non-stretch tape measure in the middle of the last rib to just above the hipbones, and hip circumference (HC) was measured around the widest portion of the buttocks. By dividing the WC (cm) by the HC (cm), the waist-to-hip ratio (WHR) was obtained, and by dividing the weight (kg) by height (square meter), the body mass index (BMI) was calculated. The participants' body composition was assessed using InBody 720 (Biospace, Seoul, Korea). The participants were required to follow the following conditions before the measurements: not consume any foods for at least 4 hours, at least 2 liters of water consumption the day before, at least 8 h without physical activity, and at least 12 h without coffee or alcoholic beverages and also at least 24 h without using diuretics before the assessment.

Cardiorespiratory fitness

CRF is an important test of physical fitness measurements which is related to the maximum oxygen consumption ($VO2_{Max}$). According to the gold standard, $VO2_{Max}$ is the best indicator of CRF and is measured by graded treadmill exercise tests. The participants were asked to accomplish a graded exercise protocol until exhaustion while they could breathe through a respiratory gas analyzer mask. The graded exercise test constitutes of seven steps with different treadmill settings, starting from 1.7 mph speed with 10% gradient. The speed and gradient of the treadmill are elevated every 3 minutes and the next step begins. The test was terminated when the subject was not able to keep exercising because of tiredness, pain, or any other medical symptom. Respiratory gases were analyzed by MetaLyzer3B during the test. Criteria used were as follows: (1) the respiratory exchange ratio and maximum heart rate reached 1.1 and (220_age) respectively. (2) The participant not to able to continue requested test interruption. CRF was measured with L/min, ml/min/kg body weight units. The resting metabolic rate (RMR) was measured by MetaLyzer3B and bioelectrical impedance analysis.

Dietary assessment

Participants' dietary intakes were evaluated by trained nutritionists through face-to-face interviews using a valid, reliable, semi-quantitative 168-item food frequency questionnaire (FFQ) (24). This questionnaire reported the consumption of each food item on a daily, weekly, or monthly basis during the last year, and then these data were converted into daily consumption. Finally, all foods were analyzed in terms of their energy content using modified Nutritionist IV software prepared for Iranian food (version 7.0; *N*-Squared Computing, Salem, OR, USA).

We used the method introduced by Tubong et al. to calculate empirically derived daily and meal-based DII (EDII) (25). For this purpose, we used our dataset including 522 Tehranian people aged 18–70 years, in which foods and food groups contributing to systemic inflammation were explored. In that study, low-grade systemic inflammation was evaluated by measuring circulating high-sensitive C-reactive protein (hs-CRP) concentrations. Dietary intake was evaluated by using a food frequency questionnaire, and then foods were classified into 24 food groups. Major dietary patterns were identified through factor analysis, and accordingly, three major dietary patterns including Western, healthy, and traditional dietary patterns were identified. By applying multiple linear regression analysis, a positive association between the Western dietary pattern and levels of hs-CRP, as well as a negative association between healthy and traditional dietary patterns and hs-CRP concentrations was identified (data not

TABLE 1 General characteristic of participant according to the tertile of EDII.

		EDII		
		T1	T2	T3
		89	90	89
EDII score		(−1.32, 4.23)	(4.24, 6.82)	(6.83, 27.0)
Age (year)		35.4 ± 13.4	35.3 ± 12.7	39.0 ± 19.9
Blood pressure (mmHg)	Systolic	109 ± 16.6	109 ± 22.1	114 ± 18.0
	Diastolic	68.6 ± 10.7*	70.1 ± 11.2*	73.0 ± 9.69*
WC (cm)		86.1 ± 11.3*	88.3 ± 11.8*	94.3 ± 13.2*
Weight (Kg)		68.2 ± 14.7*	71.1 ± 15.0*	78.8 ± 16.7*
BMI (kg/m^2)		24.5 ± 4.09*	25.4 ± 4.49*	26.9 ± 5.14*
FFM (kg)		47.7 ± 12.6*	48.3 ± 12.09*	54.4 ± 12.3*
FM (kg)		20.5 ± 8.45*	22.3 ± 8.87*	24.4 ± 10.5*
Sex, *n* (%)	Male	29 (24.8%)*	33 (28.2%)*	55 (47.0%)*
	Female	60 (39.7%)*	57 (37.7%)*	34 (22.5%)*
Smoking, *n* (%)	Yes	6 (30%)	5 (25%)	9 (45%)
	No	79 (34.1%)	82 (35.3%)	71 (30.6%)
	Quit	4 (28.6%)	1 (7.1%)	9(64.3%)
CVD, *n* (%)	Yes	1 (16.7%)	1 (16.7%)	4 (66.7%)
	No	88 (33.7%)	89 (34.1%)	84 (32.2%)
Respiratory disease, *n* (%)	Yes	0 (0.0%)	0 (0.0%)	3 (100%)
	No	89 (33.6%)	90 (34%)	86 (32.5%)
Physical activity, *n* (%)	Low	31 (30.4%)	42 (41.2%)	29 (28.4%)
	Moderate	38 (34.2%)	33 (29.7%)	40 (36%)
	High	20 (36.4%)	15 (27.3%)	20 (36.4%)

p-value < 0.05 was considered significant.
Significant *p*-value are showed with*.
Values are based on mean ± standard deviation or reported percentage.
One-way Anova for quantitative data and Chi-2 test for qualitative data have been used.
P_{trend} derived from polynomial regression test.
EDII, empirically dietary inflammatory index; WC, Waist circumference; BMI, body mass index; FFM, fat free mass; FM, fat mass; CVD, cardiovascular disease; WHR, waist to hip ratio; mmHg, millimeter of mercury; cm, centimeter; kg, kilogram; m, meter.
Subjects in the first tertile of EDII had EDII score between (−1.23, 4.23); second tertile: between (4.24, 6.82); third tertile: between (6.83, 27.0).

shown). Thus, eight food groups contributing to the Western dietary patterns including eggs, snacks, nuts, mayonnaise, salt, processed meat, and fried potato were considered pro-inflammatory, and 16 food groups contributing to healthy and traditional dietary patterns including vegetables, canned fish, grains, olive, fruits, fishes, bread, high-fat dairy, low-fat dairy, legumes and soy, organ meat, boiled potato, oil and butter, coffee, pickles, and sweets and dessert were considered anti-inflammatory. Then, the average daily intakes of pro- and anti-inflammatory food groups for each participant were multiplied by their given factor loadings. The overall EDII score for each participant was computed by summing up the score of each 24 pro- and anti-inflammatory food groups. Finally, the EDII score was divided by 100 to reduce the magnitude of the score. A higher EDII score (more positive) indicates a more pro-inflammatory diet, and a lower EDII score (more negative) indicates a less pro-inflammatory diet.

Statistical analysis

All the participants were grouped based on tertiles of the EDII. We used one-way ANOVA for quantitative data and chi-square tests for qualitative data, to compare general characteristics across EDII tertiles. The analysis of covariance (ANCOVA) was performed to assess the association between the EDII score with dietary intakes of the study participants after adjusting for potential confounders. The first tertile was considered the reference group. To evaluate the association between $VO2_{Max}$, VO2 (L·min), and VO2 (LBM) across tertiles of the EDII, we used multiple linear regression analysis after adjustment for covariates such as age, sex, smoking, physical activity, body mass index, and energy intake. To find the association between the EDII with high/low CRF, we used multivariable logistic regression analysis. In the first model, we adjusted for energy intake, age, and sex. In the second model, we controlled for the confounding impact

of more confounders such as age, sex, smoking, physical activity, cardiovascular disease, respiratory disease, and energy intake. In the last model, we adjusted all the variables adjusted in the second model along with the body mass index. The overall trend of odds ratios across tertiles of the EDII was calculated by considering the median of the EDII in each tertile as a continuous variable. All statistical analyses were performed with the Statistical Package for Social Sciences (SPSS) for Windows 25.0 software package (SPSS, Chicago, IL). The level of statistical significance was defined at $p < 0.05$.

Results

General characteristics of the participants based on tertiles of the EDII are shown in Table 1. The participants' mean age was 36.7 ± 13.1 years, and their mean BMI was 25.6 ± 4.67. A total of 270 apparently healthy (117 male and 153 female) participants were included in our study. There was no significant difference among the mean of systolic blood pressure, smoking status, physical activity, CVD, and respiratory disease across the tertiles of the EDII. The mean of diastolic blood pressure, WC, weight, BMI, fat-free mass (F00FM), and fat mass (FM) showed a significant increase from the first tertile to the third tertile of EDII ($p < 0.001$ for all).

Table 2 shows the dietary intakes of the study participants according to tertiles of the EDII. The intake of refined grains, vegetables, soft drinks, mayonnaise, eggs, and pickles was higher in the top tertile of the EDII than in the first tertile. By contrast, the participants consumed fewer dairy products (high-fat and low-fat dairy), fish, fruits, and juices in the top tertile of the EDII than in the first tertile.

Table 3 indicates the multivariate-adjusted means of CRF across tertiles of the EDII. In the crude model, we observed that there was a significant reduction in VO_{2Max} (ml/kg/min) across tertiles of the EDII. After adjusting for potential confounders (age, sex, smoking, physical activity, cardiovascular disease, respiratory disease, BMI, and energy), the relationship remained unchanged (p-value = 0.04). There was no significant difference in terms of VO_{2Max} (L·min) and VO_{2Max} (LBM) among tertiles of the EDII. After controlling for covariates, these associations remained non-significant.

Multivariate adjusted odds ratios and 95% confidence intervals for having better CRF across tertiles of the EDII are presented in Table 4. In the crude model, although those who were in the third tertile of the EDII were less likely to have better VO_{2Max} (ml/kg/min) [OR= 0.81; CI95%: 0.39, 1.68] than those in the first tertile, there was no association between a higher EDII and VO_{2Max} (ml/kg/min) ($p = 0.07$) after adjusting for age, sex, smoking, physical activity, cardiovascular disease, respiratory disease, and energy intake; also, this result remained non-significant.

TABLE 2 Dietary intakes of the study participants according to the tertile of EDII.

	T1	T2	T3
Range	(−1.32, 4.23)	(4.24, 6.82)	(6.83, 27.0)
Subjects, n	89	90	89
Refined grains, g/d	255 ± 115*	344 ± 160*	511 ± 310*
Whole-grains, g/d	51.1 ± 50.3	95.5 ± 106	108 ± 116
Legumes, g/d	25.3 ± 25.2	25.5 ± 29.2	58.2 ± 73.8
Red or processed meat, g/d	32.7 ± 21.8	36.3 ± 35.0	59.0 ± 48.8
Vegetables, g/d	288 ± 165	321 ± 179	492 ± 350
Poultry, g/d	50.4 ± 85.7	50.7 ± 55.7	85.8 ± 165
Organ meat, g/d	2.81 ± 4.71	2.14 ± 3.47	5.37 ± 13.9
Vegetable Oils, g/d	6.24 ± 7.08	7.05 ± 5.96	7.68 ± 7.13
Soft drinks, g/d	28.5 ± 38.5*	39.5 ± 57.4*	81.1 ± 111*
Sweets and dessert, g/d	48.7 ± 35.6	59.3 ± 40.0	78.5 ± 47.6
Salt, g/d	5.69 ± 5.50	7.14 ± 6.05	7.43 ± 5.94
Mayonnaise, g/d	2.12 ± 3.17	2.17 ± 3.05	5.09 ± 9.77
Tea and coffee, g/d	378 ± 265*	633 ± 311*	1077 ± 618*
Salty snacks, g/d	6.98 ± 9.38	8.20 ± 11.8	11.4 ± 14.0
High-fat dairy, g/d	54.4 ± 58.5	75.6 ± 79.2	172 ± 235
French-fries, g/d	4.46 ± 5.86	5.69 ± 6.33	8.71 ± 10.2
Potato, g/d	19.0 ± 17.9	21.0 ± 17.9	25.9 ± 19.2
Low-fat dairy, g/d	472 ± 422*	317 ± 208*	351 ± 258*
Fruits and juices, g/d	339 ± 213	280 ± 166	313 ± 196
Nuts, g/d	11.0 ± 10.7	11.6 ± 14.7	14.0 ± 16.1
Fish, g/d	18.8 ± 21.2*	11.8 ± 10.4*	17.4 ± 18.6*
Egg, g/d	29.7 ± 41.6*	26.8 ± 31.1*	42.0 ± 52.7*
Pickles, g/d	6.94 ± 7.73	8.83 ± 10.7	19.1 ± 26.0
Hydrogenated fats, g/d	10.8 ± 13.3	13.8 ± 17.0	19.7 ± 23.5
Olive and olive oil, g/d	4.48 ± 4.99	4.50 ± 7.01	6.10 ± 7.62

All values were adjusted for energy intake using analysis of covariance.
Values are based on mean ± standard deviation.
p-value less than 0.05 was considered significant.
Significant p-value are showed with*.
EDII, empirically dietary inflammatory index; CRF, cardiorespiratory fitness.

The linear associations between the EDII score and CRF are shown in Table 5. VO_{2Max} (ml/kg/min) had no significant linear association with EDII ($\beta = -0.11$, $p = 0.33$). Moreover, after controlling for covariates, these associations changed to significant ($\beta = -0.35$, $p = 0.001$).

Discussion

Overall, we found that higher adherence to a diet containing higher pro-inflammatory potential may be associated with less $Vo2_{Max}$ (ml/kg/min), and there is an inverse association between the EDII and $Vo2_{Max}$ (ml/kg/min).

Cardiorespiratory fitness reflects the ability of cardiovascular and respiratory systems to transport oxygen to skeletal muscle

TABLE 3 Multivariate adjusted means for CRF across tertiles of EDII.

	Tertiles of EDII					
	T1 (−1.32, 4.23)	T2 (4.24, 6.82)	T3 (6.83, 27.0)	P_1	P_2	P_3
VO_2 Max (ml/kg/min)	31.8 ± 8.60	30.9 ± 7.14	29.0 ± 7.43	0.02	0.02	0.04
VO_2 (L/min)	2.25 ±0.70	2.10 ± 0.65	2.35 ± 0.77	0.09	0.43	0.26
VO_2 (LBM)	47.1 ± 8.99	46.8 ± 7.54	48.3 ± 8.39	0.49	0.34	0.81

p-value less than 0.05 was considered significant.
Values are based on mean ± standard deviation.
P_1 derived from One-way analysis of variance.
P_2 derived from polynomial regression test.
P_3 derived from analysis of covariance.
Subjects in the first tertile of DII had DII score between (−1.32, 4.23); second tertile: between (4.24, 6.82); third tertile: between (6.83, 27.0).
EDII, empirically dietary inflammatory index; CRF, cardiorespiratory fitness.
P_{ANCOVA}, Adjusted for age, sex, smoking, physical activity; CVD, respiratory disease, BMI and energy intake.

TABLE 4 Odds ratios (ORs) and 95% confidence intervals (95% CIs) for CRF according to tertiles of EDII.

	Tertiles of EDII			
	T1 OR	T2 OR (95%CI)	T3 OR (95%CI)	*P* trend
VO_2 Max (ml/kg/min)				
Crude	1	0.93(0.51, 1.68)	0.91(0.50, 1.65)	0.07
Model 1*	1	0.79(0.39, 1.60)	0.42(0.18, 1.00)	0.06
Model 2†	1	0.90(0.46, 2.2)	0.43(0.16, 1.12)	0.01
VO_2 (L/min)				
Crude	1	0.68(0.37, 1.23)	0.95(0.53, 1.72)	0.35
Model 1	1	0.66(0.36, 1.20)	0.82(0.41, 1.63)	0.83
Model 2	1	0.64(0.35, 1.18)	0.87(0.43, 1.75)	0.96
VO_2 (LBM)				
Crude	1	0.93(0.52, 1.68)	1.00(0.55, 1.80)	0.45
Model 1	1	0.88(0.48, 1.60)	0.82(0.41, 1.64)	0.96
Model 2	1	0.88(0.48, 1.61)	0.85(0.42, 1.72)	0.84

Data are OR (95%CI).
*Adjusted for age, sex, smoking, physical activity, Cardiovascular disease, respiratory disease, energy.
†Additionally adjusted for BMI.
EDII, Empirical dietary inflammatory index; LBM, Lean body mass; CRF Cardiorespiratory Fitness.

mitochondria for energy production needed during physical activity (26). CRF is an important criterion for our mental and physical health and is affected by various factors such as age, genetics, body composition, gender, lifestyle, and the inflammatory potential of the diet (8, 26, 27). Recently, researchers have been using the EDII, instead of the DII, to measure the inflammatory potential of diet (20). The EDII is based on food groups, while the DII focuses on dietary nutrients (15).

Most available studies have used the nutrient-based DII score to assess the inflammatory potential of the diet, and they assessed the association of the DII score with various diseases, including cardiovascular disease (CVD), cancer, metabolic syndrome (Mets), and respiratory disease (2, 4, 28–30). According to the results of a prospective study, there was a positive association between the EDII and the risk of Mets in adults (16). They showed that a lower level of HDL-C and a higher level of FBG and WC were associated with a higher EDII score (16). They also indicated that there is no association between the EDII with hypertriglyceridemia and hypertension (16), while Camargo-Ramos et al. demonstrated that an increased inflammatory potential of the diet was inversely associated with an improved cardiometabolic profile, higher HDL-C, and lower Hb1Ac (31). In a 2017 study, Tabung et al. evaluated the validity of the EDII in two independent groups of men and women and found that the EDII significantly predicted the concentration of inflammatory markers (15). Also, the study of Li et al. showed that the EDII in large sample size has a positive relationship with inflammatory markers such as CRP, interleukin 6, and TNFα-R2 (20).

TABLE 5 Multiple regression analysis models exploring the association of EDII with CRF.

	β ± SE	95% CI
VO_2 Max (ml/kg/min)		
Model 1	−0.11 ± 0.12	−0.34, 0.11
Model 2	−0.35 ± 0.10*	−0.55, −0.14
VO_2 (L/min)		
Model 1	0.01 ±0.01	−0.01, 0.03
Model 2	0.001 ± 0.01	−0.02, 0.03
VO_2 (LBM)		
Model 1	0.09 ± 0.12	−0.15, 0.34
Model 2	0.03 ± 0.16	−0.30, 0.36

p-value<0.05 was considered significant.
Significant *p*-value are showed with*.
Model 1 was crude.
Model 2 adjusted for age, sex, smoking, physical activity, *CVD*, respiratory diseases; BMI and energy intake.
EDII, empirically dietary inflammatory index; CRF, cardiorespiratory fitness; SE, standard error.
β coefficient obtained from linear regression.

In a study by Wood et al., it was suggested that the Western diet may lead to systemic inflammation and subsequent inflammation of the airways and respiratory problems (32). By contrast, components of anti-inflammatory diets can have protective effects on the respiratory system in both adults and children (29, 33). Another case–control study by Wood et al. in 2015 on the adult population showed that the DII score was correlated with a systemic inflammation increase and less lung function. Each unit increase in the DII score can increase the risk of asthma by 70%. It also can reduce forced expiratory volume in 1 s by 3.44% (30).

On the contrary, some studies reported no association between the DII score and metabolic syndrome in young adults (34, 35). The results of a study by Christensen et al. in 2019 also showed a significant negative correlation among cardiorespiratory fitness, visceral fat, body fat, dietary fat, polyunsaturated and saturated fat, and CRP level (36). However, there was no association between the dietary inflammatory index and the CRP level in cancer survivors (36). Also, in a study by Asadi et al. on a middle-aged Iranian population, it was shown that there was no significant relationship between the DII and total cardiovascular disease, myocardial infarction, stable angina, or unstable angina (28).

The possible mechanisms by which an anti-inflammatory diet can reduce chronic disease or improve cardiorespiratory fitness are unclear. Despite this, it has been hypothesized that unhealthy dietary patterns with pro-inflammatory effects are associated with an innate immune response (increased production of pro-inflammatory cytokines and decreased production of anti-inflammatory cytokines). This eventually causes chronic inflammation and ultimately increases the risk of endothelial dysfunction, metabolic syndrome, cardiovascular disease, and cancer (27, 31).

Contradictory results of studies on the factors affecting CRF include different demographic characteristics of participants, other aspects of diet such as acidity and carcinogenic potential of the diet, different study design, different confounding factors, and the use of two different indicators to assess the inflammatory potential of the diet.

There are strengths in our study. The present study is the first Iranian study that examined the more complete EDII to evaluate the inflammatory potential of diet and its relationship with CRF and its components. We have used the valid 168-item FFQ that have been prepared to estimate the Iranian eating habits. In addition, we adjusted several important confounders, which could affect our results. Finally, using the EDII, instead of the DII, refers to the inflammatory potential of foods in the participants' diet. In addition, the use of an inflammatory index based on dietary groups can facilitate the recommendations of the anti-inflammatory diet by nutritionists. Also, unlike the DII, the EDII is not affected by the use of dietary supplements, which is a good advantage in clinical trials. However, the cross-sectional study we conducted has some limitations, which should be addressed in future studies. First, the study design was cross-sectional, which makes the conclusion of causality difficult. Second, we used the FFQ as a standard instrument to assess long-term dietary intake; however, estimates of food consumption from the FFQ are not precise, and there is always a probability of measurement error, although our FFQ was validated to have reasonably high validity (37). Third, the limited number of participants may have caused diminished statistical power in multivariable analyses.

Eventually, cardiorespiratory fitness appears to be affected by a variety of diet-related factors, including carcinogenicity of the diet and high acidity of the food (38–40). Although other dietary indicators, such as the alternate healthy eating index (AHEI), Dietary Approaches to Stop Hypertension (DASH), and Alternate Mediterranean Diet Score (AMED), assess the overall quality of the diet (41), the EDII specifically focuses on the potential of the diet to aid in chronic inflammation (15, 20). In this study, we only assessed the inflammatory aspect of the diet, but in future, more comprehensive studies with more detailed designs are needed.

Conclusion

In summary, we found that a higher empirical DII is associated with increased $VO2_{Max}$ after adjusting for confounding variables. The importance of following a healthy diet with a lower inflammatory index and having higher CRF should be considered as part of a healthy lifestyle

approach, and longitudinal studies are needed to confirm our findings.

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.

Ethics statement

The studies involving human participants were reviewed and approved by Tehran University of Medical Sciences (Ethic Number: IR.TUMS.VCR.REC.1396.4085). The patients/participants provided their written informed consent to participate in this study.

Author contributions

NP and SS-B contributed to conception and design of the research. ME, SD, and NB contributed to acquisition, analysis, or interpretation of the data. HS and NP drafted the manuscript. KD and SS-B critically revised the manuscript. SS-B agrees to be fully accountable for ensuring the integrity and accuracy of the work. All authors read and approved the final manuscript.

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Conflict of interest

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

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References

1. Pahwa R, Goyal A, Bansal P, Jialal I. *Chronic Inflammation*. Treasure Island (FL): StatPearls Publishing LLC. (2021).

2. Wirth MD, Burch J, Shivappa N, Violanti JM, Burchfiel CM, Fekedulegn D, et al. Association of a dietary inflammatory index with inflammatory indices and metabolic syndrome among police officers. *J Occup Environ Med.* (2014) 56:986–9. doi: 10.1097/JOM.0000000000000213

3. Phosat C, Panprathip P, Chumpathat N, Prangthip P, Chantratita N, Soonthornworasiri N, et al. Elevated C-reactive protein, interleukin 6, tumor necrosis factor alpha and glycemic load associated with type 2 diabetes mellitus in rural Thais: a cross-sectional study. *BMC Endocr Disord.* (2017) 17:44. doi: 10.1186/s12902-017-0189-z

4. Ruiz-Canela M, Bes-Rastrollo M, Martínez-González MA. The role of dietary inflammatory index in cardiovascular disease, metabolic syndrome and mortality. *Int J Mol Sci.* (2016) 17:1265. doi: 10.3390/ijms17081265

5. Singh N, Baby D, Rajguru JP, Patil PB, Thakkannavar SS, Pujari VB. Inflammation and cancer. *Ann Afr Med.* (2019) 18:121–6. doi: 10.4103/aam.aam_56_18

6. Lontchi-Yimagou E, Sobngwi E, Matsha TE, Kengne AP. Diabetes mellitus and inflammation. *Curr Diab Rep.* (2013) 13:435–44. doi: 10.1007/s11892-013-0375-y

7. Carrizales-Sepúlveda EF, Ordaz-Farías A, Vera-Pineda R, Flores-Ramírez R. Periodontal disease, systemic inflammation and the risk of cardiovascular disease. *Heart Lung Circ.* (2018) 27:1327–34. doi: 10.1016/j.hlc.2018.05.102

8. Carnethon MR, Gidding SS, Nehgme R, Sidney S, Jacobs Jr DR, Liu K, Cardiorespiratory fitness in young adulthood and the development of cardiovascular disease risk factors. *Jama.* (2003) 290:3092–100. doi: 10.1001/jama.290.23.3092

9. Arias E, Smith BL. Deaths: preliminary data for 2001. *Natl Vital Stat Rep.* (2003) 51:1–44.

10. Shahinfar H, Ghanbari M, Jalilpiran Y, Payande N, Shahavandi M, Babaei N, et al. Interaction between major dietary patterns and cardiorespiratory fitness on metabolic syndrome in Iranian adults: a cross-sectional study. *Nutr J.* (2021) 20:36. doi: 10.1186/s12937-021-00695-4

11. Myers J, Kokkinos P, Nyelin E. Physical activity, cardiorespiratory fitness, and the metabolic syndrome. *Nutrients.* (2019) 11:1652. doi: 10.3390/nu11071652

12. Metsios GS, Moe RH, Kitas GD. Exercise and inflammation. *Best Pract Res Clin Rheumatol.* (2020) 34:101504. doi: 10.1016/j.berh.2020.101504

13. Church TS, Barlow CE, Earnest CP, Kampert JB, Priest EL, Blair SN. Associations between cardiorespiratory fitness and C-reactive protein in men. *Arteriosc Thromb Vasc Biol.* (2002) 22:1869–76. doi: 10.1161/01.ATV.0000036611.77940.F8

14. Hojan K, Kwiatkowska-Borowczyk E, Leporowska E, Górecki M, Ozga-Majchrzak O, Milecki T, et al. Physical exercise for functional capacity, blood immune function, fatigue, and quality of life in high-risk prostate cancer patients during radiotherapy: a prospective, randomized clinical study. *Eur J Phys Rehabil Med.* (2016) 52:489–501.

15. Tabung FK, Smith-Warner SA, Chavarro JE, Fung TT, Hu FB, Willett WC, et al. An empirical dietary inflammatory pattern score enhances prediction of circulating inflammatory biomarkers in adults. *J Nutr.* (2017) 147:1567–77. doi: 10.3945/jn.117.248377

16. Shakeri Z, Mirmiran P, Khalili-Moghadam S, Hosseini-Esfahani F, Ataie-Jafari A, Azizi F. Empirical dietary inflammatory pattern and risk of metabolic syndrome and its components: Tehran lipid and glucose study. *Diabetol Metab Syndr.* (2019) 11:16. doi: 10.1186/s13098-019-0411-4

17. Soltani S, Moslehi N, Hosseini-Esfahani F, Vafa M. The association between empirical dietary inflammatory pattern and metabolic phenotypes in overweight/obese adults. *Int J Endocrinol Metab.* (2018) 16:e60048-e. doi: 10.5812/ijem.60048

18. Shivappa N, Hébert JR, Steck SE, Safari A, Sedaghat F, Rashidkhani B. Dietary inflammatory index and odds of colorectal cancer in a case- control study from Iran. *Asian Pac J Cancer Prev.* (2018) 19:1999–2006.

19. Namazi N, Larijani B, Azadbakht L. Dietary inflammatory index and its association with the risk of cardiovascular diseases, metabolic syndrome, and mortality: a systematic review and meta-analysis. *Horm Metab Res.* (2018) 50:345–58. doi: 10.1055/a-0596-8204

20. Li J, Lee DH, Hu J, Tabung FK Li Y, Bhupathiraju SN, et al. Dietary inflammatory potential and risk of cardiovascular disease among men and women in the U. S. *J Am Coll Cardiol.* (2020) 76:2181–93. doi: 10.1016/j.jacc.2020.09.535

21. Floegel A, Wientzek A, Bachlechner U, Jacobs S, Drogan D, Prehn C, et al. Linking diet, physical activity, cardiorespiratory fitness and obesity to serum metabolite networks: findings from a population-based study. *Int J Obesity.* (2014) 38:1388-96. doi: 10.1038/ijo.2014.39

22. Moghaddam MHB, Aghdam F, Asghari Jafarabadi M, Allahverdipour H, Nikookheslat S, Safarpour S. The Iranian version of international physical activity questionnaire (IPAQ) in Iran: content and construct validity, factor structure, internal consistency and stability. *World Appl Sci J.* (2012) 18:1073–80.

23. Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European prospective investigation into cancer and nutrition (EPIC) study. *Public Health Nutr.* (2003) 6:407–13. doi: 10.1079/PHN2002439

24. Mirmiran P, Esfahani FH, Mehrabi Y, Hedayati M, Azizi F. Reliability and relative validity of an FFQ for nutrients in the Tehran lipid and glucose study. *Public Health Nutr.* (2010) 13:654–62. doi: 10.1017/S1368980009991698

25. Tabung FK, Smith-Warner SA, Chavarro JE, Wu K, Fuchs CS, Hu FB, et al. Development and validation of an empirical dietary inflammatory index. *J Nutr.* (2016) 146:1560–70. doi: 10.3945/jn.115.228718

26. Raghuveer G, Hartz J, Lubans DR, Takken T, Wiltz JL, Mietus-Snyder M, et al. Cardiorespiratory fitness in youth: an important marker of health: a scientific statement from the american heart association. *Circulation.* (2020) 142:e101–e18. doi: 10.1161/CIR.0000000000000866

27. Shivappa N, Godos J, Hébert JR, Wirth MD, Piuri G, Speciani AF, et al. Dietary inflammatory index and cardiovascular risk and mortality-a meta-analysis. *Nutrients.* (2018) 10:200. doi: 10.3390/nu10020200

28. Asadi Z, Yaghooti-Khorasani M, Ghazizadeh H, Sadabadi F, Mosa-Farkhany E, Darroudi S, et al. Association between dietary inflammatory index and risk of cardiovascular disease in the Mashhad stroke and heart atherosclerotic disorder study population. *IUBMB Life.* (2020) 72:706–15. doi: 10.1002/iub.2172

29. Hosseini B, Berthon BS, Wark P, Wood LG. Effects of fruit and vegetable consumption on risk of asthma, wheezing and immune responses: a systematic review and meta-analysis. *Nutrients.* (2017) 9. doi: 10.3390/nu9040341

30. Wood LG, Shivappa N, Berthon BS, Gibson PG, Hebert JR. Dietary inflammatory index is related to asthma risk, lung function and systemic inflammation in asthma. *Clin Exp Allerg.* (2015) 45:177–83. doi: 10.1111/cea.12323

31. Camargo-Ramos CM, Correa-Bautista JE, Correa-Rodríguez M, Ramírez-Vélez R. Dietary inflammatory index and cardiometabolic risk parameters in overweight and sedentary subjects. *Int J Environ Res Public Health.* (2017) 14:1104. doi: 10.3390/ijerph14101104

32. Wood LG, Gibson PG. Dietary factors lead to innate immune activation in asthma. *Pharmacol Ther.* (2009) 123:37–53. doi: 10.1016/j.pharmthera.2009.03.015

33. Seyedrezazadeh E, Moghaddam MP, Ansarin K, Vafa MR, Sharma S, Kolahdooz F. Fruit and vegetable intake and risk of wheezing and asthma: a systematic review and meta-analysis. *Nutr Rev.* (2014) 72:411–28. doi: 10.1111/nure.12121

34. Carvalho CA, Silva AAM, Assunção MCF, Fonseca PCA, Barbieri MA, Bettiol H, et al. The dietary inflammatory index and insulin resistance or metabolic syndrome in young adults. *Nutrition.* (2019) 58:187–93. doi: 10.1016/j.nut.2018.07.014

35. Ren Z, Zhao A, Wang Y, Meng L, Szeto IM-Y, Li T, et al. Association between Dietary Inflammatory Index, C-reactive protein and metabolic syndrome: a cross-sectional study. *Nutrients.* (2018) 10:831. doi: 10.3390/nu10070831

36. Christensen MA, Smoak P, Lisano JK, Hayward R, Coronado C, Kage K, et al. Cardiorespiratory fitness, visceral fat, and body fat, but not dietary inflammatory index, are related to C-reactive protein in cancer survivors. *Nutr Health.* (2019) 25:195–202. doi: 10.1177/02601060198 41840

37. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol.* (1985) 122:51–65. doi: 10.1093/oxfordjournals.aje.a114086

38. Han E, Kim G, Hong N, Lee YH, Kim DW, Shin HJ, et al. Association between dietary acid load and the risk of cardiovascular disease: nationwide surveys (KNHANES 2008-2011). *Cardiovasc Diabetol.* (2016) 15:122. doi: 10.1186/s12933-016-0436-z

39. Battino M, Forbes-Hernández TY, Gasparrini M, Afrin S, Cianciosi D, Zhang J, et al. Relevance of functional foods in the Mediterranean diet: the role of olive oil, berries and honey in the prevention of cancer and cardiovascular diseases. *Crit Rev Food Sci Nutr.* (2019) 59:893–920. doi: 10.1080/10408398.2018.1526165

40. Hejazi E, Emamat H, Sharafkhah M, Saidpour A, Poustchi H, Sepanlou S, et al. Dietary acid load and mortality from all causes, CVD and cancer: results from the Golestan Cohort study. *Br J Nutr.* (2022) 128:237–43. doi: 10.1017/S0007114521003135

41. Sotos-Prieto M, Bhupathiraju SN, Mattei J, Fung TT Li Y, Pan A, et al. Changes in diet quality scores and risk of cardiovascular disease among US men and women. *Circulation.* (2015) 132:2212–9. doi: 10.1161/CIRCULATIONAHA.115.017158

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Association between visceral adiposity index and kidney stones in American adults: A cross-sectional analysis of NHANES 2007–2018

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Objective: To explore the association between Visceral Adiposity Index (VAI) and kidney stones in an American adult population.

Materials and methods: National Health and Nutrition Examination Survey (NHANES) datasets from 2007 to 2018 were used. Participants aged ≥20 years who reported kidney stone history and VAI were included. Weighted proportions, multivariable analysis, generalized additive model (GAM), and spline smoothing were used to evaluate the associations between VAI and kidney stones by adjusting gender, age, race, education, marital status, poverty income ratio, smoking, alcohol, high blood pressure, diabetes, congestive heart failure, cancer, vigorous activity, moderate activity, HEI2015 total score, and energy.

Results: Totally 13,871 American adults were included. All the participants were divided by the VAI into four groups according to the quartile: Q1 (11.96–42.89), Q2 (42.90–74.45), Q3 (74.45–131.43), and Q4 (131.45–611.34). The mean ± standard deviation of the VAI in the four groups were Q1 (29.07 ± 8.22), Q2 (57.53 ± 8.81), Q3 (99.52 ± 16.25), and Q4 (225.92 ± 95.83). In the fully adjusted multivariable model, VAI was positively correlated with urolithiasis [odds ratio (OR) = 1.001; 95% confidence interval (CI) 1.000–1.001]. Compared with the first quartile of VAI, the population in the fourth quartile of VAI had a higher prevalence of kidney stones (OR = 1.329; 95% CI 1.104–1.600). Subgroup analysis detected no significant interaction effect after adjusting for covariates.

Conclusion: The value of VAI is positively correlated with the prevalence of kidney stones, which suggest VAI can be used to assess the potential risk of the prevalence of kidney stones.

KEYWORDS

VAI, NHANES, kidney stones, association, cross-sectional analysis

Introduction

Urinary calculi are relatively common in urinary system–related diseases. The incidence of urinary calculi is about 1–20% and increases year by year (1). Survey data from eight countries show that the annual incidence of kidney stones is about 114/100,000–720/100,000, and the prevalence is about 1.7–14.8% (2). Unfortunately, the causes for the high incidence and recurrence rates of urinary calculi are not clear yet, which indirectly leads to difficulties in the prevention and treatment of urinary calculi. The kidney as an important organ in the body retains or excretes most of the body's metabolites through the filtration and reabsorption function of renal tubular epithelial cells. Disorders of metabolic balance in the body and damages to renal tubular epithelial cells caused by local accumulation of metabolites may be important causes for the formation of urinary calculi (3).

With the changes in dietary structure and daily lifestyle, obesity has gradually become a major problem that plagues human health. Obesity not only brings about body fat accumulation and affects body appearance but also can cause visceral fat accumulation and affect the functions of the corresponding organs. Obesity is related to most of the chronic diseases in the body and is a risk factor for hyperuricemia (4). In addition to relevant cardiovascular diseases caused by obesity, excessive body fat accumulation may increase uric acid production in the serum and inhibit its excretion, eventually leading to uric acid metabolism disorder and affecting kidney function (5). The obesity-caused changes in body mass index (BMI) and waist circumference are positively associated with the risk of kidney stone hospitalization, and the enlarged waist circumference is an independent risk factor for increased risk of kidney stone hospitalization (2).

Visceral Adiposity Index (VAI), a new index used to assess the visceral fat level, is used clinically to assess organ fat. VAI evaluates visceral fat levels by combining waist circumference, BMI, triglycerides, and high-density lipoprotein (6). VAI is closely related to hyperuricemia regardless of the type of metabolic obesity (5). In patients with type 2 diabetes, VAI is also connected with urinary albumin. Compared with the triglyceride/high-density lipoprotein-cholesterol (TG/HDL-c) ratio, VAI has a similar predictive power for the risk of albuminuria (7). VAI is a fast and reliable indicator for evaluating early kidney injury in patients with type 2 diabetes (8). Studies have reported that VAI is also strongly associated with CKD and BPH (9, 10).

The calculation of VAI includes TG, WC, HDL, and BMI. These components are closely related to metabolism, and these components or metabolism are closely related to kidney stones (2, 11). As a comprehensive index containing these components, VAI is closely related to metabolism. Nevertheless, there is limited evidence about the relationship between VAI and kidney stones. Therefore, we investigated the association between VAI and nephrolithiasis by analyzing National Health and Nutrition Examination Survey (NHANES) cross-sectional data. We hypothesize that lower VAI is associated with a lower prevalence of kidney stones.

Materials and methods

Study design and participants

The NHANES conducted by the Centers for Disease Control and Prevention (CDC) is a research project that evaluates the health and nutritional status of Americans participating in the survey through interviews, examinations, and laboratory research. The design, data collection procedures, sample weights, and informed consents are elaborated at the National Center for Health Statistics (NCHS), from which related data are publicly available (12).

We extracted data from the NHANES database at 6 consecutive periods from 2007 to 2018 (the period from 1999 to 2006 that did not include issues such as urinary stones or visceral fat assessment was excluded). We initially selected 59,842 participants and included 34,770 participants over the age of 20 years. First, after pregnant participants (n = 372) and participants with incomplete kidney stone questionnaires (n = 91) were excluded, there were 34,307 participants left. Then by removing those with empty VAI (n = 20,155), 14,152 participants were left. Finally, 13,871 participants were included for analysis after removing outliers (less than 1% and more than 99% of data were treated as outliers). All six consecutive periods of NHANES research projects included were approved by the Research Ethics Review Committee of NCHS.

Outcome and exposure factor

The main outcome indicator was whether the participant had urinary calculi. By limiting the research time between 2007 and 2018, we screened out the questionnaires related to urinary calculi in the NHANES system that had answer "Yes" to the question "Have you/Has sample person (SP) ever had kidney stones."

The major exposure factor was the VAI, and its value was used as the primary variable. VAI was calculated based on the sex-specific mathematical model: $\mathrm{VAI} = \left(\frac{\mathrm{WC}}{36.58+(1.89 \times \mathrm{BMI})}\right) \times \left(\frac{\mathrm{TG}}{0.81}\right) \times \left(\frac{1.52}{\mathrm{HDL}}\right)$ for women, and $\mathrm{VAI} = \left(\frac{\mathrm{WC}}{39.68+(1.88 \times \mathrm{BMI})}\right) \times \left(\frac{\mathrm{TG}}{1.03}\right) \times \left(\frac{1.31}{\mathrm{HDL}}\right)$ for men (13). The values of VAI calculated thereby are listed in **Table 1**. The VAI mainly reflects the visceral fat content of the body. A higher VAI means a larger visceral fat content and predicts a higher incidence of cardiovascular diseases.

TABLE 1 Characteristics of participants divided by quartile of VAI: NHANES 2007–2018.*†

Characteristics	Q1 (11.96–42.89)	Q2 (42.90–74.45)	Q3 (74.45–131.43)	Q4 (131.45–611.34)
VAI (mean ± SD)	29.07 ± 8.22	57.53 ± 8.81	99.52 ± 16.25	225.92 ± 95.83
Age (years, mean ± SD)	45.13 ± 17.44	48.32 ± 17.07	48.72 ± 16.50	49.81 ± 15.37
20–34 (%)	35.08	25.88	23.23	19.12
35–49 (%)	24.57	27.06	29.25	30.28
50–64 (%)	23.51	27.10	27.52	31.58
≥65 (%)	16.84	19.96	20.00	19.02
Gender (%)				
Male	49.43	50.69	47.57	47.26
Female	50.57	49.31	52.43	52.74
Race (%)				
Mexican American	5.63	8.17	10.09	10.97
Other Hispanic	4.99	5.98	6.90	6.28
Non-Hispanic white	66.83	66.63	65.20	69.51
Non-Hispanic black	13.24	11.65	9.83	6.22
Other races	9.31	7.56	7.98	7.03
Poverty Income Ratio (mean ± SD)	3.18 ± 1.64	3.06 ± 1.66	2.89 ± 1.62	2.80 ± 1.63
≤1.3 (%)	17.44	19.50	20.51	23.59
>1.3 and ≤3.5 (%)	31.19	31.97	36.75	34.13
>3.5 (%)	44.08	41.73	35.51	35.13
Missing (%)	7.28	6.79	7.23	7.15
Energy (kcal)	2217.36 ± 972.27	2153.39 ± 938.39	2135.70 ± 1003.54	2153.53 ± 974.02
HEI2015_TOTAL_SCORE	52.96 ± 14.23	51.09 ± 13.99	49.23 ± 13.57	48.73 ± 12.84
Education (%)				
Less than 9th grade	3.72	5.05	5.95	7.45
9–11th grade	7.98	10.12	11.36	13.39
High school graduate	19.77	22.01	24.35	25.51
Some college	28.36	31.58	32.01	31.02
College graduate or above	40.16	31.24	26.33	22.64
Marital Status (%)				
Married	54.44	55.51	55.83	58.54
Widowed	4.28	5.27	5.38	6.80
Divorced	8.26	11.14	11.49	11.40
Separated	1.65	2.47	2.72	2.36
Never married	22.60	17.38	16.35	13.17
Living with partner	8.78	8.24	8.23	7.72
Smoking (%)				
<100 cigarettes in life	59.87	57.20	52.48	50.86
≥100 cigarettes in life	40.13	42.80	47.52	49.14
Alcohol (%)				
<12 drinks/year	14.45	17.78	17.11	21.22
≥12 drinks/year	58.17	60.37	60.09	58.40
Missing	27.38	21.85	22.79	20.38
High Blood Pressure (%)				
No	79.79	68.72	64.15	54.78
Yes	20.21	31.28	35.85	45.22
Diabetes (%)				
No	94.76	91.23	86.50	79.39
Yes	3.68	6.57	11.38	17.03
Missing	1.57	2.20	2.12	3.58

(Continued)

TABLE 1 (Continued)

Congestive Heart Failure (%)				
No	98.55	98.22	97.35	96.36
Yes	1.45	1.78	2.65	3.64
Cancer (%)				
No	90.93	90.43	90.73	88.48
Yes	9.07	9.57	9.27	11.52
Vigorous activity (%)				
No	62.54	72.10	79.78	85.45
Yes	37.46	27.90	20.22	14.55
Moderate activity (%)				
No	45.92	53.32	55.96	63.08
Yes	54.08	46.68	44.04	36.92
Kidney Stones (%)				
No	93.22	90.79	88.71	87.39
Yes	6.78	9.21	11.29	12.61

*Mean ± SD for continuous variables, and *p* value calculated by weighted *t*-test.
†% for categorical variables, and *p* value calculated by weighted Chi-square test.

Covariates

To make the association between kidney stones and VAI robust, we adjusted the following covariates: age, gender, race, marital status, education, poverty income ratio (PIR), smoking, alcohol; vigorous activity, moderate activity, HEI2015 total score, energy, and some self-reported medical conditions (all classified as yes/no). Age was categorized as 20–34, 35–49, 50–64, and >65 years. Races included Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, and other races. PIR was set at ≤1.3, >1.3 and ≤3.5, >3.5. Smoking was classified as <100 or ≥100 cigarettes in life. Alcohol drinking was defined as <12 or ≥12 alcohol drinks per year. Marital status was divided into married, widowed, divorced, separated, never married, and living with a partner. Education level included less than 9th grade, 9th–11th grade, high school graduate, some college, and college graduate or above. The medical conditions included diabetes, high blood pressure (HBP), congestive heart failure (CHF), and cancer. Dummy variables were used to indicate missing covariate values for variables with more than 2% missing data.

Statistical analyses

In NHANES, sampling weights are often used to account for more complex study designs. The interview and test weights recommended by the CDC guidelines[1] were used (12, 14–16). Continuous variables were presented as mean ± standard deviation (SD), while categorical variables were expressed as proportions. Analytical comparisons were performed using a weighted *t*-test and Chi-square test.

The association of VAI with nephrolithiasis was analyzed using logistic regression models with or without adjustment for various potential confounders. Model 1 was not adjusted. Model 2 was adjusted for age, gender, and race. Model 3 was adjusted for gender, age, race, education, marital, PIR, smoking, alcohol, HBP, diabetes, CHF, cancer, HEI2015 total score, energy, vigorous activity, and moderate activity. To better explore the association between VAI and kidney stones, we conducted multivariable logistic regression with VAI as a continuous and categorical variable (divided into quarters). The trends were estimated by treating VAI quartiles as a continuous variable. We treated less than 1% and more than 99% of data as outliers and excluded. Then, we further analyzed whether there was a nonlinear association between VAI and the risk of kidney stones by using generalized additive model (GAM) and curve fitting. If yes, a two-piecewise linear regression model was conducted to calculate the threshold effect of the VAI on kidney stones in terms of the smoothing plot and used a recursive method to automatically calculate the infection point, where the maximum model likelihood was used. Finally, subgroup analyses were performed using hierarchical logistic regression models for all potential confounders listed in the baseline table.

We used the statistical software packages R[2] (the R Foundation) and EmpowerStats[3] (X&Y Solutions Inc.) in all analyses. Two-tailed *p* values <0.05 were considered significant.

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

2 http://www.R-project.org

3 http://www.empowerstats.com

Results

Baseline characteristics

A total of 13,871 American adults were included, and divided into four quartiles according to VAI: Q1 (11.96–42.89), Q2 (42.90–74.45), Q3 (74.45–131.43), and Q4 (131.45–611.34). The mean ± SD of the VAI in the four groups are Q1 (29.07 ± 8.22), Q2 (57.53 ± 8.81), Q3 (99.52 ± 16.25), and Q4 (225.92 ± 95.83). Table 1 shows the baseline characteristics of different VAI groups. According to the VAI categories, kidney stones ever accounted for 6.78, 9.21, 11.29, and 12.61% in groups Q1, Q2, Q3, and Q4, respectively.

Multivariate regression analysis

The multivariate regression analyses with different adjustments for the effect of confounders on the correlation showed that VAI was positively correlated with kidney stones in model 1 [OR (95%CI) = 1.002 (1.001–1.003)], model 2 [1.002 (1.001–1.002)], and model 3 [1.001 (1.000–1.001)]. Besides, compared to Q1, the participants in group Q4 (131.45–611.34) had a significantly increased risk of developing kidney stones in model 1 [1.879 (1.589–2.222)], model 2 [1.1.644 (1.384–1.953)], and model 3 [1.329 (1.104–1.600)]. *P* for trend in all three models was less than.05 (Table 2).

Nonlinear analysis

The association between VAI and kidney stones was investigated by using GAM, smooth curve fitting, and piecewise linear regression (Table 3 and Figure 1). Figure 1 demonstrated the results of the fully adjusted model. We treated less than 1% and more than 99% of data as outliers and excluded. The plotting revealed that VAI and kidney stone incidence were under a curve relationship. With the increase of VAI, the risk of developing nephrolithiasis increased parabolically and leveled off gradually after VAI reached a certain value. Then, we further conducted piecewise linear regression to find the point of infection (Table 3). When VAI was <75.130, each unit increase in VAI increased 5‰ risk of developing kidney stones [1.005 (1.001–1.009)]. When VAI was >75.130, the risk of kidney stones was steady [1.000 (1.000–1.001)]. *P* for likelihood-ratio test was less than 0.05. These results indicate that the association between VAI and kidney stones is nonlinear.

TABLE 3 Results of binary logistic regression and piecewise linear regression.*

Outcome: kidney stones	Adjusted OR (95% CI)	*p* value
Fitting by binary logistic regression model	1.001 (1.000, 1.001)	0.0082
Fitting by piecewise linear regression model		
Inflection point	75.130	
VAI < 75.130	1.005 (1.001, 1.009)	0.0084
VAI > 75.130	1.000 (1.000, 1.001)	0.3078
Log likelihood ratio test	0.025	

*All models were adjusted for: gender; age; race; education; marital; PIR; smoking; alcohol; HBP; diabetes; CHF; cancer; vigorous activity; moderate activity, energy, and HEI2015 total score.

Subgroup or interaction analyses

According to the piecewise linear regression analysis, when VAI was <75.130, VAI was positively correlated with the risk of kidney stones (p = 0.0084) and the relationship was approximately linear. We performed subgroup and interaction analyses with VAI as a categorical variable (divided into quarters), for the part of VAI < 75.130, with model 3. The plotting uncovered that in subgroup analysis, compared Q4 with Q1, no significant interaction effect was detected after adjusting for covariates (Figure 2). Same results were found in models 1 and 2 (Supplementary Figures 1, 2).

TABLE 2 Association of VAI with kidney stones.

Exposure	Model 1*	Model 2†	Model 3‡
VAI (continuous)	1.002 (1.001, 1.003) <0.00001	1.002 (1.001, 1.002) <0.00001	1.001 (1.000, 1.001) 0.00818
Quartile of VAI			
Q1 (4.20–42.09)	Ref	Ref	Ref
Q2 (42.09–73.63)	1.347 (1.129, 1.608) 0.00095	1.247 (1.042, 1.491) 0.01587	1.173 (0.973, 1.414) 0.09432
Q3 (73.64–130.54)	1.661 (1.400, 1.970) < 0.00001	1.501 (1.262, 1.787) < 0.00001	1.322 (1.101, 1.588) 0.00280
Q4 (130.56–611.34)	1.879 (1.589, 2.222) < 0.00001	1.644 (1.384, 1.953) < 0.00001	1.329 (1.104, 1.600) 0.00263
P for trend	< 0.00001	<0.00001	0.00164

*Model 1: not adjusted.
†Model 2: adjusted for gender; age; race.
‡Model 3: adjusted for gender; age; race; education; marital; PIR; smoking; alcohol; HBP; diabetes; CHF; cancer; vigorous activity; moderate activity, energy, and HEI2015 total score.

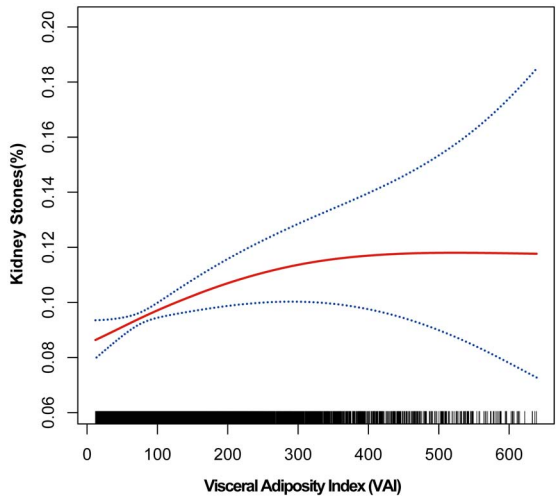

FIGURE 1
Smooth curve fitting of kidney stones and VAI Smooth curve fitting was performed using GAM to explore the association between kidney stones and VAI.

Discussion

We investigated the association between VAI and the risk of developing kidney stones by analyzing large population data in NHANES. Results demonstrate that kidney stones are closely related to VAI, showing a positive correlation. In other words, higher VAI was associated with a higher prevalence of kidney stones. For those with VAI <75.130, each unit increase in VAI is associated with a 5‰ increase in the risk of kidney stones. For those with VAI >75.130, however, increasing VAI shows a trend toward an increased risk of kidney stones, although not significant. Therefore, we present a close relationship between the increase in VAI and the higher risk of kidney stones. VAI may be a practical indicator for clinical assessment of the risk of kidney stones.

As an indicator of the visceral fat level in the body, VAI can more accurately assess the visceral fat content, which is closely related to the health status of most human bodies. Visceral fat accumulation indirectly reflects the abnormality of matter and energy metabolism in the body. Compared with indicators such as BMI and WC, VAI has a stronger predictive ability for metabolic disorders (5). Reportedly, VAI has the potential value of identifying metabolic disorder syndromes (17). VAI even plays an important role in assessing ED, lung function, cardiovascular diseases, diabetes, chronic kidney disease (CKD), and the degree of hepatic steatosis (10, 13, 18–21).

Kidney stones are a multifactorial disease. Reportedly, disturbance of Ca^{2+} level in the body caused by abnormal parathyroid function can exacerbate the formation of kidney stones (22, 23). In addition to the above factors, metabolism plays an important role in the formation of kidney stones. Some scholars believe that diet structure and diet type are inseparable from the formation of kidney stones. Adhering to normal BMI, drinking enough water, eating more fruits and vegetables, eating more low-fat dairy products and adequate calcium intake, avoiding regular sugar-sweetened beverages, and maintaining a healthy lifestyle all can help reduce the incidence of kidney stones rate over 50% (24). Metabolic syndrome, characterized by various metabolic disorders in the body, can increase urinary calcium, uric acid, and oxalate excretion, and reduce urinary citrate excretion, leading to the formation of calcium oxalate and uric acid stones (25–27). Obesity is often a manifestation of abnormal lipid metabolism in the body, and a higher proportion of fat is positively associated with a higher risk of kidney stones in both men and women (28, 29). At the same time, different lipoprotein levels in the body affect different stone types, such as the significant prevalence of uric acid stones caused by high-density lipoprotein and triglyceride levels (11). Animal experiments show that peritubular fat accumulation can increase the accumulation of local pro-inflammatory adipocytokines and macrophages, thereby increasing the formation of kidney stones (30). In addition, the formation of kidney stones was significantly increased in a mouse model after the lipid metabolism-related protein fatty acid binding protein 4 (FABP4) was knocked out (31).

Due to the high prevalence and recurrence rates, kidney stones have brought enormous economic pressure to the health prevention and treatment system. Problems such as renal colic and renal function damage that accompany kidney stones have greatly challenged human health. Therefore, the prevention of kidney stones is an indispensable part of the current health prevention and treatment system. This study is based on a large population-based analysis of the association between VAI and

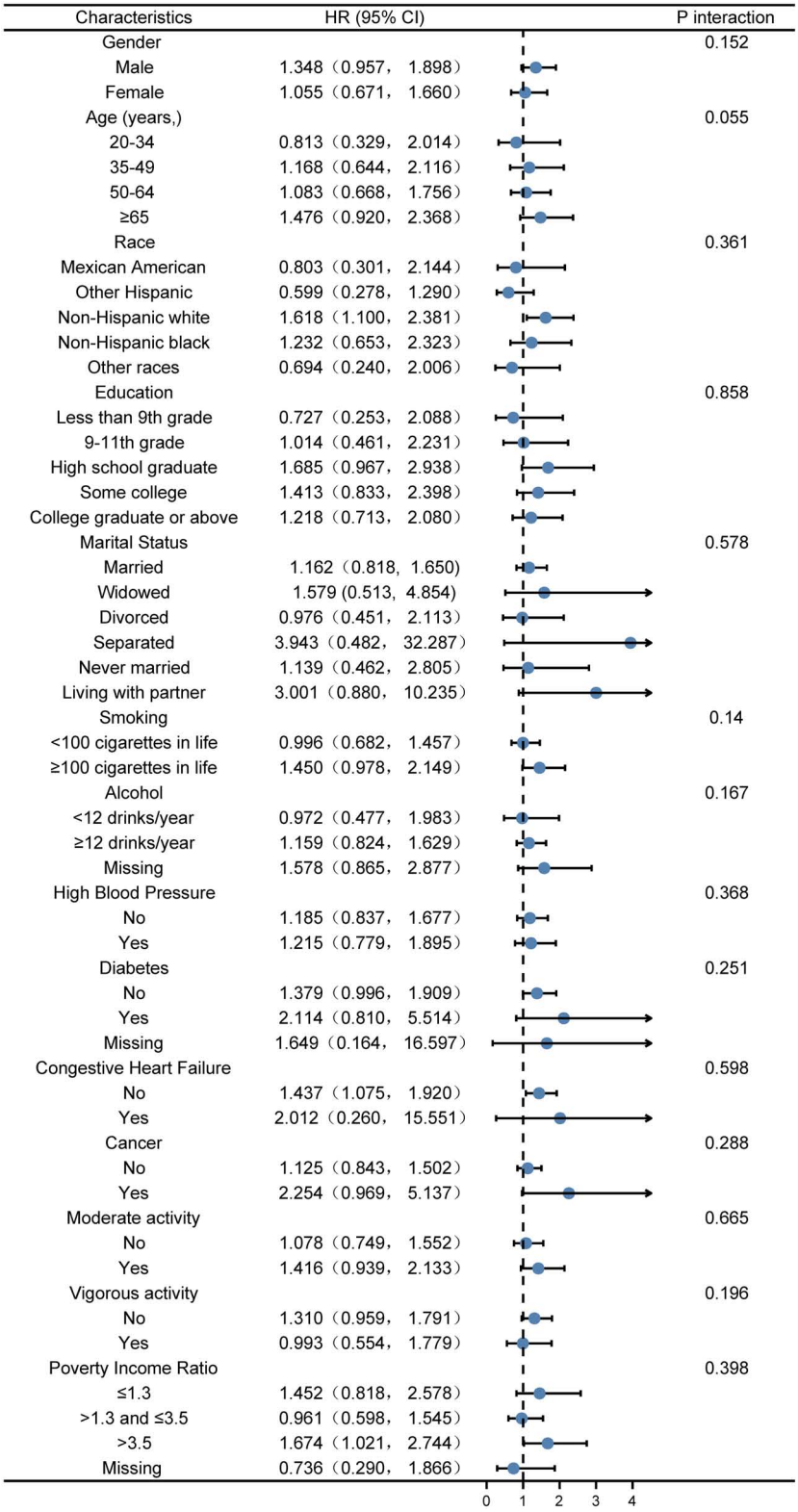

FIGURE 2
Stratified logistic regression analysis to identify variables that modify the correlation between VAI and kidney stones A subgroup and interaction analyses with VAI as a categorical variable (divided into quarters), for the part of VAI <75.130, compared Q4 with Q1. Adjusted for gender, age, race, poverty income ratio, education, marital status, smoking, alcohol, vigorous activity, moderate activity, diabetes, HBP, CHF, cancer, energy, and HEI2015 total score. The model is not adjusted for the variable itself in each stratification.

kidney stone prevalence. Results show a certain relationship between VAI and the prevalence of kidney stones, which theoretically supports the use of VAI to assess the potential risk of developing kidney stones in the body. However, the study still has certain limitations. First, due to the cross-sectional study design, we cannot draw a causal relationship between VAI and kidney stone prevalence. Furthermore, despite the adjustment for some potential confounders, we still cannot completely rule out the confounding caused by some unknown variables.

Conclusion

Visceral Adiposity Index is inversely associated with the prevalence of kidney stones, suggesting that maintaining a lower VAI is related to a lower risk of kidney stones. VAI may be a practical indicator for clinical assessment of the risk of kidney stones. Nevertheless, more high-quality prospective studies are needed to clarify the underlying mechanism between VAI and nephrolithiasis.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the National Center for Health Statistics (NCHS) Research Ethics Review Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JW and JHW: conceptualization and methodology. SY, JHW, and ZZY: data acquisition. JHW, SY, YJB, ZZY, JWC, and YFX: software and formal analysis. JHW and SY: writing—original draft. JW: data curation and supervision. All authors: writing—review and editing and read and approve the final manuscript.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.994669/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Stratified logistic regression analysis to identify variables that modify the correlation between VAI and kidney stones. A subgroup and interaction analyses with VAI as a categorical variable (divided into quarters), for the part of VAI <75.130, compared Q4 with Q1. Not adjusted.

SUPPLEMENTARY FIGURE 2
Stratified logistic regression analysis to identify variables that modify the correlation between VAI and kidney stones. A subgroup and interaction analyses with VAI as a categorical variable (divided into quarters), for the part of VAI <75.130, compared Q4 with Q1. Adjusted for gender; age and race. The model is not adjusted for the variable itself in each stratification.

References

1. Duan X, Kong Z, Mai X, Lan Y, Liu Y, Yang Z, et al. Autophagy inhibition attenuates hyperoxaluria-induced renal tubular oxidative injury and calcium oxalate crystal depositions in the rat kidney. *Redox Biol.* (2018) 16:414–25. doi: 10.1016/j.redox.2018.03.019

2. Wang H, Fan JN, Yu CQ, Guo Y, Pei P, Chen JS, et al. [Associations of body mass index and waist circumference with hospitalization risk of kidney stones in Chinese adults]. *Zhonghua Liu Xing Bing Xue Za Zhi.* (2022) 43:154–61. doi: 10.3760/cma.j.cn112338-20210311-00201

3. O'Kell AL, Grant DC, Khan SR. Pathogenesis of calcium oxalate urinary stone disease: species comparison of humans, dogs, and cats. *Urolithiasis.* (2017) 45:329–36. doi: 10.1007/s00240-017-0978-x

4. Song P, Wang H, Xia W, Chang X, Wang M, An L. Prevalence and correlates of hyperuricemia in the middle-aged and older adults in China. *Sci Rep.* (2018) 8:4314. doi: 10.1038/s41598-018-22570-9

5. Kielstein JT, Pontremoli R, Burnier M. Management of hyperuricemia in patients with chronic kidney disease: a focus on renal protection. *Curr Hypertens Rep.* (2020) 22:102. doi: 10.1007/s11906-020-01116-3

6. Dong H, Xu Y, Zhang X, Tian S. Visceral adiposity index is strongly associated with hyperuricemia independently of metabolic health and obesity phenotypes. *Sci Rep.* (2017) 7:8822. doi: 10.1038/s41598-017-09455-z

7. Li R, Li Q, Cui M, Yin Z, Li L, Zhong T, et al. Clinical surrogate markers for predicting metabolic syndrome in middle-aged and elderly Chinese. *J Diabetes Invest.* (2018) 9:411–8. doi: 10.1111/jdi.12708

8. Wei J, Liu X, Xue H, Wang Y, Shi Z. Comparisons of visceral adiposity index, body shape index, body mass index and waist circumference and their associations with diabetes mellitus in adults. *Nutrients.* (2019) 11:1580. doi: 10.3390/nu11071580

9. Bamba R, Okamura T, Hashimoto Y, Hamaguchi M, Obora A, Kojima T, et al. The visceral adiposity index is a predictor of incident chronic kidney disease: a population-based longitudinal study. *Kidney Blood Press Res.* (2020) 45:407–18. doi: 10.1159/000506461

10. Besiroglu H, Ozbek E, Dursun M, Otunctemur A. Visceral adiposity index is associated with benign prostatic enlargement in non-diabetic patients: a cross-sectional study. *Aging Male.* (2018) 21:40–7. doi: 10.1080/13685538.2017.1365833

11. Torricelli FC, De SK, Gebreselassie S, Li I, Sarkissian C, Monga M. Dyslipidemia and kidney stone risk. *J Urol.* (2014) 191:667–72. doi: 10.1016/j.juro.2013.09.022

12. Chen TC, Clark J, Riddles MK, Mohadjer LK, Fakhouri T. National Health and Nutrition Examination Survey, 2015-2018: sample design and estimation procedures. *Vital Health Stat 2.* (2020) 184:1–35.

13. He S, Yang J, Li X, Gu H, Su Q, Qin L. Visceral adiposity index is associated with lung function impairment: a population-based study. *Respir Res.* (2021) 22:2. doi: 10.1186/s12931-020-01599-3

14. Chen TC, Parker JD, Clark J, Shin HC, Rammon JR, Burt VL. National Health and Nutrition Examination Survey: estimation procedures, 2011-2014. *Vital Health Stat 2.* (2018) 177:1–26.

15. Mirel LB, Mohadjer LK, Dohrmann SM, Clark J, Burt VL, Johnson CL, et al. National Health and Nutrition Examination Survey: estimation procedures, 2007-2010. *Vital Health Stat 2.* (2013) 159:1–17.

16. Johnson CL, Dohrmann SM, Vd K, Diallo MS, Clark J, Mohadjer LK, et al. National Health and Nutrition Examination Survey: national youth fitness survey estimation procedures, 2012. *Vital Health Stat 2.* (2014) 168:1–25.

17. Vizzuso S, Del Torto A, Dilillo D, Calcaterra V, Di Profio E, Leone A, et al. Visceral Adiposity Index (VAI) in children and adolescents with obesity: no association with daily energy intake but promising tool to identify metabolic syndrome (MetS). *Nutrients.* (2021) 13:413. doi: 10.3390/nu13020413

18. Dursun M, Besiroglu H, Cakir SS, Otunctemur A, Ozbek E. Increased visceral adiposity index associated with sexual dysfunction in men. *Aging Male.* (2018) 21:187–92. doi: 10.1080/13685538.2017.1406468

19. Choi MH, Choi JI, Park MY, Rha SE, Oh SN, Jung SE, et al. Validation of intimate correlation between visceral fat and hepatic steatosis: quantitative measurement techniques using CT for area of fat and MR for hepatic steatosis. *Clin Nutr.* (2018) 37:214–22. doi: 10.1016/j.clnu.2016.12.006

20. Wang J, Jin X, Chen K, Yan W, Wang A, Zhu B, et al. Visceral adiposity index is closely associated with urinary albumin-creatinine ratio in the Chinese population with prediabetes. *Diabetes Metab Res Rev.* (2021) 37:e3424. doi: 10.1002/dmrr.3424

21. Murai J, Nishizawa H, Otsuka A, Fukuda S, Tanaka Y, Nagao H, et al. Low muscle quality in Japanese type 2 diabetic patients with visceral fat accumulation. *Cardiovasc Diabetol.* (2018) 17:112. doi: 10.1186/s12933-018-0755-3

22. Bilezikian JP, Bandeira L, Khan A, Cusano NE. Hyperparathyroidism. *Lancet.* (2018) 391:168–78. doi: 10.1016/S0140-6736(17)31430-7

23. Bergwitz C, Miyamoto KI. Hereditary hypophosphatemic rickets with hypercalciuria: pathophysiology, clinical presentation, diagnosis and therapy. *Pflugers Arch.* (2019) 471:149–63. doi: 10.1007/s00424-018-2184-2

24. 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

25. Kohjimoto Y, Sasaki Y, Iguchi M, Matsumura N, Inagaki T, Hara I. Association of metabolic syndrome traits and severity of kidney stones: results from a nationwide survey on urolithiasis in Japan. *Am J Kidney Dis.* (2013) 61:923–9. doi: 10.1053/j.ajkd.2012.12.028

26. Sakhaee K, Maalouf NM. Metabolic syndrome and uric acid nephrolithiasis. *Semin Nephrol.* (2008) 28:174–80. doi: 10.1016/j.semnephrol.2008.01.010

27. Mulay SR, Honarpisheh MM, Foresto-Neto O, Shi C, Desai J, Zhao ZB, et al. Mitochondria permeability transition versus necroptosis in oxalate-induced AKI. *J Am Soc Nephrol.* (2019) 30:1857–69. doi: 10.1681/ASN.2018121218

28. Abufaraj M, Siyam A, Xu T, Imm K, Cao C, Waldoer T, et al. Association between body fat mass and kidney stones in US adults: analysis of the National Health and Nutrition Examination Survey 2011-2018. *Eur Urol Focus.* (2022) 8:580–7. doi: 10.1016/j.euf.2021.03.010

29. 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

30. Taguchi K, Okada A, Hamamoto S, Iwatsuki S, Naiki T, Ando R, et al. Proinflammatory and metabolic changes facilitate renal crystal deposition in an obese mouse model of metabolic syndrome. *J Urol.* (2015) 194:1787–96. doi: 10.1016/j.juro.2015.07.083

31. 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

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Longitudinal association of dietary acid load with kidney function decline in an older adult population with metabolic syndrome

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Background: Diets high in acid load may contribute to kidney function impairment. This study aimed to investigate the association between dietary acid load and 1-year changes in glomerular filtration rate (eGFR) and urine albumin/creatinine ratio (UACR).

Methods: Older adults with overweight/obesity and metabolic syndrome (mean age 65 ± 5 years, 48% women) from the PREDIMED-Plus study who had available data on eGFR ($n = 5,874$) or UACR ($n = 3,639$) at baseline and after 1 year of follow-up were included in this prospective analysis. Dietary acid load was estimated as potential renal acid load (PRAL) and net endogenous acid production (NEAP) at baseline from a food frequency questionnaire. Linear and logistic regression models were fitted to evaluate the associations between baseline tertiles of dietary acid load and kidney function outcomes. One year-changes in eGFR and UACR were set as the primary outcomes. We secondarily assessed ≥ 10% eGFR decline or ≥10% UACR increase.

Results: After multiple adjustments, individuals in the highest tertile of PRAL or NEAP showed higher one-year changes in eGFR (PRAL, β: −0.64 ml/min/1.73 m^2; 95% CI: −1.21 to −0.08 and NEAP, β: −0.56 ml/min/1.73 m^2; 95% CI: −1.13 to 0.01) compared to those in the lowest category. No associations with changes in UACR were found. Participants with higher levels of PRAL and NEAP had significantly higher odds of developing ≥10% eGFR decline (PRAL, OR: 1.28; 95% CI: 1.07–1.54 and NEAP, OR: 1.24; 95% CI: 1.03–1.50) and ≥10 % UACR increase (PRAL, OR: 1.23; 95% CI: 1.04–1.46) compared to individuals with lower dietary acid load.

Conclusions: Higher PRAL and NEAP were associated with worse kidney function after 1 year of follow-up as measured by eGFR and UACR markers in an older Spanish population with overweight/obesity and metabolic syndrome.

KEYWORDS

kidney function, chronic kidney disease (CKD), glomerular filtration rate (GFR), net endogenous acid production (NEAP), potential renal acid load (PRAL), dietary acid load, albuminuria, renal nutrition

Introduction

Impaired renal function is a common condition in older individuals with comorbidities as diabetes, hypertension or obesity that usually predicts the onset of Chronic Kidney Disease (CKD) (1). In the last few years, there has been a growing concern about this disease since it has a huge impact worldwide affecting around 700 million people (2). In addition, CKD is linked to several complications, such as cardiovascular events, hospitalization and/or premature death (2, 3). Consequently, appropriate and affordable prevention measures are required to preserve renal function, especially in high-risk populations (1). Prevention measures could also reduce the severe impact of CKD on the wellbeing of individuals and health systems (3–5).

Dietary habits appear to be one of the major modifiable risk factors markedly influencing renal impartment and its progression to CKD (5, 6). Additionally, the role of diet in preserving the acid-base balance of the body has recently become more relevant, given the emerging evidence linking dietary acid load with the development of different chronic diseases (7, 8), including CKD (9). It has been previously documented that healthy dietary patterns provide an alkaline environment in the body (10, 11) since plant-based food such as vegetables, fruit and some nuts or legumes have the capacity of inducing a basic environment (12). However, red and processed meats as well as ultra-processed foods are acid-producing (9, 12). Thus, these foods might be implied in the onset of a low-grade metabolic acidosis state, thereby, resulting in faster progression of kidney disease (11, 13). Overall, potential renal acid load (PRAL) and net endogenous acid production (NEAP) are the most common and suitable indexes used to estimate the acid load of the diet (9, 11). Considering the aforementioned evidence, following a healthy diet characterized by a low acid load may be a useful preventive strategy against kidney dysfunction.

To date, results from epidemiological studies focused on dietary acid load and kidney function or CKD development are inconsistent (9) and this relationship needs to be further explored. In some studies, an association between higher levels of PRAL and/or NEAP indexes and an estimated-glomerular filtration rate (eGFR) decline or higher risk of incident CKD (14–18) has been reported, but others have observed no such associations (19, 20). Also, the quality of evidence is moderate as most of the studies were mainly cross-sectional (14–17, 21, 22), and only a few were longitudinal studies (18–20). Furthermore, since most research has been conducted in healthy young or middle-aged individuals or in patients with advanced CKD, little is known about the potentially harmful association between dietary acid load and kidney function of older populations with underlying comorbid conditions. In addition, analyses assessing dietary acid load on kidney function have rarely been conducted in Mediterranean populations at high cardiovascular risk. Hence, as more scientific evidence and longitudinal studies in this field are required, we prospectively investigated the association between PRAL and NEAP and 1-year changes in two markers of kidney function decline, eGFR and Urine Albumin/Creatinine Ratio (UACR), in a large Spanish cohort of older adults with overweight/obesity and metabolic syndrome (MetS).

Materials and methods

Study population and design

The present study is a prospective analysis of baseline and 1-year data within the framework of the PREvención con DIeta MEDiterránea (PREDIMED)-Plus trial. Briefly, the PREDIMED-Plus is an ongoing, parallel-group, randomized and controlled clinical trial aiming to assess the effect of an intensive weight loss intervention on cardiovascular disease (CVD) morbidity and mortality. An energy-restricted Mediterranean diet (MedDiet), physical activity promotion and behavioral support are compared to usual care advice in 6,874 older adults enrolled between 2013 and 2016 by 23 Spanish recruitment centers. Eligible participants were

Abbreviations: BMI, Body Mass Index; CKD, Chronic Kidney Disease; CI, Confidence Interval; E, Energy; FFQ, Food Frequency Questionnaire; GFR, Glomerular Filtration Rate; MedDiet, Mediterranean Diet; MetS, Metabolic Syndrome; METS, Metabolic Equivalent Task; NEAP, Net Endogenous Acid Production; PRAL, Potential Renal Acid Load; PREDIMED, Prevención con Dieta Mediterránea; SCr, Serum Creatinine; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration equation for Caucasian individuals; OR, Odds Ratios; UACR, Urine Albumin/Creatinine Ratio.

men aged 55–75 years and women aged 60–75 years with overweight or obesity [Body Mass Index (BMI) 27–40 kg/m^2], who satisfied at least 3 criteria for the MetS (23). Further details of the inclusion and exclusion criteria and the study design have been described elsewhere (24). A detailed explanation of the protocol is also available at https://www.predimedplus.com. This trial was registered on the International Standard Randomized Controlled Trial registry (https://www.isrctn.com/ISRCTN89898870) with number 89898870 in July of 2014. The final study protocol and procedures were approved following the standards of the Declaration of Helsinki by the Institutional Review Boards of participating centers and all participants provided written informed consent.

For the current study, participants without completed food frequency questionnaire (FFQ) information and reporting implausible total energy intake (men $<$ 800 and $>$4,000 kcal/day and women $<$ 500 and $>$3,500 kcal/day) at baseline were excluded (n = 227) from the analyses (25). We also excluded participants who died (n = 11) or were lost to follow-up (n = 16) during the first year. Moreover, participants with missing data on eGFR (n = 746) or UACR (n = 2,981) at baseline and/or at the 1-year assessment were excluded when eGFR or UACR were the outcomes, respectively. Therefore, a final sample of 5,874 participants for eGFR and 3,639 participants for UACR were analyzed (Supplementary Figure 1).

Assessment of dietary intake and dietary acid load

To evaluate dietary intake, trained dieticians administered a 143-item FFQ, based on a previously validated one for the Spanish population (26), in face-to-face interviews at baseline. Each participant was asked about their frequency of consumption during the preceding year of each specific item, which had nine possible answers ranging from never to more than 6 times per day. The typical portion size of each item was subsequently transformed into grams or milliliters per day, as appropriate. Two Spanish food composition databases were referenced to calculate total daily energy and nutrient intake (27, 28).

Dietary acid load was estimated at baseline using individual nutritional data obtained from the FFQ. Previously published methods proposed by Remer and Manz (29) and Frassetto et al. (8) were applied for the calculation of PRAL and NEAP scores, respectively. PRAL (mEq/day) = 0.4888 × protein intake (g/day) + 0.0366 × phosphorus (mg/day) − 0.0205 × potassium (mg/day) − 0.0125 × calcium (mg/day) − 0.0263 × magnesium (mg/day). NEAP (mEq/day) = 54.5 × protein (g/day)/potassium (mEq/day) − 10.2.

Ascertainment of the outcome

Serum creatinine (SCr) levels and urinary creatinine and albumin concentrations were determined using routine laboratory methods from blood and spot morning urine samples collected at baseline and 1-year following overnight fasting. For the current study, 1-year changes in eGFR and UACR were considered our primary outcomes. We indirectly determined eGFR from SCr using the Chronic Kidney Disease Epidemiology Collaboration equation for Caucasian individuals (CKD-EPI) (30) and the UACR was calculated by dividing urine albumin (mg/l) by urine creatinine concentrations (mg/l). UACR values were truncated at 500 mg/g to minimize the influence of outliers. There were 21 observations $>$ 500 mg/g at baseline and 24 at 1 year that were $>$500 mg/g and subsequently set to 500 mg/g. One-year changes in both eGFR and UACR were calculated by subtracting values at 1 year minus values at baseline. Secondary outcomes were $\geq$10% eGFR decline and $\geq$10% UACR increase following a 1-year follow-up. These were estimated by applying the formula: [(1-year eGFR or UACR − baseline eGFR or UACR)/baseline eGFR or UACR]*100. Participants were categorized as those with a $\geq$10% or $<$10% eGFR decline (31) or with a $\geq$10% or $<$10% increase in UACR.

Covariate assessment

At baseline, trained PREDIMED-Plus staff collected socio-demographic and lifestyle information including age, sex, educational level, physical activity, smoking status, as well as medication use and history of disease using several questionnaires or reviewing medical records. Moreover, adherence to the energy-reduced MedDiet was evaluated using a validated 17-item MedDiet questionnaire (32). Compliance with each item of the MedDiet questionnaire was scored with one point and non-compliance with 0. Thereafter, a cut-off point based on the median of the score was determined by dividing individuals into those with high adherence to a MedDiet ($\geq$9 points) or a low adherence ($<$9 points). Moreover, other cut-off points were tested arbitrarily and defined as the highest tertiles or quartiles (in both cases high adherence was observed to be $\geq$12 points). Total daily energy intake and sodium intake were estimated according to data from the FFQ. Anthropometric variables were measured in duplicate and resting blood pressure was measured in triplicate using an automated digital device (Omron-HEM297705C). BMI was calculated as weight in kilograms divided by the square of height in meters. In our analysis, white blood cell count was used to assess inflammation (leucocytes $>$ 10 × 10^9/L).

Statistical analyses

For the present report, we used the PREDIMED-Plus database generated in December 2020. Participants were categorized into tertiles of PRAL and NEAP. One-way ANOVA and chi-square tests were used to evaluate differences among tertiles of PRAL and NEAP for the baseline characteristics of the study population. Descriptive data were expressed as means ± SD for continuous variables and percentages (%) and numbers for categorical variables.

Multivariate linear regression models were performed to examine the associations between tertiles of PRAL and NEAP at baseline and 1-year changes in eGFR (ml/min/1.73 m^2) and UACR (mg/g). For these associations, PRAL and NEAP were also analyzed as continuous variables (both for each 1-SD increase). β-coefficients and 95% confidence intervals (CIs) were assessed using two different models: Model 1 was adjusted for sex and age; and Model 2 was further adjusted for study center (categorized into quartiles by number of participants), intervention group (treatment/control), BMI (kg/m^2), smoking status (never/current/former smoker), educational level (primary education/secondary education/graduate), leisure-time physical activity (METs/min/week, tertiles), diabetes prevalence (yes/no), hypertension prevalence (yes/no), hypercholesterolemia prevalence (yes/no), angiotensin-converting enzyme inhibitors (ACEis) (yes/no), angiotensin II receptor blockers (ARBs) (yes/no), MedDiet adherence (high/low adherence), energy intake (kcal/day, tertiles), sodium intake (mg/day, tertiles) and high leukocyte levels (yes/no). Moreover, odds ratios (OR) and their 95% CIs were calculated for the association between tertiles of NEAP and PRAL and ≥10% eGFR decline and ≥10% UACR increase at 1-year of follow-up adjusting for the same confounders as mentioned in model 2. The first tertile was used as a reference category in all regression models. Additionally, linear regression models were further adjusted for baseline eGFR (ml/min/1.73 m^2) or baseline UACR (mg/g) depending on the main outcome. Variance inflation factors (VIFs) were used to assess collinearity for the multivariable models and, as VIFs were <2.5, none of the covariates needed to be removed. All analyses were conducted with robust estimates of the variance to correct for possible intra-cluster correlation. Intra-cluster was defined as the participants who shared the same household. To assess the linear trend, the median value of each tertile of PRAL and NEAP were modeled as continuous variables.

We also conducted subgroup analyses for the 1-year changes in eGFR and UACR stratifying by baseline categories of eGFR (≥90; 60–90; <60 ml/min/1.73 m^2) and UACR (<30; ≥30 mg/g). Interaction between tertiles of PRAL and NEAP with categories of eGFR, UACR, and energy-reduced MedDiet adherence (high/low), as well as the intervention/control group were checked in the fullest multivariable model using likelihood ratio tests and non-significant results were observed. In a sensitivity analysis, we repeated our main analysis investigating the association between PRAL and NEAP with 1-year changes in eGFR and UACR after excluding individuals with eGFR < 60 ml/min/1.73 m^2 or with UACR > 300 mg/g at baseline. In addition, as a supplementary analysis, we evaluated the association between dietary acid load and ≥5% eGFR decline and ≥5% UACR increase following the same procedure mentioned previously. Statistical analyses were conducted using Stata/SE software, version 14.0 (StataCorp, College Station, TX) and significance level was set at a 2-tailed $p < 0.05$.

Results

Table 1 shows the baseline characteristics of the study population according to tertiles of PRAL and NEAP. In general, participants with higher values of PRAL and NEAP at baseline were more likely to be younger, men, have a higher BMI, smoke, have a higher educational level, and were less likely to exercise. Participants in the highest tertiles of PRAL and NEAP also had higher levels of creatinine and eGFR than those in the lowest tertile. In terms of mediations, participants in the highest tertiles of PRAL and NEAP were more likely to have used insulin, ACEis treatment, and took less antihypertensive and ARB drugs. Furthermore, individuals in the highest tertile of NEAP were more likely to have type 2 diabetes. However, no significant differences were observed between tertiles of PRAL nor NEAP regarding the UACR or CKD. Concerning dietary assessment, adherence to an energy-reduced MedDiet was lower in individuals with higher dietary acid load levels than those in the lowest tertile of PRAL and NEAP. Moreover, participants in the highest tertile of PRAL and NEAP had a lower intake of vegetable/animal protein ratio, carbohydrates and fiber while they had a higher energy, protein and fat consumption than those with low values of both dietary acid load indexes. Similar trends were observed when baseline consumption of food groups across tertiles of PRAL and NEAP were analyzed (Supplementary Table 1). Supplementary Table 2 presents further information regarding macronutrient and micronutrient intake, especially those related to dietary acid load, at 1-year of follow-up. Baseline characteristics according to included and excluded participants from the eGFR or UACR analyses are described in Supplementary Table 3.

The association (β-coefficient; 95% CI) between tertiles of PRAL and NEAP and 1-year changes in eGFR and UACR are displayed in Table 2. In the most adjusted model, PRAL showed a significant inverse association with 1-year changes in eGFR (β: −0.17 ml/min/1.73 m^2; 95% CI: −0.71 to 0.36 for T2 vs. T1, β: −0.64 ml/min/1.73 m^2; 95% CI: −1.21 to −0.08 for T3 vs. T1). We found similar results when PRAL and NEAP were analyzed as continuous variables (PRAL: β: −0.25 ml/min/1.73 m^2; 95% CI: −0.47 to −0.03 for each

TABLE 1 Baseline characteristics of the study population with data on eGFR at 1-year follow-up by tertiles of PRAL and NEAP (n = 5,874).

		PRAL (mEq/d)				NEAP (mEq/d)			
	Total n = 5,874	**T1** n = 1,958	**T2** n = 1,958	**T3** n = 1,958	p-value	**T1** n = 1,958	**T2** n = 1,958	**T3** n = 1,958	p-value
PRAL, mEq/day	−5.4 ± 15.6	–	–	–	–	−21.4 ± 11.3	−5.0 ± 5.4	10.1 ± 8.7	<0.01
NEAP, mEq/day	36.9 ± 8.1	29.0 ± 3.8	36.4 ± 2.8	45.6 ± 6.0	<0.01	–	–	–	–
Age, years	65.0 ± 4.9	65.7 ± 4.7	65.1 ± 5.0	64.2 ± 4.9	<0.01	65.7 ± 4.7	65.3 ± 4.9	64.1 ± 4.9	<0.01
Women, % (n)	48.0 (2,818)	52.7 (1, 31)	49.1 (961)	42.2 (826)	<0.01	56.0 (1,097)	48.9 (957)	39.0 (764)	<0.01
Intervention group, % (n)	49.4 (2,901)	49.3 (966)	50.3 (984)	48.6 (951)	0.57	50.0 (978)	49.9 (976)	48.4 (947)	0.54
BMI, kg/m^2	32.5 ± 3.4	32.4 ± 3.4	32.4 ± 3.4	32.8 ± 3.5	<0.01	32.3 ± 3.4	32.5 ± 3.4	32.7 ± 3.5	<0.01
PA, METS/min/week	2,528.0 ± 2,350.4	2,740.2 ± 2,483.6	2,526.2 ± 2,342.2	2,317.7 ± 2,198.8	<0.01	2,681.7 ± 2,434.1	2,547.4 ± 2,373.8	2,355.1 ± 2,228.2	<0.01
Smoking status, % (n)					<0.01				<0.01
Never smoked	44.4 (2,605)	47.9 (939)	45.5 (891)	39.6 (775)		49.9 (976)	44.7 (875)	38.5 (754)	
Former smoker	43.0 (2,528)	40.3 (789)	42.3 (828)	46.5 (911)		38.6 (756)	43.0 (842)	47.5 (930)	
Current smoker	12.6 (741)	11.8 (230)	12.2 (239)	13.9 (272)		11.5 (226)	12.3 (241)	14.0 (274)	
Education level, % (n)					<0.01				<0.01
Primary education	49.22 (2,891)	54.9 (1,075)	49.2 (963)	43.6 (853)		54.0 (1,058)	50.0 (979)	44.6 (854)	
Secondary education	29.18 (1,714)	25.2 (494)	28.9 (565)	33.4 (655)		25.6 (501)	28.3 (555)	33.6 (658)	
College/university	21.60 (1,269)	19.9 (374)	22.0 (430)	23.0 (450)		20.4 (399)	21.7 (424)	22.8 (446)	
Creatinine	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	<0.01	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	<0.01
eGFR, ml/min/1.73 m^2	84.2 ± 13.9	83.6 ± 13.6	84.7 ± 13.9	84.3 ± 14.4	0.04	83.5 ± 13.6	84.9 ± 13.7	84.1 ± 14.5	<0.01
UACR, mg/g	16.8 ± 48.9	16.2 ± 45.0	16.8 ± 50.0	17.5 ± 51.5	0.78	16.4 ± 46.4	15.9 ± 47.0	18.2 ± 53.1	0.42
CKD, % (n)	4.4 ± 3.7	6.5 (126)	6.5 (128)	6.8 (133)	0.79	6.6 (129)	5.8 (113)	7.6 (145)	0.10
Type 2 diabetes, % (n)	30.6 (1,797)	28.9 (567)	30.7 (601)	32.1 (629)	0.10	28.8 (564)	30.4 (595)	32.6 (638)	0.04
Hypertension, % (n)	84.1 (4,941)	85.1 (1,666)	84.5 (1,654)	82.8 (1,621)	0.13	85.1 (1,667)	84.4 (1,653)	82.8 (1,621)	0.12
Hypercholesterolemia, % (n)	69.7 (4,096)	69.4 (1,359)	69.2 (1,356)	70.5 (1,381)	0.63	70.2 (1,375)	69.3 (1,356)	69.7 (1,365)	0.80
Hypertriglyceridemia, % (n)*	39.7 (2,327)	40.7 (795)	39.1 (763)	42.5 (831)	0.10	40.8 (795)	39.2 (765)	42.4 (829)	0.13
Low HDL, % (n)^y	40.8 (2,389)	38.5 (751)	39.4 (768)	41.3 (808)	0.18	39.1 (763)	39.0 (760)	41.1 (804)	0.30

(Continued)

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TABLE 1 (Continued)

		PRAL (mEq/d)				NEAP (mEq/d)			
	Total $n = 5,874$	**T1** $n = 1,958$	**T2** $n = 1,958$	**T3** $n = 1,958$	***p*-value**	**T1** $n = 1,958$	**T2** $n = 1,958$	**T3** $n = 1,958$	***p*-value**
Medication use, % (*n*)	51.8 (3, 42)	52.6 (1, 30)	51.7 (1, 13)	51.0 (999)	0.66	52.8 (1, 35)	51.9 (1, 17)	50.6 (990)	0.35
Lipid-lowering drugs									
Oral blood glucose-lowering drugs	26.0 (1,528)	25.2 (494)	26.3 (516)	26.5 (518)	0.62	25.3 (495)	25.7 (504)	27.0 (529)	0.44
Insulin treatment	4.1 (239)	3.5 (68)	3.7 (73)	5.0 (98)	0.03	3.2 (64)	4.1 (81)	4.8 (94)	0.05
Antihypertensive drugs	78.7 (4,625)	81.7 (1,599)	77.9 (1,525)	76.7 (1,501)	<0.01	81.0 (1,585)	79.1 (1,549)	76.2 (1,491)	<0.01
ARBs	36.3 (2,131)	39.6 (776)	34.9 (683)	34.3 (672)	<0.01	39.5 (774)	35.2 (689)	34.1 (668)	<0.01
ACEis	30.2 (1,775)	28.6 (559)	31.2 (611)	30.9 (605)	0.11	27.9 (546)	32.0 (624)	30.9 (605)	0.02
Dietary assessment	8.5 ± 2.7	9.2 ± 2.6	8.4 ± 2.7	7.9 ± 2.5	<0.01	9.3 ± 2.6	8.6 ± 2.6	7.7 ± 2.5	<0.01
erMedDiet score, 17-points									
Energy intake, kcal/d	2,370.5 ± 548.9	2,366.1 ± 537.9	2,303.1 ± 531.8	2,442.3 ± 567.8	<0.01	2,277.5 ± 531.2	2,278.6 ± 533.1	2,455.7 ± 562.5	<0.01
Protein intake, % energy	16.7 ± 2.8	16.1 ± 2.6	16.7 ± 2.7	17.4 ± 3.0	<0.01	16.1 ± 2.7	16.9 ± 2.7	17.3 ± 2.9	<0.01
Vegetal /animal protein ratio, g/d	0.5 ± 0.2	0.67 ± 0.27	0.56 ± 0.19	0.48 ± 0.17	<0.01	0.68 ± 0.28	0.56 ± 0.19	0.49 ± 0.17	<0.01
Fat intake, % energy	39.6 ± 6.5	38.4 ± 6.4	39.7 ± 6.4	40.8 ± 6.5	<0.01	38.5 ± 6.5	39.5 ± 6.3	40.9 ± 6.5	<0.01
Carbohydrate intake, % energy	40.5 ± 6.8	42.4 ± 6.6	40.4 ± 6.5	38.7 ± 6.8	<0.01	42.4 ± 6.8	40.5 ± 6.4	38.6 ± 6.8	<0.01
Fiber intake, g/day	26.1 ± 8.7	30.4 ± 9.1	25.2 ± 7.8	22.8 ± 7.5	<0.01	29.9 ± 9.5	26.4 ± 7.9	22.2 ± 7.0	<0.01
Potassium intake, mg/day	4,477.0 ± 1,079.6	5,108.6 ± 1,124.3	4,313.1 ± 898.2	4,009.2 ± 884.4	<0.01	4,953.4 ± 1,189.3	4,501.7 ± 924.5	3,975.8 ± 866.0	<0.01
Calcium intake, mg/day	1,034.0 ± 347.0	1,062.8 ± 353.6	999.2 ± 327.9	1,040.1 ± 355.9	<0.01	1,030.0 ± 350.5	1,049.6 ± 337.1	1,022.5 ± 352.7	0.04
Magnesium intake, mg/day	420.4 ± 108.2	457.7 ± 112.5	407.6 ± 102.2	396.0 ± 99.3	<0.01	446.2 ± 117.8	425.2 ± 102.5	389.8 ± 95.6	<0.01
Phosphorus intake, mg/day	1,759.1 ± 419.9	1,750.1 ± 429.1	1,703.8 ± 401.9	1,823.5 ± 419.7	<0.01	1,713.1 ± 438.3	1,783.5 ± 403.4	1,780.8 ± 413.6	<0.01
Sodium intake, mg/day	2,430.0 ± 774.8	2,272.5 ± 736.8	23,183.0 ± 679.8	2,699.4 ± 828.6	<0.01	2,187.8 ± 712.4	2,412.4 ± 689.3	2,689.6 ± 832.1	<0.01

ACEis, Angiotensin-Converting Enzyme Inhibitors; ARBs, Angiotensin II receptor blockers; eGFR, Estimated Glomerular Filtration Rate; erMedDiet, energy-restricted Mediterranean diet; HDL, High-Density Lipoprotein; NEAP, Net Endogenous Acid Production; MET, Metabolic Equivalent of Task T, tertile; BMI, Body Mass Index; PRAL, Potential Renal Acid Load; PA, Physical activity; eGFR, estimated Glomerular Filtration Rate; CKD, Chronic Kidney Disease (eGFR< 60 ml/min/1.73 m^2); UACR, Urine Albumin/Creatinine Ratio. Values are presented as percentages (n) for categorical variables and means ± standard deviations for continuous variables. P-value was calculated by chi-square or one-way analysis of variance test for categorical and continuous variables, respectively.

[*] Fasting triglyceride concentration ≥150 mg/dL or specific treatment for lipid abnormality.

[y] HDL concentration <40 mg/dL in men and <50 mg/dL in women or specific treatment for lipid abnormality.

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1-SD increment. NEAP: β: −0.28 ml/min/1.73 m^2; 95% CI: −0.51 to −0.05 for each 1-SD increment). Results remained essentially the same after adding 1-year BMI change to the most adjusted model (data not shown). PRAL and NEAP were not significantly associated with UACR changes after 1-year of follow-up after modeling them as tertiles, nor as continuous variables. In the sensitivity analyses, excluding individuals with <60 ml/min/1.73 m^2 of eGFR or with >300 mg/g of UACR did not modify the main findings for both outcomes (data not shown). When we repeated the principal analyses, stratifying by baseline categories of eGFR (≥90; 60–90; <60 ml/min/1.73 m^2) and UACR (<30; ≥30 mg/g), the results presented a similar tendency (Supplementary Table 4). In participants with eGFR ≥ 90 ml/min/1.73 m^2, significant associations were observed with eGFR changes when both dietary acid load indexes were modeled as continuous variables (PRAL: β: −0.28 ml/min/1.73 m^2; 95% CI: −0.56 to −0.01 for each 1-SD increment. NEAP: β: −0.31 ml/min/1.73 m^2; 95% CI: −0.58 to −0.03 for each 1-SD increment). The main analysis was repeated using other cut-offs points for the MedDiet score confounding factor (i.e., ≥12 points for high adherence) and similar results were found (Supplementary Table 5). We also explored the interactions between tertiles of PRAL and NEAP and the adherence to energy-reduced MedDiet, categories of eGFR and UACR, as well as intervention/control group, and no statistically significant findings were observed (all interactions, $p > 0.05$).

Figure 1 depicts the OR and 95% CI for ≥10% eGFR decline and ≥10% UACR increase according to tertiles of PRAL and NEAP. After multiple adjustments, participants in the highest tertile of PRAL and NEAP were significantly more likely to have a ≥10% eGFR decline after 1 year of follow up compared to those in the lowest tertile, with ORs of 1.28 (95% CI: 1.07–1.54) for PRAL and 1.24 (95% CI: 1.03–1.0) for NEAP. When PRAL and NEAP were modeled as continuous variables (per each 1-SD increment) higher ORs were also observed. Compared to participants with low PRAL values at baseline, participants with the highest levels had a 23% (95% CI: 1.04–1.46) higher odds of ≥10% UACR increase after 1 year of follow-up after adjusting for potential confounders. No significant associations were found between NEAP and the odds of ≥10% UACR increase or for 1-SD increment of PRAL and NEAP. When a ≥5% eGFR decline and a ≥5% UACR increase were assessed, the same results were found (Supplementary Table 6).

Discussion

The results of this prospective study conducted in older Spanish adults at high cardiovascular disease risk suggest that PRAL and NEAP are inversely associated with 1-year changes in eGFR, but not with 1-year UACR changes. Furthermore, participants with higher levels of both estimates of dietary acid load had higher odds of a ≥10% eGFR decline, and those in the highest tertile of PRAL had 23% higher odds of a ≥10% UACR increase. GFR and albuminuria are the main complementary biomarkers used in epidemiological studies to assess kidney function (3). As far as we know, this is the first study to prospectively evaluate the association between dietary acid load and kidney function concurrently assessing eGFR and UACR in a population of older adults with underlying comorbidities.

A large body of evidence has linked dietary acid load with kidney outcomes in several studies (9). However, to the best of our knowledge, there are only four cross-sectional studies and one longitudinal study investigating the potential relationship of dietary acid load with renal function defined by eGFR and/or CKD in older adults without CKD. These cross-sectional studies conducted in different cohorts of adults reported that higher dietary acid load was associated with higher odds of CKD and/or impaired kidney function as indicated by low eGFR after adjusting for multiple confounders (14, 16, 17, 33). Our observations are in accordance with these cross-sectional studies since we observed a greater eGFR decline at 1 year with higher PRAL and NEAP scores, even after adjusting for baseline eGFR and other essential confounding factors. Interestingly, our supplementary stratified analyses according to categories of eGFR, which have seldom been performed in previous studies, revealed a similar non-significant tendency to worsen kidney function with increased dietary acid load. Consistent with our findings, the prospective analysis from the cohort of the Atherosclerosis Risk in Communities (ARIC) study of 15,055 apparently healthy middle-aged participants with preserved kidney function showed that higher levels of PRAL were associated with a 13% higher risk of CKD incidence over 21 years of follow-up (18).

Regarding albuminuria, which is considered a reliable marker of kidney damage (3), preceding studies have assessed its cross-sectional association with dietary acid load obtaining inconclusive findings. In The Jackson Heart Study, there was no association between estimated Net Acid Excretion (NAE_{es}) and albuminuria (16). In contrast, the NHANES study reported a positive association between dietary acid load and albuminuria in 12,293 healthy American adults (17). Additionally, the researchers from The Uonuma CKD Cohort Study also found that higher NEAP was associated with a higher UACR and risk of albuminuria among 6,684 middle-aged Japanese adults (21). To date, no large prospective cohort study has focused on the relationship between dietary acid load and albuminuria in vulnerable older adults. In the current study, we report no association between PRAL and NEAP scores and 1-year changes in UACR. This could suggest that high dietary acid load may promote tubule-interstitial injury rather than glomerular damage. Nevertheless, we were not able to check this tubular damage hypothesis since spot/24 h total proteinuria data were not available in our dataset (34). However, it is worthwhile to mention that when UACR was also assessed as an increase ≥ 10%

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TABLE 2 Multivariable-adjusted β-coefficients and 95% CI of 1-year changes in eGFR (ml/min/1.73 m^2) or in UACR (mg/g) across tertiles and per 1-SD increment of baseline PRAL and NEAP.

	PRAL (mEq/d)				
	T1	**T2**	**T3**	***p* for trend**	**Continuous (1 SD**)**
	***n* = 1,958**	***n* = 1,958**	***n* = 1,958**		***n* = 5,874**
Δ in eGFR, ml/min/1.73 m^2	−0.69 (−1.07 to −0.31)	−0.86 (−1.24 to −0.49)	−1.34 (−1.72 to −0.95)		
Model 1	0 (Ref.)	−0.16 (−0.70 to 0.37)	−0.52 (−1.06 to 0.03)	0.062	−0.21 (−0.42 to 0.01)
Model 2	0 (Ref.)	−0.17 (−0.71 to 0.36)	−0.64 (−1.21 to −0.08)*	0.026	−0.25 (−0.47 to −0.03)*
	***n* = 1,213**	***n* = 1,213**	***n* = 1,213**		***n* = 3,639**
Δ in UACR, mg/g	4.37 (1.96 to 6.78)	2.74 (0.60 to 4.88)	1.39 (−0.62 to 3.39)		
Model 1	0 (Ref.)	−1.20 (−4.32 to 1.93)	−2.31 (−5.28 to 0.66)	0.128	−0.88 (−2.00 to 0.25)
Model 2	0 (Ref.)	−1.63 (−4.84 to 1.58)	−2.99 (−6.34 to 0.37)	0.082	−1.22 (−2.51 to 0.08)
	NEAP (mEq/d)				
	***n* = 1,958**	***n* = 1,958**	***n* = 1,958**		***n* = 5,874**
Δ in eGFR, ml/min/1.73 m^2	−0.68 (−1.06 to −0.30)	−0.97 (−1.35 to −0.60)	−1.24 (−1.63 to −0.84)		
Model 1	0 (Ref.)	−0.28 (−0.81 to 0.25)	−0.44 (−0.99 to 0.11)	0.116	−0.22 (−0.44 to −0.01)*
Model 2	0 (Ref.)	−0.30 (−0.83 to 0.24)	−0.56 (−1.13 to 0.01)	0.056	−0.28 (−0.51 to −0.05)*
	***n* = 1,213**	***n* = 1,213**	***n* = 1,213**		***n* = 3,639**
Δ in UACR, mg/g	3.92 (1.49 to 6.34)	3.09 (1.14 to 5.03)	1.49 (−0.54 to 3.53)		
Model 1	0 (Ref.)	−0.81 (−3.82 to 2.20)	−1.96 (−5.07 to 1.15)	0.214	−0.93 (−2.13 to 0.28)
Model 2	0 (Ref.)	−0.83 (−3.87 to 2.21)	−2.42 (−5.79 to 0.95)	0.154	−1.26 (−2.63 to 0.10)

eGFR, Estimated glomerular filtration rate; *NEAP*, Net Endogenous Acid Production; *T*, tertile; *PRAL*, Potential Renal Acid Load; *UACR*, Urine albumin/creatinine ratio. Model 1: adjusted for age (years), sex and baseline eGFR or baseline UACR (in continuous, depending on the main outcome). Model 2: additionally adjusted for participating center (categorized into quartiles by number of participants), intervention group (treatment/control), body mass index (kg/m^2), smoking habits (never, current or former smoker), educational level (primary, secondary education or graduate), leisure-time physical activity (METS/min/week in tertiles), diabetes prevalence (yes/no), hypertension prevalence (yes/no) and hypercholesterolemia prevalence (yes/no), ARBs (yes/no), ACEis (yes/no), Mediterranean diet adherence (high/low adherence), energy intake (kcal/day in tertiles), sodium intake (mg/g in tertiles) and high leukocytes levels (yes/no).

*p-value < 0.05.

**One SD = 15.6 mEq/d in PRAL and 8.1 mEq/d in NEAP.

after 1 year of follow-up, which is a more clinical approach, we found a significant association with PRAL. Consequently, future longitudinal studies and clinical trials would be helpful to clarify these observations related to albuminuria and dietary acid load.

Overall, our findings in conjunction with the evidence available to date, suggests that following a diet with a low acid load could be an appropriate measure to improve renal function and, accordingly, decrease the risk of CKD development and progression among older individuals from middle-aged to elderly with underlying comorbid conditions.

The potential mechanisms by which high dietary acid load may induce kidney dysfunction are unclear, though possible mechanisms have been proposed for consideration. Acid retention has been proposed to activate the intracellular renin-angiotensin system, through the previous stimulation of aldosterone production, which might be implicated in the onset or progression of kidney damage (35, 36). Moreover, metabolic acidosis appears to contribute to endothelin-1 production, which in turn could be related to tubulointerstitial injury (37–39). Besides, high dietary acid load would also induce tubular toxicity activating the complement pathway and increasing renal medullary ammonia concentrations (40–42). There is also a high probability that acid retention increases the production of oxygen-free radicals and oxidative stress (43, 44). Consequently, it is crucial for kidney health to maintain appropriate levels of acid load, and diet may play an important role in this respect (11). It should be noted that in our study individuals with high levels of dietary acid load reported higher intakes of some food groups which have been directly or indirectly implicated in kidney function damage, such as total and animal protein intake (33, 45) or sugar and sweetened products (46). By contrast, as dietary acid load increased there was a lower consumption of fiber-rich foods, including fruits, vegetables, whole-grain cereals, and nuts. Thus, the potential beneficial effects of fiber on the kidney (47) could be lacking in those individuals with high dietary acid load.

This study has some limitations that deserve to be mentioned. First, the population consisted of older Spanish individuals at high cardiovascular risk, meaning the findings may not be generalizable to other populations. Furthermore, the Mediterranean lifestyle could imply healthier habits which, at the same time, may result in different macro- and

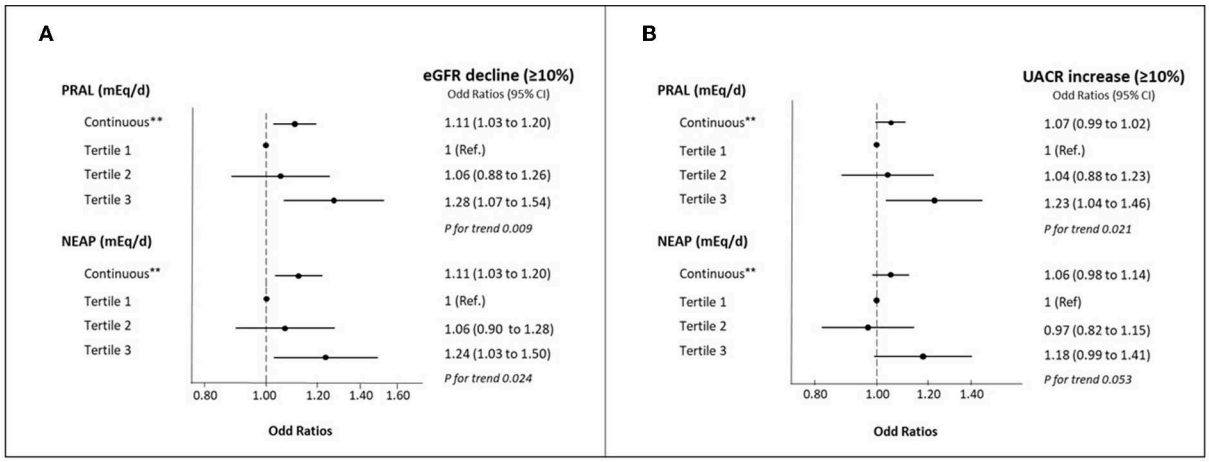

FIGURE 1
(A) Multivariable-adjusted OR (95% CIs) for ≥10% eGFR decline by tertiles of baseline PRAL and NEAP and per 1-SD increment. **(B)** Multivariable-adjusted OR (95% CIs) for ≥10% UACR increase by tertiles of baseline PRAL and NEAP and per 1-SD increment. eGFR, Estimated glomerular filtration rate; NEAP, Net Endogenous Acid Production; T, tertile; PRAL, Potential Renal Acid Load; UACR, Urine albumin/creatinine ratio. Percentage of participants with eGFR decline (>10%): tertile 1 of PRAL ($n = 296$; % = 15.1), tertile 2 of PRAL ($n = 304$; % = 15.5), tertile 3 of PRAL ($n = 346$; % = 17.7); tertile 1 of NEAP ($n = 297$; % = 15.2), tertile 2 of NEAP ($n = 312$; % = 15.9). tertile 3 of NEAP ($n = 337$; % = 17.2). Percentage of participants with UACR increase (>10%): tertile 1 of PRAL ($n = 539$; % = 44.4), tertile 2 of PRAL ($n = 547$; % = 45.1), tertile 3 of PRAL ($n = 597$; % = 49.2); tertile 1 of NEAP ($n = 550$; % = 45.3), tertile 2 of NEAP ($n = 539$; % = 44.4), tertile 3 of NEAP ($n = 594$; % = 49.0). All models were adjusted for age (years), sex, participating center (categorized into quartiles by number of participants), intervention group (treatment/control), body mass index (kg/m^2), smoking habits (never, current or former smoker), educational level (primary, secondary education or graduate), leisure-time physical activity (METS/min/week in tertiles), diabetes prevalence (yes/no), hypertension prevalence (yes/no) and hypercholesterolemia prevalence (yes/no), ARBs (yes/no), ACEis (yes/no), Mediterranean diet adherence (high/low adherence), energy intake (kcal/day in tertiles), sodium intake (mg/g in tertiles), and high leukocytes levels (yes/no). **one SD = 15.6 mEq/d in PRAL and 8.1 mEq/d in NEAP.

micronutrients intake related to kidney function, such as potassium-rich or low-sodium dietary intakes. Second, as PREDIMED-Plus is a randomized controlled trial, though, all the analyses were adjusted for the intervention group, the lifestyle advice that participants received could be affecting our findings. Third, dietary acid load was calculated using PRAL and NEAP from dietary nutrient intake information obtained from FFQ data. Although this questionnaire was validated and carefully administered by trained dietitians, potential measurement errors and reporting bias could be present. Fourth, while SCr-based eGFR was used as a biomarker of kidney function, as is common in most epidemiologic studies, there are other more optimal markers such as inulin, iothalamate or 24-h urinary creatinine clearance. Nevertheless, these procedures are expensive, time-consuming, and difficult measure in large populations. Finally, as in any observational study, although a substantial number of confounding factors were considered, confounding bias could not be completely ruled out and direct causality cannot be inferred. However, our study also has several strengths. Analyses were conducted using data from a large cohort, which has a wide selection of different variables to adjust the models for kidney function related-potential confounders. Moreover, it is important to highlight the prospective design that we performed and the joint assessment of two commonly used biomarkers of renal function. Another novel aspect of this study is the sensitivity and supplementary analyses conducted which gave robustness to the main results.

Conclusion

In conclusion, the current study conducted in a population of older Spanish adults with overweight/obesity and MetS shows that higher dietary acid load is associated with changes toward a worse eGFR and higher odds of ≥10% eGFR decline and ≥10% UACR increase. Nevertheless, further longitudinal and interventional studies are needed to clarify and confirm the consistency of these associations before considering a reduction in dietary acid load as part of strategies for preventing kidney function decline.

Data availability statement

There are restrictions on the availability of data for the PREDIMED-Plus trial, due to the signed consent agreements around data sharing, which only allow access to external researchers for studies following the project purposes. Requestors wishing to access the PREDIMED-Plus trial data used in this study can make a request to the PREDIMED-Plus trial Steering Committee chair:

predimed_plus_scommitte@googlegroups.com. The request will then be passed to members of the PREDIMED-Plus Steering Committee for deliberation.

Ethics statement

The studies involving human participants were reviewed and approved by the ethical standards of the Declaration of Helsinki by the Institutional Review Boards (IRBs). The patients/participants provided their written informed consent to participate in this study.

Author contributions

CV-H, NB-T, AD-L, ZV-R, IM, DC, AG, JM, ÁA-Gó, JW, JVio, DR, JL-M, RE, FT, JL, LS-M, AB-C, JT, VM-S, XP, JG, PM-M, JVid, AA-Ga, LD, ER, AG-A, RB, MF, PP-O, AA-A, EG-G, DM-U, MM, RC, EMG-G, LT-S, MD-F, EG, CO-A, OC, AG-R, CG-S, CS-O, HS, JS-S, and NB designed and conducted the research. CV-H and AD-L analyzed the data. CV-H, NB-T, AD-L, and NB wrote the article. CV-H and AD-L are the guarantors of this work and, as such, had 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 revised the manuscript for important intellectual content and read and approved the final manuscript.

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Conflict of interest

Author JS-S reported receiving research support from the Instituto de Salud Carlos III (ISCIII), Ministerio de Educación y Ciencia, Departament de Salut Pública de la Generalitat de Catalunya, the European Commission, the California Walnut Commission, Patrimonio Comunal Olivarero, La Morella Nuts, and Borges S.A; receiving consulting fees or travel expenses from California Walnut Commission, Eroski Foundation, Instituto Danone, Abbott Laboratories and Mundifarma, receiving non-financial support from Hojiblanca, Patrimonio Comunal Olivarero, and Almond Board of California; serving on the board of and receiving grant support through his institution from the International Nut and Dried Foundation

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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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.986190/full#supplementary-material

References

1. O'Sullivan ED, Hughes J, Ferenbach DA. Renal aging: causes and consequences. *J Am Soc Nephrol.* (2017) 28:407–20. doi: 10.1681/ASN.2015121308

2. GBD Chronic Kidney Disease Collaboration. Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* (2020) 395:709–33. doi: 10.1016/S0140-6736(20)30045-3

3. Levin A, Stevens PE, Bilous RW, Coresh J, De Francisco ALM, De Jong PE et al. Kidney disease: Improving global outcomes (KDIGO) CKD work group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl.* (2013) 3:1–150. doi: 10.1038/kisup.2012.73

4. Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, et al. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO)z. *Kidney Int.* (2005) 67:2089–100. doi: 10.1111/j.1523-1755.2005.00365.x

5. van Westing AC, Küpers LK, Geleijnse JM. Diet and kidney function: a literature review. *Curr Hypertens Rep.* (2020) 22:1–9. doi: 10.1007/s11906-020-1020-1

6. Abbate M, Mascaró CM, Montemayor S, Barbería-Latasa M, Casares M, Gómez C, et al. Energy expenditure improved risk factors associated with renal function loss in nafld and mets patients. *Nutrients.* (2021) 13:1–24. doi: 10.3390/nu13020629

7. Murakami K, Sasaki S, Takahashi Y, Uenishi K, Yamasaki M, Hisatomi Y, et al. Association between dietary acid-base load and cardiometabolic risk factors in young Japanese women. *Br J Nutr.* (2008) 100:642–51. doi: 10.1017/S0007114508901288

8. Frassetto LA, Todd KM, Morris RC, Sebastian A. Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. *Am J Clin Nutr.* (1998) 68:576–83. doi: 10.1093/ajcn/68.3.576

9. Mofrad MD, Daneshzad E, Azadbakht L. Dietary acid load, kidney function and risk of chronic kidney disease: a systematic review and meta-analysis of observational studies. *Int J Vitam Nutr Res.* (2021) 91:343–55. doi: 10.1024/0300-9831/a000584

10. Bach KE, Kelly JT, Campbell KL, Palmer SC, Khalesi S, Strippoli GFM. Healthy dietary patterns and incidence of CKD: a meta-analysis of cohort studies. *Clin J Am Soc Nephrol.* (2019) 14:1441–9. doi: 10.2215/CJN.00530119

11. Scialla JJ, Anderson CAM. Dietary acid load: a novel nutritional target in chronic kidney disease? *Adv Chronic Kidney Dis.* (2013) 20:141–9. doi: 10.1053/j.ackd.2012.11.001

12. Remer T. Influence of diet on acid-base balance. (2000) 13:221–6. doi: 10.1046/j.1525-139x.2000.00062.x

13. Kanda E, Ai M, Kuriyama R, Yoshida M, Shiigai T. Dietary acid intake and kidney disease progression in the elderly. *Am J Nephrol.* (2014) 39:145–52. doi: 10.1159/000358262

14. Mirmiran P, Yuzbashian E, Bahadoran Z, Asghari G, Azizi F. Dietary acid-base load and risk of chronic kidney disease in adults: Tehran lipid and glucose study. *Iran J Kidney Dis.* (2016) 10:119–25.

15. Ko BJ, Chang Y, Ryu S, Kim EM, Lee MY, Hyun YY, et al. Dietary acid load and chronic kidney disease in elderly adults: protein and potassium intake. *PLoS ONE.* (2017) 12:1–10. doi: 10.1371/journal.pone.0185069

16. Banerjee T, Tucker K, Griswold M, Wyatt SB, Harman J, Young B, et al. Dietary potential renal acid load and risk of albuminuria and reduced kidney function in the Jackson heart study. *J Ren Nutr.* (2018) 28:251–8. doi: 10.1053/j.jrn.2017.12.008

17. Banerjee T, Crews DC, Wesson DE, Tilea A, Saran R, Rios Burrows N, et al. Dietary acid load and chronic kidney disease among adults in the United States. *BMC Nephrol.* (2014) 15:1–12. doi: 10.1186/1471-2369-15-137

18. Rebholz CM, Coresh J, Grams ME, Steffen LM, Anderson CAM, Appel LJ, et al. Dietary acid load and incident chronic kidney disease: results from the ARIC study. *Am J Nephrol.* (2015) 42:427–35. doi: 10.1159/000443746

19. Scialla JJ, Appel LJ, Astor BC, Miller ER, Beddhu S, Woodward M, et al. Net endogenous acid production is associated with a faster decline in GFR in African Americans. *Kidney Int.* (2012) 82:106–12. doi: 10.1038/ki.2012.82

20. Scialla JJ, Asplin J, Dobre M, Chang A, Lash J, Kallem RR, et al. Higher net acid excretion is associated with lower risk of kidney disease progression in patients with diabetes. *Kidney Int.* (2018) 91:204–15. doi: 10.1016/j.kint.2016.09.012

21. Kabasawa K, Hosojima M, Takachi R, Nakamura K, Ito Y, Saito A, et al. Association of estimated dietary acid load with albuminuria in Japanese adults: a cross-sectional study. *BMC Nephrol.* (2019) 20:1–10. doi: 10.1186/s12882-019-1352-8

22. So R, Song S, Lee JE, Yoon HJ. The association between renal hyperfiltration and the sources of habitual protein intake and dietary acid load in a general population with preserved renal function: the KoGES study. *PLoS ONE.* (2016) 11:e0166495. doi: 10.1371/journal.pone.0166495

23. Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; National heart, lung, and blood institute; American heart association; World heart federation; International. *Circulation.* (2009) 120:1640–5. doi: 10.1161/CIRCULATIONAHA.109.192644

24. Martínez-González MA, Buil-Cosiales P, Corella D, Bulló M, Fitó M, Vioque J, et al. Cohort profile: design and methods of the PREDIMED-Plus randomized trial. *Int J Epidemiol.* (2019) 48:387–8o. doi: 10.1093/ije/dyy225

25. Willett WC, Howe R, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr.* (1997) 65:1220S—8S. doi: 10.1093/ajcn/65.4.1220S

26. Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, Toledo E, et al. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. *Br J Nutr.* (2010) 103:1808–16. doi: 10.1017/S0007114509993837

27. Moreiras O, Carvajal A, Cabrera L CC. *Tablas de composición de alimentos" Food Composition Tables" Pirámide*. Madrid: Pirámide (2005).

28. Mataix Verdú J. *Tabla de composición de alimentos [Food Composition Tables]*. Granada: Universidad de Granada (2003).

29. Remer T, Manz F. Potential renal acid load of foods and its influence on urine pH. *J Am Diet Assoc.* (1995) 95:791–7. doi: 10.1016/S0002-8223(95)00219-7

30. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro 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

31. Levey AS, Inker LA, Matsushita K, Greene T, Willis K, Lewis E, et al. GFR decline as an end point for clinical trials in CKD: a scientific workshop sponsored by the national kidney foundation and the US food and drug administration. *Am J Kidney Dis.* (2014) 64:821–35. doi: 10.1053/j.ajkd.2014.07.030

32. Schröder H, Zomeño MD, Martínez-González MA, Salas-Salvadó J, Corella D, Vioque J, et al. Validity of the energy-restricted mediterranean diet adherence screener. *Clin Nutr.* (2021) 40:4971–9. doi: 10.1016/j.clnu.2021.06.030

33. Jee Ko G, Obi Y, Tortorici AR, Kalantar-Zadeh K. Dietary protein intake and chronic kidney disease. *Curr Opin Clin Nutr Metab Care.* (2017) 20:77–85. doi: 10.1097/MCO.0000000000000342

34. Cravedi P, Remuzzi G. Pathophysiology of proteinuria and its value as an outcome measure in chronic kidney disease. *Br J Clin Pharmacol.* (2013) 76:516–23. doi: 10.1111/bcp.12104

35. Ng HY, Chen HC, Tsai YC, Yang YK, Lee C Te. Activation of intrarenal renin-angiotensin system during metabolic acidosis. *Am J Nephrol.* (2011) 34:55–63. doi: 10.1159/000328742

36. Remuzzi G, Perico N, Macia M, Ruggenenti P. The role of renin-angiotensin-aldosterone system in the progression of chronic kidney disease. *Kidney Int.* (2005) 68:57–65. doi: 10.1111/j.1523-1755.2005.09911.x

37. Khanna A, Simoni J, Hacker C, Duran MJ, Wesson DE. Increased endothelin activity mediates augmented distal nephron acidification induced by dietary protein. *J Am Soc Nephrol.* (2004) 15:2266–2275. doi: 10.1097/01.ASN.0000138233.78329.4E

38. Wesson DE, Simoni J, Broglio K, Sheather S. Acid retention accompanies reduced GFR in humans and increases plasma levels of endothelin and aldosterone. *Am J Physiol Ren Physiol.* (2011) 300:830–7. doi: 10.1152/ajprenal.00587.2010

39. Phisitkul S, Khanna A, Simoni J, Broglio K, Sheather S, Rajab MH, et al. Amelioration of metabolic acidosis in patients with low GFR reduced kidney endothelin production and kidney injury, and better preserved GFR. *Kidney Int.* (2010) 77:617–23. doi: 10.1038/ki.2009.519

40. Nath KA, Hostetter MK, Hostetter TH. Increased ammoniagenesis as a determinant of progressive renal injury. *Am J Kidney Dis.* (1991) 17:654–7. doi: 10.1016/S0272-6386(12)80344-1

41. Nath KA, Hostetter MK, Hostetter TH. Pathophysiology of chronic tubulo-interstitial disease in rats. *J Clin Investig.* (1985) 76:667–75. doi: 10.1172/JCI112020

42. Nagami GT, Hamm LL. Regulation of acid-base balance in chronic kidney disease. *Adv Chronic Kidney Dis.* (2017) 24:274–9. doi: 10.1053/j.ackd.2017.07.004

43. Irazabal M, Torres V. ROS and redox signaling in chronic kidney disease. *Ann Intern Med.* (2020) 162:ITC1–ITC14. doi: 10.7326/AITC201506020

44. Katholi RE, Woods WT, Taylor GJ, Deitrick CL, Womack KA, Katholi CR, et al. Oxygen free radicals and contrast nephropathy. *Am J Kidney Dis.* (1998) 32:64–71. doi: 10.1053/ajkd.1998.v32.pm9669426

45. Lew QLJ, Jafar TH, Koh HWL, Jin A, Chow KY, Yuan JM, et al. Red meat intake and risk of ESRD. *J Am Soc Nephrol.* (2017) 28:304–12. doi: 10.1681/ASN.2016030248

46. Karalius VP, Shoham DA. Dietary sugar and artificial sweetener intake and chronic kidney disease: a review. *Adv Chronic Kidney Dis.* (2013) 20:157–64. doi: 10.1053/j.ackd.2012.12.005

47. Chiavaroli L, Mirrahimi A, Sievenpiper JL, Jenkins DJA, Darling PB. Dietary fiber effects in chronic kidney disease: a systematic review and meta-analysis of controlled feeding trials. *Eur J Clin Nutr.* (2015) 69:761–8. doi: 10.1038/ejcn.2014.237

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Relationship between dietary selenium intake and serum thyroid function measures in U.S. adults: Data from NHANES 2007–2012

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Although numerous studies have explored the relationship between selenium intake and thyroid diseases, few epidemiological studies have investigated the association between selenium intake and thyroid hormones. Therefore, we conducted this analysis to investigate the association between dietary selenium intake and thyroid hormones. Our sample included 5,575 adults (age $\geq$ 20) years from the National Health and Nutrition Examination Survey (NHANES) 2007–2012. Thyroid hormones, including total triiodothyronine (T3), total thyroxine (T4), free T3 (FT3), free T4 (FT4), and thyroid-stimulating hormone (TSH), were detected. Multivariable linear regression models showed that log10-transformed selenium intake (LogSe) was negatively correlated with TT4 ($\beta = -0.383$, 95% CI: −0.695, −0.070) and TT4/TT3 ($\beta = -0.003$, 95% CI: −0.006, −0.0004) in U.S. adults. Besides, additional stratified analyses by sex demonstrated that LogSe was negatively associated with TT4 ($\beta = -0.007$, 95% CI: −0.013, −0.001) and TT4/TT3 ($\beta = -0.664$, 95% CI: −1.182, −0.146) and positively associated with FT4/TT4 ($\beta = 0.031$, 95% CI: 0.004, 0.059) in male adults. Meanwhile, subgroup analysis by iodine status showed that LogSe was negatively associated with TT4 ($\beta = -0.006$, 95% CI: −0.011, −0.002), FT4/FT3 ($\beta = -0.011$, 95% CI: −0.023, −0.00002) and TT4/TT3 ($\beta = -0.456$, 95% CI: −0.886, −0.026) in iodine sufficiency but not in iodine deficiency adults. Our results demonstrated that the increased dietary selenium intake was negatively correlated with TT4 and TT4/TT3 in U.S. adults. Furthermore, the association between dietary selenium intake and thyroid hormones was more pronounced in males and iodine sufficiency adults.

KEYWORDS

selenium, thyroid hormones, NHANES, sex, iodine status

Introduction

Thyroid hormones, mainly including triiodothyronine (T3) and thyroxine (T4), are present in numerous tissues and play important roles in maintaining the normal development of the brain, heart, and other organs by controlling energy expenditure and thermogenesis, modulating lipid profiles, and maintaining the normal reproductive function, etc (1). The biosynthesis and secretion of thyroxine (T4) and triiodothyronine (T3) in the thyroid are regulated by thyroid-stimulating hormone (TSH). In turn, the synthesis and release of TSH from the pituitary could also be regulated by T4 and T3 *via* a negative feedback loop. T4 and T3 exist in free and binding forms in peripheral blood, and the former enters target cells to exert their biological functions. In addition, T4 can be converted to T3 under the action of type 1 and 2 iodothyronine deiodinases (DIO1, DIO2) (2). Studies have shown that both thyroid hormone deficiency and excess lead to serious diseases. Thyroid hormone deficiency is associated with hypertension, dyslipidemia, and coronary heart disease (CHD) (3, 4), while thyroid hormone excess is associated with atrial fibrillation (AF) and heart failure (HF) (5).

Selenium, an essential micronutrient, enters the human food chain through plants, seafood, and animal products (6). According to a recent study, the individual dietary selenium intake ranged from 7 to 4,990 μg/day due to varying selenium content in the soil in which crops were grown (7). Schwarz and Foltz demonstrated that a low concentration of selenium could help prevent hepatic necrosis as early as 1957, which was the first study to demonstrate the nutritional value of selenium (8). In recent years, some studies started to study the relationship between selenium and thyroid function because of selenium's anti-inflammatory and antioxidant roles (9). Given that increased inflammation is believed to play an important role in thyroid dysfunction, selenium as an anti-inflammatory substance mediated by selenoproteins could improve thyroid function, which might be propitious to regulate the synthesis and secretion of thyroid hormones (10). Besides that, in the process of thyroid hormone synthesis, the excessive production of H_2O_2 by thyroid follicular epithelial cells might damage the normal function of thyroid function (11), and selenium or antioxidant selenoproteins are acknowledged to scavenge H_2O_2 (12), which suggests selenium might be beneficial to ameliorating thyroid dysfunction induced by excessive H_2O_2 and ultimately influence the synthesis and secretion of thyroid hormones. Also, some previous studies found that selenium was the nucleophilic atom in the DIO1 active site, which further highlights the important role of selenium in thyroid hormones (13).

The current studies mainly explored the relationship between selenium intake and thyroid diseases. In a prospective randomized placebo-controlled clinical trial of 70 female patients with autoimmune thyroid disease who received 200 mcg sodium selenite or placebo daily for 3 months, adjuvant sodium selenite treatment reduced serum thyroid peroxidase antibodies levels by 36% (14). A cross-sectional study conducted in China found that low selenium status was associated with an increased risk of autoimmune thyroiditis, subclinical hypothyroidism, hypothyroidism, and enlarged thyroid (15). A longitudinal study conducted in Brazil also reported that dietary selenium intake was inversely associated with subclinical hypothyroidism (16). However, fewer studies have examined the association between selenium and thyroid hormones, and current findings are inconsistent. A cross-sectional study in coastal fishermen and inland subjects from Latvia found higher plasma selenium level was associated with lower TSH, but not T3 and T4 (17). In a randomized controlled trial among 491 Danes, Kristian et al. found that selenium supplementation could affect thyroid function by reducing serum TSH and FT4 concentrations (18). In contrast, two other studies, one in the UK, and the other in New Zealand, found no association between selenium intervention and thyroid hormone concentrations (19, 20).

Based on the above background, this study aimed to evaluate the cross-sectional association between dietary selenium intake and serum thyroid hormone with data from the National Health and Nutrition Examination Survey (NHANES).

Materials and methods

Study population

The National Health and Nutrition Examination Surveys (NHANES) was a nationwide and ongoing cross-sectional survey conducted among the non-institutionalized US population. To assemble a sample of participants who were representative of the civilian non-institutionalized U.S. population, a repeated 2-year cycle survey with a complex multistage probability sampling design was used. Detailed information about the survey design and methods has been described elsewhere (21). The NHANES protocol was approved by the National Center for Health Statistics (NCHS) Research Ethics Review Board.

Two thousand seven to two thousand eight, 2009–2010, and 2011–2012 NHANES cycles were selected, and a total of 10,548 participants with complete thyroid function data constituted the study sample. We excluded participants under the minimum criteria on dietary recall status ($n = 1,758$), at the age of 19 years or below ($n = 1,528$), with the missing value of covariates [education levels, household income, body mass index (BMI), urine iodine concentration (UIC), serum cotinine, and drinking ($n = 1,135$)]. Also, pregnant women ($n = 49$) and participants with thyroid disease or thyroid cancer ($n = 503$) were excluded. Finally, a total of 5,575 participants were included in the present study. These participants represented a weighted population of 73.5 million non-institutionalized US

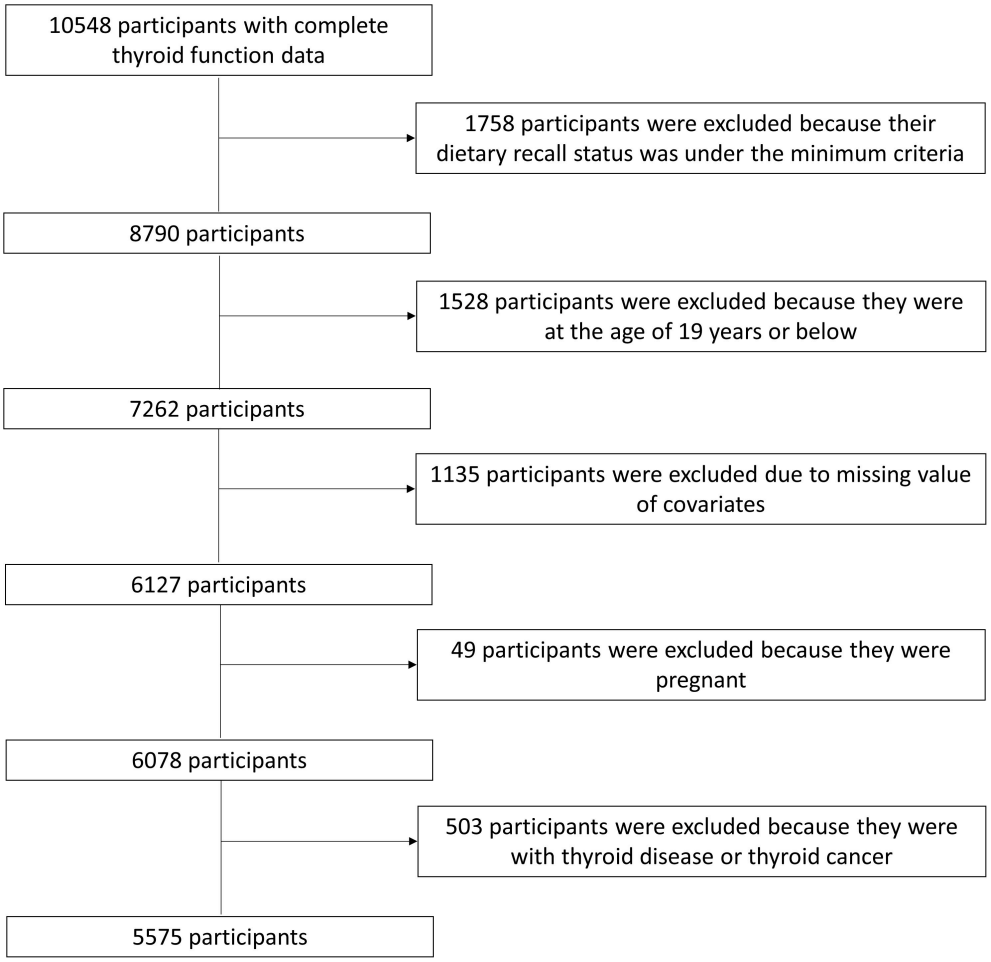

FIGURE 1
Flowchart of the study population.

adults. The flowchart of sample selection was presented in Figure 1.

Determination of serum thyroid hormones

During the physical examinations, whole blood specimens were collected into a red-top 15-mL vacutainer tube and then centrifuged, after which approximately 1 mL serum was collected for further biochemical examinations. Serum total T4 (TT4), total T3 (TT3), and free T3 (FT3) were determined using a competitive binding immunoenzymatic assay. Serum free T4 (FT4) was determined using a two-step enzyme immunoassay. Serum thyroid-stimulating hormone (TSH) was determined using a 3rd generation, two-site immunoenzymatic ("sandwich") assay. Meanwhile, the ratios of FT4/FT3 and TT4/TT3 were calculated to reflect the metabolic level of peripheral T4, while the ratios of FT4/TT4 and FT3/TT3 were acquired to reveal the binding level of thyroid hormones with thyroid hormone-binding proteins (22).

Dietary selenium intake assessment

In NHANES, dietary intake information was assessed by two reliable 24-h dietary recall interviews. The first dietary recall interview was conducted in the mobile examination center (MEC) and the second interview was conducted by telephone 3–10 days later. The intakes of dietary selenium during the 24-h period prior to the interview were calculated based on the University of Texas Food Intake Analysis System and U.S. Department of Agriculture (USDA) Survey Nutrients Database. Then, the mean value of selenium intake from the two 24-h dietary recall interviews was adopted as the final dietary selenium intake.

Covariates

Potential covariates included age (20–39, 40–64, and ≥ 65) (23), sex (male, female), race (non-Hispanic White, non-Hispanic Black, Mexican American, and other races) (24), education level (less than a high school diploma, high school graduate/GED, some college/AA degree, and college graduate or more) (25), household income (family income to poverty (FPL) ≤ 1.3, 1, 3–3.5, and ≥ 3.5) (26), marital status (never married, married or living with a partner, and the other) (27), BMI (≤ 24.9, 25–29.9, and ≥ 30 kg/m^2) (28), serum cotinine (< 1, 1–9.9, and ≥ 10 ng/mL) (1, 29), drinking (< 12, ≥ 12 times/year) (30), urine iodine concentration (≤100 and > 100 ug/L) (31), and fasting time (≤ 10 and > 10 h) (1).

Demographic data such as age, sex, race, education level, household income, and marital status were collected in an in-home interview. The body weight (kg) and height (m) were measured during mobile physical examination, and the BMI was calculated as BMI = weight/height^2. Serum cotinine was measured using isotope dilution-high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (ID HPLC-APCI MS/MS). Drinking status was ascertained *via* questionnaires. Urine iodine concentration was detected using inductively coupled plasma dynamic reaction cell mass spectroscopy (ICP-DRC-MS). In addition, fasting time was acquired by questionnaires before blood collection.

Statistical analysis

In order to generate nationally representative estimates, SDMVPSU, and SDMVSTRA procedures were used to interpret NHANES's complex survey design, and WTMEC2YR was used to provide weight for all data.

The continuous variables were shown as means ± standard deviations, and the categorical variables were presented as counts (percentages). Initially, the Scott–Rao chi-square test was used to compare dietary selenium intake levels of different groups. Then, the one-way analysis of variance (ANOVA) was used to assess FT4, TT4, FT3, TT3, TSH, and ratios of thyroid hormones (FT4/FT3, TT4/TT3, FT4/TT4, and FT3/TT3) differences among different dietary selenium intake groups. Whereafter, two multivariable linear regression models were used to explore the association of dietary selenium intake with levels of FT4, TT4, FT3, TT3, TSH, FT4/FT3, TT4/TT3, FT4/TT4, and FT3/TT3. Due to right skewness, we log10-transformed dietary selenium intake to approximate normality assumptions. Model 1 (unadjusted) did not include any covariates. Model 2 was adjusted for sex, age, race, education level, household income, marital status, BMI, serum cotinine level, drinking, urine iodine concentration, and fasting time. Considering the effects of sex and urine iodine condition on thyroid function indices, we performed subgroup analyses to explore whether the association between dietary selenium intake and thyroid hormones was modified by sex and urine iodine condition. In addition, we carried out several restricted cubic spline (RCS) analyses to explore the non-linear dose-response relationship between dietary selenium intake and thyroid hormones in the whole and subgroup adults, and five knots were placed at the 5th, 25th, 50th, 75th, and 95th percentiles.

All statistical analyses were performed using STATA software (version 16.0), and R software (version 4.1.0, R Foundation for Statistical Computing). *P*-values and confidence intervals (CI) were reported two-sided without adjustment for multiple testing. The $p < 0.05$ was the significance criterion in the Scott–Rao chi-square test, ANOVA, and RCS analyses. Confidence intervals that do not contain 0 were considered to indicate statistical significance in multivariable linear regression models.

Results

As shown in Table 1, participants were categorized according to their dietary selenium intake status. The chi-squared tests revealed dietary selenium intake was associated with sex, age, education level, marital status, household income, serum cotinine, drinking, and urine iodine concentration. The ANOVA analysis revealed that participants in the fourth quartile had significantly lower TT4, FT4/FT3, TT4/TT3, and higher FT3, TT3, FT4/TT4, and FT3/TT3 when compared to other groups.

In the unadjusted model (Table 2), dietary selenium intake, described as LogSe, negatively correlated with TT4 ($\beta = -0.746$, 95% CI: −1.019, −0.472), FT4/FT3 ($\beta = -0.292$, 95% CI: −0.401, −0.182), and TT4/TT3 ($\beta = -0.009$, 95% CI: −0.011, −0.007), while positively correlated with FT3 ($\beta = 0.227$, 95% CI: 0.178, 0.275), FT4/TT4 ($\beta = 0.105$, 95% CI: 0.067, 0.143), and FT3/TT3 ($\beta = 0.001$, 95% CI: 0.0004, 0.002). When the models were further adjusted for potential confounders, the associations with TT4 ($\beta = -0.383$, 95% CI: −0.695, −0.070) and TT4/TT3 ($\beta = -0.003$, 95% CI: −0.006, −0.0004) remained, but the association with FT3 ($\beta = -0.129$, 95% CI: −0.064, 0.039), FT4/FT3 ($\beta = -0.111$, 95% CI: −0.235, 0.012), FT4/TT4 (β= 0.021, 95% CI: −0.023, 0.065), and FT3/TT3 ($\beta = 0.000$, 95% CI: −0.001, 0.001) were no longer present.

Subgroup analyses by sex (Table 3) showed that the negative association between LogSe and TT4 ($\beta = -0.007$, 95% CI: −0.013, −0.001) and TT4/TT3 ($\beta = -0.664$, 95% CI: −1.182, −0.146) tended to be stronger in male adults compared to female adults. In addition, there was also a positive correlation between LogSe and FT4/TT4 ($\beta = 0.031$, 95% CI: 0.004, 0.059) in male adults.

Moreover, subgroup analysis by iodine status (Table 4) showed that LogSe was negatively associated with TT4 ($\beta = -0.006$, 95% CI: −0.011, −0.002), FT4/FT3 ($\beta = -0.011$,

TABLE 1 Characteristics of study participants.

Characteristics	Dietary selenium intake (mcg/day)				*p*-value
	Q1 (0.000–77.950)	Q2 (77.950–105.550)	Q3 (105.550–140.100)	Q4 (140.100–523.450)	
No. weighted %	1,560 (25.000)	1,411 (25.020)	1,362 (25.010)	1,242 (24.970)	
Sex					**<0.001**
Male	498 (27.200)	644 (41.100)	856 (61.200)	1,007 (82.600)	
Female	1,062 (72.800)	767 (58.900)	506 (38.300)	235 (17.400)	
Age					**<0.001**
20–39	416 (31.000)	450 (36.200)	469 (37.800)	516 (42.900)	
40–64	656 (47.300)	567 (43.400)	614 (48.400)	569 (49.400)	
≥65	488 (21.700)	394 (20.400)	279 (13.800)	157 (7.700)	
Race					0.063
Non-Hispanic white	731 (70.900)	700 (71.900)	674 (72.100)	598 (71.800)	
Non-Hispanic black	369 (12.800)	276 (9.900)	257 (9.500)	237 (9.500)	
Mexican American	234 (7.300)	211 (7.400)	208 (7.500)	196 (8.600)	
Other races	226 (8.900)	224 (10.800)	223 (10.800)	211 (10.100)	
Education level					**<0.001**
<High school	532 (23.600)	368 (16.500)	317 (15.600)	280 (15.800)	
High school graduate/GED	381 (25.800)	339 (24.100)	316 (23.000)	269 (22.400)	
Some college/AA degree	391 (28.900)	405 (30.800)	397 (30.600)	357 (29.200)	
College graduate or more	256 (21.700)	299 (28.600)	332 (30.800)	336 (32.600)	
Marital status					**<0.001**
Never married	249 (16.400)	231 (17.700)	221 (16.200)	251 (20.600)	
Married or living with a partner	881 (60.400)	833 (61.600)	885 (68.500)	812 (68.100)	
Others	430 (23.300)	347 (20.700)	256 (15.200)	179 (11.300)	
Household income					**<0.001**
≤1.3 FPL	567 (25.000)	450 (22.200)	361 (16.100)	340 (18.200)	
1.3–3.5 FPL	610 (38.800)	533 (35.000)	515 (33.800)	447 (32.300)	
>3.5 FPL	383 (36.200)	428 (42.800)	486 (50.100)	455 (49.500)	
BMI status					0.224
≤24.9	428 (32.100)	404 (31.900)	345 (26.500)	363 (28.300)	
25–29.9	537 (33.500)	493 (34.300)	503 (37.200)	404 (35.300)	
>30	595 (34.400)	514 (33.900)	514 (36.300)	475 (36.400)	
Serum cotinine					**0.037**
<1 ng/mL	1,106 (69.500)	1,006 (71.700)	980 (73.300)	816 (66.300)	
1–9.9 ng/mL	68 (4.000)	59 (3.700)	53 (4.000)	66 (5.800)	
≥10 ng/mL	386 (26.500)	346 (24.700)	329 (22.700)	360 (27.900)	
Drinking					**<0.001**
<12 times/year	570 (31.300)	405 (23.700)	285 (17.600)	224 (14.600)	
≥12 times/year	990 (68.700)	1,006 (76.300)	1,077 (82.400)	1,018 (85.400)	
Urine iodine concentration					**0.025**
>100 ug/L	1,038 (63.100)	962 (66.700)	917 (68.200)	876 (70.500)	
≤100 ug/L	522 (36.900)	449 (33.300)	445 (31.800)	366 (29.500)	
Fasting time					0.855
≤10 h	1,003 (64.800)	930 (66.800)	879 (66.000)	819 (65.600)	
>10 h	557 (35.200)	481 (33.200)	483 (34.000)	423 (34.400)	
FT4 (pmol/L)	10.150 ± 0.043	10.246 ± 0.052	10.100 ± 0.047	10.118 ± 0.051	0.138

(Continued)

TABLE 1 (Continued)

Characteristics	Dietary selenium intake (mcg/day)				*p*-value
	Q1 (0.000–77.950)	Q2 (77.950–105.550)	Q3 (105.550–140.100)	Q4 (140.100–523.450)	
TT4 (ug/dL)	7.922 ± 0.041	7.920 ± 0.040	7.650 ± 0.040	7.559 ± 0.043	**<0.001**
FT3 (pg/mL)	3.137 ± 0.010	3.161 ± 0.010	3.186 ± 0.017	3.270 ± 0.010	**<0.001**
TT3 (ng/dL)	113.606 ± 0.609	114.748 ± 0.630	112.279 ± 0.581	115.228 ± 0.626	**0.003**
TSH (mIU/I)	2.045 ± 0.053	1.945 ± 0.059	2.015 ± 0.052	1.870 ± 0.034	0.069
FT4/FT3	3.276 ± 0.017	3.273 ± 0.017	3.210 ± 0.017	3.125 ± 0.018	**<0.001**
TT4/TT3	0.072 ± 0.000	0.071 ± 0.000	0.070 ± 0.000	0.067 ± 0.000	**<0.001**
FT4/TT4	1.308 ± 0.006	1.318 ± 0.006	1.349 ± 0.007	1.365 ± 0.007	**<0.001**
FT3/TT3	0.028 ± 0.000	0.028 ± 0.000	0.029 ± 0.000	0.029 ± 0.000	**<0.001**

Q1, Quartile 1; Q2, Quartile 2; Q3, Quartile 3; Q4, Quartile 4. FPL, family income to poverty; BMI, body mass index; FT4, free thyroxine; TT4, total thyroxine; FT3, free triiodothyronine; TT3, total triiodothyronine; TSH, thyroid-stimulating hormone. The bold values indicate statistically significant differences.

TABLE 2 Association between dietary selenium intake and serum thyroid hormones in U.S. adults in NHANES 2007–2012.

	Model 1		Model 2	
	β (95% CI)	*p*-value	*β* (95% CI)	*p*-value
FT4	−0.146 (−0.469, 0.176)	0.366	−0.347 (−0.707, 0.013)	0.059
TT4	**−0.746 (−1.019, −0.472)**	**<0.001**	**−0.383 (−0.695, −0.070)**	**0.017**
FT3	**0.227 (0.178, 0.275)**	**<0.001**	−0.129 (−0.064, 0.039)	0.616
TT3	1.595 (−2.353, 5.543)	0.421	−1.807 (−6.464, 2.849)	0.439
TSH	−0.384 (−0.851, 0.084)	0.105	−0.232 (−0.679, 0.215)	0.303
FT4/FT3	**−0.292 (−0.401, −0.182)**	**<0.001**	−0.111 (−0.235, 0.012)	0.077
TT4/TT3	**−0.009 (−0.011, −0.007)**	**<0.001**	**−0.003 (−0.006, −0.0004)**	**0.024**
FT4/TT4	**0.105 (0.067, 0.143)**	**<0.001**	0.021 (−0.023, 0.065)	0.348
FT3/TT3	**0.001 (0.0004, 0.002)**	**0.005**	0.000 (−0.001, 0.001)	0.707

FT4, free thyroxine; TT4, total thyroxine; FT3, free triiodothyronine; TT3, total triiodothyronine; TSH, thyroid-stimulating hormone. Model 1 (unadjusted) did not include any covariates. Model 2 was adjusted for sex, age, race, education level, household income, marital status, BMI, serum cotinine level, drinking, urine iodine concentration, and fasting time. The bold values indicate statistically significant differences.

95% CI: −0.023, −0.00002), and TT4/TT3 ($\beta = -0.456$, 95% CI: −0.886, −0.026) in iodine sufficiency but not in iodine deficiency adults.

In addition, we further used the restricted cubic splines to estimate the dose-response relationship between LogSe and thyroid hormones (Figure 2). Overall, there was not any departure from linearity in TT4 (*P for non-linearity* = 0.708) and TT4/TT3 (*P for non-linearity* = 0.670) of whole adults, TT4 (*P for non-linearity* = 0.203), TT4/TT3 (*P for non-linearity* = 0.796), and FT4/TT4 (*P for non-linearity* = 0.072) of male adults, and TT4 (*P for non-linearity* = 0.715), FT4/FT3 (*P for non-linearity* = 0.095), and TT4/TT3 (*P for non-linearity* =0.663) of iodine-sufficient adults.

Discussion

Based on a nationally representative survey of non-institutionalized US adults, we found inverse correlations between LogSe and TT4 and TT4/TT3 in U.S. adults. LogSe was also negatively associated with FT4/FT3 and positively associated with FT3, FT4/TT4, and FT3/TT3 in U.S. adults. However, these correlations were no longer significant when the model was further adjusted for potential confounders. When the subgroup analysis was performed according to sex, we found that LogSe was negatively correlated with TT4 and TT4/TT3 while positively correlated with FT4/TT4 within male adults. When subgroup analysis was carried out according to urine iodine concentration, we found that LogSe was negatively associated with TT4, FT4/FT3, and TT4/TT3 within iodine sufficiency adults.

Previous research showed that low selenium status was associated with an increased risk of thyroid disease (32, 33). However, the association between selenium and thyroid hormones remains unknown. Contempré et al. found that selenium supplementation caused a decrease in serum T4 concentrations without a concomitant increase in serum TSH

TABLE 3 Association between dietary selenium intake and thyroid hormones in U.S. adults after subgroup analysis by sex.

Outcomes	Male		Female	
	β (95% CI)	*p*-value	β (95% CI)	*p*-value
FT4	−0.002 (−0.006, 0.002)	0.346	−0.005 (−0.011, 0.001)	0.106
TT4	**−0.007 (−0.013, −0.001)**	**0.032**	−0.003 (−0.008, 0.002)	0.242
FT3	−0.000 (−0. 021, 0.020)	0.975	−0.006 (−0.022, 0.011)	0.481
TT3	−0.000 (−0.000, 0.000)	0.756	−0.000 (−0.001, 0.000)	0.472
TSH	−0.002 (−0.007, 0.003)	0.404	−0.002 (−0.007, 0.003)	0.375
FT4/FT3	−0.007 (−0.018, 0.004)	0.205	−0.009 (−0.026, 0.007)	0.262
TT4/TT3	**−0.664 (−1.182, −0.146)**	**0.013**	−0.091 (−0.627, 0.444)	0.734
FT4/TT4	**0.031 (0.004, 0.059)**	**0.027**	−0.015 (−0.052, 0.022)	0.409
FT3/TT3	0.196 (−1.513, 1.904)	0.819	0.060 (−1.331, 1.452)	0.931

FT4, free thyroxine; TT4, total thyroxine; FT3, free triiodothyronine; TT3, total triiodothyronine; TSH, thyroid-stimulating hormone. Model was adjusted for sex, age, race, education level, household income, marital status, BMI, serum cotinine level, drinking, urine iodine concentration, and fasting time. The bold values indicate statistically significant differences.

TABLE 4 Association between dietary selenium intake and thyroid hormones in U.S. adults after subgroup analysis by urine iodine concentration.

Outcomes	>100 ug/L		≤ 100 ug/L	
	β (95% CI)	*p*-value	β (95% CI)	*p*-value
FT4	−0.004 (−0.008, 0.000)	0.052	−0.002 (−0.008, 0.004)	0.436
TT4	**−0.006 (−0.011, −0.002)**	**0.009**	−0.004 (−0.012, 0.004)	0.319
FT3	0.000 (−0.009, 0.009)	0.963	−0.010 (−0.041, 0.020)	0.495
TT3	−0.000 (−0.001, 0.000)	0.302	0.000 (−0.000, 0.000)	0.909
TSH	−0.001 (−0.004, 0.003)	0.698	−0.004 (−0.010, 0.002)	0.217
FT4/FT3	**−0.011 (−0.023, −0.00002)**	**0.050**	−0.005 (−0.023, 0.013)	0.606
TT4/TT3	**−0.456 (−0.886, −0.026)**	**0.038**	−0.434 (−0.962, 0.093)	0.104
FT4/TT4	0.012 (−0.015, 0.039)	0.382	0.013 (−0.030, 0.056)	0.548
FT3/TT3	0.496 (−0.657, 1.649)	0.392	−0.541 (−3.326, 2.243)	0.698

FT4, free thyroxine; TT4, total thyroxine; FT3, free triiodothyronine; TT3, total triiodothyronine; TSH, thyroid-stimulating hormone. Model was adjusted for sex, age, race, education level, household income, marital status, BMI, serum cotinine level, drinking, urine iodine concentration, and fasting time. The bold values indicate statistically significant differences.

in healthy children (34). Consistent with the above findings, our results showed that LogSe was negatively correlated with serum TT4 but not with TSH in U.S. adults. The biological mechanism of the negative correlation between dietary selenium intake and TT4 has not been fully clarified. That may be because dietary selenium intake could increase type I deiodinase activity, which eventually reduces the concentration of T4 in the serum (35). Meanwhile, we also identified that LogSe negatively correlated with TT4/TT3, which further reflected that dietary selenium intake could contribute to the metabolism of peripheral T4. To fully understand the potential mechanism, further experiments *in vivo* and *in vitro* are needed in future studies.

Furthermore, the present study also found sex differences in the relationship between dietary selenium intake and thyroid hormone levels. For male adults, LogSe was negatively associated with TT4 and TT4/TT3 while positively correlated with FT4/TT4. Previous studies have demonstrated that estrogens could increase iodine uptake, thyroperoxidase activity, thyroglobulin expression, and modulate TSH levels (36–38). Moreover, estrogens also influence thyroid gland redox status by regulating nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4) and dual oxidase 2 (DUOX2) activity and expression (37). Selenium might have a much weaker effect on thyroid hormones than estrogens, suggesting that the effect of dietary selenium intake on thyroid hormones may be more significant in male adults. However, the sex differences between dietary selenium intake and thyroid hormone remain elusive, and further studies are needed to explore possible mechanisms.

Iodine is also an essential micronutrient for the thyroid gland to synthesize thyroid hormones (39). Previous studies have identified that both iodine deficiency and iodine excess may lead to thyroid dysfunction (40, 41). Thus, we carried out subgroup analyses stratified by urine iodine concentration. The results showed that LogSe was negatively associated with TT4, FT4/FT3, and TT4/TT3 in iodine

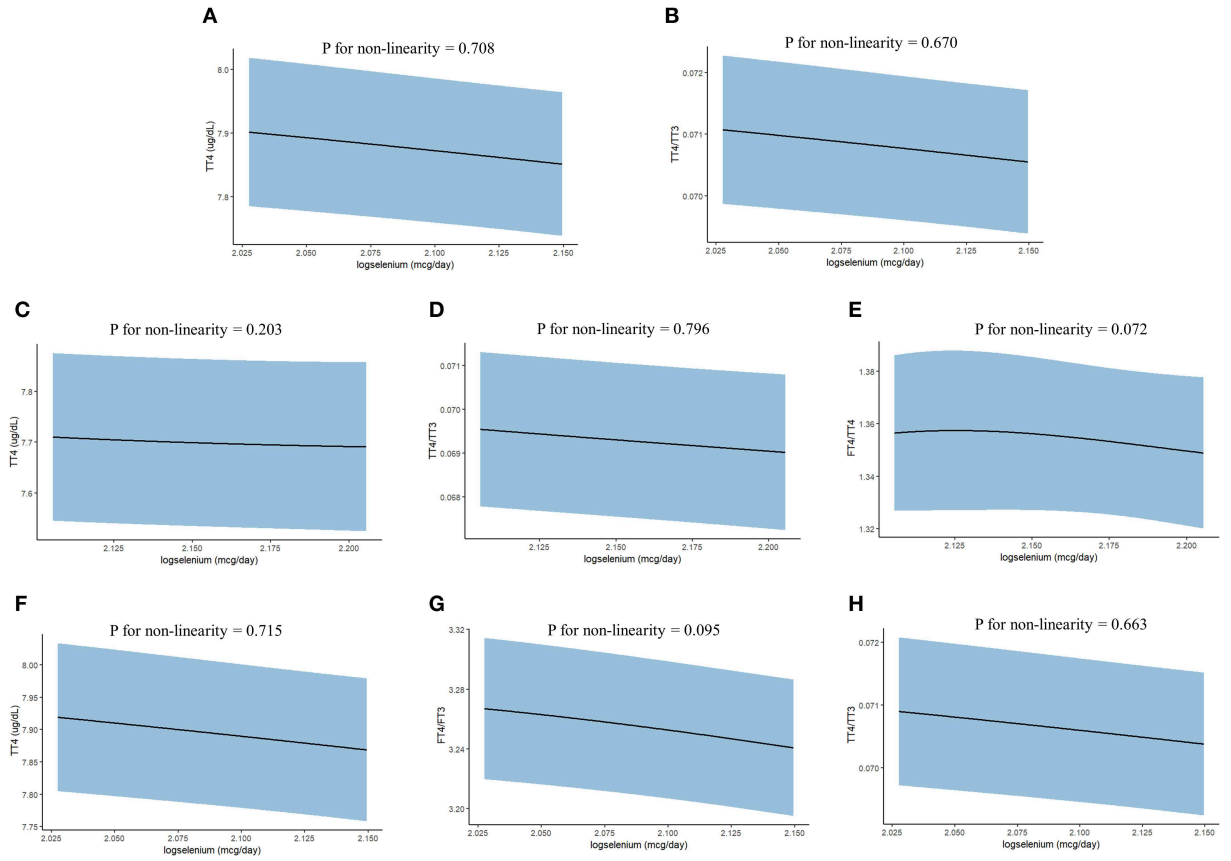

FIGURE 2
The dose-response relationship between dietary selenium intake and thyroid hormones in the whole and subgroup adults from NHANES 2007–2012. **(A)** TT4 of whole adults; **(B)** TT4/TT3 of whole adults; **(C)** TT4 of male adults; **(D)** TT4/TT3 of male adults; **(E)** FT4/TT4 of male adults; **(F)** TT4 of iodine-sufficient adults; **(G)** FT4/FT3 of iodine-sufficient adults; and **(H)** TT4/TT3 of iodine-sufficient adults. Point estimates (solid line) and 95% confidence intervals (blue area) were estimated by restricted cubic splines analysis with knots placed at the 5th, 25th, 50th, 75th, and 95th percentile. Models were adjusted for sex, age, race, education level, household income, marital status, BMI, serum cotinine level, drinking, urine iodine concentration, and fasting time.

sufficiency adults. Nevertheless, these connections were not observed in iodine-deficient adults. The major reason for explaining this result is that iodine deficiency results in reduced circulating TT3 and TT4 and increased TSH, which weakens the effect of dietary selenium intake on thyroid hormones (42).

Selenium is an essential nutrient element, which is rich in organ meats and seafood, followed by grain, cereals, and dairy products (43). However, it is worth noting that although selenium possesses various biological actions, such as anti-inflammatory and anti-oxidant properties, excessive selenium intake may also lead to harmful consequences. Multiple reports showed that high exposure to selenium was associated with increased risks of type 2 diabetes and non-alcoholic fatty liver disease (44, 45). Our results showed that LogSe was negatively correlated with serum TT4. Meanwhile, both thyroid hormone deficiency and excess may lead to a number of deleterious consequences including hyperthyroidism, hypothyroidism, thyroid inflammation, thyroid nodules, and thyroid cancer (46, 47). Furthermore, a diet intervention study showed that the high selenium diet could induce a subclinical hypothyroid response, while the low selenium diet could cause a subclinical hyperthyroid response (48). Therefore, to avoid excess risk, it is recommended that selenium-rich foods should be consumed carefully, considering individual dietary requirements.

The strength of the present study was that this was the first large population-based study to date to reveal the relationship between dietary selenium intake and thyroid hormones in U.S. adults to the best of our knowledge, and our findings might be a complement to the literature regarding the association between selenium and thyroid health. However, the study also had some limitations. First, as with any cross-sectional study, we cannot ascertain causality between dietary selenium and thyroid hormone. Second, dietary data were collected using two days of 24-h dietary recall survey, which

might cause an underestimation or overestimation of diet selenium consumption. Third, the data on dietary selenium supplementation was not assessed due to substantial missing data. Lastly, we excluded 4,973 participants due to missing values for covariates, complete information about dietary selenium intake, or were under the minimum criteria on dietary recall status, which might decrease the generalizability of our results.

In conclusion, this study demonstrated that the increased dietary selenium intake was negatively correlated with TT4 and TT4/TT3 in U.S. adults. Furthermore, the association between dietary selenium intake and thyroid hormones was more pronounced in males and iodine sufficiency adults. However, further large-scale prospective studies are needed to confirm these findings in different populations.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

FL and JN contributed to the conception and design of this study. FL, KW, and M-GD performed the statistical analysis. FL, QF, XL, and YY wrote the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Conflict of interest

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

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References

1. Sun Y, Xia PF, Korevaar TIM, Mustieles V, Zhang Y, Pan XF, et al. Relationship between blood trihalomethane concentrations and serum thyroid function measures in US adults. *Environ Sci Technol.* (2021) 55:14087–94. doi: 10.1021/acs.est.1c04008

2. Oppenheimer JH, Schwartz HL, Surks MI. Propylthiouracil inhibits the conversion of L-thyroxine to L-triiodothyronine. An explanation of the antithyroxine effect of propylthiouracil and evidence supporting the concept that triiodothyronine is the active thyroid hormone. *J Clin Invest.* (1972) 51:2493–7. doi: 10.1172/JCI107063

3. Rodondi N, den Elzen WP, Bauer DC, Cappola AR, Razvi S, Walsh JP, et al. Subclinical hypothyroidism and the risk of coronary heart disease and mortality. *Jama.* (2010) 304:1365–74. doi: 10.1001/jama.2010.1361

4. Pearce EN. Update in lipid alterations in subclinical hypothyroidism. *J Clin Endocrinol Metab.* (2012) 97:326–33. doi: 10.1210/jc.2011-2532

5. Biondi B. Mechanisms in endocrinology: heart failure and thyroid dysfunction. *Eur J Endocrinol.* (2012) 167:609–18. doi: 10.1530/EJE-12-0627

6. Rayman MP. Selenium and human health. *Lancet.* (2012) 379:1256–68. doi: 10.1016/S0140-6736(11)61452-9

7. Winther KH, Rayman MP, Bonnema SJ, Hegedüs L. Selenium in thyroid disorders—essential knowledge for clinicians. *Nat Rev Endocrinol.* (2020) 16:165–76. doi: 10.1038/s41574-019-0311-6

8. Ammerman CB, Miller SM. Selenium in ruminant nutrition: a review. *J Dairy Sci.* (1975) 58:1561–77. doi: 10.3168/jds.S0022-0302(75)84752-7

9. Tang KK Li HQ, Qu KC, Fan RF. Selenium alleviates cadmium-induced inflammation and meat quality degradation via antioxidant and anti-inflammation in chicken breast muscles. *Environ Sci Pollut Res Int.* (2019) 26:23453–9. doi: 10.1007/s11356-019-05675-0

10. Thyroid Hormones O. and inflammation. *Med Inflamm.* (2016) 2016:6757154. doi: 10.1155/2016/6757154

11. Rasool M, Malik A, Saleem S, Ashraf MAB, Khan AQ, Waquar S, et al. Role of oxidative stress and the identification of biomarkers associated with thyroid dysfunction in schizophrenics. *Front Pharmacol.* (2021) 12:646287. doi: 10.3389/fphar.2021.646287

12. Brenneisen P, Steinbrenner H, Sies H. Selenium, oxidative stress, and health aspects. *Mol Aspects Med.* (2005) 26:256–67. doi: 10.1016/j.mam.2005.07.004

13. Berry MJ, Banu L, Larsen PR. Type I iodothyronine deiodinase is a selenocysteine-containing enzyme. *Nature.* (1991) 349:438–40. doi: 10.1038/349438a0

14. Gärtner R, Gasnier BC, Dietrich JW, Krebs B, Angstwurm MW. Selenium supplementation in patients with autoimmune thyroiditis decreases thyroid peroxidase antibodies concentrations. *J Clin Endocrinol Metab.* (2002) 87:1687–91. doi: 10.1210/jcem.87.4.8421

15. Wu Q, Rayman MP, Lv H, Schomburg L, Cui B, Gao C, et al. Low population selenium status is associated with increased prevalence of thyroid disease. *J Clin Endocrinol Metab.* (2015) 100:4037–47. doi: 10.1210/jc.2015-2222

16. Andrade GRG, Gorgulho B, Lotufo PA, Bensenor IM, Marchioni DM. Dietary selenium intake and subclinical hypothyroidism: a cross-sectional analysis of the ELSA-Brasil study. *Nutrients.* (2018) 10. doi: 10.3390/nu10060693

17. Hagmar L, Persson-Moschos M, Akesson B, Schütz A. Plasma levels of selenium, selenoprotein P and glutathione peroxidase and their correlations to fish intake and serum levels of thyrotropin and thyroid hormones: a study on Latvian fish consumers. *Eur J Clin Nutr.* (1998) 52:796–800. doi: 10.1038/sj.ejcn.1600649

18. Winther KH, Bonnema SJ, Cold F, Debrabant B, Nybo M, Cold S, et al. Does selenium supplementation affect thyroid function? Results from a randomized, controlled, double-blinded trial in a Danish population. *Eur J Endocrinol.* (2015) 172:657–67. doi: 10.1530/EJE-15-0069

19. Thomson CD, McLachlan SK, Grant AM, Paterson E, Lillico AJ. The effect of selenium on thyroid status in a population with marginal selenium and iodine status. *Br J Nutr.* (2005) 94:962–8. doi: 10.1079/BJN20051564

20. Rayman MP, Thompson AJ, Bekaert B, Catterick J, Galassini R, Hall E, et al. Randomized controlled trial of the effect of selenium supplementation on thyroid function in the elderly in the United Kingdom. *Am J Clin Nutr.* (2008) 87:370–8. doi: 10.1093/ajcn/87.2.370

21. Wang K, Zhao Y, Nie J, Xu H, Yu C, and Wang S. Higher HEI-2015 Score is associated with reduced risk of depression: result from NHANES 2005–2016. *Nutrients.* (2021) 13. doi: 10.3390/nu13020348

22. Zhai W, Huang Z, Chen L, Feng C, Li B, Li T. Thyroid endocrine disruption in zebrafish larvae after exposure to mono-(2-ethylhexyl) phthalate (MEHP). *PLoS ONE.* (2014) 9:e92465. doi: 10.1371/journal.pone.0092465

23. Carney RM, Freedland KE. Depression and coronary heart disease. *Nat Rev Cardiol.* (2017) 14:145–55. doi: 10.1038/nrcardio.2016.181

24. Tsai J, Homa DM, Gentzke AS, Mahoney M, Sharapova SR, Sosnoff CS, et al. Exposure to secondhand smoke among non-smokers—United States, 1988–2014. *MMWR Morb Mortal Wkly Rep.* (2018) 67:1342–6. doi: 10.15585/mmwr.mm6748a3

25. Scholes S, Bann D. Education-related disparities in reported physical activity during leisure-time, active transportation, and work among US adults: repeated cross-sectional analysis from the national health and nutrition examination surveys, 2007 to 2016. *BMC Public Health.* (2018) 18:926. doi: 10.1186/s12889-018-5857-z

26. Montgomery J, Lu J, Ratliff S, Mezuk B. Food insecurity and depression among adults with diabetes: results from the national health and nutrition examination survey (NHANES). *Diabet Educ.* (2017) 43:260–71. doi: 10.1177/0145721717699890

27. Liu X, Chen Y, Boucher NL, Rothberg AE. Prevalence and change of central obesity among US Asian adults: NHANES 2011-2014. *BMC Public Health.* (2017) 17:678. doi: 10.1186/s12889-017-4689-6

28. Dreimüller N, Lieb K, Tadić A, Engelmann J, Wollschläger D, Wagner S. Body mass index (BMI) in major depressive disorder and its effects on depressive symptomatology and antidepressant response. *J Affect Disord.* (2019) 256:524–31. doi: 10.1016/j.jad.2019.06.067

29. Jain RB, Wang RY. Association of caffeine consumption and smoking status with the serum concentrations of polychlorinated biphenyls, dioxins, and furans in the general U.S. population: NHANES 2003-2004. *J Toxicol Environ Health A.* (2011) 74: 1225–39. doi: 10.1080/15287394.2011.587105

30. Dong X, Li S, Sun J, Li Y, Zhang D. Association of coffee, decaffeinated coffee and caffeine intake from coffee with cognitive performance in older adults: national health and nutrition examination survey (NHANES) 2011–2014. *Nutrients*. (2020) 12. doi: 10.3390/nu12030840

31. Webster GM, Rauch SA, Marie NS, Mattman A, Lanphear BP, Venners SA. Cross-sectional associations of serum perfluoroalkyl acids and thyroid hormones in U.S. adults: variation according to TPOAb and iodine status (NHANES 2007–2008). *Environ Health Perspect*. (2016) 124: 935–42. doi: 10.1289/ehp.1409589

32. Dumont JES. the thyroid, and the endocrine system. *Endocr Rev.* (2005) 26:944–84. doi: 10.1210/er.2001-0034

33. Rasmussen LB, Schomburg L, Köhrle J, Pedersen IB, Hollenbach B, Hög A, et al. Selenium status, thyroid volume, and multiple nodule formation in an area with mild iodine deficiency. *Eur J Endocrinol.* (2011) 164:585–90. doi: 10.1530/EJE-10-1026

34. Contempré B, Duale NL, Dumont JE, Ngo B, Diplock AT, Vanderpas J. Effect of selenium supplementation on thyroid hormone metabolism in an iodine and selenium deficient population. *Clin Endocrinol.* (1992) 36:579–83. doi: 10.1111/j.1365-2265.1992.tb02268.x

35. Schomburg L, Köhrle J. On the importance of selenium and iodine metabolism for thyroid hormone biosynthesis and human health. *Mol Nutr Food Res.* (2008) 52:1235–46. doi: 10.1002/mnfr.200700465

36. Qiu Y, Hu Y, Xing Z, Fu Q, Zhu J, Su A. Birth control pills and risk of hypothyroidism: a cross-sectional study of the national health and nutrition examination survey, 2007-2012. *BMJ Open.* (2021) 11:e046607. doi: 10.1136/bmjopen-2020-046607

37. Miranda RA, de Moura EG, Soares PN, Peixoto TC, Lopes BP, de Andrade CBV, et al. Thyroid redox imbalance in adult Wistar rats that were exposed to nicotine during breastfeeding. *Sci Rep.* (2020) 10:15646. doi: 10.1038/s41598-020-72725-w

38. Fortunato RS, Ferreira AC, Hecht F, Dupuy C, Carvalho DP. Sexual dimorphism and thyroid dysfunction: a matter of oxidative stress? *J Endocrinol.* (2014) 221:R31–40. doi: 10.1530/JOE-13-0588

39. Jain RB. Association between thyroid profile and perfluoroalkyl acids: data from NHNAES 2007–2008. *Environ Res.* (2013) 126:51–9. doi: 10.1016/j.envres.2013.08.006

40. Sun Y, Chen C, Liu GG, Wang M, Shi C, Yu G, et al. The association between iodine intake and semen quality among fertile men in China. *BMC Public Health.* (2020) 20:461. doi: 10.1186/s12889-020-08547-2

41. Abu BAZ, Oldewage-Theron W, Aryeetey RNO. Risks of excess iodine intake in Ghana: current situation, challenges, and lessons for the future. *Ann N Y Acad Sci.* (2019) 1446:117–38. doi: 10.1111/nyas.13988

42. Murray KN, Wolf JC, Spagnoli ST, Lains D, Budrow N, Kent ML. Reversibility of proliferative thyroid lesions induced by iodine deficiency in a laboratory zebrafish colony. *Zebrafish.* (2018) 15:558–65. doi: 10.1089/zeb.2018.1603

43. Rayman MP. Food-chain selenium and human health: emphasis on intake. *Br J Nutr.* (2008) 100:254–68. doi: 10.1017/S0007114508939830

44. Siddiqi SM, Sun C, Wu X, Shah I, Mehmood A. The correlation between dietary selenium intake and type 2 diabetes: a cross-sectional population-based study on north chinese adults. *Biomed Res Int.* (2020) 2020:8058463. doi: 10.1155/2020/8058463

45. Yang Z, Yan C, Liu G, Niu Y, Zhang W, Lu S, et al. Plasma selenium levels and non-alcoholic fatty liver disease in Chinese adults: a cross-sectional analysis. *Sci Rep.* (2016) 6:37288. doi: 10.1038/srep37288

46. Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, et al. Guidelines for the treatment of hypothyroidism: prepared by the american thyroid association task force on thyroid hormone replacement. *Thyroid.* (2014) 24:1670–751. doi: 10.1089/thy.2014.0028

47. Awad SM, Zohny YM, Ali SA, Mahgoub S, Said AM. Design, synthesis, molecular modeling, and biological evaluation of novel thiouracil derivatives as potential antithyroid agents. *Molecules*. (2018) 23. doi: 10.3390/molecules23112913

48. Hawkes WC, Keim NL. Dietary selenium intake modulates thyroid hormone and energy metabolism in men. *J Nutr.* (2003) 133:3443–8. doi: 10.1093/jn/133.11.3443

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Association of dietary inflammatory index with chronic kidney disease and kidney stones in Iranian adults: A cross-sectional study within the Ravansar non-communicable diseases cohort

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Chronic inflammation plays a central role in the pathogenesis of chronic kidney disease (CKD). The association of dietary inflammatory index (DII) with CKD remains underexplored. Thus, the present study aimed to determine the association between the DII, risk of CKD, and kidney stone formation using the data from the Ravansar non-communicable diseases (RaNCD) cohort study conducted in Kermanshah, Iran. The cross-sectional study was conducted using the recruitment phase data of the RaNCD cohort study comprising 9,824 individuals with an age range of 35–65 years. Food frequency questionnaires (FFQ) were used to evaluate the association between diet and DII scores. Renal function was assessed using estimated glomerular filtration rate (eGFR), blood urea nitrogen (BUN), and serum creatinine (Cr) level. CKD was defined based on eGFR. The prevalence of kidney stones was evaluated by participants' self-report. A total of 1,791 participants (18.24%) had kidney stones, while a majority were in the first quartile (27.69%). Out of 9,824 subjects, 1,747 subjects (eGFR: 18.50 ml/min per 1.73 m2; 95% CI: 17.72–19.30) had CKD. A significant trend for eGFR across all quartiles (Qs) of DII was observed. The odds ratio of CKD in the fourth quartile (pro-inflammatory diet) was 4.38-times higher than in the first quartile (anti-inflammatory diet) of DII (95% CI = 3.58–5.36). Women were found to be more likely to have less eGFR than men in the DII Qs. Collectively, the findings indicated that consumption of a pro-inflammatory

diet was associated with a high occurrence of CKD. As a matter of interest, the results also revealed that a pro-inflammatory diet had no significant correlation with kidney stone development.

KEYWORDS

dietary inflammatory index, chronic kidney disease, glomerular filtration rate, kidney stones, cohort study

Introduction

Chronic kidney disease (CKD) is pre-epidemic on a global scale (1). Pooled estimates indicate that the prevalence of CKD ranges from 8 to 13% globally (2). Typically, since CKD affects the structure of the kidney, it progressively leads to renal dysfunction (2). The estimated glomerular filtration rate (eGFR) is direct evidence and a general measure of kidney function which lowers with CKD (3). CKD is divided into five stages by kidney disease outcomes quality initiative (KDOQI) (4) using eGFR per ml/min per 1·73 m^2 as follows: stage 1 (normal or increased GFR $\geq$ 90), stage 2 (mildly decreased GFR 60–89), stage 3 (moderately decreased GFR 30–59), stage 4 (severely decreased GFR 15–29), and stage 5 (kidney failure, GFR $<$ 15) (3–5).

The pathophysiological process of CKD is defined by an underlying chronic pro-inflammatory state (6, 7). Since chronic inflammation could be reflective of dietary quantity and quality, the dietary intakes in CKD patients and the regulation of chronic inflammation have been demonstrated by previous studies (8). A healthy diet rich in fibers, omega 3 fatty acids, vitamins, nuts, and fish, and containing low amounts of sodium and saturated fatty acids has been associated with improved renal function and lower hazard of albuminuria and CKD (9–11). On the contrary, the intake of simple carbohydrates, saturated fats, and trans-fats, which are in general thought to have pro-inflammatory effects, are often associated with higher rates of age-adjusted mortality in individuals with CKD (8).

The Dietary Inflammatory Index (DII) is a relatively new index that is built upon the association between diet and inflammation, appraises the overall inflammatory potentials of the dietary components, and is standardized for assessing the average dietary intake globally (12). The DII has been successfully used for several conditions including chronic systemic inflammation, obesity, diabetes, cancer, cardiovascular diseases (CVD), and fatty liver disease (12, 13).

Despite the success of DII in associating diet and inflammation with multiple diseases, research activities in related dietary habits and kidney health remain critically undertaken. Thus, understanding the role of inflammation in CKD in relation to diet can foster the development of effective prophylactic and therapeutic strategies against the underlying inflammation in CKD patients. While the inflammatory pathophysiology may not be similar in patients with chronic renal failure, persistent low-grade inflammation has been hypothesized as a risk factor for CKD (14). Dietary intake may contribute to the development of an inflammatory condition and trigger the risk of CKD (5, 9, 10). Chronic inflammation also plays an important role in the pathogenesis of kidney stone formation (15, 16). Several inflammatory biomarkers [e.g., c-reactive protein (CRP), P-selectin] usually increase in the urine of individuals with kidney stones. Furthermore, it is well-established that kidney stone formation is attributed to unhealthy dietary patterns (17) and an anti-inflammatory diet may be associated with improved kidney function (18). However, no studies have investigated the relationship between DII and kidney stone formation.

Since the onset and progression of CKD are associated with a chronic pro-inflammatory state, we hypothesized that higher DII scores, indicating a more pro-inflammatory food intake, were associated with an incidence of increased CKD and poorer clinical outcomes. Therefore, in the current study, we aimed to determine the association between DII and renal function and kidney stone formation in Ravansar non-communicable diseases (RaNCD) cohort study, Kermanshah, Iran.

Materials and methods

Study design

This cross-sectional study was conducted using data from the recruitment phase of a prospective study called the RaNCD cohort study. The RaNCD study is a dimension of the prospective epidemiological study in Iran (PERSIAN), which was conducted on different ethnic groups of the Iranian population in collaboration with the Ministry of Health and Medical Education, Iran. Ravansar, with a population of about 50,000, is one of the Kurdish provinces in the northwestern region of Kermanshah, Iran. The preliminary phase of the RaNCD began in November 2014 and ended in February 2017, during which 10,047 people participated in the study after informed consent. Further details and protocol of the RaNCD cohort study have been proactively published elsewhere (19, 20).

Patients with a background of cancer, thyroid disease, fatty liver, stroke, and end-stage renal disease (ESRD) were excluded due to the possibility of altered dietary patterns. In addition, subjects taking medications such as herbs, corticosteroids, and multivitamin supplements were not included in the study, as these supplements could affect nutritional outcomes for chronic renal failure. Patients with questionable total daily energy intake (less than 500 kcal/day and more than 4,200 kcal/d) were excluded from the study.

Data collection and measurement

Information about participants in the RaNCD cohort study was gathered by a trained master at the cohort center (Ravansar cohort center).

Assessment of renal function

CKD was characterized by renal abnormalities or a GFR $<$ 60 ml/min/1.73 m^2 (1.0 mL/s/1.73 m^2) for more than 90-d. Kidney irregularities were diagnosed by pathological problems or markers of dysfunction, including abnormalities in blood or urine tests (21). Diet modification equation in kidney disease 4 (MDRD4) was used to measure eGFR using serum creatinine (Cr), age, gender, and ethnicity as follows (22):

eGFR (mL/min per 1.73 m^2) = 175 $\times$ serum creatinine (μmol/L)–1.154 $\times$ age (years)–0.203 $\times$ 0.742 (if female) $\times$ 1.213 (if black). Abnormal stages of CKD were classified as follows: stage 3 (30 eGFR $\leq$ 59 mL/min/1.73 m^2); stage 4 (15 eGFR 29 ml/min/1.73 m^2); stage 5 (eGFR $<$ 15 mL/min/1.73 m^2) (23, 24).

Kidney stone

All the cases with kidney stones in the RaNCD cohort study by self-report were confirmed by clinical records.

Dietary assessment

The standardized 118-item 1-y FFQ used in a national cohort study—PERSIAN—was used to assess the dietary patterns (19). Updated nutritional databases were used to assess the amount of nutrient intake (19). The FFQ was used to determine the DII and was presented as part of the previous study (12). The DII was formulated by studying articles that were published between 1950 and 2010 and which were centered around the link between a series of food parameters and 6 inflammatory markers *viz.* IL (interleukin)-1β, IL-4, IL-6, IL-10, CRP, and tumor necrosis factor-α (TNF-α). Similarly, 45 nutritional parameters were recognized including macronutrients, micronutrients, flavonoids, and other foods, which can influence the outcomes of inflammation. The inflammatory potential of each parameter was evaluated based on their impact on the expansion, reduction, or elimination of various inflammatory markers. Foods having pro-inflammatory potentials were rated + 1, anti-inflammatory foods were rated −1, and foods with no effects on inflammation were given a score of zero. The DII values can range from −8.87 (highest anti-inflammatory score) to + 7.98 (highest pro-inflammatory score). Based on the mean admission and global SD, z-score and percentage were determined for each parameter. The inflammatory score for each of the nutritional parameters was determined and the total DII score was obtained from the set of inflammatory scores. A more negative DII score indicates the most potent pro-inflammatory diet, and a more positive DII score indicates the most impressive pro-inflammatory diet (7–12).

The nature of the diet was examined using the 2015 health eating index (HEI), which evaluates 13 food groups, including 9 adequate components and 4 moderate components. Using principal component analysis (PCA), subjects were economically categorized as poorest, middle class, richest, and rich (25).

Clinical measurements

Diagnosis of diabetes included fasting blood glucose (FBS) levels of at least 126 mg/dL or cases treated with hypoglycemic drugs. People with a systolic blood pressure of at least 140 mmHg and diastolic blood pressure of at least 90 mmHg or who were being treated with medication for high blood pressure (BPH) were considered to have BPH. In this study, dyslipidemia was also considered a problem in serum lipid profile indices, including one or more of the following: low-density lipoprotein (LDL) $>$ 130 mg/dL, high-density lipoprotein (HDL) $<$ 45 mg/dL, triglycerides (TG) $>$ 150 mg/dL, total cholesterol $>$ 200 mg/dL, or taking lipid-lowering drugs such as amlodipine, atorvastatin, clofibrate, fenofibrate, gemfibrozil, lovastatin, and simvastatin. Blood urea nitrogen (BUN) and serum creatinine (Cr) concentrations were measured using enzymatic techniques. The one-to-one questionnaire was used to assess participants' physical activity. The questionnaire consisted of 22 questions that assessed individual activity on an hourly or minute-per-day basis. At last, information from the questionnaire was extracted and used on the basis of metabolic equivalents (METS)/hour per day.

Statistical analysis

Data were presented using mean ± S.D for quantitative variables and frequency and percentage for qualitative variables. Raw ORs with 95% CIs were used to analyze the relationship between DII and CKD. The relationship between the factors was assessed using univariate and multivariate

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TABLE 1 Characteristics of the participants according to the quartiles of dietary inflammatory index.

Variable		Total	Q1 (anti-inflammatory)	Q2	Q3	Q4 (pro-inflammatory)	*P*-value*
Total (%)		9,824 (100)	2,456 (25.0)	2,456 (25.0)	2,456 (25.0)	2,456 (25.0)	
Mean (min, max)		−0.84 (−5.00, 4.64)	−2.82 (−5.00, −2.02)	−1.51 (−2.08, −1.02)	−0.45 (−1.02, 0.25)	1.40 (0.25, 4.64)	
Gender	Male	4,610 (46.93)	1,518 (32.93)	1,389 (30.13)	1,049 (22.75)	654 (14.19)	<**0.001**
	Female	5,214 (53.07)	938 (17.99)	1,067 (20.46)	1,407 (26.99)	1,802 (34.56)	
Age group	35–45	4,298 (43.75)	1,172 (27.27)	1,198 (27.87)	1,074 (24.99)	854 (19.87)	<**0.001**
	46–55	3,284 (33.43)	850 (25.88)	779 (23.72)	479 (21.36)	821 (25.00)	
	56–65	2,242 (22.82)	434 (25.00)	479 (21.36)	548 (24.44)	781 (34.83)	
Education level	Illiterate	2,435 (24.79)	389 (15.98)	452 (18.56)	602 (24.72)	992 (40.74)	<**0.001**
	1–5 years	3,762 (38.29)	844 (22.43)	937 (24.91)	989 (26.29)	992 (26.37)	
	6–9 years	1,629 (16.58)	502 (30.82)	468 (28.73)	4,069 (24.92)	253 (15.53)	
	10.12 years	1,224 (12.46)	425 (34.72)	363 (29.66)	293 (23.94)	143 (11.68)	
	>13ψεαρσ	774 (7.88)	296 (38.24)	236 (30.49)	166 (21.45)	76 (9.82)	
Place of residence	City	5,806 (59.10)	1,912 (32.93)	1,610 (27.73)	1,344 (23.15)	940 (16.19)	<**0.001**
	Village	4,018 (40.90)	544 (13.54)	846 (21.06)	1,112 (27.68)	15,169 (37.73)	
Physical Activity (MET-hours per day)	24–36.5	2,724 (24.74)	694 (25.48)	699 (25.66)	6,849 (25.11)	647 (23.75)	<**0.001**
	36.6–44.9	5,073 (51.66)	1,188 (23.42)	1,209 (23.83)	1,285 (25.33)	1,391 (27.42)	
	≥45	2,023 (20.60)	573 (28.32)	547 (27.04)	485 (23.97)	418 (20.66)	
Smoking status	No	7,866 (80.27)	1,897 (24.12)	1,910 (24.25)	2,002 (25.45)	2,057 (26.15)	<**0.001**
	Current	1,130 (11.53)	311 (27.52)	324 (28.67)	273 (24.16)	222 (19.65)	
	Former	804 (8.20)	239 (29.73)	216 (26.87)	177 (22.01)	172 (21.39)	
BMI (kg/m^2)	<18.9	164 (1.68)	21 (12.80)	34 (20.73)	52 (31.71)	57 (34.76)	<**0.001**
	19–24.9	2,683 (27.52)	594 (22.14)	638 (23.78)	676 (25.20)	775 (28.89)	
	25–29.9	4,241 (43.51)	1,078 (25.42)	1,115 (26.29)	1,052 (24.81)	996 (23.49)	
	30–34.9	2,087 (21.41)	574 (27.50)	519 (24.87)	509 (24.39)	485 (23.24)	
	≥35	573 (5.88)	167 (29.14)	135 (23.56)	143 (24.96)	128 (22.34)	
HEI	1st quintile (poorest)	2,071 (21.09)	143 (6.90)	342 (16.51)	585 (28.25)	1,001 (48.330	<**0.001**
	2nd quintile	1,890 (19.24)	287 (15.19)	481 (25.45)	537 (28.41)	585 (30.95)	
	3rd quintile	2,115 (21.54)	497 (23.5)	579 (27.38)	582 (27.52)	457 (21.61)	
	4th quintile	1,845 (18.79)	634 (34.36)	5,499 (29.76)	414 (22.44)	248 (13.44)	
	5th quintile (highest)	1,900 (19.35)	895 (47.11)	505 (26.58)	337 (17.74)	163 (8.58)	
Kidney stone	No	8,025 (81.74)	1,958 (24.40)	2,030 (25.30)	2,001 (24.93)	2,036 (25.37)	**0.01**
	Yes	1,791 (18.24)	498 (27.69)	424 (23.67)	418 (23.34)	418 (23.34)	
Type 2 diabetes	No	8,964 (91.79)	2,239 (24.98)	2,243 (25.02)	2,236 (25.06)	2,246 (25.06)	**0.98**
	Yes	802 (8.21)	200 (24.94)	198 (24.69)	205 (25.56)	199 (24.81)	
Hypertension	No	8,251 (84.18)	2,111 (25.58)	2,126 (25.77)	2,043 (24.76)	1971 (23.89)	<**0.001**
	Yes	1,551 (15.82)	341 (21.99)	328 (21.15)	405 (26.11)	477 (30.75)	
eGFR	Mean ± SD	76.1 ± 14.1	80.0 ± 13.4	78.3 ± 14.3	75.5 ± 13.7	70.7 ± 13.4	<**0.001**
Creatinine (mg/dL)	Mean ± SD	0.99 ± 0.18	0.98 ± 0.17	0.99 ± 0.19	0.98 ± 0.17	0.99 ± 0.17	**0.9**
Urea	Mean ± SD	13.56 ± 4.2	13.91 ± 4.1	13.7 ± 4.1	13.3 ± 3.9	13.2 ± 2.3	**0.4**

*p-value < 0.05 were considered statistically significant and bold values showed the P-value results.

logistic regression models. Variables with $P < 0.3$ in the univariate analysis were included in the multivariate model. Then, the variables with $P > 0.05$ were removed using the forward or reverse method. The fractional polynomial method was performed to quantitatively associate the effect of DII with the odds ratio of CKD. To estimate the effect of DII on CKD and kidney stones, we entered confounding and then adjusted variables for diabetes and hypertension, age, gender, smoking status, body mass index (BMI), education level, and physical activity. The effect of the DII was then evaluated. The fractional polynomial is a regular polynomial alternative method that provides flexible parameterization for continuous variables. All analyzes were performed using Stata software version 14.1 (Stata

Corp., College Station, TX, United States) with a 95% confidence interval.

Ethical approval

The convention of this study was supported by the ethics committee of the Kermanshah University of Medical Science (IR. KUMS.REC.1394. 318).

Results

In the present study, of the 10,047 individuals enrolled in the RaNCD cohort, the status of CKD and other related markers was recorded for 9,824 (97%) participants. Out of these, 5,214 (53.07%) were female participants. The mean ± SD age of women and men was 48.2 ± 8.3 and 47.7 ± 8.1 years, respectively. Of the participants, 2,683 (27.52%) had a normal BMI and 26.1% had low-physical activity (36 > −MET/h per day) (Table 1).

The average DII score In this study was −0.84 ± 1.6, ranging from −5.00 (diet with the lowest pro-inflammatory potential) to + 4.64 (diet with the highest pro-inflammatory potential).

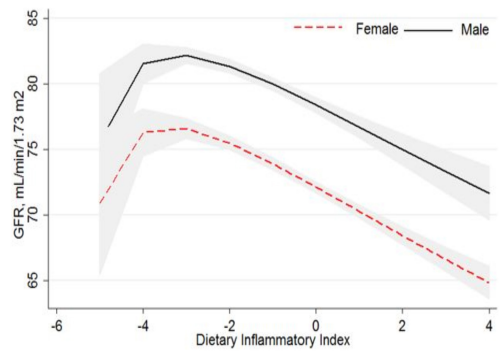

FIGURE 1
Association of glomerular filtration rate and dietary inflammatory index.

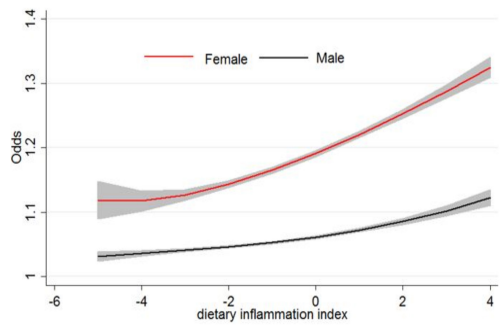

FIGURE 2
Odds of chronic kidney disease and dietary inflammatory index.

Table 1 summarizes the general characteristics of subjects with CKD in the DII Qs. There was no significant difference in the prevalence of diabetes ($p = 0.98$) between the DII Qs.

According to the participants' self-reported data, 18.24% of them had kidney stones, and most of them (27.69%) were in the first quarter. In addition, as the inflammation of the diet increased, a decreasing trend in the incidence of kidney stones was observed among the study participants. However, no significant differences were found in the incidence of kidney stones between the quartiles ($P = 0.01$) (Table 1).

In this study, out of 9,824 subjects 1,747 subjects [eGFR: 18.50 mL/min per 1.73 m2; 95% confidence interval (CI): 17.72–19.30] had CKD. The prevalence of kidney stones was evaluated by participants' self-report. A total of 1,791 participants (18.24%) had kidney stones, and most of them were in the first quartile (27.69%).

The highest prevalence of kidney stones was observed in the first quartile (diet with the highest anti-inflammatory potential). No significant differences were found in creatinine and urea concentrations across the Qs or DII score (Table 1).

The average eGFR in men (80.2 ± 13.1) was higher than in women (72.6 ± 13.5) in the study population. Figure 1 shows the mean of eGFR over Qs of DII. A significant decreasing trend was observed for eGFR across Qs of DII in men and women. Subjects with a higher DII had lower eGFR and the trend was similar in both genders.

It is likely that a pro-inflammatory diet increases the risk of developing CKD. According to our findings, which can be seen in the crude model, the risk of CKD in quartile 4 was 4.38 times higher than that in quartile 1 (OR = 4.38, 95% CI: 3.58–5.36). Although the odds ratio decreased compared to the crude model after controlling for confounding factors, the association between DII and CKD remained significant. Moreover, after adjusting for all major confounding factors, the probability of chronic renal failure in subjects in the fourth quartile (pro-inflammatory) was 1.92 (95% CI: 1.52–2.42) times higher than Q1 (anti-inflammatory) (Table 2).

As shown in Figure 2, an increased risk of being in the higher stage of CKD was found among those in the top Qs of DII (P for trend = 0.03). The results also revealed that women were more likely to get CKD than men.

Discussion

The present study was conducted in an Iranian Kurdish population to assess the relationship between DII and the likelihood of CKD progression in a large general population in Iran. We have shown that DII scores are directly related to CKD risk. The odds ratio of CKD in the fourth quarter was 4.38 times higher than in the first quarter of DII. These findings remain in accordance with prior studies (11, 15, 26). Mihai et al. indicated that in patients with

TABLE 2 Bivariate and the multivariate association between DII and CKD.

Variable	Mean (min-max)	Crude OR (CI 95%)	Model 1* OR (CI 95%)	Model 2† OR (CI 95%)	Model3‡ OR (CI 95%)
DII	Q1 (anti-inflammatory)	1	1	1	1
	Q2	1.39 (1.10–1.75)	1.25 (0.98–1.59)	1.13 (0.98–1.44)	1.14 (0.89–1.45)
	Q3	2.31 (1.86–2.86)	1.75 (1.40–2.19)	1.48 (1.17–1.87)	1.44 (1.14–1.82)
	Q4 (pro-inflammatory)	4.38 (3.58–5.36)	2.49 (2.01–3.08)	1.95 (1.54–2.46)	1.92 (1.52–2.42)

*Model 1: Adjusted for baseline age, gender, smoking status, BMI, place, education level, and physical activity.
†Model 2: In addition, adjusted for HEI.
‡Model 3: In addition, adjusted for kidney stone, diabetes, and high blood pressure.

CDK, consuming a diet with pro-inflammatory potential may trigger disease progression (15). Another observational study examining the likelihood of harmful effects of pro-inflammatory diets on kidney health suggested that the anti-inflammatory properties of the diet are crucial for preventing kidney failure in American adults (27). A cohort study recommended that a pro-inflammatory diet, as assessed by the DII, was associated with systemic inflammation and impaired renal function in older Swedish populations (11). The significant mediating role of serum CRP in the association between the DII and kidney function proposes that inflammation is a likely mechanism through which diet may prompt kidney dysfunction (11). Therefore, an anti-inflammatory diet appears to be a reasonable prophylactic strategy for limiting the risk of CKD. Overall, previous evidence has shown that diet quality can develop outcomes in patients with chronic renal failure (28). A pro-inflammatory diet is not only effective in reducing the burden of disease in patients with chronic kidney failure but also in preventing kidney cancer. Shivappa et al. showed that higher DII scores for pro-inflammatory diets were associated with a higher risk of kidney cancer in the American population (29). An anti-inflammatory diet high in fruits and whole grains was associated with lower urinary albumin to creatinine ratio (ACR) (30), whereas a pro-inflammatory diet such as animal-based foods was associated with higher ACR across all quartiles (31). Furthermore, a greater intake of animal fats and sodium is associated with the onset of micro albuminuria, while a greater intake of carotenoids, which have an anti-inflammatory effect, is associated with an increase in GFR (32). One of the potential mechanisms adding to the relationship between DII and the risk of renal disease is the impact of diet-related chronic inflammation in the upregulation of various pro-inflammatory mediators like TGF-β, TNF-α, IL-6, and CRP (14). In contrast, others have shown that the DII score is unrelated to serum hs-CRP and biomarkers of kidney function in elderly patients (26). Nevertheless, previous reports suggest that dietary habits are associated with CRF according to major community registries (9). The DII has a good overall ability to assess nutritional and inflammatory status to reduce morbidity and mortality in patients with chronic renal failure (33).

In the present study, we also observed significantly fewer kidney stones in subjects in Q1 of the DII compared to the later Qs. It has been suggested that the formation of kidney stones could be attributed to poor dietary habits (16, 17). An earlier study showed that adherence to an unhealthy dietary pattern that is rich in red meats and high-fat dairy products is related to kidney stone formation (17). Moreover, adherence to the Mediterranean dietary pattern (18) and DASH (dietary approaches to stop hypertension) diet (16) with a low DII, which includes a high intake of fruits, vegetables, and low-fat dairy products and a low intake of total fat, are associated with decreased kidney stone formation. Earlier studies likewise propose that individuals with kidney stones are inclined to CKD at later stages of life (34, 35).

Our study showed that there was no significant difference in the prevalence of diabetes between the Qs of the DII. In contrast to this study, Nikniaz et al. showed that the DII score was related to total metabolic syndrome (MetS) and FBS after adjusting for all covariates in Iranian adults (36). In a cross-sectional study, the upper DII quartile (Q4) was positively associated with the prevalence of MetS in men and in postmenopausal women (37). Another study showed that an increased pro-inflammatory diet is associated with poor glucose homeostasis (38).

Despite the fact that hypertension and CKD coexist frequently (39), findings of our study demonstrated that patients with HBP as the highest consumer of an anti-inflammatory diet. This finding does not corroborate any previous study. Phillips et al. proposed that expanded admission to a pro-inflammatory diet is related to higher blood pressure among an Irish population (38). Ramallal et al. likewise showed a significant association between DII and hypertension in a Spanish population (40). Our results also showed that women had a higher risk of CKD progression than men consistent with a previous study (41). It has been proposed that the distinction in sexual orientation in CKD can be largely attributed to differences in predictors of renal function due to urinary tract infection, especially in women (42).

At last, our study consists of certain limitations. First, we calculated the DII using 29 diet items, and data on 16 diet items were not available in this study. Second, due to the cross-sectional design of the present study, it was not possible to investigate the causal relationship between DII and the

progression of CKD. Nevertheless, the greatest strength of the present study was that it aimed to determine the relationship between DII and CKD among the Kurdish population in Iran with large sample size. Other strengths of this study are the high quality of data collection, population-based study design, and adjustment for all known confounders such as age, sex, smoking status, BMI, location, level of education, and physical activity. Using DII instead of inflammatory markers to assess the effect of inflammation may help directly measure the impact of diet on clinical outcomes through inflammation and reduce the overall cost of the study. The calculation of the DII by an inexpensive and non-invasive method (FFQ) makes it possible to evaluate the inflammatory properties of the dietary components.

Conclusion

In conclusion, the lowest quartiles of DII were at a reduced risk of being in the highest stage of CKD and improved renal function in a large general population. Considering the role of diet through its antioxidant properties in the occurrence of diseases such as CKD, it is recommended that the DII should be taken into account to help prevent, control, and treat CKD, with an emphasis on the use of antioxidant diets as part of prophylactic dietary strategies.

Data availability statement

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

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YP, NK, and FN designed the study. YP completed the entire study. JM and LS collected and analyzed the data. JM and HF prepared the manuscript. MM conducted the statistical analysis. All of the authors edited the manuscript.

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Conflict of interest

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

Publisher's note

References

1. Ruggenenti P, Cravedi P, Remuzzi G. Mechanisms and treatment of CKD. *J Am Soc Nephrol.* (2012) 23:1917–28. doi: 10.1681/ASN.2012040390

2. Levey AS, Coresh J. Chronic kidney disease. *Lancet.* (2012) 379:165–80. doi: 10.1016/S0140-6736(11)60178-5

3. Levey AS, Coresh J, Bolton K. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis.* (2002) 39:S1–266.

4. Wong H, Mylrea K, Feber J, Drukker A, Filler G. Prevalence of complications in children with chronic kidney disease according to KDOQI. *Kidney Int.* (2006) 70:585–90. doi: 10.1038/sj.ki.5001608

5. Remuzzi G, Benigni A, Remuzzi A. Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. *J Clin Invest.* (2006) 116:288–96. doi: 10.1172/JCI27699

6. Raphael KL, Zhang Y, Wei G, Greene T, Cheung AK, Beddhu S. Serum bicarbonate and mortality in adults in NHANES III. *Nephrol Dialy Trans.* (2013) 28:1207. doi: 10.1093/ndt/gfs609

7. Ahluwalia N, Andreeva V, Kesse-Guyot E, Hercberg S. Dietary patterns, inflammation and the metabolic syndrome. *Diab Metab.* (2013) 39:99–110. doi: 10.1016/j.diabet.2012.08.007

8. Mirmiran P, Esfahani FH, Mehrabi Y, Hedayati M, Azizi F. Reliability and relative validity of an FFQ for nutrients in the tehran lipid and

glucose study. *Public Health Nutr.* (2010) 13:654–62. doi: 10.1017/S13689800099 91698

9. Smyth A, Griffin M, Yusuf S, Mann JF, Reddan D, Canavan M, et al. Diet and major renal outcomes: a prospective cohort study. The NIH-AARP diet and health study. *J Renal Nutrit.* (2016) 26:288–98. doi: 10.1053/j.jrn.2016.01.016

10. Weinstein SJ, Vogt TM, Gerrior SA. Healthy eating index scores are associated with blood nutrient concentrations in the third national health and nutrition examination survey. *J Am Diet Assoc.* (2004) 104:576–84. doi: 10.1016/ j.jada.2004.01.005

11. Xu H, Sjögren P, Ärnlöv J, Banerjee T, Cederholm T, Risérus U, et al. A proinflammatory diet is associated with systemic inflammation and reduced kidney function in elderly adults. *J Nutr.* (2015) 145:729–35. doi: 10.3945/jn.114.205187

12. Shivappa N, Prizment AE, Blair CK, Jacobs DR Jr., Steck SE, Hébert JR. Dietary inflammatory index and risk of colorectal cancer in the Iowa women's health study. *Cancer Epidemiol Prevent Biomark.* (2014) 23:2383–92. doi: 10.1158/ 1055-9965

13. Darbandi M, Hamzeh B, Ayenepour A, Rezaeian S, Najafi F, Shakiba E, et al. Anti-inflammatory diet consumption reduced fatty liver indices. *Sci Rep.* (2021) 11:1–8.

14. Ayeneh A, Moradinazar M, Samadi M, Hamzeh B, Najafi F, Karimi S, et al. Association of dietary inflammatory index with cardiovascular disease in kurdish adults: results of a prospective study on Ravansar non-communicable diseases. *BMC Cardiov Dis.* (2020) 20:1–8. doi: 10.1186/s12872-020-01707-7

15. Mihai S, Codrici E, Popescu ID, Enciu AM, Albulescu L, Necula LG, et al. Inflammation-related mechanisms in chronic kidney disease prediction, progression, and outcome. *J Immunol Res.* (2018) 2018:373. doi: 10.1155/2018/ 2180373

16. Noori N, Honarkar E, Goldfarb DS, Kalantar-Zadeh K, Taheri M, Shakhssalim N, et al. Urinary lithogenic risk profile in recurrent stone formers with hyperoxaluria: a randomized controlled trial comparing DASH (dietary approaches to stop hypertension)-style and low-oxalate diets. *Am J Kidney Dis.* (2014) 63:456– 63. doi: 10.1053/j.ajkd.2013.11.022

17. Maddahi NS, Mirzaei K, Aghamir SMK, Modaresi SS, Yekaninejad MS. Major dietary patterns and kidney stone formation among Iranian men. *J Nutr Sci Diete.* (2017) 2017:11–7.

18. Leone A, Fernández-Montero A, de la Fuente-Arrillaga C, Martínez-González MÁ, Bertoli S, Battezzati A, et al. Adherence to the mediterranean dietary pattern and incidence of nephrolithiasis in the seguimiento universidad de navarra follow-up (SUN) cohort. *Am J Kidney Dis.* (2017) 70:778–86. doi: 10.1053/j.ajkd. 2017.06.027

19. Pasdar Y, Najafi F, Moradinazar M, Shakiba E, Karim H, Hamzeh B, et al. Cohort profile: Ravansar non-communicable disease cohort study: the first cohort study in a kurdish population. *Int J Epidemiol.* (2019) 48:682–3. doi: 10.1093/ije/ dyy296

20. Poustchi H, Eghtesad S, Kamangar F, Etemadi A, Keshtkar AA, Hekmatdoost A, et al. Prospective epidemiological research studies in Iran (the PERSIAN cohort study): rationale, objectives, and design. *Am J Epidemiol.* (2018) 187:647–55. doi: 10.1093/aje/kwx314

21. Jamshidi P, Najafi F, Mostafaei S, Shakiba E, Pasdar Y, Hamzeh B, et al. Investigating associated factors with glomerular filtration rate: structural equation modeling. *BMC Nephrol.* (2020) 21:30. doi: 10.1186/s12882-020-1686-2

22. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Int Med.* (2006) 145:247–54. doi: 10.7326/0003-4819-145-4-200608150-00004

23. Eknoyan G, Levin NWK. /DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis.* (2002) 39:S1–266.

24. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Int Med.* (1999) 130:461–70. doi: 10.7326/0003-4819-130-6-199903160-00002

25. 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 Dietet.* (2018) 118:1591–602. doi: 10.1016/j.jand.2018.05.021

26. Tajik S, Eimeri S, Mansouri S. Association between dietary inflammatory index and kidney function in elderly population: results from a cross-sectional study. *Nutr Food Sci.* (2019) 49:491–503.

27. Mazidi M, Shivappa N, Wirth MD, Hebert JR, Kengne AP. Greater dietary inflammatory index score is associated with higher likelihood of chronic kidney disease. *Br J Nutr.* (2018) 120:204–9. doi: 10.1017/S0007114518001071

28. Fernandes AS, Ramos CI, Nerbass FB, Cuppari L. Diet quality of chronic kidney disease patients and the impact of nutritional counseling. *J Renal Nutr.* (2018) 28:403–10. doi: 10.1053/j.jrn.2017.10.005

29. Shivappa N, Blair CK, Prizment AE, Jacobs DR Jr., Hébert JR. Dietary inflammatory index and risk of renal cancer in the Iowa women's health study. *Eur J Nutr.* (2018) 57:1207–13. doi: 10.1007/s00394-017-1403-5

30. Nettleton JA, Steffen LM, Palmas W. Associations between microalbuminuria and animal foods, plant foods, and dietary patterns in the multiethnic study of atherosclerosis. *Am J Clin Nutr.* (2008) 87:1825–36. doi: 10.1093/ajcn/87.6. 1825

31. Lin J, Hu FB, Curhan GC. Associations of diet with albuminuria and kidney function decline. *Clin J Am Soc Nephrol.* (2010) 5:836–43. doi: 10.2215/CJN. 08001109

32. Gopinath B, Harris D, Flood V. A better diet quality is associated with a reduced likelihood of CKD in older adults. *Nutr Metab Cardiov Dis.* (2013) 23:937–43. doi: 10.1016/j.numecd.2012.07.003

33. Kizil M, Tengilimoglu-Metin MM, Gumus D. Dietary inflammatory index is associated with serum C-reactive protein and protein energy wasting in hemodialysis patients: a cross-sectional study. *Nutr Res Pract.* (2016) 10:404–10. doi: 10.4162/nrp.2016.10. 4.404

34. Saucier NA, Sinha MK, Liang KV. Risk factors for CKD in persons with kidney stones: a case-control study in olmsted county, minnesota. *Am J Kidney Dis.* (2010) 55:61–8.

35. Rule AD, Krambeck AE, Lieske JC. Chronic kidney disease in kidney stone formers. *Clin J Am Soc Nephrol.* (2011) 6:2069–75. doi: 10.2215/CJN.106 51110

36. Nikniaz L, Nikniaz Z, Shivappa N. The association between dietary inflammatory index and metabolic syndrome components in Iranian adults. *Primary Care Diab.* (2018) 12:467–72. doi: 10.1016/j.pcd.2018.07.008

37. Kim H-Y, Lee J, Kim J. Association between dietary inflammatory index and metabolic syndrome in the general korean population. *Nutrients.* (2018) 10:648. doi: 10.3390/nu10050648

38. Phillips C, Shivappa N, Hébert J. Dietary inflammatory index and biomarkers of lipoprotein metabolism, inflammation and glucose homeostasis in adults. *Nutrients.* (2018) 10:1033. doi: 10.3390/nu10081033

39. Sinha AD, Agarwal R. The complex relationship between CKD and ambulatory blood pressure patterns. *Adv Chronic Kidney Dis.* (2015) 22:102–7. doi: 10.1053/j.ackd.2015.01.003

40. Ramallal R, Toledo E, Martínez-González MA. Dietary inflammatory index and incidence of cardiovascular disease in the SUN cohort. *PLoS One.* (2015) 10:e0135221. doi: 10.1371/journal.pone.0135221

41. Iseki K. Gender differences in chronic kidney disease. *Kidney Int.* (2008) 74:415–7. doi: 10.1038/ki.2008.261

42. Hsiao C-Y, Yang H-Y, Hsiao M-C. Risk factors for development of acute kidney injury in patients with urinary tract infection. *PLoS One.* (2015) 10:105642. doi: 10.1016/j.clineuro.2019.105642

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Association of dietary phytochemical index with metabolically unhealthy overweight/obesity phenotype among Iranian women: A cross-sectional study

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Background: Phytochemicals have been recently studied as adjuvants for the treatment of obesity. No study has investigated the association of phytochemical-rich foods with metabolically unhealthy overweight/obesity phenotype (MUOW/O). This study aimed to determine the association of dietary phytochemical index (DPI) with MUOW/O based on Karelis criteria among Iranian female adults.

Methods: In this cross-sectional study, a total of 228 overweight and obese women aged 18–48 years were included. Anthropometric measurements were evaluated for all participants. A validated 147-item Food Frequency Questionnaire (FFQ) was used for dietary assessment. DPI was calculated as [dietary energy derived from phytochemical-rich foods (kcal)/total daily energy intake (kcal)] × 100. Participants' body composition and biochemical parameters of Karelis criteria [triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), insulin, and high-sensitivity C-reactive protein (hs-CRP)] were determined.

Results: The mean age of the study participants was 36.69 ± 9.20, and the mean DPI score was 26.23 ± 9.48 among participants with MUOW/O phenotype. After controlling for potential confounders, women in the highest tertile of DPI had lower odds for MUOW/O phenotype [odds ratio (OR): 0.23, 95% confidence interval (CI): 0.07–0.68, P = 0.008] compared to the lowest tertile. Among the components of Karelis criteria, homeostatic model assessment for insulin resistance (HOMA-IR) was significantly associated with MUOW/O phenotype in the fully adjusted model (OR: 0.29, 95% CI: 0.10–0.79, P = 0.01).

Conclusion: We found a significant association between DPI and MUOW/O phenotype in Iranian women. Prospective studies are needed to confirm these findings.

KEYWORDS

phytochemical index, obesity, metabolically healthy, obesity phenotypes, overweight

Background

Obesity is a prevalent disease that is described as an epidemic with its globally increasing high rate (1). It is estimated that almost 2.1 billion people are either overweight or obese around the world (2). Obesity is caused by a constant positive energy balance, which may stem from various genetic, social, and personal factors (2, 3). Obesity is strongly linked to multiple metabolic disorders, including diabetes mellitus (DM), cardiovascular diseases (CVDs), hypertension, dyslipidemia, and inflammation-related conditions (4, 5).

Despite these facts, not all obese people develop metabolic dysfunction (6). It has been recognized that some obese individuals have a favorable metabolic profile, including blood pressure, lipid and hormonal profile, insulin sensitivity, and a lower risk of CVDs (7, 8). This subcategory of obesity is defined as metabolically healthy obesity (MHO) phenotype (7). The prevalence of MHO is controversial; however, based on different criteria and populations, it is estimated to be 6–75% (9, 10). This phenotype results from a complex interaction of multiple factors, e.g., genetics, environment, lifestyle, and diet (6, 11, 12). On the contrary, the metabolically unhealthy obesity (MUO) phenotype is connected with at least two or more metabolic disorders and more susceptibility to CVDs (13). A 10-year follow-up demonstrated that almost half the people with MHO phenotype would develop one or more metabolic abnormalities (14). Thus, MHO is a rather transient phenotype (10, 14, 15). Several criteria have been suggested to define MHO phenotypes (16, 17). Karelis criteria (18) measure triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), high-sensitivity C-reactive protein (hs-CRP), and homeostatic model assessment for insulin resistance (HOMA-IR) to determine MHO. In the current study, Karelis criteria have been used since it considers both insulin resistance and inflammation as practical measurements of health evaluation in the obese population (18) and demands at least four of the proposed criteria to introduce an individual as having MHO phenotype (19).

A significant factor that affects the metabolic health of obese individuals is diet (20). Among dietary factors, foods rich in phytochemical compounds such as fruits, vegetables, and whole grains are associated with weight control and the prevention of chronic and metabolic diseases, e.g., CVDs, type 2 DM, and metabolic syndrome (21). Phytochemicals, also called natural secondary plant metabolites, include a large group of compounds, such as phenolic compounds, organosulfur compounds, flavonoids, carotenoids, and alkaloids (22–24). It has been shown that phytochemicals regulate carbohydrate and lipid metabolism and have anti-inflammatory and anti-oxidative characteristics (25). Since it is quite troublesome to determine the amount of phytochemical intake in large populations (26), McCarty (27) proposed a practical tool, the phytochemical index (PI), which is defined as the percentage of calorie intake derived from phytochemical-rich foods. Previous studies have assessed the association between PI and metabolic syndrome, overweight/obesity, insulin resistance, lipid profile, and hypertension (25, 26, 28–30). These studies reported that PI was inversely related to hypertension, dyslipidemia, obesity, insulin resistance development, and prevalence of metabolic syndrome. However, we are not aware of any study examining PI in metabolically healthy and unhealthy individuals according to Karelis criteria. Therefore, this study aimed to evaluate the association of PI with MHO and MUO phenotypes among Iranian women.

Abbreviations: ANCOVA, analysis of covariance; ANOVA, one-way analysis of variance; BIA, bioelectrical impedance analyzer; BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; DM, diabetes mellitus; DPI, dietary phytochemical index; ELISA, enzyme-linked immunosorbent assay; FFM, fat-free mass; FM, fat mass; FFQ, Food Frequency Questionnaire; GOD/PAP, glucose oxidase phenol 4-aminoantipyrine peroxidase; GPO/PAP, glycerol-3-phosphate oxidase phenol 4-aminoantipyrine peroxidase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IPAQ, International Physical Activity Questionnaire; LDL-C, low-density lipoprotein cholesterol; MET, metabolic equivalent; MHO, metabolically healthy obesity; MUO, metabolically unhealthy obesity; OGTT, oral glucose tolerance test; OR, odds ratio; PA, physical activity; TG, triglyceride; TUMS, Tehran University of Medical Sciences; WC, waist circumference; WHR, waist to hip ratio.

Materials and methods

Study subjects

This cross-sectional study is conducted on 228 overweight/obese women from Tehran, Iran, referred

to health centers affiliated to the Tehran University of Medical Sciences (TUMS) during 2017–1019. A multistage cluster sampling method was used to recruit the study population. Participants were selected based on the following inclusion criteria: age 18–48 years, body mass index (BMI) $\geq$25 kg/m^2, and no ongoing weight loss program or taking weight loss supplements. Participants with CVDs, diabetes, cancer, kidney disease, thyroid disease, acute and chronic diseases, currently menopause, pregnant/lactating, or taking antidiabetic, antihypertensive, lipid-lowering medications, and weight loss supplements were excluded. Subjects who had a daily energy intake lower than 800 or higher than 4,200 kcal were also excluded. All participants signed informed written consent before their entrance to the study. The study protocol was approved by the Ethics Committee of TUMS with the following identification: IR.TUMS.MEDICINE.REC.1399.636.

Measurement of biochemical parameters

After 10–12 h of fasting, all blood samples were collected into tubes containing 0.1% EDTA. Samples were immediately centrifuged for 10 min at 3,000 rpm, aliquoted, and stored at a temperature of −80°C until analysis. An AutoAnalyzer BT1500 (Selectra 2; Vital Scientific, Spankeren, Netherlands) was used to analyze all the samples. Plasma glucose was measured by the glucose oxidase phenol 4-aminoantipyrine peroxidase (GOD/PAP) method. The glycerol-3-phosphate oxidase phenol 4-aminoantipyrine peroxidase (GPO-PAP) method was used to assess serum TG. Total cholesterol was measured by an endpoint enzymatic method. LDL-C and HDL-C were assayed by the direct method and immunoinhibition. An immunoturbidimetric assay measured the level of hs-CRP as a marker of inflammation. All kits were purchased from Pars Azmoon (Pars Azmoon Inc., Tehran, Iran). Also, the enzyme-linked immunosorbent assay (ELISA) method (Human Insulin ELISA Kit, DRG Pharmaceuticals, GmbH, USA) was used for serum insulin measurement.

Definition of metabolic health and its components

According to Karelis criteria, metabolic health status was defined as follows: TG $\leq$1.7 mmol/L, HDL $\geq$1.3 mmol/L and no treatment, LDL $\leq$2.6 mmol/L and no treatment, hs-CRP $\leq$3.0 mg/L, and HOMA-IR $\leq$2.7. The presence of four or more of the features is a diagnosis of metabolic health. Thus, according to metabolic health, participants are classified into two groups, namely, MHO and MUO (18).

HOMA-IR calculation

We calculated insulin resistance using HOMA-IR as the following formula: fasting insulin (mUI/L) × fasting glucose (mmol/L)/22.5 (31).

Assessment of dietary intake and phytochemical index calculation

Dietary intake was examined using a validated 147-item semi-quantitative Food Frequency Questionnaire (FFQ) (32). The FFQ contains a list of foods commonly consumed by Iranians in standard serving sizes. In face-to-face interviews, participants were asked to report their average frequency of food intakes. Finally, we converted the daily intake of all food items to grams per day using household measures.

Phytochemical index was calculated based on the method developed by McCarty {PI = [daily energy derived from phytochemical-rich foods (kcal)/total daily energy intake (kcal)] × 100} (27). This index included phytochemical-rich foods such as whole grains, fruits and vegetables, nuts and seeds, legumes, soy products, and olive and olive oil. The potato was not included due to its low phytochemical content, and it is often consumed as a starch component rather than a vegetable. Natural fruit and vegetable juices and tomato sauce were considered in PI due to their high phytochemical content. We also added tea and coffee as rich sources of phytochemicals. Phenolic compounds, including flavonoids, phenolic acids, hydroxycinnamic acids, lignans, tyrosol esters, stilbenoids, isoprenoids, and organosulfur compounds (allyl sulfurs, isothiocyanate) are taken into account in PI.

Body composition analysis

A multi-frequency bioelectrical impedance analyzer (BIA), InBody 770 Scanner (Inbody Co., Seoul, South Korea), was used for body composition assessment. Fat-free mass (FFM), fat mass (FM), and waist-to-hip ratio (WHR) were recorded by this analyzer. As the manufacturer's instructions required, participants had to take their shoes, coats, and sweaters off, stand on the balance scale, and hold the handles of the machine. In addition, subjects were required to abstain from intense exercise and intake of a meal or excessive fluid before the analysis. Body composition analysis was conducted in a fasting condition in the morning after emptying the bladder (33).

Assessment of other variables

Information on age, education (primary/secondary/university), marriage (single/married), smoking status

(smoker/non-smoker), family history of obesity (yes/no), and occupation (employed/unemployed) was obtained.

In terms of anthropometric measurements, weight was measured to the nearest 100 g using digital scales with minimal clothes, and height was measured to the nearest 0.5 cm by a tape meter mounted on the wall while the subject was standing in a normal position without shoes. BMI was calculated by dividing weight (kg) by height squared (m^2). Waist circumference (WC) was measured by a tape at the level midway between the lower rib margin and the iliac crest at the end of normal expiration to the nearest 0.5 cm when subjects were standing.

The level of physical activity (PA) was assessed using the International Physical Activity Questionnaire (IPAQ). Participants reported the frequency and duration of severe, moderate, jogging, and sedentary PA during the past 7 days, and the level of PA was expressed as metabolic equivalent hours per week (METs/h/week). METs were classified as low (<600 MET-min/week), moderate (600–3,500 MET-min/week), and vigorous (>3,500 MET-min/week).

Statistical analysis

Kolmogorov–Smirnov's test was used to evaluate the normal distribution of the data. One-way analysis of variance (ANOVA) was used to determine differences in continuous variables across tertiles of dietary phytochemical index (DPI). The Chi-square test was applied to examine the distribution of participants in terms of categorical variables across tertiles of DPI. Data on quantitative characteristics were reported as the mean ± SD and data on qualitative characteristics were expressed as a percentage. The differences between the dietary intakes of participants based on the obesity phenotypes were assessed by one-way ANOVA. For evaluating these differences after adjustment of age (continuous), BMI (continuous), PA (low, moderate, and high), and total energy intake (continuous), the analysis of covariance (ANCOVA) test was used. Binary logistic regression was applied to examine the associations of the DPI (independent variable) with the MUO (dependent variable) and components of Karelis criteria (dependent variable). DPI was categorized as tertiles, and MUO and the five components of Karelis Criteria were categorized as binary variables in the models. SPSS software was used (version 24; SPSS Inc., Chicago, IL, USA) for all statistical analyses. P-value < 0.05 was considered statistically significant.

Results

The general characteristics of the 228 study participants across tertiles of DPI are shown in Table 1. Compared to the participants in the lowest tertile, participants in the highest tertile were older ($P < 0.001$), more likely to have lower weight ($P = 0.03$) and height ($P = 0.004$), lower FM ($P = 0.03$), FFM ($P = 0.02$), and more likely to be non-smokers ($P = 0.01$).

The dietary intakes of participants according to obesity phenotypes are presented in Table 2. Only cholesterol intake was significantly higher among subjects with MUH status ($P = 0.02$) even after adjusting for total energy intake ($P = 0.04$).

Crude and multivariable-adjusted odd ratios (ORs) with 95% confidence intervals (CIs) for MUH phenotype across tertiles of DPI are provided in Table 3. No significant association was found between MUH phenotype and DPI in the crude model. After adjusting for age, BMI, total energy intake, and PA, those in the highest tertile had lower odds for MUH phenotype (OR: 0.29, 95% CI: 0.11–0.76, $P = 0.01$). After further adjustments for marital status, occupation, education, socioeconomic status, weight loss history, and family size, individuals in the highest tertile of DPI still had 77% lower odds for MUH phenotype compared to the lowest tertile (OR: 0.23, 95% CI: 0.07–0.68, $P = 0.008$).

Table 4 shows multivariate-adjusted models with 95% CIs for components of Karelis criteria among tertiles of DPI. Compared with the subjects in the lowest tertile of DPI, those in the highest tertile had 58% lower odds of having HOMA-IR (OR: 0.42, 95% CI: 0.18–0.97, $P = 0.04$). This association remained significant after additionally controlling for marital status, occupation, education, socioeconomic status, weight loss history, and family size (OR: 0.29, 95% CI: 0.10–0.79, $P = 0.01$). For TG level, in the fully adjusted model, the participants in the highest tertile of DPI had marginally lower odds for TG concentrations above 1.7 mmol/L (OR: 0.35, 95% CI: 0.12–1.03, $P = 0.05$). No significant association was detected between DPI and other components of Karelis criteria.

Discussion

Little has been understood about the association of DPI, a dietary index that is a simple and practical method for assessing dietary phytochemical intake, with metabolic health status in overweight/obese individuals. Although prior investigations have found an inverse association between DPI and metabolic disorders, including hypertension, dyslipidemia, obesity, insulin resistance, and metabolic syndrome, there is no study investigating the DPI in overweight/obese metabolically healthy and unhealthy individuals according to Karelis criteria. As far as we know, this is the first research project evaluating the relationship between DPI and MHO and MUO phenotypes among overweight/obese Iranian females. Based on our findings, DPI was found to be inversely correlated with MUO phenotype, HOMA-IR, and TG levels even after adjustment for potential confounders, while no association was observed between DPI and serum concentrations of HDL, LDL, and hs-CRP.

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TABLE 1 General characteristics of study participants by tertiles of DPI.

		Tertiles of DPI			
	Total (*n* = 228)	**T1 (*n* = 76)**	**T2 (*n* = 76)**	**T3 (*n* = 76)**	***P*-value[a]**
Demographic variables					
Age (years)	36.69 ± 9.20	33.51 ± 8.27	37.61 ± 9.22	39.97 ± 9.26	<0.001
Weight (kg)	81.17 ± 12.26	83.24 ± 13.26	81.03 ± 11.74	79.24 ± 11.48	0.03
Height (cm)	161.15 ± 5.88	162.08 ± 5.56	161.57 ± 5.61	159.80 ± 6.25	0.004
Body composition					
BMI (kg/m^2)	31.27 ± 4.30	31.84 ± 4.95	30.95 ± 3.66	31.02 ± 4.16	0.18
FM (kg)	34.73 ± 8.74	36.28 ± 9.93	34.27 ± 7.69	33.63 ± 8.29	0.03
FFM (kg)	46.50 ± 5.66	47.32 ± 5.40	46.69 ± 5.99	45.48 ± 5.47	0.02
WC (cm)	99.59 ± 10.07	101.01 ± 10.72	99.47 ± 9.43	98.28 ± 9.91	0.09
WHR	0.93 ± 0.05	0.93 ± 0.05	0.93 ± 0.05	0.93 ± 0.05	0.42
Biochemical parameters					
FBS (mmol/L)	4.86 ± 0.53	4.83 ± 0.49	4.86 ± 0.51	4.87 ± 0.58	0.89
TG (mmol/L)	1.38 ± 0.79	1.33 ± 0.82	1.37 ± 0.78	1.42 ± 0.78	0.76
LDL-C (mmol/L)	2.45 ± 0.62	2.37 ± 0.61	2.48 ± 0.60	2.50 ± 0.65	0.40
HDL-C (mmol/L)	1.21 ± 0.28	1.19 ± 0.25	1.22 ± 0.28	1.20 ± 0.30	0.69
Total-C (mmol/L)	4.78 ± 0.93	4.69 ± 0.99	4.83 ± 0.87	4.82 ± 0.95	0.56
HOMA	3.34 ± 1.28	3.23 ± 1.18	3.30 ± 1.35	3.48 ± 1.30	0.46
Inflammatory marker					
hs-CRP (mg/L)	4.31 ± 4.65	4.61 ± 4.51	4.18 ± 4.80	4.19 ± 4.66	0.81
Qualitative variables					
Marital status (%)					
Married	72.2	65.9	77.7	73.1	0.10
Single	27.8	34.1	22.3	26.9	
Family size (%)					
≤4		86.8	79	87.3	0.12
>4		13.2	21	12.7	
Education (%)					
≤Primary	4.6	6.2	3.1	4.6	0.65
Secondary	40.6	37.2	40.8	43.8	
University	54.8	56.6	56.2	51.5	
Occupation (%)					
Unemployed	59	63.8	60.5	52.7	0.18
Employed	41	36.2	39.5	47.3	
Smoking (%)					
Yes	6.9	12.3	4.6	3.9	0.01
No	93.1	87.7	95.4	96.1	
Economic status (%)					
Poor	22.9	18.5	25.4	24.8	0.67
Medium	48.5	52.4	47.6	45.6	
High	28.6	29	27	29.6	
Weight loss history (%)					
Yes	54.5	49.6	56.8	57	0.42
No	45.5	50.4	43.2	43	

(Continued)

TABLE 1 (Continued)

		Tertiles of DPI			
	Total (n = 228)	T1 (n = 76)	T2 (n = 76)	T3 (n = 76)	P-value[a]
Family history of obesity (%)					
Yes	71	72.1	67.7	73.2	0.60
No	29	27.9	32.3	26.8	
Physical activity (%)[b]					
Low	50	56.0	42.9	51.2	0.14
Moderate	45.3	41.3	53.8	40.7	
High	4.7	2.7	3.3	8.1	

DPI, dietary phytochemical index; BMI, body mass index; FM, fat mass; FFM, fat free mass; PBF, percent body fat; WC, waist circumference; WHR, waist to hip ratio; FBS, fasting blood sugar; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Total-C, total cholesterol; HOMA, homeostatic model assessment; hs-CRP, high-sensitivity C-reactive protein. P-value less than 0.05 was considered significant.
All values are mean ± SD or reported percentage.
[a] P-values result from ANOVA for quantitative variables and χ^2 test for qualitative variables.
[b] Physical activity was classified as low <600 MET-h/week, moderate = 600–3,500 MET-h/week, and high >3,500 MET-h/week.

TABLE 2 Dietary intake of MHO and MUO participants.

	MHO (n = 64)	MUO (n = 164)	P-value[a]	P-value[b]
Total energy (kcal/day)	2, 516.37 ± 725.67	2, 636.87 ± 751.67	0.27	–
Carbohydrate (g/day)	363.26 ± 124.21	374.42 ± 117.81	0.53	0.28
Protein (g/day)	83.29 ± 25.78	90.13 ± 28.47	0.09	0.19
Fat (g/day)	89.77 ± 27.81	95.35 ± 33.56	0.24	0.62
Total fiber (g/day)	45.35 ± 19.98	44.49 ± 17.82	0.75	0.19
SFA (g/day)	26.23 ± 9.78	28.56 ± 11.66	0.16	0.37
PUFA (g/day)	19.21 ± 7.04	20.27 ± 9.00	0.40	0.72
MUFA (g/day)	29.28 ± 8.82	31.65 ± 11.62	0.14	0.32
Cholesterol (mg/day)	225.83 ± 82.04	259.32 ± 105.13	0.02	0.04
Vitamin A (RAE/day)	725.76 ± 349.76	807.93 ± 435.35	0.18	0.34
Vitamin E (mg/day)	16.31 ± 7.23	17.58 ± 9.22	0.32	0.50
Vitamin C (mg/day)	191.31 ± 124.73	199.48 ± 134.42	0.67	0.88
Calcium (mg/day)	1, 115.47 ± 420.35	1, 172.18 ± 416.70	0.36	0.81
Magnesium (mg/day)	443.31 ± 143.05	469.90 ± 150.17	0.22	0.58
Potassium (mg/day)	4, 216.38 ± 1, 632.89	4, 416.48 ± 1, 546.65	0.39	0.96
Zinc (mg/day)	12.29 ± 4.08	13.26 ± 4.31	0.12	0.25
Whole grains (g/day)	56.86 ± 53.47	63.50 ± 62.17	0.45	0.63
Fruits (g/day)	557.51 ± 398.24	565.57 ± 367.16	0.88	0.59
Vegetables (g/day)	425.25 ± 243.12	455.95 ± 279.76	0.44	0.66
Nuts and seeds (g/day)	15.61 ± 16.91	15.60 ± 17.86	0.99	0.64
Legumes (g/day)	48.81 ± 53.05	46.41 ± 31.25	0.67	0.55
Soy sources (g/day)	4.96 ± 8.14	6.06 ± 9.29	0.41	0.43
Olive and olive oil (g/day)	4.11 ± 6.16	4.88 ± 7.58	0.47	0.53
Tea and coffee (g/day)	740.02 ± 575.90	776.88 ± 909.48	0.76	0.88
Phytochemical index	28.15 ± 12.06	26.23 ± 9.48	0.21	0.49

MHO, metabolic healthy obesity; MUO, metabolic unhealthy obesity; SFA, saturated fatty acids; PUFA, poly unsaturated fatty acids; MUFA, mono unsaturated fatty acids. P-value less than 0.05 was considered significant. All values are mean ± SD.
[a] P-values result from ANOVA.
[b] P-values adjusted for energy intake.

In accordance with the obtained results, a longitudinal study performed by Mirmiran et al. (34) concluded that the highest quartile category of DPI was inversely associated with 3-year changes in weight, WC, and body adiposity index among 1,983 Tehranian adults. A recent research project evaluated the relationship between DPI and metabolic syndrome components in the Korean population and proposed that the highest DPI quintile was significantly related to a lower prevalence of

TABLE 3 Multivariate adjusted odds ratios (OR) and 95% confidence intervals (CI) for MUO phenotypes across tertiles of DPI.

MUO phenotype	Tertiles of DPI				
	T1 (n = 76)	T2 (n = 76)		T3 (n = 76)	
		OR (95% CI)	P-value	OR (95% CI)	P-value
Model 1[a]	1	1.05 (0.48, 2.28)	0.89	0.57 (0.29, 1.14)	0.11
Model 2[b]	1	0.48 (0.11, 1.98)	0.31	0.29 (0.11, 0.76)	0.01
Model 3[c]	1	0.36 (0.07, 1.81)	0.21	0.23 (0.07, 0.68)	0.008

MUO, metabolically unhealthy obesity; DPI, dietary phytochemical index. Results are presented as OR and 95% CI.
[a]Unadjusted.
[b]Adjusted for age, BMI, total energy intake, and physical activity.
[c]Additionally adjusted for marital status, occupation, education, socioeconomic status, weight loss history, and family size.

TABLE 4 Multivariate adjusted odds ratios (OR) and 95% confidence intervals (CI) for Karelis criteria components across tertiles of DPI.

	Tertiles of DPI				
	T1 (n = 76)	T2 (n = 76)		T3 (n = 76)	
		OR (95% CI)	P-value	OR (95% CI)	P-value
HOMA-IR					
Model 1[a]	1	0.69 (0.34, 1.40)	0.31	0.49 (0.25, 0.95)	0.03
Model 2[b]	1	0.72 (0.21, 2.50)	0.61	0.42 (0.18, 0.97)	0.04
Model 3[c]	1	0.43 (0.10, 1.83)	0.25	0.29 (0.10, 0.79)	0.01
hs-CRP (mg/L)					
Model 1	1	1.70 (0.89, 3.24)	0.10	1.08 (0.57, 2.04)	0.79
Model 2	1	1.79 (0.54, 5.91)	0.33	0.89 (0.38, 2.06)	0.78
Model 3	1	3.00 (0.77, 11.56)	0.11	1.10 (0.42, 2.88)	0.84
LDL-C (mmol/L)					
Model 1	1	0.70 (0.36, 1.35)	0.29	1.03 (0.56, 1.91)	0.90
Model 2	1	1.08 (0.34, 3.43)	0.89	1.12 (0.49, 2.51)	0.78
Model 3	1	0.71 (0.19, 2.61)	0.61	0.95 (0.38, 2.36)	0.91
HDL-C (mmol/L)					
Model 1	1	0.84 (0.42, 1.64)	0.61	0.66 (0.35, 1.26)	0.21
Model 2	1	0.53 (0.16, 1.70)	0.28	0.58 (0.25, 1.32)	0.20
Model 3	1	0.40 (0.10, 1.50)	0.17	0.43 (0.17, 1.11)	0.08
TG (mmol/L)					
Model 1	1	0.71 (0.34, 1.47)	0.36	0.64 (0.31, 1.31)	0.22
Model 2	1	0.68 (0.19, 2.43)	0.55	0.54 (0.21, 1.37)	0.19
Model 3	1	0.42 (0.10, 1.80)	0.24	0.35 (0.12, 1.03)	0.05

DPI, dietary phytochemical index; HOMA-IR, homeostatic model assessment for insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride.
[a]Unadjusted.
[b]Adjusted for age, BMI, total energy intake, and physical activity.
[c]Additionally adjusted for marital status, occupation, education, socioeconomic status, weight loss history, and family size.

metabolic syndrome and its components, including abdominal obesity, hyperglycemia, hypertriglyceridemia, and high blood pressure, although WC and the concentrations of HDL did not alter significantly among DPI quintiles (30). Also, a cross-sectional study by Bahadoran et al. (35) disclosed that higher intakes of phytochemical-rich foods are correlated with 66 and 36% reduced risk of abdominal obesity and hypertriglyceridemia as the main cardiometabolic risk factors in 2,567 Iranian adults aged 19–70 years. In the same study, the mean serum HDL level had increasing trends across the increase of phytochemical intake categories, and also participants in the upper quartile of DPI had a lower weight and WC (35). Furthermore, another cross-sectional study examining the association between DPI and serum concentrations of hs-CRP, a marker of low-grade systemic inflammation released by adipose tissue, found no association between hs-CRP and DPI

among 170 premenopausal women, while there were significant associations between hs-CRP and several central obesity indices (36). Moreover, Golzarand et al. (25) reported an inverse association between DPI and TG, TC, and non-HDL cholesterol levels among the Iranian population aged 19–70 years in a Tehran Lipid and Glucose prospective study. In addition, DPI was found to be negatively associated with FBS levels and the 2-h oral glucose tolerance test (OGTT) even after controlling for confounders, including BMI, PA, and dietary intake of energy, fiber, carbohydrate, fat, and protein in a case-control study conducted on 300 Iranian adults (37). The discrepancies between the findings of the mentioned studies may be caused by different nationalities, physiological status, lifestyles, and age groups of participants.

The mechanisms of action of phytochemicals, non-toxic and cost-effective bioactive compounds compared to synthetic alternatives, on obesity and its related indices have been studied by several *in vitro* and *in vivo* models (38). Phytochemicals exert anti-obesogenic activities by targeting various obesity-related pathways and regulatory functions, including inhibition of dietary lipid digestion through attenuating pancreatic lipase activities, which are considered as main enzymes responsible for the digestion and absorption of lipids, inhibition of adipogenesis and differentiation of preadipocytes, stimulation of existing adipocytes apoptosis (38–43). Also, phytochemicals are shown to exert weight-lowering effects by elevating energy expenditure through activating brown adipogenesis, browning of white adipose tissue, and upregulating the expression of thermogenic genes, induction of lipolytic pathways, and appetite modulation through regulating various satiety-related hormonal and neurological signals such as the sympathetic nervous system, dopamine, histamine, and serotonin receptors, and adrenalin, leptin, and ghrelin levels in human bodies (38–43).

Despite several remarkable strengths of the present study, including relatively appropriate sample size, adjustment for numerous probable confounders that could potentially affect the results, and examining the relationship between DPI and metabolic phenotypes of obesity among overweight/obese Iranian females for the first time, our findings should be interpreted in the context of some weaknesses. First, as the most significant limitation, the current study cannot exhibit the causal association between DPI, MHO, and MUO phenotypes based on its cross-sectional nature. Second, there was no information about the duration of obesity that has been suggested to affect participants' metabolic health status. Third, DPI was computed using calories from consumed food items, and thereby food items containing zero calories, such as spices, which are good sources of phytochemicals, could not be considered. Fourth, we used retrospective dietary data by FFQ, which may cause errors in calculating DPI due to the participants' recall bias. Finally, racial homogeneity was also a limitation of the current research.

Conclusion

Dietary phytochemical index was found to be inversely correlated with MUO phenotype, HOMA-IR, and TG levels even after adjustment for potential confounders. Further prospective cohort studies with larger sample sizes are proposed to elucidate the association between DPI and metabolic phenotypes of obesity among overweight/obese subjects.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Tehran University of Medical Sciences (TUMS) with the following identification: IR.TUMS.MEDICINE.REC.1400.696. The patients/participants provided their written informed consent to participate in this study.

Author contributions

SP was responsible for conceptualization. SP and AM analyzed the data. SP, FN, and SN took the lead in writing the manuscript. AM and KM were responsible for review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

References

1. Moreno-Perez, B, Benito E, Civera M, Alabadi B, Martinez-Hervas S, Peiro M, et al. Postprandial triglyceridemia is modulated by insulin resistance but not by grade of obesity in abdominal and morbid obese subjects. *Int J Clin Pract.* (2020) 75:e13776.

2. Smith KB, Smith MS. Obesity statistics. *Prim Care.* (2016) 43:121–35.

3. Lopez-Contreras IN, Vilchis-Gil J, Klunder-Klunder M, Villalpando-Carrion S, Flores-Huerta S. Dietary habits and metabolic response improve in obese children whose mothers received an intervention to promote healthy eating: randomized clinical trial. *BMC Public Health.* (2020) 20:1240. doi: 10.1186/s12889-020-09339-4

4. Thomsen SB, Gjesing AP, Rathcke CN, Ekstrom CT, Eiberg H, Hansen T, et al. Associations of the Inflammatory marker YKL-40 with measures of obesity and dyslipidaemia in individuals at high risk of type 2 diabetes. *PLoS One.* (2015) 10:e0133672. doi: 10.1371/journal.pone.0133672

5. Kawser Hossain M, Abdal Dayem A, Han J, Yin Y, Kim K, Kumar Saha S, et al. Molecular Mechanisms of the anti-obesity and anti-diabetic properties of flavonoids. *Int J Mol Sci.* (2016) 17:569.

6. Oh CM, Park JH, Chung HS, Yu JM, Chung W, Kang JG, et al. Effect of body shape on the development of cardiovascular disease in individuals with metabolically healthy obesity. *Medicine (Baltimore).* (2020) 99:e22036. doi: 10.1097/MD.0000000000022036

7. Frey S, Patouraux S, Debs T, Gugenheim J, Anty R, Iannelli A. Prevalence of NASH/NAFLD in people with obesity who are currently classified as metabolically healthy. *Surg Obes Relat Dis.* (2020) 16:2050–7. doi: 10.1016/j.soard.2020.07.009

8. Rahimi H, Yuzbashian E, Zareie R, Asghari G, Djazayery A, Movahedi A, et al. Dietary approaches to stop hypertension (DASH) score and obesity phenotypes in children and adolescents. *Nutr J.* (2020) 19:112. doi: 10.1186/s12937-020-00631-y

9. Rey-Lopez J, De Rezende L, Pastor-Valero M, Tess B. The prevalence of metabolically healthy obesity: a systematic review and critical evaluation of the definitions used. *Obes Rev.* (2014) 15:781–90. doi: 10.1111/obr.12198

10. Hosseinpanah F, Tasdighi E, Barzin M, Mahdavi M, Ghanbarian A, Valizadeh M, et al. The association between transition from metabolically healthy obesity to metabolic syndrome, and incidence of cardiovascular disease: Tehran lipid and glucose study. *PLoS One.* (2020) 15:e0239164. doi: 10.1371/journal.pone.0239164

11. Stefan N, Häring H-U, Hu FB, Schulze MB. Metabolically healthy obesity: epidemiology, mechanisms, and clinical implications. *Lancet Diabetes Endocrinol.* (2013) 1:152–62.

12. Mathew H, Farr OM, Mantzoros CS. Metabolic health and weight: understanding metabolically unhealthy normal weight or metabolically healthy obese patients. *Metab Clin Exp.* (2016) 65:73–80.

13. Tang HN, Xiao F, Chen YR, Zhuang SQ, Guo Y, Wu HX, et al. Higher serum neuropeptide Y levels are associated with metabolically unhealthy obesity in obese chinese adults: a cross-sectional study. *Mediat Inflamm.* (2020) 2020:7903140. doi: 10.1155/2020/7903140

14. Appleton SL, Seaborn CJ, Visvanathan R, Hill CL, Gill TK, Taylor AW, et al. Diabetes and cardiovascular disease outcomes in the metabolically healthy obese phenotype: a cohort study. *Diabetes Care.* (2013) 36:2388–94.

15. Lin H, Zhang L, Zheng R, Zheng Y. The prevalence, metabolic risk and effects of lifestyle intervention for metabolically healthy obesity: a systematic review and meta-analysis: a PRISMA-compliant article. *Medicine.* (2017) 96:e8838. doi: 10.1097/MD.0000000000008838

16. Alam I, Ng TP, Larbi A. Does inflammation determine whether obesity is metabolically healthy or unhealthy? The aging perspective. *Mediat Inflamm.* (2012) 2012:456456.

17. Tian S, Xu Y, Dong H. The effect of metabolic health and obesity phenotypes on risk of hypertension: a nationwide population-based study using 5 representative definitions of metabolic health. *Medicine.* (2018) 97:e12425. doi: 10.1097/MD.0000000000012425

18. 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.

19. Truthmann J, Mensink GB, Bosy-Westphal A, Scheidt-Nave C, Schienkiewitz A. Metabolic health in relation to body size: changes in prevalence over time between 1997-99 and 2008-11 in Germany. *PLoS One.* (2016) 11:e0167159. doi: 10.1371/journal.pone.0167159

20. Boonchaya-Anant P, Apovian CM. Metabolically healthy obesity—does it exist? *Curr Atherosc Rep.* (2014) 16:441. doi: 10.1007/s11883-014-0441-1

21. Dingeo G, Brito A, Samouda H, Iddir M, La Frano MR, Bohn T. Phytochemicals as modifiers of gut microbial communities. *Food Funct.* (2020) 11:8444–71.

22. Vazquez-Prieto MA, Miatello RM. Organosulfur compounds and cardiovascular disease. *Mol Aspects Med.* (2010) 31:540–5.

23. Sivakumar D, Chen L, Sultanbawa Y. A comprehensive review on beneficial dietary phytochemicals in common traditional Southern African leafy vegetables. *Food Sci Nutr.* (2018) 6:714–27. doi: 10.1002/fsn3.643

24. Singh VK, Arora D, Ansari MI, Sharma PK. Phytochemicals based chemopreventive and chemotherapeutic strategies and modern technologies to overcome limitations for better clinical applications. *Phytother Res.* (2019) 33:3064–89. doi: 10.1002/ptr.6508

25. Golzarand M, Mirmiran P, Bahadoran Z, Alamdari S, Azizi F. Dietary phytochemical index and subsequent changes of lipid profile: a 3-year follow-up in Tehran Lipid and glucose study in Iran. *ARYA Atheroscler.* (2014) 10:203–10.

26. Golzarand M, Bahadoran Z, Mirmiran P, Sadeghian-Sharif S, Azizi F. Dietary phytochemical index is inversely associated with the occurrence of hypertension in adults: a 3-year follow-up (the Tehran Lipid and Glucose Study). *Eur J Clin Nutr.* (2015) 69:392–8. doi: 10.1038/ejcn.2014.233

27. Mccarty MF. Proposal for a dietary "phytochemical index". *Med Hypotheses.* (2004) 63:813–7.

28. Bahadoran Z, Mirmiran P, Tohidi M, Azizi F. Dietary phytochemical index and the risk of insulin resistance and beta-cell dysfunction: a prospective approach in Tehran lipid and glucose study. *Int J Food Sci Nutr.* (2015) 66:950–5. doi: 10.3109/09637486.2015.1111867

29. Im J, Kim M, Park K. Association between the phytochemical index and lower prevalence of obesity/abdominal obesity in Korean adults. *Nutrients.* (2020) 12:2312.

30. Kim M, Park K. Association between phytochemical index and metabolic syndrome. *Nutr Res Pract.* (2020) 14:252–61.

31. Mirzaei K, Hossein-Nezhad A, Keshavarz S, Eshaghi S, Koohdani F, Saboor-Yaraghi A, et al. Insulin resistance via modification of PGC1α function identifying a possible preventive role of vitamin D analogues in chronic inflammatory state of obesity. A double blind clinical trial study. *Miner Med.* (2014) 105:63–78.

32. Mirmiran P, Esfahani FH, Mehrabi Y, Hedayati M, Azizi F. Reliability and relative validity of an FFQ for nutrients in the Tehran lipid and glucose study. *Public Health Nutr.* (2010) 13:654–62. doi: 10.1017/S1368980009991698

33. Mirzababaei A, Sajjadi SF, Ghodoosi N, Pooyan S, Arghavani H, Yekaninejad MS, et al. Relations of major dietary patterns and metabolically unhealthy overweight/obesity phenotypes among Iranian women. *Diabetes Metab Syndrome Clin Res Rev.* (2019) 13:322–31. doi: 10.1016/j.dsx.2018.09.012

34. Mirmiran P, Bahadoran Z, Golzarand M, Shiva N, Azizi F. Association between dietary phytochemical index and 3-year changes in weight, waist circumference and body adiposity index in adults: Tehran Lipid and Glucose study. *Nutr Metab.* (2012) 9:108. doi: 10.1186/1743-7075-9-108

35. Bahadoran Z, Golzarand M, Mirmiran P, Saadati N, Azizi F. The association of dietary phytochemical index and cardiometabolic risk factors in adults: Tehran Lipid and glucose study. *J Hum Nutr Diet.* (2013) 26(Suppl. 1):145–53. doi: 10.1111/jhn.12048

36. Edalati S, Alipour B, Rashidkhani B. Association between dietary intake of phytochemicals and hs-CRP in healthy women from Tehran: a holistic approach using dietary phytochemical index. *Nutr Food Sci Res.* (2018) 5:11–6.

37. Abshirini M, Mahaki B, Bagheri F, Siassi F, Koohdani F, Sotoudeh G. Higher intake of phytochemical-rich foods is inversely related to prediabetes: a case-control study. *Int J Prev Med.* (2018) 9:64–64. doi: 10.4103/ijpvm.IJPVM_145_18

38. Ahmad B, Friar EP, Vohra MS, Garrett MD, Serpell CJ, Fong IL, et al. Mechanisms of action for the anti-obesogenic activities of phytochemicals. *Phytochemistry.* (2020) 180:112513.

39. Belza A, Frandsen E, Kondrup J. Body fat loss achieved by stimulation of thermogenesis by a combination of bioactive food ingredients: a placebo-controlled, double-blind 8-week intervention in obese subjects. *Int J Obes.* (2007) 31:121–30. doi: 10.1038/sj.ijo.0803351

40. Klok MD, Jakobsdottir S, Drent ML. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. *Obes Rev.* (2007) 8:21–34.

41. Yun JW. Possible anti-obesity therapeutics from nature–a review. *Phytochemistry.* (2010) 71:1625–41. doi: 10.1016/j.phytochem.2010.07.011

42. Morton GJ, Meek TH, Schwartz MW. Neurobiology of food intake in health and disease. *Nat Rev Neurosci.* (2014) 15:367–78.

43. Azhar Y, Parmar A, Miller CN, Samuels JS, Rayalam S. Phytochemicals as novel agents for the induction of browning in white adipose tissue. *Nutr Metab.* (2016) 13:1–11. doi: 10.1186/s12986-016-0150-6

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Dietary total antioxidant capacity is inversely associated with the odds of non-alcoholic fatty liver disease in people with type-2 diabetes

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Background: This study was conducted to evaluate possible associations between Dietary Total Antioxidant Capacity (DTAC) and odds of non-alcoholic fatty liver disease (NAFLD) in people with type-2 diabetes mellitus (T2DM).

Materials and methods: We recruited two hundred people with T2DM, and evaluated their liver steatosis using Fibroscan. Dietary intakes of participants were assessed using a validated food frequency questionnaire. DTAC was computed *via* ferric reducing antioxidant power (FRAP).

Results: In the crude model, no statistically significant association was found between DTAC and the odds of NAFLD in people with diabetes. However, after adjustment for potential confounders including age, gender, diabetes duration, smoking status, physical activity, BMI, waist circumference, and energy, the most reduced adjusted OR was indicated for the third tertile *vs.* the first one (OR: 0.28, 95% CI: 0.09–0.81, P = 0.02), meaning that diabetic patients in the third tertile of DTAC had 72% decreased risk of NAFLD in comparison to those in the first one. The relationship was remained significant after additional adjustment for HOMA-IR, HbA1c, serum Triglyceride (TG), and low-density lipoprotein-cholesterol (LDL) levels (OR: 0.29, 95% CI: 0.09–0.93,

P = 0.03). Importantly, a dose-response pattern was demonstrated for DTAC and risk of NAFLD (P = 0.04).

Conclusion: Higher DTAC was related with a decreased risk of NAFLD in individuals with diabetes.

KEYWORDS

NAFLD, diabetes, diet, DTAC, antioxidant

Introduction

Non-alcoholic fatty liver disease (NAFLD), an obesity complication commonly overlooked, is defined by the deposition of more than 5% fat in the liver not resulting from other identifiable factors such as alcohol consumption or viral hepatitis, ranging from hepatic steatosis to fibrosis and related cirrhosis (1, 2). Non-alcoholic steatohepatitis (NASH) is a more severe form of NAFLD featured by inflammation, hepatocyte necrosis, and regularly fibrosis (3). As a major cause of liver disease globally, NAFLD and NASH give rise to a substantial burden (4). A recent meta-analysis of 86 studies in 2016, estimated the prevalence of NAFLD in general population worldwide at 25.24%, whereas several meta-analyses demonstrated that in 2017 the prevalence was twofold (54–59.67%) in people with type 2 diabetes mellitus (T2DM) (2, 5, 6). The link between NAFLD and T2DM has been clearly identified, and can be described by IR and hyperinsulinemia resulting in defective lipid metabolism and the accumulation of fatty acids in the liver (7). Moreover, in people suffering from T2DM and NASH, the process of oxidative stress is increased, compared to T2DM patients without NAFLD (8, 9). T2DM furthermore enhances the susceptibility to advanced NAFLD including NASH, liver fibrosis, and hepatocellular carcinoma (4). Therefore, it seems vital to identify and monitor these high-risk patients.

The gold standard procedure for distinguishing NAFLD is liver biopsy, however, is impossible to apply an invasive and expensive technique in such a great number of T2DM patients (10). Consequently, it is of benefit in identifying people suffering from NAFLD using simple methods, in order to detect those likely to progress to NASH or advanced liver disease (10). Nutrition is known as a main modifiable environmental factor in the development and management of NAFLD (11). A few researchers have clarified that antioxidant intake plays a crucial role in protecting against oxidative damage and relevant inflammatory complications in people with NAFLD (12). Currently, dietary total antioxidant capacity (DTAC) is regarded as a useful index to investigate the whole antioxidant capacity of foods (13). In comparison to a simple sum of certain dietary antioxidants, DTAC provides the cumulative capability of the total dietary antioxidants (13). Accumulating evidence suggests that DTAC is inversely associated with adverse health consequences such as cardiovascular diseases, T2DM, cancer deaths, and obesity (14–17). A recent case-control study declared that high DTAC is significantly related to decreased risk of NAFLD (12). Additionally, patients with greater DTAC indicated lower odds of NAFLD in comparison to lower amounts of DTAC (18).

Overall, the present study focused on the changes in blood glucose, lipid profile, transaminases, and IR among T2DM patients with and without NAFLD in order to present potential prognostic markers for identifying diabetic patients being at high risk of NAFLD. Furthermore, people with T2DM need to specifically be considered in the assessment of potential dietary prevention strategies for NAFLD. Nevertheless, to the best of the authors' knowledge, the association between DTAC and the development of NAFLD in T2DM patients has not yet been investigated. Thus, the present study was conducted to evaluate probable association between DTAC and odds of NAFLD in T2DM patients.

Materials and methods

Study population

This cross-sectional study was conducted between April 2021 and February 2022 and was approved by the Ethics Committee of the Shahid Beheshti University of Medical Sciences (NO: IR.SBMU.NNFTRI.REC.1399.061). Eligible volunteers were selected by the use of consecutive-sampling method and provided with informed written consent, prior to study commencement.

Inclusion criteria

The study population was recruited from people with T2DM referred to the diabetes clinic affiliated with the Institute of Diabetes and Metabolism, Iran University of Medical Sciences, Tehran, Iran. Participants in the NAFLD group (n = 133) were individuals aged between 18 and 70 years old with confirmed T2DM for over 2 years and CAP (Controlled attenuation parameter) score > 270 dB/m. The non-NAFLD group (n = 67)

included individuals with 18–70 years of age and diagnosis of T2DM for over 2 years, as well as CAP score $\leq$ 270 dB/m. In addition, no participants in the NAFLD or non-NAFLD group was on insulin therapy. Body mass index (BMI) $\geq$ 23 kg/m^2 was another inclusion criterion for participants in the both study groups. NAFLD was diagnosed using the findings of Fibroscan performed by an expert physician.

Exclusion criteria

People with a history of any type of pathologically confirmed cancer, under chemotherapy or radiotherapy (due to cancer), drug use, chronic inflammatory disease, heart failure, myocardial infarction, and kidney disease were not included in the study. Moreover, Participants were excluded for recently weight-loss diet, taking weight-loss medications, pregnancy, lactation, more than 10% weight reduction during the last 6 months, history of hepatic diseases such as hepatitis, autoimmune disease, biliary disease, hereditary disorders of the liver including Wilson's disease and hemochromatosis, and using toxins or drugs affecting the liver such as NSAIDs, anti-inflammatory drugs, etc. Participants with a clear drinking history ($\geq$ 21 units/week in men and $\geq$ 14 units/week in women) were not included in the research. All of the studied patients reported that they do no drink alcohol at all. The flowchart of participants selection is shown in **Figure 1**.

Overall, anthropometric, and physical activity evaluations

Required information about age, sex, smoking status, duration of diabetes, and use of supplements were collected *via* a standard questionnaire. In order to measure CAP score, transient elastography (TE) equipped with M and XL probes (Fibroscan®) was used. A digital scale (Seca, Germany) was used to evaluate individuals' weight (kg), unshod and to the nearest 100 g. Height was measured using a tape measure and in a standing position to the nearest 0.5 cm. Finally, BMI was calculated by dividing weight (kg) by the square of height (meters). In order to determine the level of physical activity during the last 7 days, the International Physical Activity Questionnaire (IPAQ) short form was applied, and it was expressed as metabolic equivalent task (MET)-min/week (19). The validity and reliability of this questionnaire have been already examined in Iranian adult women. Blood pressure was measured on the basis of standard protocols using an automatic sphygmomanometer (OMRON, Mannheim, Germany).

Laboratory tests

Venous blood samples were collected after 10–12 h of overnight fasting. Enzymatic colorimetric method was used to measure the levels of fasting blood glucose. Serum levels of Triglyceride (TG), Total Cholesterol (TC), and high-density lipoprotein (HDL) were calculated by the use of enzymatic assays and standard biochemical kits (Pars Azmun Co., Iran). Between- and within-run coefficient of variations were less than 6.2%. Low-density lipoprotein (LDL) was calculated by modified version of Friedewald equation (20). ECLIA method and Roche Diagnostics kits (Roche Cobas 6000 analyzer) were used to measure serum insulin. HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) was calculated by the following equation: [fasting insulin (μU/mL) $\times$ fasting glucose (mmol/L)]/22.5 (21). QUICKI (Quantitative Insulin Sensitivity Check Index) was computed as $1/[\log(\text{fasting insulin in } \mu\text{U/mL}) + \log(\text{fasting glucose in mg/dL})]$ (22). TyG (Triglyceride and glucose) index was determined as Ln [TG (mg/dL) $\times$ fasting glucose (mg/dL)/2] (23).

Dietary assessment and calculation of dietary total antioxidant capacity

Dietary intakes of participants during the last year were examined using a semi-quantitative food frequency questionnaire (FFQ) with 147 item which its reliability and validity in Iranian population have been previously evaluated (24). An expert dietitian, being totally unaware of the participants' situation (regarding having NAFLD), administered the FFQ *via* face-to-face interviews. Subjects reported how often they consumed each food item during the last year and household measures were used to convert usual intakes to grams per day (25). Subsequently, Nutritionist 4 software (First Databank Inc., Hearst Corp., San Bruno, CA, USA) modified for Iranian foods determined the amount of total energy and dietary nutrients consumed every day. In the present study, DTAC was calculated *via* ferric reducing antioxidant power (FRAP). In order to obtain the total antioxidant capacity (TAC) (in mmol) of each food, published databases with antioxidant capacity of foods measured by the use of FRAP, were applied. When TAC of food items was unavailable, the amount of the closest comparable food was allocated (26). In order to calculate DTAC (in mmol), the daily intake of each food item over all products and units of antioxidant content (derived from an antioxidant index database) were summed (27).

Statistical analysis

The normality of data was assessed using the Kolmogorov–Smirnov test and the histogram chart. By the use of the independent Student's *t*-test and Mann–Whitney test, general characteristics with normal and abnormal distributions were compared between the study groups, respectively, and for qualitative variables, X^2 test was applied. Next, subjects were

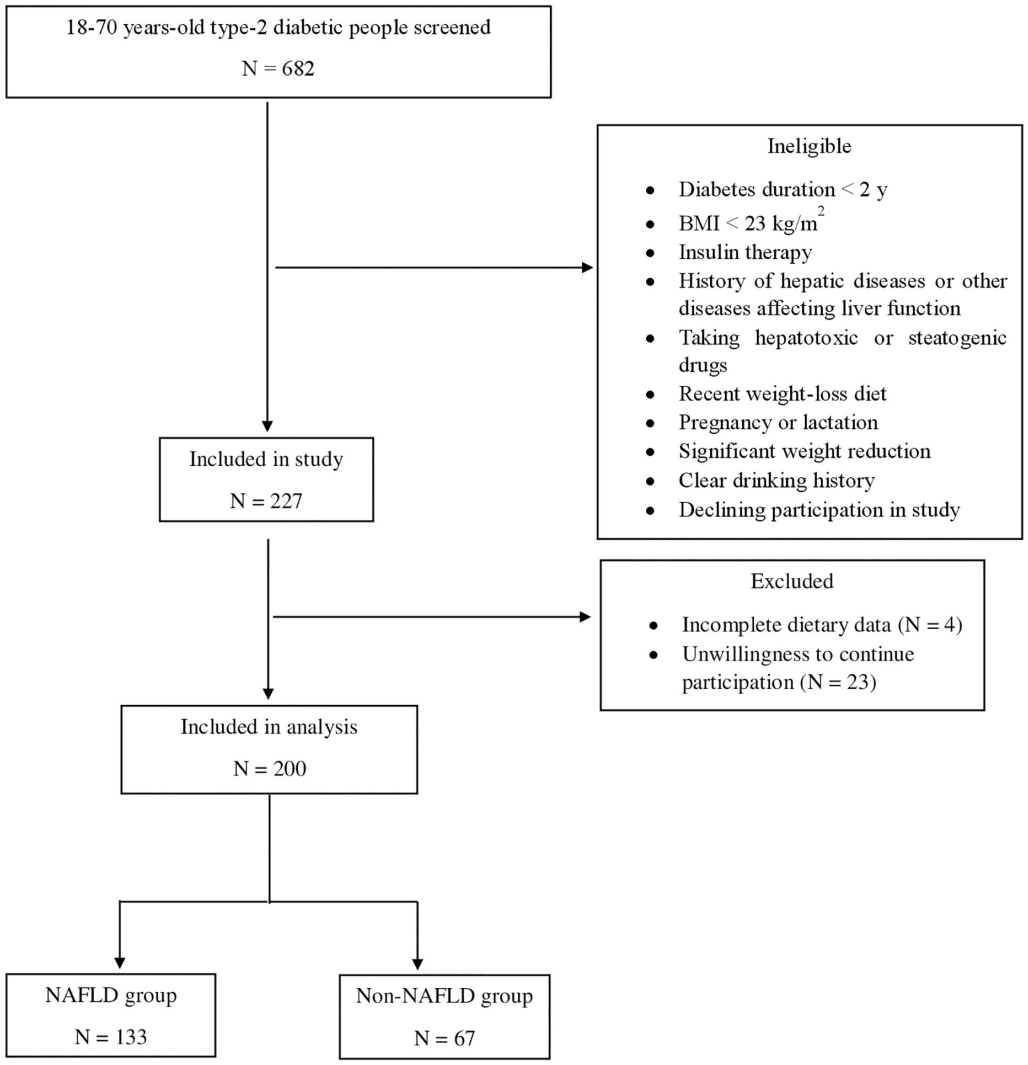

FIGURE 1
Flowchart of the study's participants.

categorized into tertiles of DTAC. General characteristics, biochemical parameters, and dietary intakes across tertiles of DTAC were evaluated by the use of Kruskal–Wallis test and analysis of covariance (ANCOVA) for continuous variables and X^2 test for categorical variables.

Quantitative and qualitative variables were reported as mean ± SD (standard deviation) and percentages, respectively. To assess the association between biochemical factors and NAFLD in diabetic patients, binary logistic regression in different models was used. In addition, multiple logistic regression models were applied to determine unadjusted and adjusted ORs for DTAC. In all analyses, the first tertile of biochemical factors or DTAC was regarded as the reference category. A broad range of confounders was controlled to examine whether the association was independent of them. All the statistical analyses were conducted using SPSS (SPSS Inc., version 25). *P* values less than 0.05 were considered as significant.

Results

Overall characteristics of individuals across the study groups are presented in Table 1. No statistically significant difference was found in age between the NAFLD and non-NAFLD groups. Among the diabetic patients with NAFLD and without NAFLD, 58.64 and 44.77 % were women, respectively. In terms of BMI, subjects in the NAFLD group significantly presented greater BMI compared with those in the non-NAFLD group. Besides, diabetic patients with NAFLD (NAFLD group) higher levels of TC, TG, LDL, transaminases, HbA1c, and TyG index, in comparison to those without NAFLD. Moreover, IR was more

TABLE 1 Baseline characteristics of type 2 diabetic patients with or without non-alcoholic fatty liver disease (NAFLD)[†].

Variables	NAFLD group (n = 133)	Non-NAFLD group (n = 67)	*P-value*[‡]
Age (y)	52.19 ± 9.06	52.24 ± 9.75	0.84
Gender (female) (%)	58.64	44.77	0.07
Current smokers (%)	18.04	16.41	0.84
Diabetes duration (y)	8 ± 5.26	10 ± 6.77	0.27
Weight (kg)	81.4 ± 15.08	72.7 ± 10.91	< 0.001
BMI (kg/m^2)	30.07 ± 4.06	26.17 ± 3.42	< 0.001
SBP (mmHg)	123 ± 14.55	125 ± 16.03	0.58
DBP (mmHg)	78 ± 10.42	75 ± 9.02	0.11
Physical activity (MET-min/week)	950.83 ± 1757.85	738.06 ± 683.27	0.37
FBS (mg/dL)	150.53 ± 59.22	148.79 ± 60.81	0.36
TC (mg/dL)	153.68 ± 51.75	132.52 ± 35.69	0.002
TG (mg/dL)	179.98 ± 173.01	141.69 ± 128.45	0.005
HDL (mg/dL) (n = 197)	49.53 ± 12.51	49.31 ± 13.53	0.6
LDL (mg/dL) (n = 197)	77.54 ± 27.77	66.37 ± 23.6	0.005
SGPT (IU/L)	20.05 ± 10.62	16.49 ± 7.28	0.02
SGOT (IU/L)	21.38 ± 9.05	18.82 ± 8.53	0.04
HbA1c (%) (n = 198)	7.92 ± 1.85	7.33 ± 1.64	0.01
Insulin (μU/mL) (n = 197)	8.77 ± 6.56	5.73 ± 3.36	< 0.001
QUICKI (n = 197)	0.33 ± 0.03	0.35 ± 0.02	< 0.001
HOMA–IR (n = 197)	3.23 ± 2.79	1.89 ± 0.94	< 0.001
TyG index	4.02 ± 0.31	3.91 ± 0.3	0.02
DTAC (mmol)	16.1 ± 10.48	15.11 ± 5.77	0.44

[†]Values are mean ± SD, unless indicated. [‡]*P-values* were obtained from Mann Whitney *U* test or chi-square test, where appropriate unless QUICKI and TyG index for which they were obtained from independent sample test. BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MET, metabolic equivalents; FBS, fasting blood glucose; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein-cholesterol; LDL, low-density lipoprotein-cholesterol; SGPT, serum glutamate pyruvate transaminase; SGOT, serum glutamic-oxaloacetic transaminase; HbA1c, hemoglobin A1c; QUICKI, quantitative insulin-sensitivity check index; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; TyG index, Triglyceride-glucose index; DTAC, dietary total antioxidant capacity.

prevalent among them. The two groups, however, showed no significant differences in current smoking, diabetes duration, blood pressure, physical activity, FBS, HDL, and DTAC.

Among categories of DTAC, a significant difference was found in terms of age, diastolic blood pressure (DBP), and insulin levels. However, BMI, physical activity, blood pressure, diabetes duration, and biochemical parameters did not differ significantly across categories of DTAC. We found that individuals in the top tertile had significantly higher intakes of monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), dietary fiber, fruits, vegetables, low-fat dairy products, and poultry. Additionally, the intake of whole grains, legumes, nuts, seeds, green/black tea, and coffee was significantly higher in them (Table 2).

TABLE 2 Characteristics, biochemical parameters, and dietary intakes of study participants across tertiles of dietary total antioxidant capacity[†].

Variables	Dietary total antioxidant capacity[‡]			*P-value*[§]
	T1	T2	T3	
Subjects, n	66	67	67	
Age (y)	52.05 ± 8.52	50.09 ± 10.05	54.48 ± 8.77	0.02
Gender (female) (%)	63.63	49.25	49.25	0.15
Current smokers (%)	12.12	26.86	13.43	0.04
Diabetes duration (y)	9 ± 5.43	8 ± 5.78	9.64 ± 6.17	0.13
SBP (mmHg)	125 ± 16.39	121 ± 11.66	124 ± 16.48	0.4
DBP (mmHg)	80 ± 10.7	75 ± 8.75	76 ± 9.86	0.01
Weight (kg)	76.72 ± 13.06	80.87 ± 17.29	77.86 ± 12.2	0.51
BMI (kg/m^2)	28.71 ± 3.88	29.29 ± 4.93	28.29 ± 3.9	0.63
Physical activity (MET-min/week)	591.51 ± 497.34	1194.74 ± 2249.06	848.11 ± 1091.26	0.64
FBS (mg/dL)	153.58 ± 68.04	142.75 ± 52.05	153.58 ± 58.03	0.44
SGOT (IU/L)	20.7 ± 9.08	21.7 ± 10.05	19.16 ± 7.43	0.34
SGPT (IU/L)	19.7 ± 12.64	19.22 ± 8.61	17.66 ± 7.3	0.66
TC (mg/dL)	154.82 ± 62.17	140.85 ± 36.65	144.24 ± 40.94	0.32
TG (mg/dL)	171.67 ± 206.59	159.45 ± 100.46	170.42 ± 158.55	0.93
HDL (mg/dL)	50.29 ± 10.18	47 ± 13.45	51.09 ± 14.28	0.14
LDL (mg/dL)	78.41 ± 28.07	70 ± 26.56	72.89 ± 25.74	0.31
HbA1c (%)	7.77 ± 1.72	7.63 ± 1.81	7.76 ± 1.87	0.67
Insulin (μU/mL)	6.74 ± 4.18	9.81 ± 8.13	6.63 ± 3.6	0.01
HOMA-IR	2.54 ± 2.28	3.36 ± 3.05	2.42 ± 1.6	0.05
TyG index	3.99 ± 0.32	3.97 ± 0.28	3.99 ± 0.33	0.87
QUICKI	0.34 ± 0.03	0.33 ± 0.03	0.34 ± 0.03	0.07
Dietary intakes				
Carbohydrate (% of energy)	57.2 ± 8.61	56.73 ± 9.04	58.95 ± 10.44	0.26
Fat (% of energy)	31.95 ± 7.92	32.35 ± 11.16	31.15 ± 11.75	0.54
Protein (% of energy)	14.1 ± 2.56	18.67 ± 32.63	18.68 ± 34.99	0.25
SFA (% of energy)	10.25 ± 4.01	10.77 ± 6.64	9.7 ± 5.83	0.37
MUFA (g/d)	22.97 ± 10.54	30.03 ± 10.9	34.75 ± 18.88	< 0.001
PUFA (g/d)	14.3 ± 8.02	19.12 ± 7.26	22.66 ± 15.07	< 0.001
Dietary fiber (g/d)	31.53 ± 16.12	45.76 ± 21.82	48.77 ± 20.74	< 0.001
Whole grains (g/d)	98.39 ± 91.84	180.55 ± 154.94	173.9 ± 167.63	0.004
Low-fat dairy products (g/d)	158.27 ± 139.5	211.5 ± 204.88	278.33 ± 262.1	0.01
High-fat dairy products (g/d)	52.45 ± 111.43	67.62 ± 109.17	56.62 ± 104.96	0.07
Fish (g/d)	5.16 ± 8.41	5.87 ± 6.58	6.74 ± 9	0.24
Fruits (g/d)	355.78 ± 209.1	508.85 ± 302.39	773.31 ± 560.78	< 0.001

(Continued)

TABLE 2 (Continued)

Variables	Dietary total antioxidant capacity‡			*P-value*§
	T1	T2	T3	
Vegetables (g/d)	349.63 ± 215.55	403.91 ± 225.24	489.04 ± 285.82	0.005
Green/black tea (g/d)	400.47 ± 247.29	724.41 ± 324.88	1449.62 ± 1159.82	< 0.001
Nuts (g/d)	6.23 ± 7.97	7.51 ± 9.12	10.83 ± 11.78	0.002
Legumes (g/d)	21.29 ± 24.28	31.22 ± 23.53	33.25 ± 47.27	0.003
Red meats (g/d)	14.27 ± 18.51	21.82 ± 19.18	19.85 ± 28.89	0.001
Organ meats (g/d)	1.85 ± 2.87	4.32 ± 8.58	3.92 ± 8.94	0.16
Poultry (g/d)	24.2 ± 19.01	37.2 ± 46.17	49.2 ± 55.78	< 0.001
Coffee (g/d)	16.73 ± 27.26	28.22 ± 63.4	100.59 ± 218.5	0.02
Sweets (g/d)	2.15 ± 3.58	2.41 ± 3.33	3.12 ± 7.27	0.09
Oil and olive oil (g/d)	3.87 ± 6.15	3.71 ± 5.96	6.79 ± 10.87	0.18
Seeds (g/d)	3.8 ± 8.04	6.29 ± 12.2	13.74 ± 36.05	0.03
Salt (g/d)	7.52 ± 7.25	4.12 ± 5.32	6.01 ± 5.77	0.001
Sugar-sweetened beverages (g/d)	17.36 ± 54.15	23.37 ± 63.5	15.19 ± 33.65	0.3

†Values are mean ± SD, unless indicated. ‡Individuals in the first tertile of DTAC had DTAC score less than 11.55; second tertile: between 11.55 and 16.68 and third tertile: more than 16.68. §*P-values* were obtained from Kruskal-Wallis test or chi-square test, where appropriate unless QUICKI and TyG index for which they were obtained from one-way ANOVA. SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, Body mass index; MET, metabolic equivalents; FBS, fasting blood glucose; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamate pyruvate transaminase; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein-cholesterol; LDL, low-density lipoprotein-cholesterol; HbA1c, hemoglobin A1c; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; TyG index, Triglyceride-glucose index; QUICKI, quantitative insulin-sensitivity check index; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Multiple logistic regression analysis was applied to examine whether certain biochemical factors were independently and significantly associated with the presence of NAFLD in people with diabetes (Table 3). For all T2DM patients, LDL-C, HbA1c, HOMA-IR, and TyG index were significantly associated with greater odds of having NAFLD, according to the fully adjusted model. On the other hand, the analysis showed that high QUICKI was a protective factor for NAFLD. Specifically, those with LDL (OR = 3.08, 95% CI: 1.16–8.16, P = 0.02) levels in tertile 3 had significantly increased odds for NAFLD, independently of age, gender, diabetes duration, smoking status, physical activity, BMI, waist circumference, HOMA-IR, HbA1c, and energy. Additionally, compared to tertile 1, HbA1c levels in tertile 3 were independently associated with a significantly increased odd for NAFLD (OR = 2.92, 95% CI: 1.14–7.45, P = 0.02). Similarly, T2DM patients with higher amounts of HOMA-IR (> 2.7) had significantly 5.33 times the odds (OR = 5.33, 95% CI: 1.83–15.5, P = 0.002) of NAFLD compared to T2DM patients with lower amounts (< 1.62) after controlling for the potential confounders. Also, diabetic patients with higher levels of

TABLE 3 Crude and multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for non-alcoholic fatty liver disease (NAFLD) in participants with type 2 diabetes across tertiles of biochemical parameters.

Model	Odds ratio (95% CI)			*P*-trend†
	T1	T2	T3	
		FBS, mg/dL		
	< 121	121–149.98	149.98 <	
Subjects, n	68	65	67	
Model 1	1 (Ref)	1.97 (0.94–4.11)	1.43 (0.7–2.89)	0.3
Model 2	1 (Ref)	2.45 (1.02–5.85)	1.9 (0.77–4.71)	0.13
		TC, mg/dL		
	< 124.98	124.98–156	156 <	
Subjects, n	66	68	66	
Model 1	1 (Ref)	1.19 (0.59–2.37)	3 (1.37–6.54)	0.006
Model 2	1 (Ref)	0.83 (0.34–2.04)	3.18 (1.22–8.26)	0.02
Model 3	1 (Ref)	0.75 (0.29–1.9)	2.26 (0.82–6.2)	0.13
		TG, mg/dL		
	< 102	102–162.96	162.96 <	
Subjects, n	68	65	67	
Model 1	1 (Ref)	2.02 (0.99–4.15)	2.46 (1.18–5.1)	0.01
Model 2	1 (Ref)	2.06 (0.89–4.77)	2.01 (0.85–4.73)	0.08
Model 3	1 (Ref)	2.06 (0.84–5)	1.8 (0.72–4.48)	0.16
		HDL-C, mg/dL		
	< 42.99	42.99–54	54 <	
Subjects, n	65	69	63	
Model 1	1 (Ref)	1.53 (0.74–3.15)	1.16 (0.56–2.39)	0.66
Model 2	1 (Ref)	1.78 (0.72–4.37)	1.02 (0.4–2.61)	0.97
Model 3	1 (Ref)	1.91 (0.73–4.97)	1.31 (0.48–3.53)	0.59
		LDL-C, mg/dL		
	< 60	60–80	80 <	
Subjects, n	67	65	65	
Model 1	1 (Ref)	1.57 (0.78–3.16)	3.44 (1.58–7.47)	0.002
Model 2	1 (Ref)	1.28 (0.54–3.03)	4.03 (1.58–10.29)	0.004
Model 3	1 (Ref)	1.07 (0.43–2.63)	3.08 (1.16–8.16)	0.02
		SGOT, IU/L		
	< 16	16–22	22 <	
Subjects, n	72	58	70	
Model 1	1 (Ref)	0.86 (0.42–1.75)	1.63 (0.79–3.35)	0.19
Model 2	1 (Ref)	0.57 (0.23–1.39)	1.64 (0.7–3.83)	0.25
Model 3	1 (Ref)	0.58 (0.22–1.48)	1.46 (0.59–3.6)	0.43
		SGPT, IU/L		
	< 13	13–20	20 <	
Subjects, n	67	61	72	
Model 1	1 (Ref)	0.97 (0.48–1.99)	2.05 (0.98–4.27)	0.05
Model 2	1 (Ref)	1.33 (0.57–3.11)	2.38 (0.97–5.81)	0.06
Model 3	1 (Ref)	1.36 (0.55–3.37)	1.91 (0.75–4.88)	0.24
		HbA1c, %		
	< 6.7	6.7–8	8 <	
Subjects, n	65	66	68	
Model 1	1 (Ref)	1.54 (0.76–3.13)	2.92 (1.37–6.23)	0.34

(Continued)

TABLE 3 (Continued)

Model	Odds ratio (95% CI)			*P*-trend†
	T1	T2	T3	
Model 2	1 (Ref)	1.47 (0.63–3.45)	2.92 (1.14–7.45)	0.02
		Fasting insulin, μU/mL		
	< 4.59	4.59–8.39	8.39 <	
Subjects, n	66	66	66	
Model 1	1 (Ref)	2.07 (1.02–4.22)	3.95 (1.81–8.6)	< 0.001
Model 2	1 (Ref)	1.57 (0.67–3.68)	2.2 (0.86–5.64)	0.09
		HOMA-IR		
	< 1.62	1.62–2.7	2.7 <	
Subjects, n	66	66	66	
Model 1	1 (Ref)	0.17 (0.7–0.4)	0.26 (0.11–0.62)	< 0.001
Model 2	1 (Ref)	1 (0.43–2.31)	5.33 (1.83–15.5)	0.004
		TyG index		
	< 3.83	3.83–4.06	4.06 <	
Subjects, n	66	67	67	
Model 1	1 (Ref)	1.95 (0.96–3.99)	2.45 (1.17–5.1)	0.01
Model 2	1 (Ref)	1.99 (0.86–4.63)	2.99 (1.22–7.32)	0.01
		QUICKI		
	< 0.32	0.32–0.35	0.35 <	
Subjects, n	66	66	66	
Model 1	1 (Ref)	0.26 (0.11–0.62)	0.17 (0.07–0.4)	< 0.001
Model 2	1 (Ref)	0.18 (0.06–0.55)	0.18 (0.06–0.54)	0.004

Model 1: Crude. Model 2: Adjusted for age (continuous), sex (male/female), physical activity (continuous), diabetes duration (continuous), current smoking (yes/no), BMI (continuous), waist circumference (continuous), and energy intake (kcal/d). Model 3: This model was additionally adjusted for HOMA-IR (continuous) and HbA1c (continuous). †Binary logistic regression models were employed to obtain odds ratios (ORs) and 95% CIs. The overall trend of ORs was examined by the use of tertiles of the biochemical parameters as an ordinal variable in the model.

QUICKI had reduced OR in NAFLD than those with lower levels (OR = 0.18, 95% CI: 0.06–0.54, P = 0.002). Another parameter independently associated with an increased odds of having NAFLD was TyG index (OR: 2.99, 95% CI: 1.22–7.32; P = 0.01).

Multivariable-adjusted odds ratios and 95% CIs for NAFLD by tertiles of DTAC are indicated in Table 4. In the crude model, DTAC was not significantly associated with the odds of NAFLD in people with diabetes. However, after adjustment for potential confounders including age, gender, diabetes duration, smoking status, physical activity, BMI, waist circumference, and energy, the lowest adjusted OR was observed for the last tertile vs. the first one (OR: 0.28, 95% CI: 0.09–0.81, P = 0.02), meaning that diabetic patients in the highest tertile of DTAC had 72 % decreased risk of NAFLD compared with those in the lowest tertile. The association remained significant after additional adjustment for HOMA-IR, HbA1c, TG, and LDL levels (OR: 0.29, 95% CI: 0.09–0.93, P = 0.03). Importantly, a dose-response pattern was demonstrated for DTAC and risk of NAFLD (P = 0.04).

TABLE 4 Crude and multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for non-alcoholic fatty liver disease (NAFLD) in type 2 diabetes patients across tertiles of dietary total antioxidant capacity.

	Dietary total antioxidant capacity			*P*-trend†
	T1 (n = 66) < 11.55	T2 (n = 67) 11.55–16.68	T3 (n = 67) 16.68 <	
	OR	OR (95 % CI)	OR (95 % CI)	
Model 1	1 (Ref)	0.66 (0.31–1.4)	0.51 (0.24–1.07)	0.07
Model 2	1 (Ref)	0.38 (0.14–0.99)	0.28 (0.09–0.81)	0.02
Model 3	1 (Ref)	0.41 (0.14–1.15)	0.29 (0.09–0.93)	0.04

Model 1: Crude. Model 2: Adjusted for age (continuous), sex (male/female), physical activity (continuous), diabetes duration (continuous), current smoking (yes/no), BMI (continuous), waist circumference (continuous), and energy intake (kcal/d). Model 3: Further adjustments were made for HOMA-IR (continuous), HbA1c (continuous), triglycerides (continuous), and LDL (continuous) levels. †Binary logistic regression models were employed to obtain odds ratios (ORs) and 95% CIs. The overall trend of ORs was examined by the use of tertiles of the DTAC as an ordinal variable in the model.

Discussion

The present cross-sectional study investigated the associations between DTAC and NAFLD in people with diabetes. According to our results, patients in the NAFLD group (diabetes with NAFLD) had a higher BMI and serum levels of TC, TG, LDL, transaminases and HbA1C than the non-NAFLD group (diabetes without NAFLD). These patients also showed higher degrees of IR. The risk of NAFLD was significantly higher in the third tertile of LDL and HbA1c compared to the first tertile. The risk of NAFLD also increased significantly with increasing IR and decreasing insulin sensitivity. After fully adjustment for age, sex, physical activity, diabetes duration, current smoking, BMI, waist circumference, energy intake, Triglycerides, LDL, HbA1c, and HOMA-IR, risk of NAFLD was significantly lower in patients with higher DTAC. However, no significant difference was observed in DTAC score between the study groups. To the best of our knowledge, this is the first study reporting the inverse associations between DTAC and odds of NAFLD in people with diabetes.

Dietary total antioxidant capacity (DTAC) is a measure of the antioxidant capacity of the diet and is considered a good indicator of diet quality (28). An inverse association has been reported between DTAC and numerous chronic diseases including cardiovascular diseases (29), diabetes (16, 30), metabolic syndrome (31) and NAFLD (32, 33). Similar metabolic abnormalities are common among these disorders, which are exacerbated by combination of diabetes and NAFLD. Previously, an inverse association of antioxidant-rich dietary patterns, including the Dietary Approaches to Stop Hypertension (DASH) diet (34–36), plant-based diet (37, 38), with the risk of NAFLD and diabetes has been reported

separately. All of these diets are rich in antioxidant compounds and therefore have antioxidant effects.

Oxidative stress is involved in the pathogenesis of NAFLD by promoting IR, dyslipidemia (39). Antioxidant-rich foods/diets might be effective in reinforcing antioxidant defense by reducing lipid peroxidation, cellular and organ damage, and insulin sensitivity (40). Increasing the antioxidant capacity of cells improves glucose and lipid metabolism and reduces the risk of NAFLD (39, 40).

The findings of this study showed that the risk of NAFLD in people with diabetes increased significantly with increasing IR. Study groups were significantly different in terms of insulin resistance and sensitivity, although this difference was not found among DTAC tertiles. IR is a mediator in the effect of oxidative stress on lipid profile (41). Another factor that may play a role in DTAC and NAFLD risk reduction is fiber, which contributes to weight loss, improved insulin sensitivity, dyslipidemia, and glycemic control (42).

Although dietary antioxidants play a role in reducing obesity and related disorders by inhibiting fat absorption, stimulating adipose tissue catabolism, inhibiting the proliferation and differentiation of adipocytes (43), no significant difference in weight was observed between the three tertiles of DTAC. However, it has been suggested that the association of DTAC with cardiovascular risk factors is independent of weight (44).

In this study, no difference was observed between DTAC between NAFLD and non-NAFLD groups, which is contrary to previous findings in diabetic patients (45) and NAFLD patients (33). However, in these studies, the comparisons were made between patients and healthy individuals, while in our study, diabetic people with and without NAFLD were compared. Oxidative stress reduces insulin secretion by damaging the mitochondria of pancreatic beta cells and causes diabetes (46, 47).

The present study has several limitations. First, due to the cross-sectional nature of the study design, cause and effect relationships could not be confirmed. The use of United States Department of Agriculture (USDA) food composition table was another limitation of the study due to incomplete database for the content of Iranian food antioxidant. The validity of DTAC obtained from the FFQ in the Iranian diet has not been examined previously. Also, the use of FFQ is associated with recall bias.

In conclusion, higher DTAC was associated with a decreased odds of having NAFLD in people with diabetes.

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 Shahid Beheshti University of Medical Sciences, Tehran, Iran. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MS conceived and designed the study, performed the statistical analysis, interpreted the data, and drafted the manuscript. SS and AM contributed to the sampling selection schedule. ZY and MSh drafted the manuscript. HP contributed to the data interpretation. MK, FA-S, AH, and MSh made critical revisions to the manuscript. MK and AH contributed to the study supervision. All authors have read and approved the final version to be published.

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Conflict of interest

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

Publisher's note

References

1. Moore MP, Cunningham RP, Dashek RJ, Mucinski JM, Rector RS. A fad too far? Dietary strategies for the prevention and treatment of NAFLD. *Obesity.* (2020) 28:1843–52. doi: 10.1002/oby.22964

2. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology.* (2016) 64:73–84. doi: 10.1002/hep.28431

3. Barb D, Bril F, Kalavalapalli S, Cusi K. Plasma fibroblast growth factor 21 is associated with severity of nonalcoholic steatohepatitis in patients with obesity and type 2 diabetes. *J Clin Endocrinol Metab.* (2019) 104:3327–36. doi: 10.1210/jc.2018-02414

4. Lange NF, Graf V, Caussy C, Dufour J-F. PPAR-targeted therapies in the treatment of non-alcoholic fatty liver disease in diabetic patients. *Int J Mol Sci.* (2022) 23:4305. doi: 10.3390/ijms23084305

5. Dai W, Ye L, Liu A, Wen SW, Deng J, Wu X, et al. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: a meta-analysis. *Medicine.* (2017) 96:e8179. doi: 10.1097/MD.0000000000008179

6. Atan NAD, Koushki M, Motedayen M, Dousti M, Sayehmiri F, Vafaee R, et al. Type 2 diabetes mellitus and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Gastroenterol Hepatol Bed Bench.* (2017) 10(Suppl. 1):S1.

7. Alsabaani AA, Mahfouz AA, Awadalla NJ, Musa MJ, Al Humayed SM. Non-alcoholic fatty liver disease among type-2 diabetes mellitus patients in Abha City, South Western Saudi Arabia. *Int J Environ Res Public Health.* (2018) 15:2521. doi: 10.3390/ijerph15112521

8. Klisic A, Isakovic A, Kocic G, Kavaric N, Jovanovic M, Zvrko E, et al. Relationship between oxidative stress, inflammation and Dyslipidemia with fatty liver index in patients with type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes.* (2018) 126:371–8. doi: 10.1055/s-0043-118667

9. Casoinic F, Sampelean D, Buzoianu AD, Hancu N, Baston D. Serum levels of oxidative stress markers in patients with type 2 diabetes mellitus and non-alcoholic steatohepatitis. *Roman J Intern Med.* (2016) 54:228–36. doi: 10.1515/rjim-2016-0035

10. Silaghi CA, Silaghi H, Colosi HA, Craciun AE, Farcas A, Cosma DT, et al. Prevalence and predictors of non-alcoholic fatty liver disease as defined by the fatty liver index in a type 2 diabetes population. *Clujul Med.* (2016) 89:82–8. doi: 10.15386/cjmed-544

11. Talenezhad N, Mirzavandi F, Rahimpour S, Amel Shahbaz AP, Mohammadi M, Hosseinzadeh M. Empirically derived dietary pattern and odds of non-alcoholic fatty liver diseases in overweight and obese adults: a case-control study. *BMC Gastroenterol.* (2022) 22:158. doi: 10.1186/s12876-022-02222-z

12. Salehi-Sahlabadi A, Mokari A, Elhamkia M, Farahmand F, Jabbari M, Hekmatdost A. Dietary total antioxidant capacity and risk of non-alcoholic fatty liver disease: a case-control study. *J Res Health Sci.* (2020) 20:e00486. doi: 10.34172/jrhs.2020.18

13. Kim D, Han A, Park Y. Association of dietary total antioxidant capacity with bone mass and osteoporosis risk in Korean women: analysis of the Korea national health and nutrition examination survey 2008-2011. *Nutrients.* (2021) 13:1149. doi: 10.3390/nu13041149

14. Mirzababaei A, Shiraseb F, Abaj F, Khosroshahi RA, Tavakoli A, Koohdani F, et al. The effect of dietary total antioxidant capacity (DTAC) and Caveolin-1 gene variant interaction on cardiovascular risk factors among overweight and obese women: a cross-sectional investigation. *Clin Nutr.* (2021) 40:4893–903. doi: 10.1016/j.clnu.2021.07.013

15. Zamani B, Daneshzad E, Azadbakht L. Dietary total antioxidant capacity and risk of gastrointestinal cancers: a systematic review and meta-analysis of observational studies. *Arch Iran Med.* (2019) 22:328–35. doi: 10.1017/S1368980019001125

16. Mancini FR, Affret A, Dow C, Balkau B, Bonnet F, Boutron-Ruault M-C, et al. Dietary antioxidant capacity and risk of type 2 diabetes in the large prospective E3N-EPIC cohort. *Diabetologia.* (2018) 61:308–16. doi: 10.1007/s00125-017-4489-7

17. Zujko ME, Witkowska AM, Waśkiewicz A, Piotrowski W, Terlikowska KM. Dietary antioxidant capacity of the patients with cardiovascular disease in a cross-sectional study. *Nutr J.* (2015) 14:1–13. doi: 10.1186/s12937-015-0005-4

18. Sohouli M, Fatahi S. Associations between dietary total antioxidant capacity and risk of nonalcoholic fatty liver disease (NAFLD) in adults: a case-control study. *Nutr Food Sci Res.* (2020) 7:1–7. doi: 10.29252/nfsr.7.3.1

19. Moghaddam MB, Aghdam FB, Jafarabadi MA, Allahverdipour H, Nikookheslat SD, Safarpour S. The Iranian version of international physical activity questionnaire (IPAQ) in Iran: content and construct validity, factor structure, internal consistency and stability. *World Appl Sci J.* (2012) 18:1073–80.

20. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* (1972) 18:499–502. doi: 10.1093/clinchem/18.6.499

21. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care.* (2000) 23:57–63. doi: 10.2337/diacare.23.1.57

22. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab.* (2000) 85:2402–10. doi: 10.1210/jcem.85.7.6661

23. Simental-Mendía LE, Rodríguez-Morán M, Guerrero-Romero F. The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. *Metab Syndr Relat Disord.* (2008) 6:299–304. doi: 10.1089/met.2008.0034

24. Esmaillzadeh A, Mirmiran P, Azizi F. Whole-grain intake and the prevalence of hypertriglyceridemic waist phenotype in Tehranian adults. *Am J Clin Nutr.* (2005) 81:55–63. doi: 10.1093/ajcn/81.1.55

25. Ghaffarpour M, Houshiar-Rad A, Kianfar H. The manual for household measures, cooking yields factors and edible portion of foods. *Tehran Nashre Olume Keshavarzy.* (1999) 7:42–58.

26. Zhou S, Wang X, Tan Y, Qiu L, Fang H, Li W. Association between vitamin C intake and glioma risk: evidence from a meta-analysis. *Neuroepidemiology.* (2015) 44:39–44. doi: 10.1159/000369814

27. Carlsen MH, Halvorsen BL, Holte K, Bøhn SK, Dragland S, Sampson L, et al. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. *Nutr J.* (2010) 9:1–11. doi: 10.1186/1475-2891-9-3

28. Kourlaba G, Panagiotakos DB. Dietary quality indices and human health: a review. *Maturitas.* (2009) 62:1–8. doi: 10.1016/j.maturitas.2008.11.021

29. Bahadoran Z, Golzarand M, Mirmiran P, Shiva N, Azizi F. Dietary total antioxidant capacity and the occurrence of metabolic syndrome and its components after a 3-year follow-up in adults: Tehran lipid and glucose study. *Nutr Metab.* (2012) 9:70. doi: 10.1186/1743-7075-9-70

30. van der Schaft N, Schoufour JD, Nano J, Kiefte-de Jong JC, Muka T, Sijbrands EJ, et al. Dietary antioxidant capacity and risk of type 2 diabetes mellitus, prediabetes and insulin resistance: the Rotterdam study. *Eur J Epidemiol.* (2019) 34:853–61. doi: 10.1007/s10654-019-00548-9

31. Hermsdorff HHM, Puchau B, Volp ACP, Barbosa KB, Bressan J, Zulet M, et al. Dietary total antioxidant capacity is inversely related to central adiposity as well as to metabolic and oxidative stress markers in healthy young adults. *Nutr Metab.* (2011) 8:1–8. doi: 10.1186/1743-7075-8-59

32. Salehi-Sahlabadi A, Mokari A, Elhamkia M, Farahmand F, Jabbari M, Hekmatdoost A. Dietary total antioxidant capacity and risk of non-alcoholic fatty liver disease: a case-control study. *J Res Health Sci.* (2020) 20:e00486.

33. Sohouli MH, Fatahi S, Sayyari A, Olang B, Shidfar F. Associations between dietary total antioxidant capacity and odds of non-alcoholic fatty liver disease (NAFLD) in adults: a case–control study. *J Nutr Sci.* (2020) 9:e48. doi: 10.1017/jns.2020.39

34. Liese AD, Bortsov A, Günther AL, Dabelea D, Reynolds K, Standiford DA, et al. Association of DASH diet with cardiovascular risk factors in youth with diabetes mellitus: the SEARCH for diabetes in Youth study. *Circulation.* (2011) 123:1410–7. doi: 10.1161/CIRCULATIONAHA.110.955922

35. Liese AD, Nichols M, Sun X, D'Agostino RB Jr., Haffner SM. Adherence to the DASH Diet is inversely associated with incidence of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes Care.* (2009) 32:1434–6. doi: 10.2337/dc09-0228

36. Doustmohammadian A, Clark CC, Maadi M, Motamed N, Sobhrakhshankhah E, Ajdarkosh H, et al. Favorable association between Mediterranean diet (MeD) and DASH with NAFLD among Iranian adults of the Amol Cohort study (AmolCS). *Sci Rep.* (2022) 12:1–9. doi: 10.1038/s41598-022-06035-8

37. Alferink LJ, Erler NS, de Knegt RJ, Janssen HL, Metselaar HJ, Darwish Murad S, et al. Adherence to a plant-based, high-fibre dietary pattern is related to regression of non-alcoholic fatty liver disease in an elderly population. *Eur J Epidemiol.* (2020) 35:1069–85. doi: 10.1007/s10654-020-00627-2

38. Qian F, Liu G, Hu FB, Bhupathiraju SN, Sun Q. Association between plant-based dietary patterns and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA Intern Med.* (2019) 179:1335–44. doi: 10.1001/jamainternmed.2019.2195

39. de Oliveira DG, de Faria Ghetti F, Moreira APB, Hermsdorff HHM, de Oliveira JM, de Castro Ferreira LEVV. Association between dietary total antioxidant capacity and hepatocellular ballooning in nonalcoholic steatohepatitis: a cross-sectional study. *Eur J Nutr.* (2019) 58:2263–70.

40. Liu W, Baker SS, D Baker R, Zhu L. Antioxidant mechanisms in nonalcoholic fatty liver disease. *Curr Drug Targets.* (2015) 16:1301–14. doi: 10.2174/1389450116666150427155342

41. Videla LA, Rodrigo R, Araya J, Poniachik J. Insulin resistance and oxidative stress interdependency in non-alcoholic fatty liver disease. *Trends Mol Med.* (2006) 12:555–8. doi: 10.1016/j.molmed.2006.10.001

42. Galisteo M, Duarte J, Zarzuelo A. Effects of dietary fibers on disturbances clustered in the metabolic syndrome. *J Nutr Biochem.* (2008) 19:71–84. doi: 10.1016/j.jnutbio.2007.02.009

43. Barnard ND, Scialli AR, Turner-McGrievy G, Lanou AJ, Glass J. The effects of a low-fat, plant-based dietary intervention on body weight, metabolism, and insulin sensitivity. *Am J Med.* (2005) 118:991–7. doi: 10.1016/j.amjmed.2005.03.039

44. Mozaffari H, Daneshzad E, Surkan PJ, Azadbakht L. Dietary total antioxidant capacity and cardiovascular disease risk factors: a systematic review of observational studies. *J Am Coll Nutr.* (2018) 37:533–45. doi: 10.1080/07315724.2018.1441079

45. Zujko ME, Witkowska AM, Górska M, Wilk J, Kretowski A. Reduced intake of dietary antioxidants can impair antioxidant status in type 2 diabetes patients. *Pol Arch Med Wewn.* (2014) 124:599–607. doi: 10.20452/pamw.2497

46. Franzini L, Ardigò D, Zavaroni I. Dietary antioxidants and glucose metabolism. *Curr Opin Clin Nutr Metab Care.* (2008) 11:471–6. doi: 10.1097/MCO.0b013e328303be79

47. Tiedge M, Lortz S, Drinkgern J, Lenzen S. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes.* (1997) 46:1733–42. doi: 10.2337/diabetes.46.11.1733

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Differential influences of serum vitamin C on blood pressure based on age and sex in normotensive individuals

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Aim: Hypertension is among the most prevalent chronic diseases with diverse etiology, affecting over 1 billion people globally. In numerous studies, vitamin C inversely correlated with blood pressure and was suspected to have antihypertensive properties. Currently, there is conflicting evidence regarding the relationship between vitamin C and blood pressure, with most studies being conducted on hypertensive subjects. The principal objective of this project was to investigate the relationship between vitamin C and blood pressure in normotensive adult subjects.

Methods: A total of 2,533 individuals aged 20 years and above were enrolled in the present study from the National Health and Nutrition Examination Survey (NHANES) 2017-2018. Outcome variables were systolic blood pressure (SBP) and diastolic blood pressure (DBP). Serum vitamin C was regarded as an independent variable. EmpowerStats software and R (version 3.4.3) were used to examine the association between vitamin C and SBP or DBP.

Results: Vitamin C was reversely correlated with both SBP (β = −0.02, 95% CI: −0.03 to −0.00, p = 0.0306) and DBP (β = −0.02, 95% CI: −0.04 to −0.01, p = <0.0011) after adjusting all covariates. This reverse relationship may be affected by a number of factors, including a person's gender, age, race, and ethnicity. A U-shaped association between vitamin C and SBP in females and an inverted one between vitamin C and DBP in males were detected, respectively. We further calculated the inflection points at 90.3 μmol/L for females and 40 μmol/L for males. It is somewhat surprising that a reverse U-shaped distribution between vitamin C and SBP and DBP in people over 50 was detected, and the point of inflection of vitamin C were all located at 40 μ mol/L.

Conclusion: Vitamin C was negatively correlated with both SBP and DBP in this cross-sectional analysis. However, a U-shaped relationship and an inverted one were also observed in certain people, which implied that, though vitamin C is considered a vital antioxidant, maintaining vitamin C at appropriate levels may be beneficial according to different populations.

KEYWORDS

vitamin C, blood pressure, hypertension, U-shaped relationship, normotensive

Introduction

Hypertension is among the most prevalent chronic diseases, affecting over 1 billion people globally (1). Concomitantly, the rapidly increasing morbidity of hypertension, which can progress to other cardiovascular diseases and cerebrovascular conditions, severely impairs quality of life around the world (2–4). With advancements in medicine and socioeconomics, recent decades have witnessed a significant improvement in the treatment and control rate of hypertension, especially in developed countries. However, multiple studies are still urgently needed to develop novel and alternative therapeutics and interventions, thus reducing the prevalence of hypertension to a large degree.

The etiology of hypertension is complex and multifactorial, arising from the interplay of lifestyles, physical activities, living environment, and genetic factors. Moreover, numerous population-based epidemiological studies have recognized that multiple dietary factors were associated with hypertension in recent years (5). For instance, extensive evidence has demonstrated that inappropriate consumption of calcium (6, 7), phosphorus (8, 9), sodium (7, 10, 11), potassium (11, 12), magnesium (13, 14) was intricately related with blood pressure. Additionally, various clinical and animal experimental studies have repeatedly revealed that dietary vitamin C and serum vitamin C were associated with blood pressure (15–19). A meta-analysis that included 18 studies found that serum vitamin C was negatively correlated with systolic blood pressure (SBP) and diastolic blood pressure (DBP) (15). Ashor et al. (18) found that vitamin C supplementation can decrease peripheral pulse wave velocity, SBP, and mean arterial pressure in the elderly. Consistent with these conclusions, numerous relevant studies have detected this inverse relationship (16, 17).

The benefit of vitamin C is attributed partly to antioxidative stress response and anti-inflammatory cytokines, thus reducing endothelial cells oxidative damage following arterioles injury in the progression of hypertension (20–22). Therefore, some researchers believe that vitamin C supplementation may contribute significantly to preventing hypertension (18, 23). Nevertheless, some studies have reached a diametrically opposite conclusion (24, 25). A Mendelian randomized study showed that vitamin C supplementation might not aid in preventing any cardiovascular diseases (24). Similar to this study, observational research suggested that although supplementation of fruits and vegetables can increase the concentrations of serum vitamin C and other beneficial substances, it has no anti-oxidative stress effect in 12 weeks (25). In addition, the current research on the relationship between vitamin C and blood pressure was mainly carried out in hypertensive subjects, which may be affected by the use of antihypertensive drugs and the cause of hypertension. Therefore, we use NHANES for the first time to discuss the potential relationship between serum vitamin C and blood pressure in normotensive cases and to develop a promising strategy to prevent hypertension in the pre-clinical stage.

Materials and methods

Study population

The dataset in the present study was received from the National Health and Nutrition Examination Survey (NHANES) collected from 2017 to 2018, which contained cross-sectional socio-demographic, dietary, and medical records obtained by questionnaires, standard physical examinations, and laboratory tests conducted in authoritative laboratories. The NHANES database, a population-based survey conducted by the National Center for Health Statistics (NCHS), is a publicly used data set used to record the health status and related personal and lifestyle characteristics of all civilians in the United States. A multi-stage, complex clusters, probabilistic sample design is used for data acquisition and analysis to achieve nationally

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; NHANES, National Health and Nutrition Examination Survey; NCHS, National Center for Health Statistics; MEC, Mobile Inspection Center; BMI, body mass index; MIL, maximum inflation level; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; TP, total protein; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; Cr, creatinine; TG, triglyceride; TC, total cholesterol; LDL_C, LDL-cholesterol; γGT, gamma-glutamyl transferase; sUA, serum uric acid.

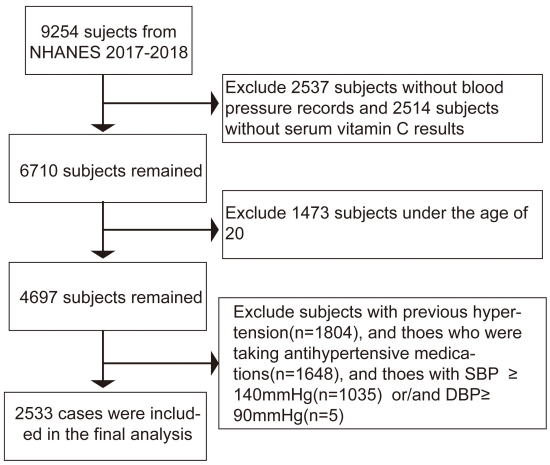

FIGURE 1
Flow chart of participants selection in the present study.

representative, rather than a simple random sample from the general US population. In particular, the Centers for Disease Control and Prevention (CDC) is responsible for preparing and disseminating data files to provide full access to the data (26–28).

In the 2017-2018 cycle, there were 9,254 subjects, of which 6,717 cases had valid blood pressure records, and 6,740 individuals were tested for serum vitamin C. We excluded certain participants as follows:(1) individuals with missing serum Vitamin C or blood pressure;(2) those who were diagnosed with hypertension in the past or take antihypertensive medications now;(3) those with SBP $\geq$ 140 mmHg or/and DBP $\geq$ 90mmHg. A total of 2,533 subjects were enrolled in the final study. The flow chart of study subjects is shown in **Figure 1**. NCHS Ethics Review Board supported the research. Furthermore, written informed consent was received from each subject (29).

Variables

The dependent variable and independent variable of the present study were blood pressure, including SBP and DBP, and serum vitamin C, respectively. Vitamin C was tested and recorded in authoritative laboratories using standard procedures (Details of the test method can be found in **Supplementary material 1**).

For blood pressure measurement: After 5 min of resting quietly in the seat, once the participant's maximum inflation level (MIL) is determined, three consecutive blood pressure readings will be obtained. If the blood pressure measurement is interrupted or incomplete, a fourth attempt would be made. All blood pressure measurements were conducted in the Mobile Inspection Center (MEC). The absolute blood pressure is the average of three valid measurements.

Besides, the following variables were included in the present study: age, race/ethnicity, sex, marital status, education level, ratio of family income to poverty, alcohol consumption, smoking, body mass index (BMI), waist circumference, pulse, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (Cr), globulin, glycohemoglobin, calcium, triglyceride (TG), total cholesterol (TC), LDL-cholesterol (LDL), HDL-cholesterol (HDL), fasting blood glucose (FBG), gamma-glutamyl transferase (γGT), total protein (TP) and serum uric acid (sUA).

We excluded subjects with missing independent or dependent variables. For missing continuous variables, we use the median to fill in. For missing categorical variables, we separate the missing group as a group. All the covariate acquisition processes and any detailed information can be found at www.cdc.gov/nchs/nhanes/.

Statistical analyses

R statistical programming language (version 3.4.3)[1] and EmpowerStats software[2] were applied to perform statistical analysis. A two-sided $p < 0.05$ was considered to be statistically significant. We used the weighted analysis as recommended by the NCHS Analysis Guide to maintain national representation. The continuous variables were characterized by mean $\pm$ standard deviation, or as median and interquartile range, as appropriate. The categorical variables were presented as a percentage. The *P*-value was calculated using a weighted chi-squared test for categorical variables and a weighted linear regression model for continuous variables.

The association between vitamin C and SBP or DBP was evaluated by multivariable linear regression analysis. To further analyze the relationship between vitamin C and SBP or DBP, we used the following three models: Model 1: No adjustment for variables; Model 2: Adjusted for sex, age, and race/ethnicity; Model 3: Adjusted for sex, age, race/ethnicity, marital status, education level, ratio of family income to poverty, BMI, smoking states, pulse, ALT, ALP, AST, BUN, globulin, Cr, TG, TC, LDL, HDL, glycohemoglobin, TP, FBG, γGT, and sUA.

For further analyses, we performed subgroup analysis stratified by sex and age subsequently. A weighted generalized additive model and a smooth curve fitting were performed to address non-linearity between vitamin C and SBP or DBP. When non-linearity was discovered, we first calculated the vital inflection point using a recursive algorithm and then performed a weighted two-piecewise linear regression model on both sides of the inflection point.

1 http://www.Rproject.org
2 http://www.empowerstats.com

Results

Table 1 shows the general description of weighed characteristics of all 2533 subjects enrolled in the study based on the quartiles of vitamin C (Q1:1.87-32.1, Q2: 32.1-51.6, Q3:51.6-68.1, Q4:68.1-191). Of all these participants, the average age was 42.11 ± 15.39 years old,46.76% were males, 53.24% were females, 10.94% were Mexican Americans,61.74% were Non-Hispanic Whites, 9.19% were Non-Hispanic Blacks, and 18.13% were other race/ethnicity. Among the four groups stratified by quartile of vitamin C, ALT, ALP, AST, Cr, globulin, GGT, calcium, TP, UA, BMI, waist circumference, pulse, SBP, DBP, AGE, ratio of family income to poverty, glycohemoglobin, FBG, HDL_C, TG were all of great statistical significance ($p < 0.05$). Moreover, alcohol consumption, smoking states, education levels, marital status, diabetes rate were comparable among the quartile groups.

Vitamin C and SBP were reversely correlated in the fully adjusted model (β = −0.02, 95% CI: −0.03 to -0.00, $p = 0.0306$). The test for trend among the vitamin C quartile groups was statistically significant ($p = 0.037$). In sub-analysis stratified by age, sex, race/ethnicity, this negative association was observed only in non-Hispanic White [β = −0.03, 95% CI: −0.06 to −0.01, $p = 0.0088$] (Table 2 and Figure 2).

Moreover, a reverse association between vitamin C and DBP in the fully-adjusted model was observed (β = −0.02, 95% CI: −0.04 to −0.01, $p<0.0011$). The trend remained to be of statistical significance among the vitamin C quartile groups as well ($p < 0.001$). Additional sub-analysis stratified by age, sex, race/ethnicity showed this negative relationship existed in both male (β = −0.02,95% CI: −0.04 to −0.00, $p = 0.0231$) and female (β = −0.02,95% CI: −0.04 to −0.04, $p = 0.0024$), and in those older than 50 years old (β = −0.04,95% CI: −0.06 to −0.02, $p<0.0011$) as well as in non-Hispanic White (β = −0.03,95% CI: −0.05 to −0.01, $p = 0.0020$) (Table 3 and Figure 3).

Additionally, we also performed a weighted generalized additive model and a smooth curve fitting stratified by age and sex to detect the non-linear association between vitamin C and SBP as well as DBP and further confirm the results. A U-shaped association and a reverse one were detected between vitamin C and SBP in females and those older than 50years, respectively. We further calculated the inflection points at 90.3μmol/L for females and 40μmol/L for age ≥ 50 years (Table 4 and Figures 4, 5).

Furthermore, we have also observed an inverted U-shaped distribution between vitamin C and DBP in males and those older than 50years. The inflection point calculated by a recursive algorithm of vitamin C in these groups was all 40 μmol/L (Table 5 and Figures 6, 7).

Finally, according to the smoothing plot, we applied a two-piecewise linear regression model to examine vitamin C's threshold effect on SBP and DBP (Tables 4, 5).

Discussion

To our knowledge, this is the first analysis to examine the relationship between vitamin C and blood pressure in NHANES. Several significant findings were uncovered in this cross-sectional study. The most prominent finding to emerge from the present study is that vitamin C was negatively associated with SBP and DBP. Sex, age, race/ethnicity might be major contributors affecting this reverse relationship. By quartile of serum vitamin C, we found that those in the highest quartile had 1.84 and 2.08 mmHg lower systolic and diastolic blood pressure, respectively, than those in the lowest quartile. A U-shaped association between vitamin C and SBP in females and an inverted one between vitamin C and DBP in males were detected, respectively. We further calculated the inflection points at 90.3 μmol/L for females and 40 μmol/L for males. It is somewhat surprising that a reverse U-shaped distribution between vitamin C and SBP and DBP in people over 50 was detected, and the point of inflection of vitamin C were all located at 40 μmol/L.

Hypertension or high blood pressure, a primary cause of disability and mortality and a leading risk factor for cardiovascular diseases globally, is a complex human disorder with diverse etiology (1, 30, 31). Continually improving and standardized management and monitoring of blood pressure are extraordinary to humans. The early detection and treatment of prehypertensive or hypertensive populations has a major role to play in reducing hypertension incidence. It is the first study to demonstrate that serum vitamin C concentration is associated with elevated blood pressure in healthy populations, as well as a non-curve relationship for specific populations, which implies that lifestyle interventions may have implications for reducing hypertension prevalence. Several observational and interventional studies have demonstrated that multiple dietary factors were associated with an increased risk of developing hypertension and might be served as a potential antihypertensive therapeutic target (5, 32–34). A Chinese longitudinal national study indicated that lower or higher calcium intake during adolescence could increase the risk of hypertension in adulthood (34). Besides, a few scholars believe that vitamin C supplementation may significantly reduce the risk of major cardiovascular events and hypertension (18, 23, 35, 36). A meta-analysis also reported that the administration of vitamin C improved the systolic left ventricular function in heart failure patients (37).

Previous studies have confirmed that vitamin C is a common antioxidant with potential tissue protection and antihypertensive effects (15, 18, 23, 38). The blood pressure lowing potential of vitamin C supplementation has been frequently reported recently in various studies (15, 17, 19, 39). In a meta-analysis conducted by Ran et al. (15), the research staff reported that serum vitamin C concentrations in

TABLE 1 Description of the participants included in the study.

Quartile of vitamin C	Q1 (1.87-32.1) *N* = 629	Q2 (32.1-51.6) *N* = 637	Q3 (51.6-68.1) *N* = 630	Q4 (68.1-191) *N* = 637	ALL *N* = 2533	*P*-value
Age (years old)	40.76 ± 14.71	40.09 ± 14.15	41.85 ± 14.80	45.43 ± 17.04	42.11 ± 15.39	<0.0001
Sex (%)						<0.0001
Male	57.38	50.31	51.27	28.79	46.76	
Female	42.62	49.69	48.73	71.21	53.24	
Race/Ethnicity (%)						<0.0001
Mexican American	9.06	15.28	10.98	8.99	10.94	
Other race	15.44	22.20	19.95	15.38	18.13	
Non-Hispanic Whites	67.73	48.86	60.16	68.60	61.74	
Non-Hispanic Blacks	7.78	13.65	8.91	7.03	9.19	
ALT (IU/L)	24.09 ± 19.87	22.01 ± 14.96	21.31 ± 12.04	19.68 ± 12.23	21.74 ± 15.15	<0.0001
ALP (IU/L)	80.87 ± 32.70	75.73 ± 23.27	70.26 ± 20.81	70.72 ± 22.46	74.28 ± 25.61	<0.0001
AST (IU/L)	22.11 ± 16.75	20.61 ± 9.11	21.36 ± 8.41	20.58 ± 7.48	21.18 ± 11.09	0.0439
BUN (mmol/L)	4.90 ± 1.57	4.99 ± 1.45	5.00 ± 1.37	5.00 ± 1.59	4.97 ± 1.50	0.5688
Cr (umol/L)	77.10 ± 18.02	76.45 ± 17.54	73.79 ± 16.92	70.87 ± 15.97	74.46 ± 17.29	< 0.0001
Globulin (g/L)	30.16 ± 4.02	30.64 ± 3.99	29.65 ± 3.44	29.09 ± 3.83	29.85 ± 3.86	<0.0001
GGT (IU/L)	34.09 ± 56.40	28.33 ± 30.75	22.06 ± 15.20	19.34 ± 16.38	25.79 ± 34.35	<0.0001
Calcium (mmol/L)	2.31 ± 0.09	2.32 ± 0.08	2.31 ± 0.08	2.32 ± 0.08	2.32 ± 0.08	0.0400
TP (g/L)	70.82 ± 4.07	71.65 ± 3.99	70.96 ± 3.66	70.55 ± 4.22	70.97 ± 4.01	<0.0001
UA (umol/L)	314.61 ± 79.31	320.20 ± 84.90	310.82 ± 78.74	280.60 ± 67.52	306.02 ± 79.11	<0.0001
BMI (kg/m^2)	30.62 ± 8.32	29.62 ± 6.59	28.45 ± 6.05	26.44 ± 5.62	28.74 ± 6.89	<0.0001
Waist circumference (cm)	102.39 ± 19.19	99.21 ± 14.89	96.61 ± 15.79	91.56 ± 14.74	97.34 ± 16.76	<0.0001
Pulse (bpm)	73.38 ± 12.25	72.58 ± 11.41	70.59 ± 9.80	71.05 ± 11.49	71.86 ± 11.31	<0.0001
SBP (mmHg)	117.35 ± 11.01	117.26 ± 10.71	115.05 ± 11.14	113.79 ± 11.35	115.79 ± 11.17	<0.0001
DBP (mmHg)	53.91 ± 7.43	53.77 ± 7.59	53.01 ± 7.35	50.84 ± 7.55	52.85 ± 7.58	<0.0001
Glycohemoglobin (%)	5.58 ± 0.88	5.57 ± 0.90	5.44 ± 0.67	5.39 ± 0.49	5.49 ± 0.75	<0.0001
FBG (mmol/L)	6.09 ± 1.04	6.12 ± 1.37	5.92 ± 0.79	5.97 ± 0.59	6.02 ± 0.98	0.0004
HDL_C (mmol/L)	1.29 ± 0.33	1.33 ± 0.38	1.42 ± 0.38	1.56 ± 0.40	1.40 ± 0.39	<0.0001
TC (mmol/L)	4.89 ± 1.05	4.94 ± 1.07	4.85 ± 0.96	4.87 ± 1.00	4.88 ± 1.02	0.4838
TG (mmol/L)	1.21 ± 0.55	1.32 ± 1.32	1.15 ± 0.60	1.14 ± 0.39	1.20 ± 0.78	0.0001
LDL_C (mmol/L)	2.86 ± 0.64	2.87 ± 0.67	2.79 ± 0.63	2.81 ± 0.57	2.83 ± 0.63	0.0746
Ratio of family income to poverty	2.81 ± 1.64	2.93 ± 1.52	3.26 ± 1.55	3.16 ± 1.54	3.05 ± 1.57	<0.0001
Alcohol consumption (%)						0.0129
Yes	24.44	18.80	21.92	26.27	22.99	
No	75.56	81.20	78.08	73.73	77.01	
Education levels (%)						<0.0001
Lower than high school	12.81	12.80	8.14	8.05	10.33	
High school	65.60	54.25	54.16	46.73	55.14	
College or above	21.59	32.95	37.70	45.16	34.51	
Missing				0.06	0.01	
Marital status (%)						0.0103
Married/cohabiting/remarried	58.03	59.76	67.92	60.23	61.61	
Unmarried/divorced/widowed	41.94	40.24	32.08	39.71	38.37	
Missing/Refused	0.03			0.06	0.02	
Diabetes (%)						0.0003
Yes	6.84	6.14	2.80	3.05	4.63	
No	93.16	93.86	97.20	96.95	95.37	
Smoking (%)						<0.0001
Yes	55.99	35.82	35.04	30.85	39.41	
No	44.01	64.18	64.96	69.15	60.59	

TABLE 2 Association between serum vitamin C (umol/L) and SBP (mmHg).

Outcome	Model 1, β (95% CI), *p*	Model 2, β (95% CI), *p*	Model 3, β (95% CI), *p*
SBP	−0.05 (−0.07, −0.03) < 0.0001	−0.05 (−0.06, −0.03) < 0.0001	−0.02 (−0.03, −0.00) 0.0306
Quartiles of vitamin C			
Q1 (1.87-32.1)	Reference	Reference	Reference
Q2 (32.1-51.6)	−0.09 (−1.35, 1.16) 0.8829	0.16 (−1.02, 1.35) 0.7876	1.17 (0.01, 2.33) 0.0489
Q3 (51.6-68.1)	−2.30 (−3.50, −1.10) 0.0002	−2.28 (−3.41, −1.15) < 0.0001	−0.74 (−1.87, 0.38) 0.1936
Q4 (68.1-191)	−3.56 (−4.77, −2.36) < 0.0001	−3.14 (−4.30, −1.98) < 0.0001	−1.84 (−2.02, −0.34) 0.0149
P for trend	<0.001	<0.001	0.037
Stratified by age			
Age < 50years	−0.10 (−0.12, −0.08) < 0.0001	−0.07 (−0.09, −0.05) < 0.0001	−0.02 (−0.04, 0.00) 0.0788
Age ≥ 50 years	−0.02 (−0.04, 0.01) 0.1592	−0.01 (−0.04, 0.01) 0.4050	−0.00 (−0.03, 0.02) 0.8273
Stratified by sex			
Male	−0.03 (−0.05, −0.00) 0.0385	−0.04 (−0.07, −0.01) 0.0022	−0.02 (−0.04, 0.01) 0.2281
Female	−0.03 (−0.06, −0.01) 0.0024	−0.05 (−0.07, −0.03) < 0.0001	−0.02 (−0.04, 0.00) 0.0827
Stratified by race/ethnicity			
Mexican American	−0.03 (−0.08, 0.02) 0.3053	−0.02 (−0.06, 0.03) 0.4547	−0.00 (−0.05, 0.05) 0.9753
Other Race/Ethnicity	−0.05 (−0.08, −0.01) 0.0075	−0.02 (−0.05, 0.01) 0.2763	−0.02 (−0.05, 0.02) 0.3313
Non-Hispanic White	−0.05 (−0.08, −0.03) 0.0001	−0.06 (−0.08, −0.03) < 0.0001	−0.03 (−0.06, −0.01) 0.0088
Non-Hispanic Black	−0.07 (−0.12, −0.03) 0.0012	−0.04 (−0.09, −0.00) 0.0473	−0.02 (−0.06, 0.03) 0.4844

Model 1: No adjustment for variables.
Model 2: Adjusted for sex, age, and race/ethnicity.
Model 3: Adjusted for sex, age, race/ethnicity, marital status, education level, ratio of family income to poverty, BMI, smoking states, pulse, ALT, ALP, AST, BUN, globulin, Cr, TG, TC, LDL, HDL, glycohemoglobin, TP, FBG, γGT, and sUA.

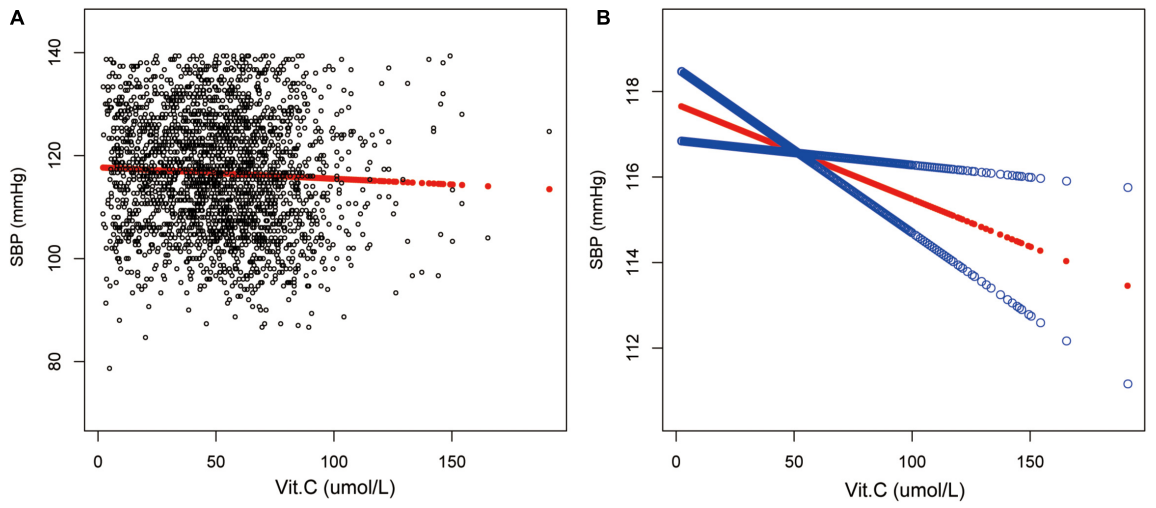

FIGURE 2
Relationship between vitamin C and SBP. **(A)** Each black point represents a sample. **(B)** The red line represents the smooth curve fit between variables. In comparison, blue bands represent the 95% CI. Sex, age, race/ethnicity, marital status, education level, ratio of family income to poverty, BMI, smoking states, pulse, ALT, ALP, AST, BUN, globulin, Cr, TG, TC, LDL, HDL, glycohemoglobin, TP, FBG, γGT and sUA were adjusted.

hypertensive subjects were 15.13 μmol/L, much lower than in normotensive individuals.

The current studies on the relationship between vitamin C and blood pressure with contradictory results were mainly carried out in participants including both normotensive and hypertensive subjects or in patients only with hypertension (17, 40, 41). Besides, as is known, antihypertensive medication used by some participants may have impacted on the relationship between serum vitamin C and blood pressure (42). On the other hand, relatively few studies have focused primarily on those with normal blood pressure. Consistent with previous studies, we also detected for the first time that

TABLE 3 Association between serum vitamin C (umol/L) and DBP (mmHg).

Outcome	Model 1, β (95% CI), *p*	Model 2, β (95% CI), *p*	Model 3, β (95% CI), *p*
DBP	−0.04 (−0.05, −0.03) < 0.0001	−0.03 (−0.04, −0.02) < 0.0001	−0.02 (−0.04, −0.01) < 0.0001
Quartiles of vitamin C			
Q1 (1.87-32.1)	Reference	Reference	Reference
Q2 (32.1-51.6)	−0.14 (−0.99, 0.71) 0.7481	0.04 (−0.80, 0.89) 0.9198	0.13 (−0.72, 0.98) 0.7642
Q3 (51.6-68.1)	−0.90 (−1.71, −0.09) 0.0294	−0.81 (−1.61, −0.00) 0.0489	−0.58 (−1.40, 0.24) 0.1663
Q4 (68.1-191)	−3.07 (−3.88, −2.25) < 0.0001	−2.58 (−3.40, −1.75) < 0.0001	−2.08 (−2.94, −1.22)<0.0001
P for trend	<0.001	<0.001	<0.001
Stratified by age			
Age < 50years	−0.04 (−0.05, −0.03) 0.0001	−0.03 (−0.04, −0.01) 0.0005	−0.01 (−0.02, 0.01) 0.2574
Age ≥ 50 years	−0.04 (−0.05, −0.02) 0.0001	−0.03 (−0.05, −0.01) 0.0007	−0.04 (−0.06, −0.02) < 0.0001
Stratified by sex			
Male	−0.03 (−0.04, −0.01) 0.0055	−0.03 (−0.05, −0.01) 0.0028	−0.02 (−0.04, −0.00) 0.0231
Female	−0.03 (−0.04, −0.02) < 0.0001	−0.03 (−0.05, −0.02) < 0.0001	−0.02 (−0.04, −0.01) 0.0024
Stratified by race/ethnicity			
Mexican American	−0.04 (−0.08, −0.01) 0.0221	−0.04 (−0.07, −0.00) 0.0338	−0.02 (−0.06, 0.01) 0.2127
Other Race/Ethnicity	−0.02 (−0.04, 0.00) 0.0529	−0.01 (−0.03, 0.01) 0.2740	−0.02 (−0.04, 0.01) 0.1600
Non-Hispanic White	−0.04 (−0.06, −0.02) < 0.0001	−0.03 (−0.05, −0.01) 0.0007	−0.03 (−0.05, −0.01) 0.0020
Non-Hispanic Black	−0.04 (−0.08, −0.01) 0.0091	−0.03 (−0.06, 0.00) 0.0658	−0.02 (−0.06, 0.01) 0.2060

Model 1: No adjustment for variables.
Model 2: Adjusted for sex, age, and race/ethnicity.
Model 3: Adjusted for sex, age, race/ethnicity, marital status, education level, ratio of family income to poverty, BMI, smoking states, pulse, ALT, ALP, AST, BUN, globulin, Cr, TG, TC, LDL, HDL, glycohemoglobin, TP, FBG, γGT and sUA.

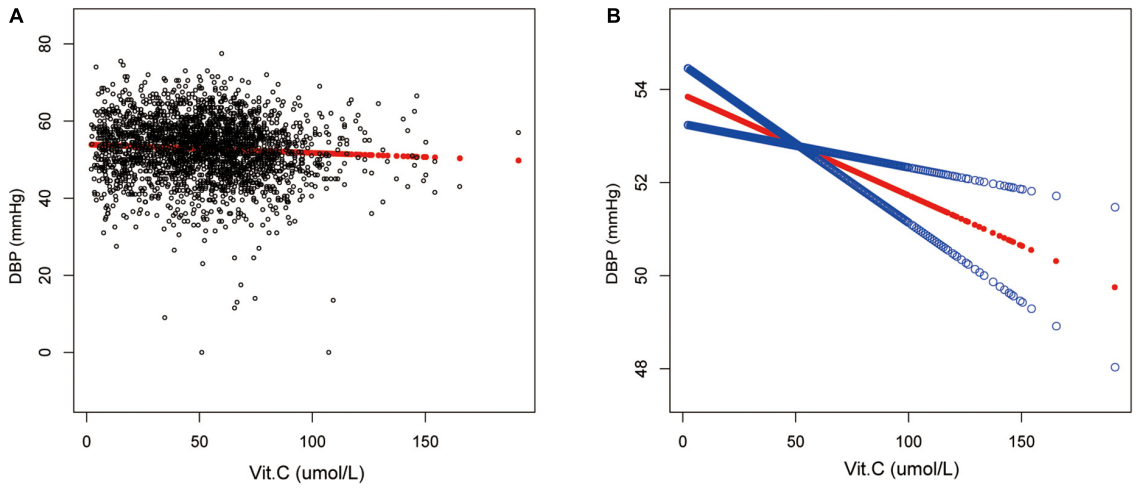

FIGURE 3
Relationship between vitamin C and DBP. **(A)** Each black point represents a sample. **(B)** The red line represents the smooth curve fit between variables. In comparison, blue bands represent the 95% CI. Sex, age, race/ethnicity, marital status, education level, ratio of family income to poverty, BMI, smoking states, pulse, ALT, ALP, AST, BUN, globulin, Cr, TG, TC, LDL, HDL, glycohemoglobin, TP, FBG, γGT, and sUA were adjusted.

serum vitamin C is reversely correlated with both SBP and DBP in normotensive subjects, which may have significant implications in the general population to reduce hypertension prevalence in the early stage by external interventions such as vitamin C supplementation.

Several mechanisms by which vitamin C may exert blood pressure lowing effect may be by downregulating antioxidative stress response and anti-inflammatory cytokines, as confirmed in a number of studies (20–22). Moreover, vitamin C may possess cardioprotective effects by ameliorating the cardiac autonomic nerve imbalance and restoring vagal and sympathetic tone to normal range. In an experimental study conducted by Fabiyi-Edebor TD (43), researchers indicated that vitamin C administration restored normal-tension in

TABLE 4 Threshold effect analysis of vitamin C and SBP using two-precise linear regression.

SBP	Adjusted β (95% CI), *p*
Female	
Fitting by a standard linear model	−0.02 (−0.04, 0.00) 0.0706
Fitting by two precise linear model	
Inflection point	90.3
Vitamin C < 90.3 umol/L	−0.03 (−0.06, −0.01) 0.0052
Vitamin C > 90.3 umol/L	0.06 (−0.11, −0.03) 0.0370
Log-likelihood ratio	<0.001
Age ≥ 50 years	
Fitting by a standard linear model	−0.01 (−0.04, 0.02) 0.5457
Fitting by two precise linear model	
Inflection point	40
Vitamin C < 40 umol/L	0.09 (0.00, 0.18) 0.0407
Vitamin C > 40 umol/L	−0.04 (−0.08, −0.00) 0.0361
Log-likelihood ratio	0.019

Sex, age, race/ethnicity, marital status, education level, ratio of family income to poverty, BMI, smoking states, pulse, ALT, ALP, AST, BUN, globulin, Cr, TG, TC, LDL, HDL, glycohemoglobin, TP, FBG, γGT and sUA were adjusted.

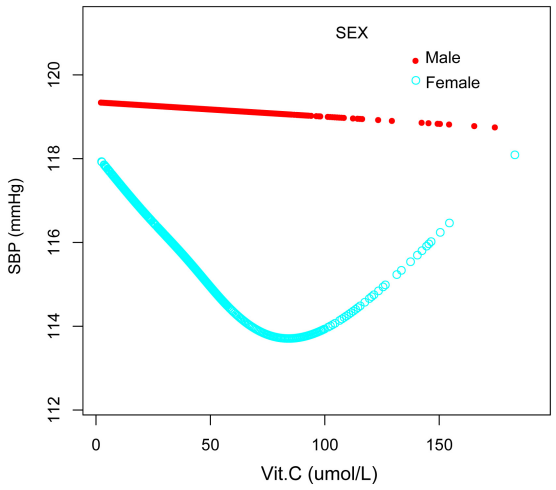

FIGURE 4
The relationship between vitamin C and SBP, stratified by sex. Age, race/ethnicity, marital status, education level, ratio of family income to poverty, BMI, smoking states, pulse, ALT, ALP, AST, BUN, globulin, Cr, TG, TC, LDL, HDL, glycohemoglobin, TP, FBG, γGT, and sUA were adjusted.

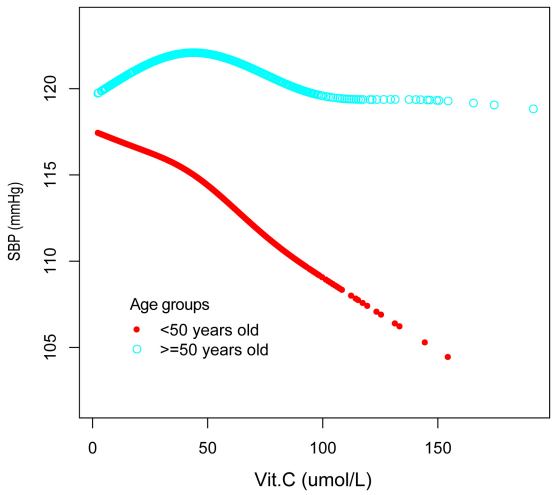

FIGURE 5
The relationship between vitamin C and SBP, stratified by age. Sex, race/ethnicity, marital status, education level, ratio of family income to poverty, BMI, smoking states, pulse, ALT, ALP, AST, BUN, globulin, Cr, TG, TC, LDL, HDL, glycohemoglobin, TP, FBG, γGT, and sUA were adjusted.

TABLE 5 Threshold effect analysis of vitamin C and DBP using two-precise linear regression.

DBP	Adjusted β (95% CI), *p*
Age ≥ 50 years	
Fitting by a standard linear model	−0.03 (−0.04, −0.01) 0.0071
Fitting by two precise linear model	
Inflection point	40
Vitamin C < 40 umol/L	−0.06 (−0.12, −0.01) 0.0268
Vitamin C > 40 umol/L	−0.01 (−0.04, 0.101) 0.0446
Log-likelihood ratio	0.014
Male	
Fitting by a standard linear model	0.02 (−0.04, −0.00) 0.0351
Fitting by two precise linear model	
Inflection point	40
Vitamin C < 40 umol/L	0.05 (−0.00, 0.10) 0.0509
Vitamin C > 40 umol/L	−0.06 (−0.09, −0.03) 0.0002
Log-likelihood ratio	0.002

Sex, age, race/ethnicity, marital status, education level, ratio of family income to poverty, BMI, smoking states, pulse, ALT, ALP, AST, BUN, globulin, Cr, TG, TC, LDL, HDL, glycohemoglobin, TP, FBG, γGT, and sUA were adjusted.

diabetic rats via ameliorating cardiac autonomic neuropathy. Furthermore, an animal study confirmed that dietary supplementation of vitamin C might play a hypotensive effect by enriching the diversity of gut microbes and reshaping their functions. To our knowledge, gut microbes and their metabolites perform important functions in cardiovascular diseases such as hypertension, myocardial fibrosis, arrhythmia, and atherosclerosis via various routes (44–46). Vitamin C as an antioxidant may counteract the effects of trimethylamine N-oxide, a metabolite derived from intestinal microbes, and exert a pharmacological effect of antihypertensive. In addition, studies have indicated that endothelial dysfunction was an underlying cause of hypertension, and vitamin C supplements restored endothelial function and corrected vascular NO deficiency that could account for its antihypertensive effect (47, 48). Alternatively, vitamin C has been reported to act as a vasodilator, possibly by increasing nitric oxide bioavailability, thereby affecting blood pressure (49). Vitamin C appears

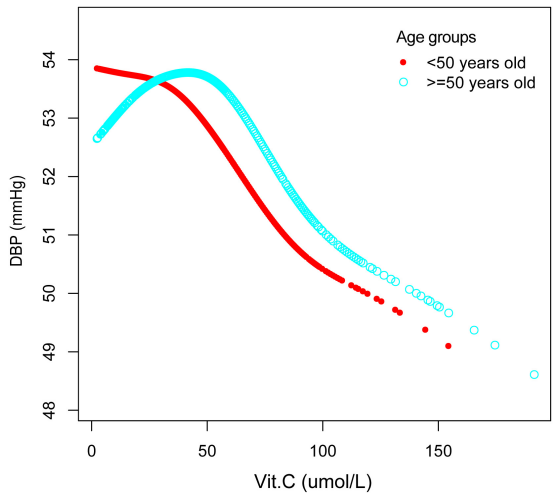

FIGURE 6
The association between vitamin C and DBP, stratified by sex. Age, race/ethnicity, marital status, education level, ratio of family income to poverty, BMI, smoking states, pulse, ALT, ALP, AST, BUN, globulin, Cr, TG, TC, LDL, HDL, glycohemoglobin, TP, FBG, γGT, and sUA were adjusted.

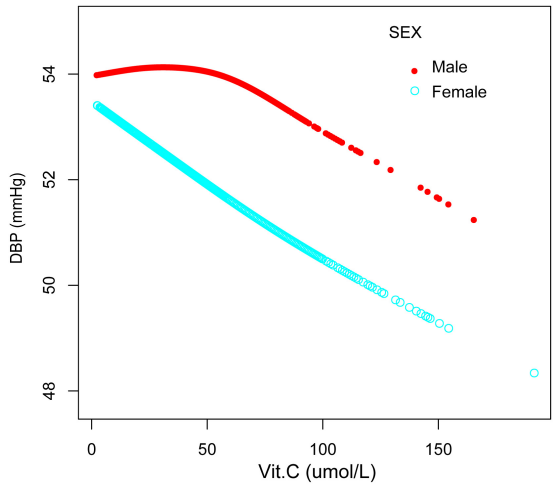

FIGURE 7
The association between vitamin C and DBP, stratified by age. Sex, race/ethnicity, marital status, education level, ratio of family income to poverty, BMI, smoking states, pulse, ALT, ALP, AST, BUN, globulin, Cr, TG, TC, LDL, HDL, glycohemoglobin, TP, FBG, γGT, and sUA were adjusted.

to be associated with a reduction in vascular sensitivity to noradrenaline and an increase in endothelium-dependent relaxation due to increased nitric oxide bioavailability, according to a study (50).

The present analyses indicate that the association between vitamin C and blood pressure remains controversial (51), and we speculate that the reason for this paradoxical phenomenon may be related to population differences. Therefore, we performed a stratified analysis and found that different concentrations of vitamin C had different effects on blood pressure in different populations, which may shed light on the current conflicting results. We found an inverted U-shape association between vitamin C and blood pressure for people over 50 years old. Specifically, it is better to maintain the concentrations of vitamin C above 40 μmol/L, for higher levels of vitamin C are associated with lower blood pressure. Lower serum vitamin C concentrations (less than 40 μmol/L) are related to increased blood pressure, which may be related to weak antioxidant effects, but studies are needed to confirm further. Another significant finding is that for females, vitamin C has a U-shaped relationship with SBP, while for males, maintaining serum vitamin C concentrations higher than 40 μmol/L is related to lower DBP. It is the first time revealing a gender difference in the relationship between vitamin C and blood pressure. The reason for this gender difference, according to our speculation, may be related to the interaction between a variety of sex hormones and maybe also associated with an insufficient research sample size. However, more prospective research is needed in the future to explore the underlying causes.

We thought that different serum vitamin C levels should be controlled or intervened to better manage blood pressure in different populations. Unfortunately, there are no studies or guidelines that discuss the optimal level of vitamin C in humans. The results of the present study will serve as a reference for future clinical management of blood pressure in various populations.

In short, our research has important clinical implications for hypertension management, especially for early interventions for those at high risk of developing hypertension. Nevertheless, there are still some shortcomings of the present study: Firstly, since this was a cross-sectional study, the individuals were not followed up, and the relationship between vitamin C and adverse outcomes and causality could not be effectively evaluated. Furthermore, this study did not exclude patients with other diseases that may interfere with blood pressure. Scholars still need to be cautious when facing the results of the study. Finally, additional potential confounding factors, such as dietary factors or physical activities were not taken into consideration.

Conclusion

Vitamin C was reversely correlated with both SBP and DBP in this cross-sectional analysis. However, a U-shaped relationship and an inverted one were also observed in certain people, which implied that, though vitamin C is considered a vital antioxidant, maintaining vitamin C at appropriate levels may be beneficial according to different populations.

Data availability statement

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

Ethics statement

This study was granted ethical approval by the National Center for Health Statistics (NCHS). This study was carried out following the ethical standards of the responsible committee on human experimentation and with the 1975 Helsinki Declaration and its later amendments. Furthermore, written informed consent was received from each subject.

Author contributions

RH, LS, and JZ contributed to the design, data analysis and interpretation, and drafting of the manuscript. YL and TL contributed to the data interpretation and critically revised the manuscript. RH and TL contributed to the conception, design, data acquisition, analysis and interpretation, and critical review of the manuscript. TL was the article's guarantor. All authors read and approved the final version of the manuscript.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.986808/full#supplementary-material

References

1. Lobo MD, Sobotka PA, Pathak A. Interventional procedures and future drug therapy for hypertension. *Eur Heart J.* (2017) 38:1101–11. doi: 10.1093/eurheartj/ehw303

2. Inoue K, Horwich T, Bhatnagar R, Bhatt K, Goldwater D, Seeman T, et al. Urinary stress hormones, hypertension, and cardiovascular events: the multi-ethnic study of atherosclerosis. *Hypertension.* (2021) 78:1640–7. doi: 10.1161/HYPERTENSIONAHA.121.17618

3. Kawada T. Cancer, hypertension and risk for cardiovascular disease. *J Hypertens.* (2021) 39:2122. doi: 10.1097/HJH.0000000000002975

4. Wang Z, Yang T, Fu H. Prevalence of diabetes and hypertension and their interaction effects on cardio-cerebrovascular diseases: a cross-sectional study. *BMC Public Health.* (2021) 21:1224. doi: 10.1186/s12889-021-11122-y

5. Zhang S, Hu L, Jiang J, Li H, Wu Q, Ooi K, et al. HMGB1/RAGE axis mediates stress-induced RVLM neuroinflammation in mice via impairing mitophagy flux in microglia. *J Neuroinflammation.* (2020) 17:15. doi: 10.1186/s12974-019-1673-3

6. Hofmeyr GJ, Seuc A, Betrán AP, Cormick G, Singata M, Fawcus S, et al. The effect of calcium supplementation on blood pressure in non-pregnant women with previous pre-eclampsia: a randomized placebo-controlled study. *Pregnancy Hypertens.* (2021) 23:91–6. doi: 10.1016/j.preghy.2020.11.012

7. Giovanella J, Wollinger LM, Capra L, Dresch F, Genro JP, Contini V. Diet-gene interaction: effects of polymorphisms in the ACE, AGT and BDKRB2 genes and the consumption of sodium, potassium, calcium, and magnesium on blood pressure of normotensive adult individuals. *Mol Cell Biochem.* (2021) 476:1211–9. doi: 10.1007/s11010-020-03983-5

8. Zhang JY, You HZ, Wang MJ, Zhang Q, Dong XY, Liu JF, et al. High-phosphorus diet controlled for sodium elevates blood pressure in healthy adults via volume expansion. *J Clin Hypertens (Greenwich).* (2021) 23:849–59. doi: 10.1111/jch.14182

9. McClure ST, Rebholz CM, Mitchell DC, Selvin E, Appel LJ. The association of dietary phosphorus with blood pressure: results from a secondary analysis of the PREMIER trial. *J Hum Hypertens.* (2020) 34:132–42. doi: 10.1038/s41371-019-0231-x

10. Robinson AT, Wenner MM, Charkoudian N. Differential influences of dietary sodium on blood pressure regulation based on race and sex. *Auton Neurosci.* (2021) 236:102873. doi: 10.1016/j.autneu.2021.102873

11. Huang L, Wang H, Wang Z, Wang Y, Zhang B, Ding G. Associations of dietary sodium, potassium, and sodium to potassium ratio with blood pressure-

regional disparities in China. *Nutrients.* (2020) 12:366. doi: 10.3390/nu12020366

12. Razavi MA, Bazzano LA, Nierenberg J, Huang Z, Fernandez C, Razavi AC, et al. Advances in genomics research of blood pressure responses to dietary sodium and potassium intakes. *Hypertension.* (2021) 78:4–15. doi: 10.1161/HYPERTENSIONAHA.121.16509

13. Chen G, Li Y, Deng G, Shrestha S, Chen F, Wei Y, et al. Associations of plasma copper, magnesium, and calcium levels with blood pressure in children: a cross-sectional study. *Biol Trace Elem Res.* (2021) 199:815–24. doi: 10.1007/s12011-020-02201-z

14. Teymoori F, Bidel Z, Nazarzadeh M. The effect of magnesium supplementation on blood pressure: multivariate meta-regression scenario. *Am J Clin Nutr.* (2018) 107:291–3. doi: 10.1093/ajcn/nqx032

15. Ran L, Zhao W, Tan X, Wang H, Mizuno K, Takagi K, et al. Association between serum vitamin c and the blood pressure: a systematic review and meta-analysis of observational studies. *Cardiovasc Ther.* (2020) 2020:4940673. doi: 10.1155/2020/4940673

16. Boonthongkaew C, Tong-Un T, Kanpetta Y, Chaungchot N, Leelayuwat C, Leelayuwat N. Vitamin C supplementation improves blood pressure and oxidative stress after acute exercise in patients with poorly controlled type 2 diabetes mellitus: a randomized, placebo-controlled, cross-over study. *Chin J Physiol.* (2021) 64:16–23. doi: 10.4103/cjp.cjp_95_20

17. Guan Y, Dai P, Wang H. Effects of vitamin C supplementation on essential hypertension: a systematic review and meta-analysis. *Medicine (Baltimore).* (2020) 99:e19274. doi: 10.1097/MD.0000000000019274

18. Ashor AW, Shannon OM, Werner AD, Scialo F, Gilliard CN, Cassel KS, et al. Effects of inorganic nitrate and vitamin C co-supplementation on blood pressure and vascular function in younger and older healthy adults: a randomised double-blind crossover trial. *Clin Nutr.* (2020) 39:708–17. doi: 10.1016/j.clnu.2019.03.006

19. Li Y, Zafar S, Salih Ibrahim RM, Chi HL, Xiao T, Xia WJ, et al. Exercise and food supplement of vitamin C ameliorate hypertension through improvement of gut microflora in the spontaneously hypertensive rats. *Life Sci.* (2021) 269:119097. doi: 10.1016/j.lfs.2021.119097

20. Fujii T, Luethi N, Young PJ, Frei DR, Eastwood GM, French CJ, et al. Effect of vitamin C, hydrocortisone, and thiamine vs hydrocortisone alone on time alive and free of vasopressor support among patients with septic shock: the VITAMINS randomized clinical trial. *JAMA.* (2020) 323:423–31. doi: 10.1001/jama.2019.22176

21. Karasavvas N, Cárcamo JM, Stratis G, Golde DW. Vitamin C protects HL60 and U266 cells from arsenic toxicity. *Blood.* (2005) 105:4004–12. doi: 10.1182/blood-2003-03-0772

22. Brain KL, Allison BJ, Niu Y, Cross CM, Itani N, Kane AD, et al. Intervention against hypertension in the next generation programmed by developmental hypoxia. *PLoS Biol.* (2019) 17:e2006552. doi: 10.1371/journal.pbio.2006552

23. Hodgson JM, Croft KD, Woodman RJ, Puddey IB, Bondonno CP, Wu JH, et al. Effects of vitamin E, vitamin C and polyphenols on the rate of blood pressure variation: results of two randomised controlled trials. *Br J Nutr.* (2014) 112:1551–61. doi: 10.1017/S0007114514002542

24. Zhu J, Ling Y, Tse LA, Kinra S, Li Y. Circulating vitamin C and the risk of cardiovascular diseases: a Mendelian randomization study. *Nutr Metab Cardiovasc Dis.* (2021) 31:2398–406. doi: 10.1016/j.numecd.2021.04.023

25. Duthie SJ, Duthie GG, Russell WR, Kyle JAM, Macdiarmid JI, Rungapamestry V, et al. Effect of increasing fruit and vegetable intake by dietary intervention on nutritional biomarkers and attitudes to dietary change: a randomised trial. *Eur J Nutr.* (2018) 57:1855–72. doi: 10.1007/s00394-017-1469-0

26. Golabi P, Gerber L, Paik JM, Deshpande R, de Avila L, Younossi ZM. Contribution of sarcopenia and physical inactivity to mortality in people with non-alcoholic fatty liver disease. *JHEP Rep.* (2020) 2:100171. doi: 10.1016/j.jhepr.2020.100171

27. Zhu Z, Hu G, Jin F, Yao X. Correlation of osteoarthritis or rheumatoid arthritis with bone mineral density in adults aged 20-59 years. *J Orthop Surg Res.* (2021) 16:190. doi: 10.1186/s13018-021-02338-0

28. Dillon CF, Weisman MH. US national health and nutrition examination survey arthritis initiatives, methodologies and data. *Rheum Dis Clin North Am.* (2018) 44:215–65. doi: 10.1016/j.rdc.2018.01.010

29. Zipf G, Chiappa M, Porter KS, Ostchega Y, Lewis BG, Dostal J. National health and nutrition examination survey: plan and operations, 1999-2010. *Vital Health Stat 1.* (2013) 1–37.

30. DeGuire J, Clarke J, Rouleau K, Roy J, Bushnik T. Blood pressure and hypertension. *Health Rep.* (2019) 30:14–21.

31. Cardoso CRL, Salles GF. Prognostic impact of home blood pressures for adverse cardiovascular outcomes and mortality in patients with resistant hypertension: a prospective cohort study. *Hypertension.* (2021) 78:1617–27. doi: 10.1161/HYPERTENSIONAHA.121.18064

32. Jafari Nasab S, Ghanavati M, Rafiee P, Bahrami A, Majidi N, Clark CCT, et al. A case-control study of dietary approaches to stop hypertension (DASH) diets, colorectal cancer and adenomas among Iranian population. *BMC Cancer.* (2021) 21:1050. doi: 10.1186/s12885-021-08786-5

33. Ghaseminasabparizi M, Nazarinia MA, Akhlaghi M. Adherence to the dietary approaches to stop hypertension dietary pattern and rheumatoid arthritis in Iranian adults. *Public Health Nutr.* (2021) 24:6085–93. doi: 10.1017/S1368980021003608

34. Sun X, Shi Z, Li Y, Xin B, Li X, Wang Y. U-shaped association between dietary calcium density intake during adolescence and hypertension in adulthood: a 20-year longitudinal nationwide study in China. *Br J Nutr.* (2021). Epub ahead of print. doi: 10.1017/S0007114521002701

35. Collins BJ, Mukherjee MS, Miller MD, Delaney CL. Effect of dietary or supplemental vitamin C intake on vitamin C levels in patients with and without cardiovascular disease: a systematic review. *Nutrients.* (2021) 13:2330. doi: 10.3390/nu13072330

36. Zhu N, Huang B, Jiang W. Targets of vitamin C with therapeutic potential for cardiovascular disease and underlying mechanisms: a study of network pharmacology. *Front Pharmacol.* (2020) 11:591337. doi: 10.3389/fphar.2020.591337

37. Ashor AW, Lara J, Mathers JC, Siervo M. Effect of vitamin C on endothelial function in health and disease: a systematic review and meta-analysis of randomised controlled trials. *Atherosclerosis.* (2014) 235:9–20. doi: 10.1016/j.atherosclerosis.2014.04.004

38. Mason SA, Keske MA, Wadley GD. Effects of vitamin C supplementation on glycemic control and cardiovascular risk factors in people with type 2 diabetes: a GRADE-assessed systematic review and meta-analysis of randomized controlled trials. *Diabetes Care.* (2021) 44:618–30. doi: 10.2337/dc20-1893

39. Das UN. Vitamin C for type 2 diabetes mellitus and hypertension. *Arch Med Res.* (2019) 50:11–4. doi: 10.1016/j.arcmed.2019.05.004

40. Moran JP, Cohen L, Greene JM, Xu G, Feldman EB, Hames CG, et al. Plasma ascorbic acid concentrations relate inversely to blood pressure in human subjects. *Am J Clin Nutr.* (1993) 57:213–7. doi: 10.1093/ajcn/57.2.213

41. Block G, Jensen CD, Norkus EP, Hudes M, Crawford PB. Vitamin C in plasma is inversely related to blood pressure and change in blood pressure during the previous year in young black and white women. *Nutr J.* (2008) 7:35. doi: 10.1186/1475-2891-7-35

42. Al-Khudairy L, Flowers N, Wheelhouse R, Ghannam O, Hartley L, Stranges S, et al. Vitamin C supplementation for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev.* (2017) 3:Cd011114. doi: 10.1002/14651858.CD011114.pub2

43. Fabiyi-Edebor TD. Vitamin C ameliorated cardiac autonomic neuropathy in type 2 diabetic rats. *World J Diabetes.* (2020) 11:52–65. doi: 10.4239/wjd.v11.i3.52

44. Verhaar BJH, Prodan A, Nieuwdorp M, Muller M. Gut microbiota in hypertension and atherosclerosis: a review. *Nutrients.* (2020) 12:2982. doi: 10.3390/nu12102982

45. Xu J, Yang Y. Gut microbiome and its meta-omics perspectives: profound implications for cardiovascular diseases. *Gut Microbes.* (2021) 13:1936379. doi: 10.1080/19490976.2021.1936379

46. Yu L, Meng G, Huang B, Zhou X, Stavrakis S, Wang M, et al. A potential relationship between gut microbes and atrial fibrillation: trimethylamine N-oxide, a gut microbe-derived metabolite, facilitates the progression of atrial fibrillation. *Int J Cardiol.* (2018) 255:92–8. doi: 10.1016/j.ijcard.2017.11.071

47. Ashor AW, Siervo M, Lara J, Oggioni C, Afshar S, Mathers JC. Effect of vitamin C and vitamin E supplementation on endothelial function: a systematic review and meta-analysis of randomised controlled trials. *Br J Nutr.* (2015) 113:1182–94. doi: 10.1017/S0007114515000227

48. Conner TS, Fletcher BD, Pullar JM, Spencer E, Mainvil LA, Vissers MCM. KiwiC for vitality: results of a randomized placebo-controlled trial testing the effects of kiwifruit or vitamin C tablets on vitality in adults with low vitamin C levels. *Nutrients.* (2020) 12:2898. doi: 10.3390/nu12092898

49. Kelly RP, Poo Yeo K, Isaac HB, Lee CY, Huang SH, Teng L, et al. Lack of effect of acute oral ingestion of vitamin C on oxidative stress, arterial stiffness or blood pressure in healthy subjects. *Free Radic Res.* (2008) 42:514–22. doi: 10.1080/10715760802087431

50. Ettarh RR, Odigie IP, Adigun SA. Vitamin C lowers blood pressure and alters vascular responsiveness in salt-induced hypertension. *Can J Physiol Pharmacol.* (2002) 80:1199–202. doi: 10.1139/y02-147

51. Morelli MB, Gambardella J, Castellanos V, Trimarco V, Santulli G. Vitamin C and cardiovascular disease: an update. *Antioxidants (Basel).* (2020) 9:1227. doi: 10.3390/antiox9121227

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Causal effect of polyunsaturated fatty acids on bone mineral density and fracture

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Background: Polyunsaturated fatty acids (PUFAs) are closely related to osteoporosis. To test their causal relationship, we conducted a Mendelian randomization (MR) analysis.

Methods: We analyzed the causal relationship between four PUFAs measures, n-3 PUFAs (n-3), n-6 PUFAs (n-6), the ratio of n-3 PUFAs to total fatty acids (n-3 pct), and the ratio of n-6 PUFAs to n-3 PUFAs (n-6 to n-3), and five measures of osteoporosis, including estimated bone mineral density (eBMD), forearm (FA) BMD, femoral neck (FN) BMD, lumbar spine (LS) BMD, and fracture, using two-sample MR analysis. In order to verify the direct effect between PUFAs and BMD, we chose interleukin-6 (IL-6), tumor necrosis factor-β (TNF-β), and bone morphogenetic proteins 7 (BMP-7), three markers or cytokines strongly related to BMD, as possible confounding factors, and analyzed the possible causal relationships between them and PUFAs or BMD by MR. Inverse variance weighting (IVW), MR-Egger, weighted and weighted median were conducted. MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) and MR-Egger regression methods were used to evaluate the potential pleiotropy of instrumental variables (IVs) and outliers were identified by MR-PRESSO. Cochran's Q statistic was used to detect the heterogeneity among IVs. Leave-one-out sensitivity analysis was used to find SNPs that have a significant impact on the results. All results were corrected by the Bonferroni correction.

Results: The IVW results showed that n-3 PUFAs (OR = 1.030, 95% CI: 1.013, 1.047, $P = 0.001$) and n-6 PUFAs (OR = 1.053, 95% CI: 1.034, 1.072, $P < 0.001$) were positively correlated with eBMD, while n-6 to n-3 (OR = 0.947, 95% CI: 0.924, 0.970, $P < 0.001$) were negatively correlated with eBMD. These casual relationships still existed after Bonferroni correction. There were positive effects of n-3 PUFAs on FA BMD (OR = 1.090, 95% CI: 1.011, 1.176, $P = 0.025$)

and LS BMD (OR = 1.056, 95% CI: 1.011, 1.104, P = 0.014), n-3 pct on eBMD (OR = 1.028, 95% CI: 1.002, 1.055, P = 0.035) and FA BMD (OR = 1.090, 95% CI: 1.011, 1.174, P = 0.025), n-6 to n-3 on LS BMD (OR = 1.071, 95% CI: 1.021, 1.124, P = 0.005); negative effects of n-3 pct on fracture (OR = 0.953, 95% CI: 0.918, 0.988, P = 0.009) and n-6 to n-3 on FA BMD (OR = 0.910, 95% CI: 0.837, 0.988, P = 0.025). However, these causal effects all disappeared after Bonferroni correction (all $P > 0.0025$). None of IL-6, TNF-β, and BMP-7 had a causal effect on PUFA and BMD simultaneously (all $P > 0.05$).

Conclusion: Evidence from this MR study supports the genetically predicted causal effects of n-3, n-6, n-3 pct, and n-6 to n-3 on eBMD. In addition, n-3 not only associate with FA BMD and LS BMD through its own level and n-6 to n-3, but also link to fracture through n-3 pct.

KEYWORDS

polyunsaturated fatty acids, osteoporosis, bone mineral density, genetic association, causal relationship, Mendelian randomization

Introduction

Osteoporosis is one of the most common systemic bone diseases in clinic, which is mainly manifested with the degradation of bone tissue microenvironment, the reduction of bone mineral density (BMD), the increase of bone fragility, and the high risk of fracture (1, 2). Osteoporosis mostly occurs in the postmenopausal women. Worldwide, fractures caused by osteoporosis can be as high as 9 million times a year (3). With the increased life expectancy of the global population and the improvement of medical and health conditions, the prevention and treatment of osteoporosis and fractures is still a common public health challenge and health care problem in the world today (4–7). It has been suggested that 20–40% of the risk of osteoporosis is caused by environmental factors such as nutrition (8).

Polyunsaturated fatty acids (PUFAs) are important immune nutrients, which play a role in nutritional treatment of a variety of diseases, including cancers, inflammatory diseases and osteoporosis (9–20). PUFAs are straight chain fatty acids with two or more double bonds, and the length of carbon chain is 18–22 carbon atoms. In PUFAs, the first unsaturated double bond is located between the 3rd and 4th carbon atoms starting from the methyl end, which is called omega-3 (n-3) PUFAs, and between the 6th and 7th carbon atoms, which is called omega-6 (n-6) PUFAs (15). Mounting evidence has suggested that PUFAs are involved in osteolysis, bone formation, bone development, bone metabolism, and metabolic bone diseases including osteoporosis and may be beneficial for skeletal health (21–29).

Recently, the relationship between PUFAs and osteoporosis has attracted a lot of attention. PUFAs intake and the ratio of n-6 to n-3 PUFAs are reported to be associated with BMD in humans (27, 30–32). Some studies found that total PUFAs, n-3 PUFAs, n-6 PUFAs intake can increase BMD, decrease the risk of fractures and beneficial for osteoporosis (30–34). A low n-6 to n-3 PUFAs ratio was proposed to be beneficial for the bone quality of rats (35). However, conclusions about the relationship between n-3 PUFAs, n-6 PUFAs, and osteoporosis are inconsistent. It was also observed that the intake of n-3 PUFAs or the ratio of n-6 to n-3 PUFAs are not associated with osteoporotic fractures, while the intake of n-6 PUFAs is positively associated with an elevated risk of fracture (32). Other study also indicated that increased intake of PUFAs is associated with greater perimenopausal femoral neck (FN) BMD loss (36). In postmenopausal women, the lower intake of marine n-3 PUFAs and the higher intake of n-6 PUFAs were observed that can decrease the risk of total fracture (33).

Clarifying the relationship between various PUFAs levels and BMD can not only further study the prevention, health care and treatment strategies of osteoporosis patients, but also can estimate the fracture risk of osteoporosis patients. It is imperative to evaluate the causal relationship between PUFAs level and BMD. However, from the above evidence, conclusions from observational studies and randomized controlled trials (RCT) about the relationship between PUFAs and osteoporosis are inconsistent, as well as the causal relationship between PUFAs and osteoporosis remains obscure.

Genome wide association study (GWAS) shows that BMD is a trait controlled by multiple genes, and is easy to be

affected by environmental factors and has the tendency of family aggregation. Some genetic determinants of fractures are regulated by lower BMD (37–40). Mendelian randomization (MR) is an advanced research using the law of free combination, which uses genetic variation as instrumental variable (IVs) (41). The free combination of alleles can reduce the confounding effect of environmental factors; the one-way relationship that genes affect traits but diseases do not change genotypes can effectively avoid reverse causal bias (42). Compared with RCT, MR can extract useful information from the existing GWAS database on a large scale, with stronger statistical ability and wider coverage (43).

Materials and methods

Study design

In this study, we used two-sample MR studies to assess the causal relationships between PUFAs and osteoporosis. We selected four measures of PUFAs, including the circulating level of n-3 PUFAs (n-3), the circulating level of n-6 PUFAs (n-6), the ratio of n-3 fatty acids to total fatty acids (n-3 pct), and the ratio of n-6 PUFAs to n-3 PUFAs (n-6 to n-3). For osteoporosis, estimated BMD (eBMD), forearm (FA) BMD, femoral neck (FN) BMD, lumbar (LS) BMD, and fracture were selected as measures.

In order to verify the direct effect between PUFAs and BMD, we chose interleukin-6 (IL-6) (44), tumor necrosis factor-β (TNF-β) (45) and bone morphogenetic proteins 7 (BMP-7) (46), three markers or cytokines strongly related to BMD, as possible confounding factors, and analyzed the possible causal relationships between them and PUFAs or BMD by MR.

Data sources and single-nucleotide polymorphism selection

Genome wide association study of polyunsaturated fatty acids

The GWAS data on PUFAs came from the latest and largest public GWAS analysis by Borges MC et al. (47). In the study, the GWAS data of n-3, n-6, n-3 pct, and n-6 to n-3 were generated from 114,999 UK Biobank participants of European ancestry using BOLT-LMM (v2.3) (**Supplementary Table 1**) (48).

Genome wide association studyof osteoporosis

Genetic associations of eBMD and fracture were obtained from the largest public GWAS of Morris et al. (1), in which eBMD GWAS data were retrieved from 426,824 individuals of European ancestry in the UK Biobank and fracture GWAS data were retrieved from 416,795 UK Biobank European ancestry participants (ncases = 53,184 and ncontrols = 373,611) (**Supplementary Table 1**).

The research by Zheng et al. (49) provided GWAS data about FA BMD, FN BMD, and LS BMD. In the research, the GWAS data for the three BMDs were obtained from 10,805, 49,988, and 44,731 individuals of European ancestry, respectively (**Supplementary Table 1**).

Genome wide association study of possible confounding factors

Genetic associations of IL-6 and TNF-β were obtained from the public GWAS of Ahola-Olli et al. (50), and the GWAS data for both were retrieved from 8,293 individuals of European ancestry. The GWAS data of BMP-7 was obtained from 3,301 individuals of European ancestry (51) (**Supplementary Table 1**).

Single-nucleotide polymorphism selection

We screened single-nucleotide polymorphisms (SNPs) strongly related to exposure factors ($P < 5 \times 10^{-8}$) from the exposure GWAS, used clustering process ($R^2 < 0.001$ and clumping distance = 10,000 kb) to eliminate linkage disequilibrium (LD) between SNPs, and excluded SNPs with minor allele frequency (MAF < 0.01), which ensured that the end result was undisturbed and feasible. The selected SNPs were matched with the outcome GWAS, and if SNP can not be found in outcome GWAS, its proxy SNP with high LD ($r^2 > 0.8$) was used instead. Finally, other SNPs were selected as IVs after the palindrome SNPs were removed.

Statistical analyses

We used four complementary and mutually corroborative methods to analyze the causal relationship between PUFAs and osteoporosis, including inverse variance weighting (IVW), MR-Egger, weighted and weighted median, among which IVW is the main analysis method. The weighted median estimator serves as an unbiased causal effect estimate when up to 50% of the instruments are invalid, by estimating the causal effect as the median of the weighted ratio estimates (52). At the same time, we used MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) and MR-Egger regression methods to evaluate the potential level pleiotropy of IVs (53, 54). Meanwhile, MR-PRESSO can also find abnormal values in IVs. After removing the abnormal values, MR-PRESSO and MR-Egger tests were performed again until there was no horizontal pleiotropic SNP in all IVs. Then, we applied Cochran's Q statistic to detect and quantify the heterogeneity among IVs (55). Leave-one-out sensitivity analysis was used to find and eliminate SNPs that have a significant impact on the results, so as to ensure the robustness of causal relationship estimation. There were four exposures (n-3, n-6, n-3pct, and n-6 to n-3) and five outcomes (eBMD, FA, FN, LS, and fracture) in this study, therefore the Bonferroni method was conducted to correct for multiple comparisons and the *P*-value was less than 0.0025 (0.05 was divided by 4 × 5) (56,

57). All statistical analyses were performed using the packages "TwoSampleMR" and "MRPRESSO" in R version 4.1.1.

Results

Instrumental variables selection

Instrumental variables of polyunsaturated fatty acids selection

After clumping process, there were 52 SNPs, 63 SNPs, 41 SNPs, and 41 SNPs strongly associated ($P < 5 \times 10^{-8}$) with the circulating level of n-3 PUFAs, the circulating level of n-6 PUFAs, n-3 pct, n-6 to n-3, respectively and no LD was screened out. MAF of all above SNPs were not less than 0.01. The main information of SNPs is listed in **Supplementary Tables 2–5**.

Instrumental variables of possible confounding factors selection

After clumping process, there were 2 SNPs and 2 SNPs strongly associated ($P < 5 \times 10^{-8}$) with TNF-β and BMP-7, respectively. There was no SNPs available at the genome wide significance threshold ($P < 5 \times 10^{-8}$) of IL-6, so we relaxed the significance threshold to $P < 5 \times 10^{-7}$, and 2 SNPs were strongly associated ($P < 5 \times 10^{-7}$) with IL-6. Among the above selected SNPs, no LD was found, and MAF of these SNPs were not less than 0.01. The main information of SNPs is provided in **Supplementary Tables 6**, **7**.

Causal relationship between polyunsaturated fatty acids and osteoporosis

n-3 polyunsaturated fatty acids on osteoporosis

From the IVW results, n-3 PUFAs had positive effects on eBMD (OR = 1.030, 95% CI: 1.013, 1.047, P = 0.001), FA BMD (OR = 1.090, 95% CI: 1.011, 1.176, P = 0.025), and LS BMD (OR = 1.056, 95% CI: 1.011, 1.104, P = 0.014), however, after further Bonferroni correction, only the effect on eBMD remained. No causal effects of n-3 PUFAs on FN BMD (OR = 0.988, 95% CI: 0.950, 1.028, P = 0.557) and fracture (OR = 0.982, 95% CI: 0.938, 1.028, P = 0.433) were observed (**Figure 1** and **Supplementary Table 8**).

n-6 polyunsaturated fatty acids on osteoporosis

The results of IVW showed a positive effect of n-6 PUFAs on eBMD (OR = 1.053, 95% CI: 1.034, 1.072, $P < 0.001$), which still persisted after Bonferroni correction. However, no causal associations were found between n-6 PUFAs and FA BMD (OR = 1.022, 95% CI: 0.925, 1.128, P = 0.673), FN BMD (OR = 1.015, 95% CI: 0.962, 1.071, P = 0.584), LS BMD (OR = 1.013, 95% CI: 0.951, 1.078, P = 0.688), and fracture (OR = 1.014, 95% CI: 0.970, 1.059, P = 0.545) (**Figure 1** and **Supplementary Table 9**).

n-3 pct on osteoporosis

The results of IVW revealed that n-3 pct had positive causal relationships with eBMD (OR = 1.028, 95% CI: 1.002, 1.055, P = 0.035) and FA BMD (OR = 1.090, 95% CI: 1.011, 1.174, P = 0.025) as well as a negative causal relationship with fracture (OR = 0.953, 95% CI: 0.918, 0.988, P = 0.009). However, these causal effects all disappeared after Bonferroni correction. There were no causal effects of n-3 pct on FN BMD (OR = 0.992, 95% CI: 0.951, 1.036, P = 0.732) and LS BMD (OR = 1.025, 95% CI: 0.969, 1.085, P = 0.384) (**Figure 1** and **Supplementary Table 10**).

n-6 to n-3 on osteoporosis

The results of IVW indicated that n-6 to n-3 had a negative effect on eBMD (OR = 0.947, 95% CI: 0.924, 0.970, $P < 0.001$), which remain persisted after Bonferroni correction and had a negative relationship with FA BMD (OR = 0.910, 95% CI: 0.837, 0.988, P = 0.025) while a positive relationship with LS BMD (OR = 1.071, 95% CI: 1.021, 1.124, P = 0.005), which both disappeared after Bonferroni correction. No causal effects of n-6 to n-3 on FN BMD (OR = 1.002, 95% CI: 0.959, 1.046, P = 0.945) and fracture (OR = 1.035, 95% CI: 0.979, 1.095, P = 0.225) were observed (**Figure 1** and **Supplementary Table 11**).

Causal relationship between possible confounding factors and polyunsaturated fatty acids

From the IVW results, BMP-7 had a negative effect on n-3 pct (OR = 0.967, 95% CI: 0.937, 0.998, P = 0.038) and a positive effect on n-6 to n-3 (OR = 1.035, 95% CI: 1.002, 1.069, P = 0.036). However, no causal effect was observed of BMP-7 on n-3 PUFAs and n-6 PUFAs (all $P > 0.05$). Moreover, no causal relationship was found between IL-6, TNF-β and all four outcomes (all $P > 0.05$) (**Figure 2** and **Supplementary Table 12**).

Causal relationship between possible confounding factors and osteoporosis

From the IVW results, no causal relationship was found between IL-6, TNF-β, BMP-7 and eBMD, FA, FN, LS as well as fracture (all $P > 0.05$) (**Figure 3** and **Supplementary Table 13**).

Pleiotropy and sensitivity analysis

The heterogeneity test did not find any heterogeneity between selected IVs of n-3 PUFAs and n-6 PUFAs.

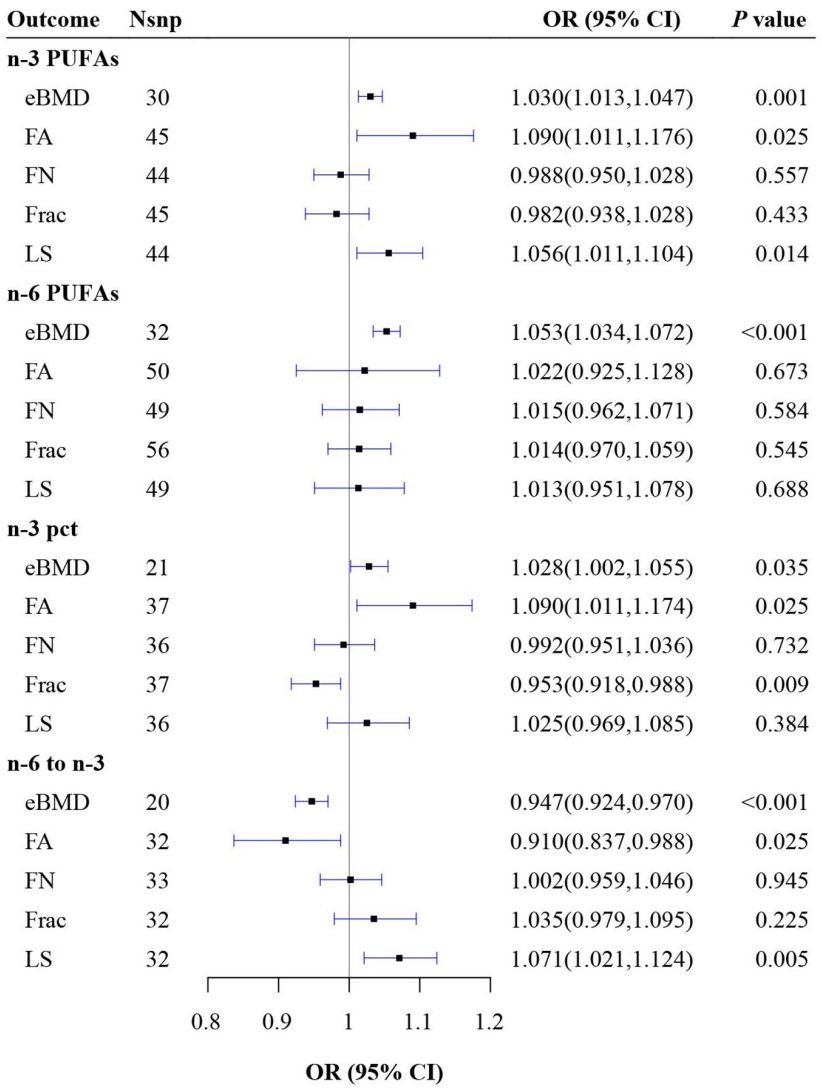

FIGURE 1
Mendelian randomization (MR) estimate results of polyunsaturated fatty acids (PUFAs) on outcomes. n-3 pct, the ratio of n-3 fatty acids to total fatty acids; n-6 to n-3, the ratio of n-6 PUFAs to n-3 PUFAs; BMD, bone mineral density; eBMD, estimated BMD; FA, forearm; FN, femoral neck; LS, lumbar.

Heterogeneity was not observed between the IVs of n-3 pct on eBMD and on FA BMD, while was found among IVs of n-3 pct on FN BMD, on fractrue and on LS BMD. No heterogeneities were found between IVs of n-6 to n-3, except for IVs of n-6 to n-3 on FN BMD (Q = 50.008, $P = 0.022$). No heterogeneity was found between the IVs of IL-6, TNF-β and BMP-7.

MR Pleiotropy RESidual Sum and Outlier global test defined SNPs with horizontal pleiotropy as outliers which were listed in **Supplementary Tables 2–7**. After removing the outliers, MR-Egger regression and MR-PRESSO global test were used to verify that there was no horizontal pleiotropy between IVs and results. Leave-one-out analysis suggested that the outcomes were not caused by any SNPs. **Supplementary Tables 8–13** and **Supplementary Figures 1–16** show the results of pleiotropy and sensitivity analysis.

Discussion

As the IVW results shown, after Bonferroni correction n-3 PUFAs and n-6 PUFAs were still positively correlated with eBMD, while n-6 to n-3 were negatively correlated with eBMD, which provides new evidence to support the relationship between PUFAs and osteoporosis.

In this study, no causal effect was observed of IL-6 and TNF-β on four measures of PUFAs and five measures of osteoporosis, suggesting that the causal effect of four measures of PUFAs

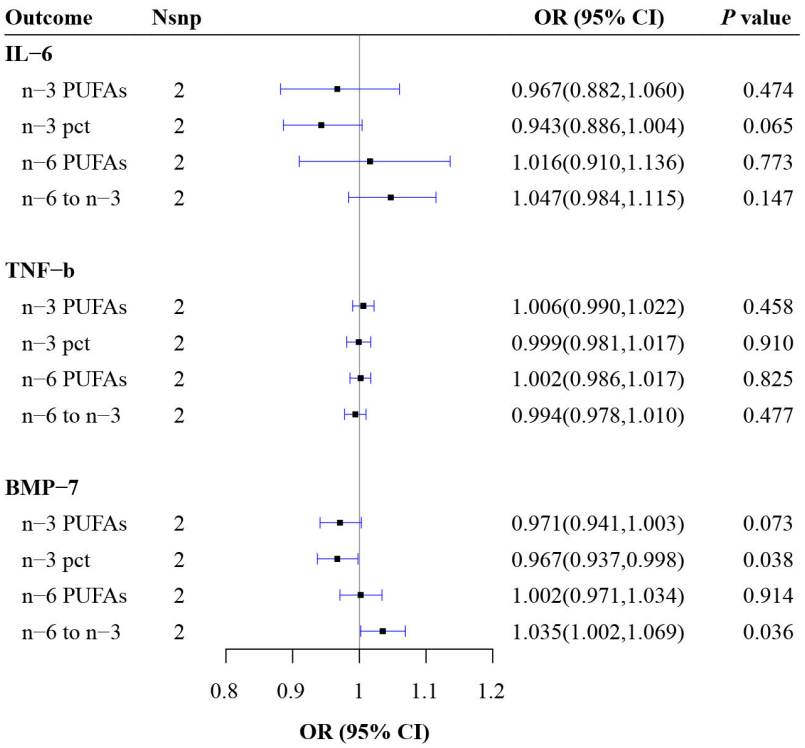

FIGURE 2
Mendelian randomization (MR) estimate results of possible confounding factors on polyunsaturated fatty acids (PUFAs). n-3 pct, the ratio of n-3 fatty acids to total fatty acids; n-6 to n-3, the ratio of n-6 PUFAs to n-3 PUFAs; IL, interleukin; TNF, tumor necrosis factor; BMP, bone morphogenetic protein.

on BMD was not affected by IL-6 and TNF-β, moreover IL-6 and TNF-β could not affect BMD by affecting PUFAs. As we all know, only in situations where the total effect, direct effect and indirect effect all act in the same direction, the exposure having indirect effect can be identified as a mediator (58). BMP-7 has a causal effect on-3 pct and n-6 to n-3, but has no causal effect on BMD, suggesting that BMP-7 can not affect BMD through PUFAs and is also not a mediator in the causal effect of PUFAs on BMD.

Recently, the relationship between PUFAs and osteoporosis has attracted a lot of attention. Some studies have found that intake of total PUFA, n-3 PUFAs, n-6 PUFAs can increase BMD and be beneficial to osteoporosis (30–34), which is mutually confirmed with our results.

These may attribute to the profitable role of PUFAs in bone formation, absorption, development and metabolism, and n-3 PUFAs can also regulate bone health by increasing osteoblast activity and decreasing osteoclast activity, promoting intestinal calcium absorption and mineral deposition during bone development (25). The intake of n-3 PUFAs has also been observed to be associated with increased bone regeneration, improved bone microstructure and strength (59–61). Osteoporosis increases the apoptosis, adipogenic differentiation, and levels of RANKL and sclerostin of bone marrow mesenchymal stem cells and osteoblasts (62). Bone mineral loss is the result of an imbalance between osteoblastic bone formation and osteoclast bone resorption. As an important n-3 PUFAs, DHA is a lipid component specific to the osteoblast membrane, which induces extensive lipid remodeling in mesenchymal stem cells, resulting in more stable membrane microdomains and thus enhanced osteogenic differentiation (63). It has been reported that dietary n-3 can reduce osteoclast formation and bone loss in ovariectomized mice (64). In rats, taking fish oil can also inhibit alveolar bone absorption and osteoclast activity (65).

In this study, n-6 to n-3 was observed to be negatively related to eBMD, which was consistent with previous studies. In previous study, a low n-6 to n-3 PUFAs ratio has also been proposed to be beneficial for the bone quality of rats (35). It has been widely documented that reducing the n-6 to n-3 PUFAs ratio can prevent bone mineral loss and prostaglandin(PG) E2 production in animal and *in vitro* cell culture experiments (66). Kelly et al. (67) also proposed that the high proportion of n-6 to n-3 PUFAs may be one of the important reasons for the increased risk of obesity and osteoporosis. According to the IVW results, the relationship between n-3 PUFAs, n-6 PUFAs, or the ratio of n-6 to n-3 PUFAs and fractures was not observed, while n-3 pct was negatively related to fractures,

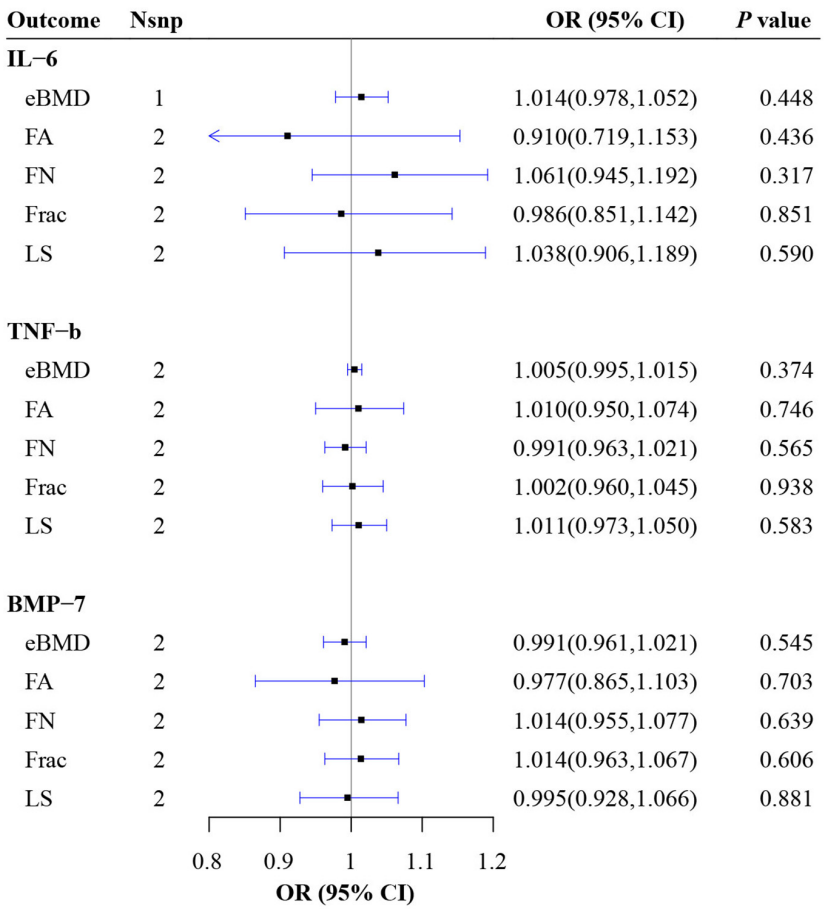

FIGURE 3
Mendelian randomization (MR) estimate results of possible confounding factors on BMD. BMD, bone mineral density; eBMD, estimated BMD; FA, forearm; FN, femoral neck; LS, lumbar; IL, interleukin; TNF, tumor necrosis factor; BMP, bone morphogenetic protein.

which suggests that it may not be the level of PUFAs who affected the fracture, but n-3 pct. These results, together with the n-6 to n-3 results, suggest that n-3 may influence BMD not only by itself or by its ratio to n-6, but also by its ratio to total fatty acids. The single item has little significance, but its proportion has important diagnostic value, which is not uncommon in clinical practice. For example, neutrophils to lymphocytes ratio (NLR) is an inflammatory index, which plays a role in the prognosis evaluation of sepsis and various diseases. A higher NLR may indicate more serious infection and worse prognosis (68, 69). The ratio of soluble fms-like tyrosine kinase 1 (sFlt-1) to placental growth factor (PlGF) can be used as a monitoring indicator of preeclampsia (PE), which is a kind of hypertensive disorder complicating pregnancy and seriously endangers the health of mothers and infants, with unpredictable outcomes (70).

Moreover, the IVW results showed that FA BMD and LS BMD, which were less affected by weight bearing, were more susceptible to n-3 and n-6 to n-3 than FN BMD which was more affected by weight bearing. Fat is digested, decomposed and metabolized into glycerin and PUFAs (71). Like vitamins and minerals, PUFAs are closely related to bone health through various ways. The distribution and accumulation of adipose tissue is extremely important for bone health. At the same time, adipose tissue secretions such as leptin, adiponectin, estrogen and osteocalcin can also act on bones. Multiple studies have shown that BMI is positively correlated with BMD, with lower BMI being thought to increase the risk of osteoporosis, while higher body weight (even obesity) protects bones (72–75). However, in recent years, this "obesity paradox" has been challenged like never before. Fat-rich bone marrow may be the cause of osteoporosis, especially in postmenopausal women (76). In 2011, the UK Fracture Liaison Service first reported that the incidence of obesity in postmenopausal women with fragility fractures can be as high as 27% (77). Two other studies were also pointed out that visceral fat was significantly associated with bone loss (78, 79). Excessive body fat, especially abdominal fat, produces inflammatory cytokines that stimulate increased bone marrow lipogenesis, increased bone resorption, decreased bone strength, and decreased bone mass (80).

Advantages and disadvantages

This study has several advantages. First of all, as a MR study, this study investigated the causal associations between different types of PUFAs and BMD in different parts, with detailed classification and comprehensive research. Secondly, we not only studied the level of PUFAs, but also the proportion of PUFAs, and all the results verified each other, and the conclusions were unified. Third, we used a strict Bonferroni correction, thus the conclusions are robust.

At the same time, this study also has some limitations. First, all GWAS data in this study were from European populations, and the representativeness of the results to the entire population remains to be determined. Second, the relationship between different doses of PUFAs and BMD has not been studied, and more detailed quantitative experiments are needed. In addition, the mediating effects of obesity, BMD and other factors still need further research.

Conclusion

This MR study establishes that n-3, n-6, n-3 pct, and n-6 to n-3 are causally associated with eBMD. In addition, n-3 not only associated with FA BMD and LS BMD through its own level and n-6 to n-3, but also associated with fracture through n-3 pct. Our findings provide new clue to further reveal the pathogenic role and therapeutic potential of PUFAs in osteoporosis.

Data availability statement

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

Author contributions

H-FP and D-GW conceived the present idea and were responsible for the design of the study. S-ST performed the statistical analysis and manuscript writing. PW and X-YW participated in acquisition of data and data analysis. K-JY, X-KY, and Z-XW participated in acquisition of data. All authors have read and approved the submitted version for publication.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.1014847/full#supplementary-material

References

1. Morris JA, Kemp JP, Youlten SE, Laurent L, Logan JG, Chai RC, et al. An atlas of genetic influences on osteoporosis in humans and mice. *Nat Genet.* (2019) 51:258–66. doi: 10.1038/s41588-018-0302-x

2. Liu J, Curtis EM, Cooper C, Harvey NC. State of the art in osteoporosis risk assessment and treatment. *J Endocrinol Invest.* (2019) 42:1149–64. doi: 10.1007/s40618-019-01041-6

3. Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. *Osteoporos Int.* (2006) 17:1726–33.

4. Reid IR. A broader strategy for osteoporosis interventions. *Nat Rev Endocrinol.* (2020) 16:333–9. doi: 10.1038/s41574-020-0339-7

5. Khosla S, Hofbauer LC. Osteoporosis treatment: recent developments and ongoing challenges. *Lancet Diabetes Endocrinol.* (2017) 5:898–907. doi: 10.1016/S2213-8587(17)30188-2

6. Compston JE, McClung MR, Leslie WD. Osteoporosis. *Lancet.* (2019) 393:364–76. doi: 10.1016/S0140-6736(18)32112-3

7. Hurley DL, Khosla S. Update on primary osteoporosis. *Mayo Clin Proc.* (1997) 72:943–9. doi: 10.4065/72.10.943

8. Weaver CM, Gordon CM, Janz KF, Kalkwarf HJ, Lappe JM, Lewis R, et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation

recommendations. *Osteoporos Int.* (2016) 27:1281–386. doi: 10.1007/s00198-015-3440-3

9. Vega OM, Abkenari S, Tong Z, Tedman A, Huerta-Yepez S. Omega-3 polyunsaturated fatty acids and lung cancer: nutrition or pharmacology? *Nutr Cancer.* (2021) 73:541–61. doi: 10.1080/01635581.2020.1761408

10. Nasir A, Bullo MMH, Ahmed Z, Imtiaz A, Yaqoob E, Jadoon M, et al. Nutrigenomics: epigenetics and cancer prevention: a comprehensive review. *Crit Rev Food Sci Nutr.* (2020) 60:1375–87. doi: 10.1080/10408398.2019.1571480

11. Ghamarzad Shishavan, N, Masoudi S, Mohamadkhani A, Sepanlou SG, Poustchi H, Hekmatdoost A, et al. The association of dietary intake and plasma fatty acid panel in pancreatic cancer patients: results from golestan cohort study. *Nutr Health.* (2022) 11:2601060221114712. doi: 10.1177/02601060221114712

12. Wawrzyniak P, Noureddine N, Wawrzyniak M, Lucchinetti E, Krämer SD, Rogler G, et al. Nutritional lipids and mucosal inflammation. *Mol Nutr Food Res.* (2021) 65:e1901269. doi: 10.1002/mnfr.201901269

13. Engelen M, Jonker R, Sulaiman H, Fisk HL, Calder PC, Deutz NEP. ω-3 polyunsaturated fatty acid supplementation improves postabsorptive and prandial protein metabolism in patients with chronic obstructive pulmonary disease: a randomized clinical trial. *Am J Clin Nutr.* (2022) 116:686–98. doi: 10.1093/ajcn/nqac138

14. Jiang H, Wang L, Wang D, Yan N, Li C, Wu M, et al. Omega-3 polyunsaturated fatty acid biomarkers and risk of type 2 diabetes, cardiovascular disease, cancer, and mortality. *Clin Nutr.* (2022) 41:1798–807. doi: 10.1016/j.clnu.2022.06.034

15. Wall R, Ross RP, Fitzgerald GF, Stanton C. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutr Rev.* (2010) 68:280–9. doi: 10.1111/j.1753-4887.2010.00287.x

16. Fritsche K. Fatty acids as modulators of the immune response. *Annu Rev Nutr.* (2006) 26:45–73. doi: 10.1146/annurev.nutr.25.050304.092610

17. Kang JX, Liu A. The role of the tissue omega-6/omega-3 fatty acid ratio in regulating tumor angiogenesis. *Cancer Metastasis Rev.* (2013) 32:201–10. doi: 10.1007/s10555-012-9401-9

18. Boston PF, Bennett A, Horrobin DF, Bennett CN. Ethyl-EPA in Alzheimer's disease–a pilot study. *Prostaglandins Leukot Essent Fatty Acids.* (2004) 71:341–6. doi: 10.1016/j.plefa.2004.07.001

19. Zhang W, Wang H, Zhang H, Leak RK, Shi Y, Hu X, et al. Dietary supplementation with omega-3 polyunsaturated fatty acids robustly promotes neurovascular restorative dynamics and improves neurological functions after stroke. *Exp Neurol.* (2015) 272:170–80. doi: 10.1016/j.expneurol.2015.03.005

20. Orchard TS, Pan X, Cheek F, Ing SW, Jackson RD. A systematic review of omega-3 fatty acids and osteoporosis. *Br J Nutr.* (2012) 107(Suppl. 2):S253–60. doi: 10.1017/S0007114512001638

21. Watkins BA, Shen CL, Allen KG, Seifert MF. Dietary (n-3) and (n-6) polyunsaturates and acetylsalicylic acid alter ex vivo PGE2 biosynthesis, tissue IGF-I levels, and bone morphometry in chicks. *J Bone Miner Res.* (1996) 11:1321–32. doi: 10.1002/jbmr.5650110917

22. Watkins BA, Lippman HE, Le Bouteiller L, Li Y, Seifert MF. Bioactive fatty acids: role in bone biology and bone cell function. *Prog Lipid Res.* (2001) 40:125–48. doi: 10.1016/S0163-7827(00)00016-3

23. Sakaguchi K, Morita I, Murota S. Eicosapentaenoic acid inhibits bone loss due to ovariectomy in rats. *Prostaglandins Leukot Essent Fatty Acids.* (1994) 50:81–4. doi: 10.1016/0952-3278(94)90151-1

24. Li Y, Seifert MF, Ney DM, Grahn M, Grant AL, Allen KG, et al. Dietary conjugated linoleic acids alter serum IGF-I and IGF binding protein concentrations and reduce bone formation in rats fed (n-6) or (n-3) fatty acids. *J Bone Miner Res.* (1999) 14:1153–62. doi: 10.1359/jbmr.1999.14.7.1153

25. Lau BY, Cohen DJ, Ward WE, Ma DW. Investigating the role of polyunsaturated fatty acids in bone development using animal models. *Molecules.* (2013) 18:14203–27. doi: 10.3390/molecules181114203

26. Hutchins-Wiese HL, Picho K, Watkins BA, Li Y, Tannenbaum S, Claffey K, et al. High-dose eicosapentaenoic acid and docosahexaenoic acid supplementation reduces bone resorption in postmenopausal breast cancer survivors on aromatase inhibitors: a pilot study. *Nutr Cancer.* (2014) 66:68–76. doi: 10.1080/01635581.2014.847964

27. Weiss LA, Barrett-Connor E, von Mühlen D. Ratio of n-6 to n-3 fatty acids and bone mineral density in older adults: the rancho bernardo study. *Am J Clin Nutr.* (2005) 81:934–8. doi: 10.1093/ajcn/81.4.934

28. Griel AE, Kris-Etherton PM, Hilpert KF, Zhao G, West SG, Corwin RL. An increase in dietary n-3 fatty acids decreases a marker of bone resorption in humans. *Nutr J.* (2007) 6:2. doi: 10.1186/1475-2891-6-2

29. Claassen N, Coetzer H, Steinmann CM, Kruger MC. The effect of different n-6/n-3 essential fatty acid ratios on calcium balance and bone in rats. *Prostaglandins Leukot Essent Fatty Acids.* (1995) 53:13–9. doi: 10.1016/0952-3278(95)90078-0

30. Coetzee M, Haag M, Kruger MC. Effects of arachidonic acid, docosahexaenoic acid, prostaglandin E(2) and parathyroid hormone on osteoprotegerin and RANKL secretion by MC3T3-E1 osteoblast-like cells. *J Nutr Biochem.* (2007) 18:54–63. doi: 10.1016/j.jnutbio.2006.03.002

31. Högström M, Nordström P, Nordström A. N-3 Fatty acids are positively associated with peak bone mineral density and bone accrual in healthy men: the NO2 Study. *Am J Clin Nutr.* (2007) 85:803–7. doi: 10.1093/ajcn/85.3.803

32. Martínez-Ramírez MJ, Palma S, Martínez-González MA, Delgado-Martínez AD, de la Fuente C, Delgado-Rodríguez M. Dietary fat intake and the risk of osteoporotic fractures in the elderly. *Eur J Clin Nutr.* (2007) 61:1114–20. doi: 10.1038/sj.ejcn.1602624

33. Orchard TS, Cauley JA, Frank GC, Neuhouser ML, Robinson JG, Snetselaar L, et al. Fatty acid consumption and risk of fracture in the Women's Health Initiative. *Am J Clin Nutr.* (2010) 92:1452–60. doi: 10.3945/ajcn.2010.29955

34. Dou Y, Wang Y, Chen Z, Yu X, Ma D. Effect of n-3 polyunsaturated fatty acid on bone health: a systematic review and meta-analysis of randomized controlled trials. *Food Sci Nutr.* (2022) 10:145–54. doi: 10.1002/fsn3.2655

35. Yang L, Yang C, Chu C, Wan M, Xu D, Pan D, et al. Beneficial effects of monounsaturated fatty acid-rich blended oils with an appropriate polyunsaturated/saturated fatty acid ratio and a low n-6/n-3 fatty acid ratio on the health of rats. *J Sci Food Agric.* (2022) 102:7172–85. doi: 10.1002/jsfa.12083

36. Macdonald HM, New SA, Golden MH, Campbell MK, Reid DM. Nutritional associations with bone loss during the menopausal transition: evidence of a beneficial effect of calcium, alcohol, and fruit and vegetable nutrients and of a detrimental effect of fatty acids. *Am J Clin Nutr.* (2004) 79:155–65. doi: 10.1093/ajcn/79.1.155

37. Richards JB, Zheng HF, Spector TD. Genetics of osteoporosis from genome-wide association studies: advances and challenges. *Nat Rev Genet.* (2012) 13:576–88. doi: 10.1038/nrg3228

38. Trajanoska K, Rivadeneira F. The genetic architecture of osteoporosis and fracture risk. *Bone.* (2019) 126:2–10. doi: 10.1016/j.bone.2019.04.005

39. Yang TL, Shen H, Liu A, Dong SS, Zhang L, Deng FY, et al. A road map for understanding molecular and genetic determinants of osteoporosis. *Nat Rev Endocrinol.* (2020) 16:91–103. doi: 10.1038/s41574-019-0282-7

40. Song YQ, Hu SD, Lin X, Meng XH, Wang X, Zhang YH, et al. Identification of PDXDC1 as a novel pleiotropic susceptibility locus shared between lumbar spine bone mineral density and birth weight. *J Mol Med.* (2022) 100:723–34. doi: 10.1007/s00109-021-02165-0

41. 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

42. 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

43. Sekula P, Del Greco MF, Pattaro C, Köttgen A. Mendelian randomization as an approach to assess causality using observational data. *J Am Soc Nephrol.* (2016) 27:3253–65. doi: 10.1681/ASN.2016010098

44. Krela-Kaźmierczak I, Szymczak-Tomczak A, Łykowska-Szuber L, Wysocka E, Michalak M, Stawczyk-Eder K, et al. Interleukin 6, osteoprotegerin, sRANKL and bone metabolism in inflammatory bowel diseases. *Adv Clin Exp Med.* (2018) 27:449–53. doi: 10.17219/acem/75675

45. Kim H, Chun S, Ku SY, Suh CS, Choi YM, Kim JG. Association between polymorphisms in tumor necrosis factor (TNF) and TNF receptor genes and circulating TNF, soluble TNF receptor levels, and bone mineral density in postmenopausal Korean women. *Menopause.* (2009) 16:1014–20. doi: 10.1097/gme.0b013e3181a039c8

46. Chen Y, Ma B, Wang X, Zha X, Sheng C, Yang P, et al. Potential functions of the BMP family in bone, obesity, and glucose metabolism. *J Diabetes Res.* (2021) 2021:6707464. doi: 10.1155/2021/6707464

47. Borges MC, Haycock PC, Zheng J, Hemani G, Holmes MV, Davey Smith G, et al. Role of circulating polyunsaturated fatty acids on cardiovascular diseases risk: analysis using Mendelian randomization and fatty acid genetic association data from over 114,000 UK Biobank participants. *BMC Med.* (2022) 20:210. doi: 10.1186/s12916-022-02399-w

48. Loh PR, Kichaev G, Gazal S, Schoech AP, Price AL. Mixed-model association for biobank-scale datasets. *Nat Genet.* (2018) 50:906–8. doi: 10.1038/s41588-018-0144-6

49. Zheng HF, Forgetta V, Hsu YH, Estrada K, Rosello-Diez A, Leo PJ, et al. Whole-genome sequencing identifies EN1 as a determinant of bone density and fracture. *Nature.* (2015) 526:112–7.

50. Ahola-Olli AV, Wurtz P, Havulinna AS, Aalto K, Pitkänen N, Lehtimäki T, et al. Genome-wide association study identifies 27 loci influencing concentrations of circulating cytokines and growth factors. *Am J Hum Genet.* (2017) 100:40–50. doi: 10.1016/j.ajhg.2016.11.007

51. Sun BB, Maranville JC, Peters JE, Stacey D, Staley JR, Blackshaw J, et al. Genomic atlas of the human plasma proteome. *Nature.* (2018) 558:73–9. doi: 10.1038/s41586-018-0175-2

52. 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

53. 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

54. 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

55. Greco MF, Minelli C, Sheehan NA, Thompson JR. Detecting pleiotropy in Mendelian randomisation studies with summary data and a continuous outcome. *Stat Med.* (2015) 34:2926–40. doi: 10.1002/sim.6522

56. Sedgwick P. Multiple hypothesis testing and Bonferroni's correction. *BMJ.* (2014) 349:g6284. doi: 10.1136/bmj.g6284

57. Curtin F, Schulz P. Multiple correlations and Bonferroni's correction. *Biol Psychiatry.* (1998) 44:775–7. doi: 10.1016/S0006-3223(98)00043-2

58. Carter AR, Sanderson E, Hammerton G, Richmond RC, Davey Smith G, Heron J, et al. Mendelian randomisation for mediation analysis: current methods and challenges for implementation. *Eur J Epidemiol.* (2021) 36:465–78. doi: 10.1007/s10654-021-00757-1

59. Martyniak K, Wei F, Ballesteros A, Meckmongkol T, Calder A, Gilbertson T, et al. Do polyunsaturated fatty acids protect against bone loss in our aging and osteoporotic population? *Bone.* (2021) 143:115736. doi: 10.1016/j.bone.2020.115736

60. Sharma T, Mandal CC. Omega-3 fatty acids in pathological calcification and bone health. *J Food Biochem.* (2020) 44:e13333. doi: 10.1111/jfbc.13333

61. Jørgensen HS, Eide IA, Jenssen T, Åsberg A, Bollerslev J, Godang K, et al. Marine n-3 polyunsaturated fatty acids and bone mineral density in kidney transplant recipients: a randomized, placebo-controlled trial. *Nutrients.* (2021) 13:2361. doi: 10.3390/nu13072361

62. Benisch P, Schilling T, Klein-Hitpass L, Frey SP, Seefried L, Raaijmakers N, et al. The transcriptional profile of mesenchymal stem cell populations in primary osteoporosis is distinct and shows overexpression of osteogenic inhibitors. *PLoS One.* (2012) 7:e45142. doi: 10.1371/journal.pone.0045142

63. Levental KR, Surma MA, Skinkle AD, Lorent JH, Zhou Y, Klose C, et al. ω-3 polyunsaturated fatty acids direct differentiation of the membrane phenotype in mesenchymal stem cells to potentiate osteogenesis. *Sci Adv.* (2017) 3:eaao1193. doi: 10.1126/sciadv.aao1193

64. Sun D, Krishnan A, Zaman K, Lawrence R, Bhattacharya A, Fernandes G. Dietary n-3 fatty acids decrease osteoclastogenesis and loss of bone mass in ovariectomized mice. *J Bone Miner Res.* (2003) 18:1206–16. doi: 10.1359/jbmr.2003.18.7.1206

65. Iwami-Morimoto Y, Yamaguchi K, Tanne K. Influence of dietary n-3 polyunsaturated fatty acid on experimental tooth movement in rats. *Angle Orthod.* (1999) 69:365–71.

66. Albertazzi P, Coupland K. Polyunsaturated fatty acids. Is there a role in postmenopausal osteoporosis prevention? *Maturitas.* (2002) 42:13–22. doi: 10.1016/s0378-5122(02)00022-1

67. Kelly OJ, Gilman JC, Kim Y, Ilich JZ. Long-chain polyunsaturated fatty acids may mutually benefit both obesity and osteoporosis. *Nutr Res.* (2013) 33:521–33. doi: 10.1016/j.nutres.2013.04.012

68. Wang K, Wang X, Du J, Liu C, Jiang Y, Zhang H, et al. Relationship between changes in the course of COVID-19 and ratio of neutrophils-to-lymphocytes and related parameters in patients with severe vs. common disease. *Epidemiol Infect.* (2021) 149:e81. doi: 10.1017/S0950268821000674

69. Templeton AJ, McNamara MG, Šeruga B, Aneja P, Ocaña A, Leibowitz-Amit R, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J Natl Cancer Inst.* (2014) 106:dju124. doi: 10.1093/jnci/dju124

70. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M, et al. Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia. *N Engl J Med.* (2016) 374:13–22. doi: 10.1056/NEJMoa1414838

71. de Carvalho C, Caramujo MJ. The various roles of fatty acids. *Molecules.* (2018) 23:2583. doi: 10.3390/molecules23102583

72. Poet JL, Galinier Pujol A, Tonolli Serabian I, Conte Devolx B, Roux H. Lumbar bone mineral density in anorexia nervosa. *Clin Rheumatol.* (1993) 12:236–9. doi: 10.1007/BF02231534

73. Asomaning K, Bertone-Johnson ER, Nasca PC, Hooven F, Pekow PS. The association between body mass index and osteoporosis in patients referred for a bone mineral density examination. *J Womens Health.* (2006) 15:1028–34. doi: 10.1089/jwh.2006.15.1028

74. Sukumar D, Schlussel Y, Riedt CS, Gordon C, Stahl T, Shapses SA. Obesity alters cortical and trabecular bone density and geometry in women. *Osteoporos Int.* (2011) 22:635–45. doi: 10.1007/s00198-010-1305-3

75. Turcotte AF, O'Connor S, Morin SN, Gibbs JC, Willie BM, Jean S, et al. Association between obesity and risk of fracture, bone mineral density and bone quality in adults: a systematic review and meta-analysis. *PLoS One.* (2021) 16:e0252487. doi: 10.1371/journal.pone.0252487

76. Menagh PJ, Turner RT, Jump DB, Wong CP, Lowry MB, Yakar S, et al. Growth hormone regulates the balance between bone formation and bone marrow adiposity. *J Bone Miner Res.* (2010) 25:757–68. doi: 10.1359/jbmr.091015

77. Compston J. Obesity and fractures in postmenopausal women. *Curr Opin Rheumatol.* (2015) 27:414–9. doi: 10.1097/BOR.0000000000000182

78. Jankowska EA, Rogucka E, Medraś M. Are general obesity and visceral adiposity in men linked to reduced bone mineral content resulting from normal ageing? A population-based study. *Andrologia.* (2001) 33:384–9. doi: 10.1046/j.1439-0272.2001.00469.x

79. Tseng YH, Huang KC, Liu ML, Shu WT, Sheu WH. Association between metabolic syndrome (MS) and bone mineral loss: a cross-sectional study in Puli Township in Taiwan. *Arch Gerontol Geriatr.* (2009) 49(Suppl. 2):S37–40. doi: 10.1016/S0167-4943(09)70011-1

80. Gkastaris K, Goulis DG, Potoupnis M, Anastasilakis AD, Kapetanos G. Obesity, osteoporosis and bone metabolism. *J Musculoskelet Neuronal Interact.* (2020) 20:372–81.

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Dietary intake of fructose increases purine *de novo* synthesis: A crucial mechanism for hyperuricemia

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Background: Fructose consumption is a potential risk factor for hyperuricemia because uric acid (UA) is a byproduct of fructose metabolism caused by the rapid consumption of adenosine triphosphate and accumulation of adenosine monophosphate (AMP) and other purine nucleotides. Additionally, a clinical experiment with four gout patients demonstrated that intravenous infusion of fructose increased the purine *de novo* synthesis rate, which implied fructose-induced hyperuricemia might be related to purine nucleotide synthesis. Moreover, the mechanistic (mammalian) target of rapamycin (mTOR) is a key protein both involved in fructose metabolism and purine *de novo* synthesis. The present study was conducted to elucidate how fructose influences mTOR and purine *de novo* synthesis in a hepatic cell line and livers of mice.

Materials and methods: RNA-sequencing in NCTC 1469 cells treated with 0- and 25-mM fructose for 24 h and metabolomics analysis on the livers of mice fed with 0- and 30-g/kg fructose for 2 weeks were assessed. Gene and protein expression of phosphoribosyl pyrophosphate synthase (PRPSAP1), Glutamine PRPP aminotransferase (PPAT), adenyl succinate lyase (ADSL), adenyl succinate synthetase isozyme-1 (Adss1), inosine-5′-monophosphate dehydrogenase (IMPDH), and guanine monophosphate synthetase (GMPS) was measured. The location of PRPSAP1 and PPAT in the liver was assessed by an immunofluorescence assay.

Results: Metabolite profiling showed that the level of AMP, adenine, adenosine, hypoxanthine, and guanine was increased significantly. RNA-sequencing showed that gene expression of phosphoribosyl pyrophosphate synthase (PRPS2), phosphoribosyl glycinamide formyl transferase (GART), AICAR transformylase (ATIC), ADSL, Adss1, and IMPDH were raised, and gene expression of adenosine monophosphate deaminase 3 (AMPD3), adenosine deaminase (ADA), 5′,3′-nucleotidase, cytosolic (NT5C), and

xanthine oxidoreductase (XOR) was also increased significantly. Fructose increased the gene expression, protein expression, and fluorescence intensity of PRPSAP1 and PPAT in mice livers by increasing mTOR expression. Fructose increased the expression and activity of XOR, decreased the expression of uricase, and increased the serum level of UA.

Conclusion: This study demonstrated that the increased purine *de novo* synthesis may be a crucial mechanism for fructose-induced hyperuricemia.

KEYWORDS

purine *de novo* synthesis, fructose, hyperuricemia, metabolomic analysis, RNA-seq analysis

Introduction

Hyperuricemia is an excessively increased uric acid (UA) level in blood. The upper limit of normal is 6.8 mg/dL (> 7 mg/dL can lead to symptoms). A high blood level of UA is associated with gout, renal dysfunction, diabetes mellitus, hypertension, and atherosclerosis (1–3). In general, hyperuricemia is caused by urate overproduction, typically due to consumption of a purine-rich diet (e.g., beer, meat, seafood), high cell turnover, or urate excretion disorders (4). However, fructose and alcohol can also increase the serum UA level, and have gained more and more attention as a potential risk factor for hyperuricemia (especially fructose) (5).

Fructose is a primary sweetener used in various industrial food products, and is also present in fresh fruits and corn syrup (6). Fructose intake has increased markedly over recent years, and consumption has exceeded physiological needs (7). In liver, fructose can be metabolized more readily than glucose because of a specific enzyme (fructokinase), which catalyzes the conversion of fructose to fructose-1-phosphate using adenosine triphosphate (ATP) as a phosphate donor (8). Fructokinase is not regulated and phosphorylates fructose as rapidly as it can, leading to depletion of intracellular ATP to generate adenosine monophosphate (AMP). AMP accumulation stimulates AMP deaminase, which results in degradation of purine nucleotide (PNs) to UA, and increases the serum UA level (9, 10): this is a well-known mechanism of fructose-induced hyperuricemia. If so, more rapid depletion of ATP induced by fructose intake only provides the precursors (PNs) for UA synthesis, which would be terminated due to fructose depletion. However, consumption of 8% fructose drinking for 8 weeks has been shown to induce stable and persistent hyperuricemia in animal models, suggesting a long-time chronic effect of fructose upon hyperuricemia (4).

Fructose intake also stimulates UA biosynthesis from amino acid precursors (1, 11). Furthermore, a clinical experiment with four gout patients demonstrated that intravenous infusion of fructose increased the rate of purine *de novo* synthesis, which implied that the mechanism of fructose-induced hyperuricemia might be related to purine nucleotide synthesis (12).

Mammalian target of rapamycin (mTOR) is a key protein involved in the metabolic effect of fructose (13). Moreover, mTOR has been reported to promote expression of the genes related to the biosynthesis of phosphoribosyl pyrophosphate (the substrate for the first reaction in the purine *de novo* synthesis) (14). Thus, understanding the regulatory effect of mTOR on purine *de novo* synthesis may provide new ideas for the rational intake of fructose as well as the prevention and treatment of metabolic diseases induced by excessive intake of fructose.

We postulated that the mechanism of fructose-induced hyperuricemia was related to the purine nucleotide synthesis and purine degradation in the liver. The present study investigated the effect of giving fructose (20, 30, and 40 g/kg, p.o.) for 2 weeks to induce a high serum-level of UA. Then, RNA-sequencing analysis and metabolomics analysis were used to ascertain the effect of fructose upon the *de novo* synthesis of purine on hepatocytes and the liver of mice. The results showed that fructose intake increased the activity of phosphoribosyl pyrophosphate synthase (PRPSAP1) and glutamine PRPP aminotransferase (PPAT) to accelerate the *de novo* synthesis of

Abbreviations: UA, uric acid; ATP, adenosine triphosphate; AMP, adenosine monophosphate; PRPSAP, phosphoribosyl pyrophosphate synthase; PPAT, Glutamine PRPP aminotransferase; IMP, inosine 5′-monophosphate; XOR, xanthine oxidoreductase; FBS, fetal bovine serum; VIP, variable importance in projection; PCA, principal components analysis; PLS-DA, partial least squares discriminant analysis; AdSS1, adenyl succinate synthetase isozyme 1; ADSL, adenyl succinate lyase; GMPS, guanine monophosphate synthetase; IMPDH, inosine-5′-monophosphate dehydrogenase; AICAR, 5-Aminoimidazole-4-carboxamide1-β-D-ribofuranoside; GART, phosphoribosyl glycinamide formyl transferase; ATIC, AICAR Tfase; AMPD3, adenosine monophosphate deaminase 3; ADA, adenosine deaminase; NT5C, 5′, 3′-nucleotidase, cytosolic; GMP, guanosine 5′-monophosphate; R5P, ribose-5-phosphate; APRT, adenine phosphoribosyl transferase; HPRT, hypoxanthine-guanine phosphoribosyl transferase; mTOR, mammalian target of rapamycin.

purine to inosine 5'-monophosphate (IMP), and drove IMP to synthesize AMP, thereby accelerating its catabolism to UA by increasing the activity of xanthine oxidoreductase (XOR). This study demonstrated increased purine *de novo* synthesis as a crucial mechanism for fructose-induced hyperuricemia.

Materials and methods

Cell culture and fructose treatment

NCTC 1469 cells (catalog number: CL-0407; Procell Life Science and Technology, Wuhan, China) were cultured in glucose (4.5 g/L)-containing Dulbecco's modified Eagle's medium with 10% (v/v) fetal bovine serum, and 1% penicillin/streptomycin solution (100 units/mL penicillin and 100 μg/mL streptomycin) in an atmosphere of 5% CO_2 and 95% humidified air at 37°C.

For fructose (purity = 99%; F0127; MilliporeSigma, Burlington, MA, USA) treatment, cells were seeded on six-well plates (6×10^5 cells per well). After reaching 80% confluence, cells were treated with medium containing fructose (0 and 25 mM) for 24 h. Then, cells were harvested for messenger (m)RNA extraction and RNA-sequencing. For the protein expression determination by western blotting, cells were seeded on six-well plates (6×10^5 cells per well) and treated with medium containing fructose (0, 1.25, 2.5, 5.0, 10, and 20 mM) for 24 h. For the mechanism detection, cells were treated with medium containing fructose (10 and 20 mM) in the presence or absence of Rapamycin at 200 nM.

RNA-sequencing

Cells in six-well plates were harvested after 24 h of treatment for extraction of total RNA using TRIzol® Reagent (254712; Ambion Life Technologies, Carlsbad, CA, USA). mRNA was enriched with poly-A selection, and 50 base pair paired-end RNA-sequencing was completed on the BGISEQ platform at Shenzhen SunV Biotech (Shenzhen, China). Raw reads were filtered using SOAP and SOAPnuke (15), Clean reads were mapped to the transcriptome of the RefSeq database using Bowtie2 (16). Gene expression was counted by RSEM (17) and normalized as transcripts per kilobase of exon model per million mapped reads. DESeq2 was employed to evaluate differential expression. Differentially expressed genes (DEGs) were identified by Benjamini Hochberg-adjusted *P* value (< 0.05) (18). Analyses of enrichment of function and metabolic pathways were undertaken based on Gene Ontology (GO[1]) and Kyoto Encyclopedia of Genes and Genomes Kyoto Encyclopedia of Genes and Genomes (KEGG[2]) databases using Database for Annotation, Visualization and Integrated Discovery[3] DAVID (19).

Animals and treatments

This study protocol was approved by the Animal Care and Use Committee of Guangdong Medical University (Zhanjiang. China).

Male C57BL/6 wild-type mice (6 weeks, 20–25 g, specific pathogen-free) were obtained from the Experimental Animal Center of Guangdong Medical University [production license: SCXK (Yue) 2018-0008] and allowed to acclimatize to the animal facility environment for 1 week before experimentation. Mice were maintained in a specific pathogen-free, temperature (24 ± 2°C)-and humidity (50 ± 5%)-controlled environment, with a standard 12 h light/dark cycle. A total of twenty-four mice were divided randomly into 4 treatment groups, including one control (mice were given distilled water) and three fructose groups (20, 30, 40 g/kg bodyweight). Each of these groups consisted of 6 mice. Fructose was prepared as a solution at 1, 1.5, and 2 g/mL concentrations. The solution was given *via* oral route twice daily at the volume of 0.5 mL (0.25 mL each time at 9:00 a.m. and 3:00 p.m.). Mice feed and water were provided *ad libitum* throughout the experiment. Additionally, the samples were collected 48 h after experiment cessation.

Uric acid level in blood

Approximately 1 mL of blood samples were collected from the angular vein of mice under anesthesia. Serum samples were obtained by centrifugation at 1,790 × *g* for 10 min at room temperature and stored in microtubes at −80°C. The UA level was determined using a commercially available kit purchased from Solarbio Life Science (Beijing, China), following the manufacturer's instructions.

Liver collection and metabolomics analysis

Each mouse's liver was harvested and washed with ice-cold, sterile 0.9% saline solution to remove blood contamination. Then, it was placed in a cryogenic vial. Liquid chromatography-mass spectrometry (LC-MS) was conducted at BioNovoGene (Suzhou, China). In brief, ∼20 mg of liver from both the control group and the 30 g/kg fructose group, respectively, was collected

1 http://geneontology.org/

2 http://www.genome.jp/

3 https://david.ncifcrf.gov/

and placed in a centrifuge tube containing magnetic beads and ground to a homogenate using 1 mL of homogenate medium (80% methanol). Then, the homogenate was placed in liquid nitrogen for 30 s and ice for 5 min (three times). The extract was obtained by centrifugation at 12,000 × *g* for 10 min at 4°C and subjected to LC-MS with an ACQUITY UPLC® HSS T3 column (1.8 μm, 2.1 × 150 mm, Ethylene Bridged Hybrid, Waters, Milford, MA, USA) (20). Samples were randomized, data acquisitions were done in one batch to eliminate system errors, and the metabolites were identified based on their molecular weight, mass spectra, and retention time. Original LC-MS data were processed and analyzed using Bio-deep Online software[4] with optimized settings. Annotation of metabolite using LC-MS data was done with the Compound Discover 3.3 (Thermo Scientific, Waltham, MA, USA) and referenced to the mzCloud database.[5] Discrimination metabolites between two classes of samples was done using a statistically significant threshold of Variable Importance in Projection (VIP) value (VIP > 1) and validated further by Student's *t*-test analysis ($P \leq 0.05$). Principal components analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were carried out with SIMCA-P software. Heatmaps were constructed using Euclidian distances and complete linkage grouping with the "heatmap" package in R language (R Institute for Statistical Computing, Vienna, Austria). Relative quantitative values of metabolites were normalized, transformed, and clustered through hierarchical clustering. Metabolite correction was assessed using Pearson's correction coefficient and constructed Cytoscape 3.2.7.[6] To further identify alternative metabolic pathways, differential metabolites were subjected to grouping and enrichment of metabolic pathways using MetaboAnalyst 4.0[7] and the KEGG database. Univariate analysis of Variance (ANOVA) was used to determine the significance of differences in contents between different groups.

Quantitative real-time PCR

mRNA expression of PRPSAP1, PPAT, and XOR was determined with the SYBR Green II Real-Time PCR kit (Thermo Fisher Scientific). Total RNA was extracted from NCTC 1469 cells and mouse livers using the RNAiso Plus kit (TaKaRa Biotechnology, Dalian, China) following manufacturer instruction. RNA integrity was checked *via* agarose gel electrophoresis with ethidium bromide. The concentration and purity of RNA were determined using an automatic microplate reader (Synergy H4, BioTek, Tokyo, Japan) at an OD 260/280 reading ratio of 1.8–2.1. After determination of the RNA concentration, total RNA (1 μg) was reverse-transcribed into complementary DNA (cDNA) using the Prime Script RT Reagent Kit (TaKaRa Biotechnology). Real-time PCR was carried out with an ABI StepOnePlus real-time PCR system (Applied Biosystems, Grand Island, Foster City, CA, USA) using the SYBR Premix Ex Taq II Reagent Kit (TaKaRa Biotechnology) under manufacturer instructions but with modification (21). The volume of the reaction system was 20 μL [10 μL of SYBR Premix Ex Taq Π, 0.4 μL of PCR forward primer (10 μM), 0.4 μL of PCR reverse primer (10 μM), 2 μL of cDNA, and 7.2 μL of RNase-free H_2O]. The primer sequences of target genes and reference genes were obtained from GenBank and are shown in **Table 1**. Relative level of mRNA expression was calculated using the $2^{-\Delta\Delta CT}$ method after normalization against the reference gene (glyceraldehyde 3-phosphate dehydrogenase; GAPDH) (22).

Western blotting

Primary antibodies against PRPSAP1 (catalog number: bs-19409R) and PPAT (bs-6359R) were purchased from Beijing Biosynthesis Biotechnology (Beijing, China). Antibody against phosphorylated (P) P-mTOR (Ser 2448, 5536S) was obtained from Cell Signaling Technology (Danvers, MA, USA). Antibodies against adenyl succinate synthetase isozyme 1 (AdSS1; sc-166401), adenyl succinate lyase (ADSL; sc-365623), guanine monophosphate synthetase (GMPS; sc-376163), and inosine-5'-monophosphate dehydrogenase (IMPDH; sc-166551) were sourced from Santa Cruz Biotechnology (Houston, TX, USA).

Liver samples were frozen in liquid nitrogen, ground into powder in a mortar, and lysed by addition of RIPA lysis buffer (Beyotime Biotechnology, Jiangsu, China). The NCTC 1469 cell lysates were prepared by harvesting cells in RIPA lysis buffer. The supernatant was centrifuged at 12,000 × *g* for 15 min at 4°C after samples had been lysed fully. Supernatants containing total proteins were quantified with a Bicinchoninic Acid Protein Kit (Meilun Biotechnology, Dalian, China); 50 μg of whole protein samples were resolved with 8, 10, or 15% polyacrylamide gel (depending on the molecular size of the proteins to be analyzed). Thereafter, immunoblotting was done by transferring resolved proteins onto polyvinylidene difluoride (PVDF) membranes in trans-buffer at 100 V for 1 or 2 h, depending on the molecular size of the protein. PVDF membranes were blocked with 5% skimmed milk or 5% bovine serum albumin (for phosphorylated protein) in TBST buffer [Tris–HCl (20 mM), pH 7.5; sodium chloride (150 mM); 0.05% Tween 20] for 2 h, washed thrice with TBST buffer for 5-min each time, and incubated overnight with primary antibodies (1:1000 dilution) at 4°C. Afterward, PVDF membranes were washed thrice with TBST buffer for 5-min each time and incubated with anti-rabbit (or anti-mouse) secondary antibodies

4 http://www.biodeep.cn/
5 http://www.mzcloud.org/
6 https://cytoscape.org/
7 http://www.metaboanalyst.ca/

TABLE 1 Primer sequences used for real-time RT-qPCR.

Primer	Forward 5′—3′	Reverse 5′—3′
PRPSAP1	ACTTATCCCAGAAAATCGCTGAC	CCACACCCACTTTGAACAATGTA
PPAT	GCGAGGAATGTGGTGTGTTTG	TTTAGGCACTGCACTCCCATC
XOR	CCGCCTTCAGAACAAGATCG	CCTTCCACAGTTGTCACAGC
HPRT	CTTCCTCCTCAGACCGCTTTT	AGCAAGTCTTTCAGTCCTGTCC
APRT	CCCGGGATTGACGTGAGTTT	GAGGGGCGAGATATCCCTGA
UOX	CAGATGAGAAACGGACCTCCC	GCCGTAGGGATTGTCGAGAG
GAPDH	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG

conjugated to horseradish peroxidase (1:2000 dilution) for 2 h at room temperature. Blots were developed in the dark using an electrochemiluminescence detection kit. Developed blots were subjected to densitometric analysis using ImageJ 1.45 (US National Institutes of Health, Bethesda, MD, USA) and expression normalized to that of GAPDH (D16H11; Cell Signaling Technology) as the internal control.

Immunohistochemistry

Paraffin-embedded liver tissues were used to measure expression of PRPSAP1 and PPAT. After deparaffinization, slides were heated in an autoclave with sodium citrate (for antigen repair), followed by 1% hydrogen peroxide (to abolish endogenous peroxidase activity), and blocked with 2% goat serum. Then, slides were incubated with primary antibodies PRPSAP1 and PPAT at 1:200 dilution overnight at 4°C, followed by incubation with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobin G (1:500 dilution; Beyotime Biotechnology) for 2 h at room temperature. Slides were counterstained with 4',6-diamidino-2-phenylindole for 5 min to stain nuclei. Coverslips were mounted on slides followed by visualization under a confocal laser scanning microscope (LSM 800, Carl Zeiss, Wetzlar Germany) using laser set at 405 and 488 nm.

Hepatic xanthine oxidoreductase activity

Approximately 0.5 g of liver tissue was used to prepare homogenates. Minced tissue was homogenized in an ice-cold phosphate buffer solution (*w/v*, 1:9) using a glass homogenate tube. The tube centrifuged at 8,000 × *g* for 10 min at 4°C. The supernatant was collected and stored at −80°C until further analyses. XOR activity was estimated using a kit (Solarbio Life Science), as reported previously (23). The protein concentration in tissue homogenates was determined using the Bicinchoninic Acid Protein Kit (24).

Statistical analysis

Quantitative data are presented as the mean ± SD of three independent experiments. Results were evaluated *via* one-way ANOVA followed by Tukey's multiple-comparison test using Prism 6 (GraphPad, San Diego, CA, USA). $P < 0.05$ was considered significant.

Results

Fructose consumption increases hepatic purine synthesis

Twelve samples (six samples from the control group and six samples from the 30-g/kg fructose group) were analyzed. Untargeted metabolomics analysis was done by LC-MS; 12 valid samples were identified and measured. Plots for PLS-DA score between the control group and fructose group were clearly different (**Figure 1A**), which suggested that metabolic biomarkers could be selected. Analysis of enrichment of metabolic pathway was conducted: enrichment was observed in "purine metabolism," "tryptophan metabolism," "phenylalanine, tyrosine, and tryptophan biosynthesis," and "biosynthesis of amino acids" (**Figure 1B**). Twenty-two metabolites related to purine synthesis were identified as having differential expression in the fructose group compared with the control group (**Figure 1C**). Fifteen metabolites were upregulated (including AMP, adenine, and adenosine) and seven metabolites were downregulated (including fructose-6-phosphate, glycylglycine, and L-glutamic acid). Correlation analysis of these metabolites showed no correlation between fructose-1-phosphate and the PNs (AMP, adenine, and adenosine) (**Figure 1D**). However, a negative correlation between glycylglycine and AMP, and a positive correlation between 5-Aminoimidazole-4-carboxamide1-β-D-ribofuranoside (AICAR) and adenosine, were found (**Figure 1D**). We explored potential diagnostic biomarkers for purine synthesis. The significantly increased metabolites of AMP, adenine, adenosine, AICAR, hypoxanthine, and guanine indicated upregulation of purine *de novo* synthesis (**Figure 1E**). Based on metabolites profiling and model

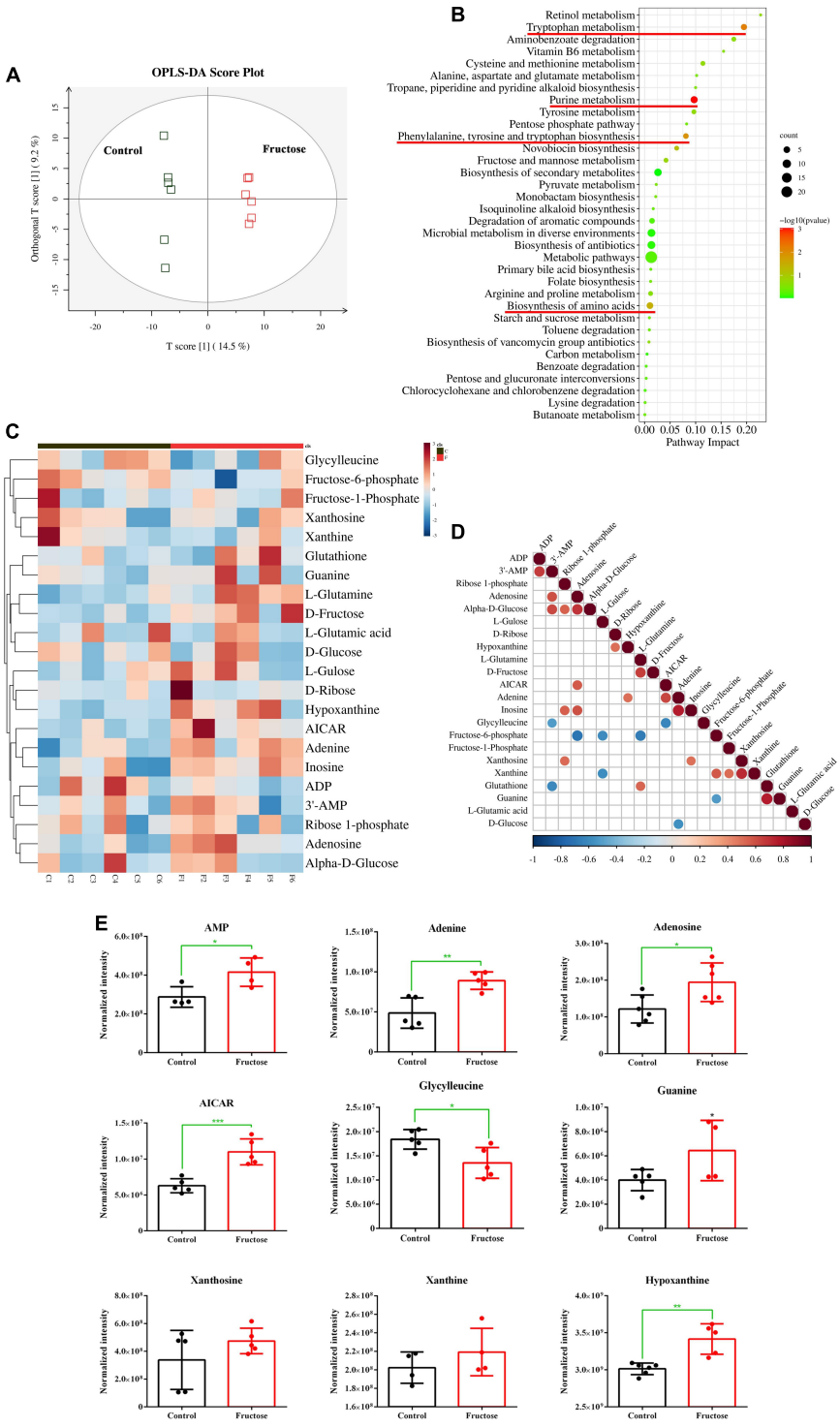

FIGURE 1

Fructose consumption increases hepatic purine synthesis **(A)** partial least squares discriminant analysis (PLS-DA) score plots for the samples remarkably separated the fructose group from the control groups. **(B)** Pathway enrichment analysis showed the pathways most significantly altered in the fructose compared with the control group (the significantly altered metabolic pathways were marked with the red line, $P < 0.05$). **(C)** Heat map of the differentially expressed metabolites related to *de novo* purine synthesis between fructose group and control group. **(D)** Thermogram of metabolite association analysis, the orange-red points represent a positive correlation between two metabolites ($P < 0.05$), and the blue-green points represent a negative correlation between two metabolites ($P < 0.05$). **(E)** The normalized intensity of 9 metabolites of purine synthesis and decomposition. The metabolite profiling was performed in mouse liver. Data are expressed as mean ± SD ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (*represents 30 g/kg fructose groups compared with the control group).

evaluation, fructose intake appeared to upregulate the purine synthesis.

Differentially expressed genes and differentially expressed pathways between fructose and the control group determined by transcriptomic analysis

Six samples (three samples in each group) of NCTC1469 cells were used for transcriptomic analysis. Transcriptomic data were downloaded from BGI with the corresponding BGISEQ platform. A total of 6,459 genes were selected as DEGs between fructose group and control group (**Figure 2A**). The top-25 enriched pathways according to the KEGG database included "purine metabolism," "carbon metabolism," and "pyrimidine metabolism" (**Figure 2B**): these pathways are associated with purine *de novo* synthesis. To further identify the role of fructose on purine synthesis, thirteen genes (purine synthesis) and eight genes (purine catabolism) were chosen for analysis. Expression of the genes involved in purine *de novo* synthesis [e.g., PRPS, phosphoribosyl glycinamide formyl transferase (GART), AICAR transfomylase (ATIC), ADSL, Adss1, and IMPDH (**Figure 2C**)] and purine catabolism [e.g., adenosine monophosphate deaminase 3 (AMPD3), adenosine deaminase (ADA), 5', 3'-nucleotidase, cytosolic (NT5C), and XOR] was upregulated significantly (**Figure 2D**). RT-qPCR showed that fructose (25 mM) consumption increased the gene expression of PRPSAP1 (**Figure 2E**) and PPAT (**Figure 2F**) significantly, but had no impact on the gene expression of hypoxanthine-guanine phosphoribosyl transferase (HPRT) (**Figure 2G**) or adenine phosphoribosyl transferase (APRT) (**Figure 2H**). These results suggested that fructose accelerated the *de novo* synthesis of purine, while promoting the catabolism of purine into UA.

Fructose increases hepatic expression of phosphoribosyl pyrophosphate synthase and Glutamine PP aminotransferase

Liquid chromatography-mass spectrometry (LC-MS) and RNA-sequencing data suggested that fructose could accelerate the purine *de novo* synthesis. To confirm this finding, expression of PRPSAP1 and PPAT (**Figure 3A**) was determined in the livers of fructose-fed mice. Gene expression of PRPSAP1 and PPAT was upregulated significantly by treatment with fructose (20, 40 g/kg) ($P < 0.05$, **Figures 3B,C**). With respect to hepatic protein expression, treatment with fructose (30, 40 g/kg) stimulated PRPSAP1 expression significantly ($P < 0.05$, **Figures 3D,E**), and PPAT expression in all three fructose groups was higher than that in the control group ($P < 0.05$) (**Figures 3D,F**). With an increase in the fructose concentration, the fluorescence intensity of PRPSAP1 and PPAT in liver slices increased gradually (**Figure 3G**). Taken together, these results demonstrated the promoting effect of fructose on the purine *de novo* synthesis.

Fructose accelerates purine *de novo* synthesis by increasing Mammalian target of rapamycin expression

An *in vitro* experiment was conducted to investigate how fructose treatment increases expression of PRPSAP1 and PPAT. Fructose treatment increased protein expression of P-mTOR, PRPSAP1, and PPAT in a dose-dependent manner, and significant effects were observed at concentrations of 5, 10, and 20 mM ($P < 0.05$) (**Figures 4A,B**). The presence of rapamycin inhibited P-mTOR expression significantly, and protein expression of PRPSAP1 and PPAT was reduced compared with that in the control group ($P < 0.05$) (**Figures 4C,D**). Fructose treatment did not affect protein expression of PRPSAP1 or PPAT in the presence of rapamycin ($P > 0.05$) (**Figures 4C,D**). Fructose treatment increased the UA level in cell supernatants significantly at concentrations of 10 and 20 mM ($P < 0.05$) (**Figure 4E**), but the UA level was not affected by fructose in the presence of rapamycin ($P > 0.05$) (**Figure 4E**). Taken together, these results suggested that fructose accelerated purine *de novo* synthesis by increasing P-mTOR expression.

Fructose promoted inosine 5'-monophosphate to adenosine monophosphate conversion and the catabolism of adenosine monophosphate to uric acid

Fructose appeared to increase IMP synthesis by promoting *de novo* synthesis of purine, but the effect of fructose on the synthesis of IMP to AMP, or guanine nucleotide (GMP) (**Figure 5A**) is not known. All three concentrations of fructose enhanced the expression of ADSS ($P < 0.05$) (**Figures 5B,C**), but did not affect the expression of ADSL. However, fructose decreased expression of IMPDH and GMPS in the liver ($P < 0.05$) (**Figures 5B,C**). These findings suggested that fructose drives IMP to synthesize AMP, not GMP. The final product of AMP decomposition is UA, and XOR is the crucial enzyme controlling this process (**Figure 5A**). Fructose increased the gene expression (**Figure 5D**) and the activity (**Figure 5E**) of XOR in the liver as compared with that in the control group ($P < 0.05$). Otherwise, fructose intake decreased the gene

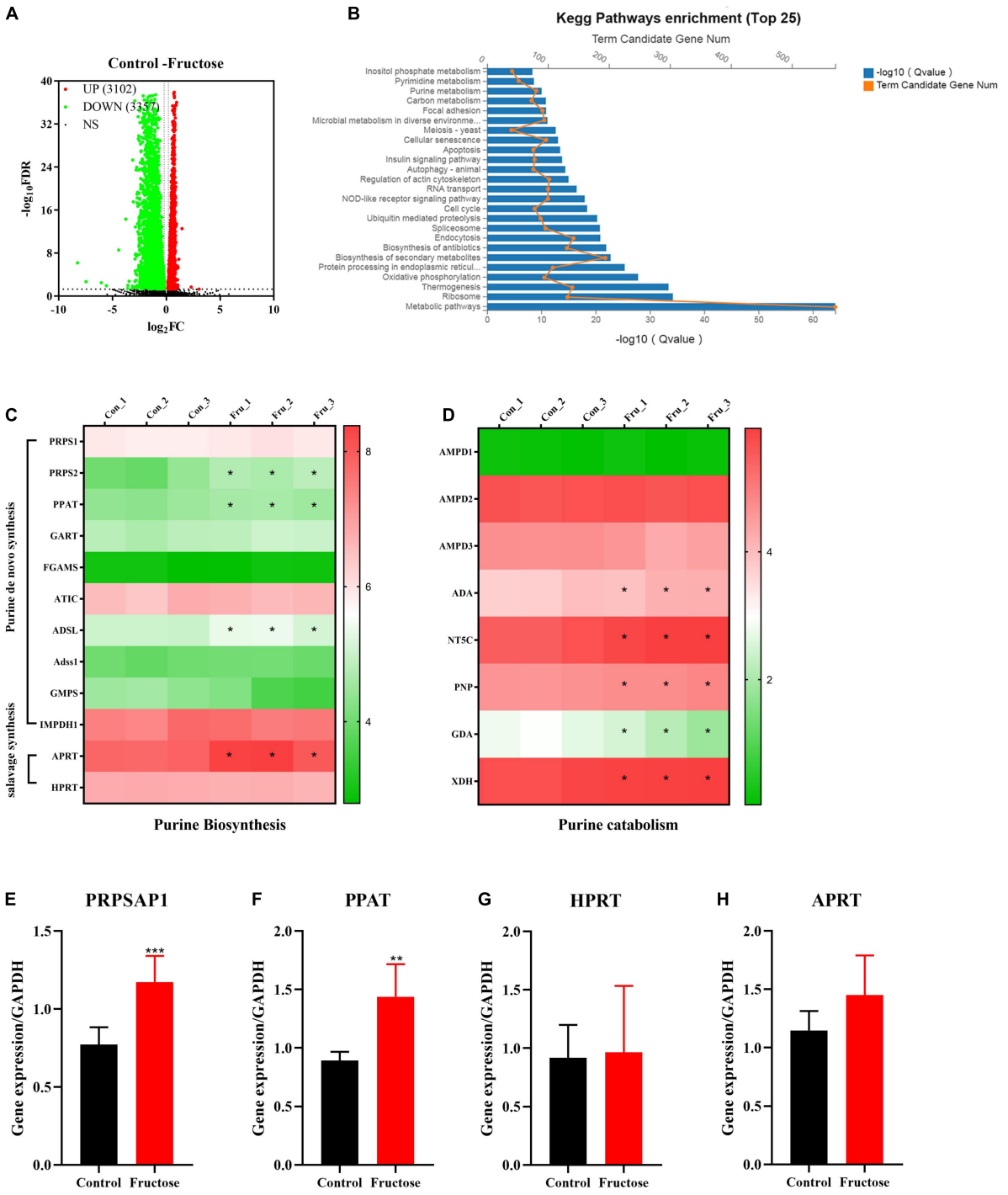

FIGURE 2
Differentially expressed genes (DEGs) and differentially expressed pathways between fructose and the control group determined by transcriptomic analysis. **(A)** Volcano plot showed the DEGs were screened between the fructose group and the control group in accordance with the corresponding BGISEQ platform. The threshold was set to $\log_2|\ FC| > 1$ and $P < 0.05$. **(B)** Pathway enrichment analysis showed the top 25 metabolism pathways most significantly altered in the fructose compared with the control group. **(C,D)** Heat map revealed gene expression differences related to purine biosynthesis and catabolism (*$P < 0.05$ represents the fructose group compared with the control group). **(E–G)** And **(H)** qPCR validation results of phosphoribosyl pyrophosphate synthase (PRPSAP1), Glutamine PRPP aminotransferase (PPAT), hypoxanthine-guanine phosphoribosyl transferase (HPRT), and adenine phosphoribosyl transferase (APRT). The RNA sequential analysis was performed in the NCTC1469 cells. Data are expressed as mean ± SD ($n = 3$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (*represents 25 mM fructose groups compared with the control group).

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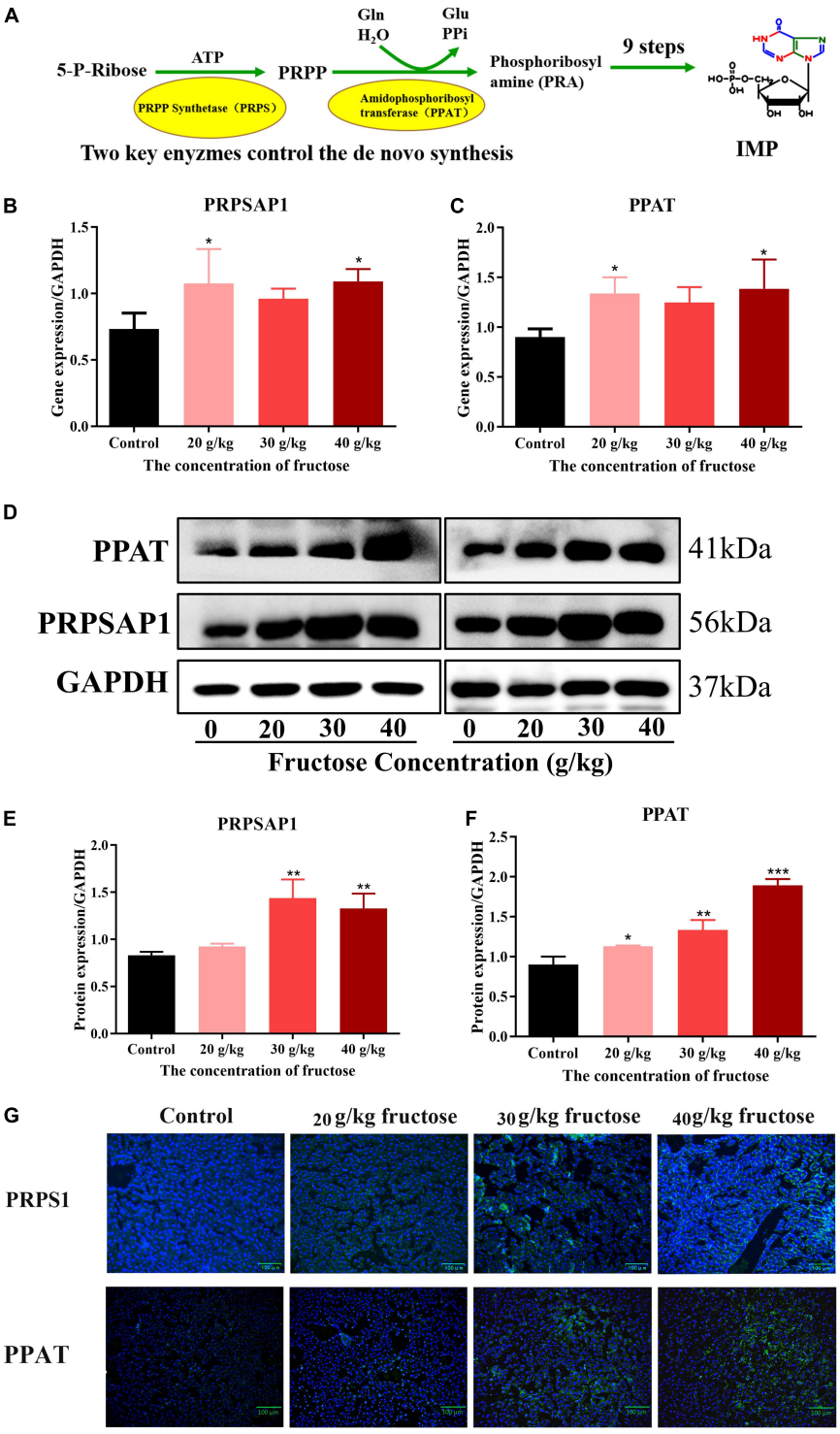

FIGURE 3
Fructose increases hepatic expression of phosphoribosyl pyrophosphate synthase (PRPSAP1) and Glutamine PRPP aminotransferase (PPAT). **(A)** The key enzymes control the *de novo* synthesis. **(B,C)** Gene expression of PRPSAP1, PPAT in reference to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). **(D)** Immunoblot analysis for PRPSAP1 and PPAT in reference to GAPDH. **(E,F)** The relative protein expression of PRPSAP1 and PPAT in regard to GAPDH. **(G)** Immunofluorescence analysis for PRPSAP1 and PPAT in the liver. Scale bars: 100 μm. Data are expressed as mean ± SD (n = 6). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (*represents the fructose group compared with the control group).

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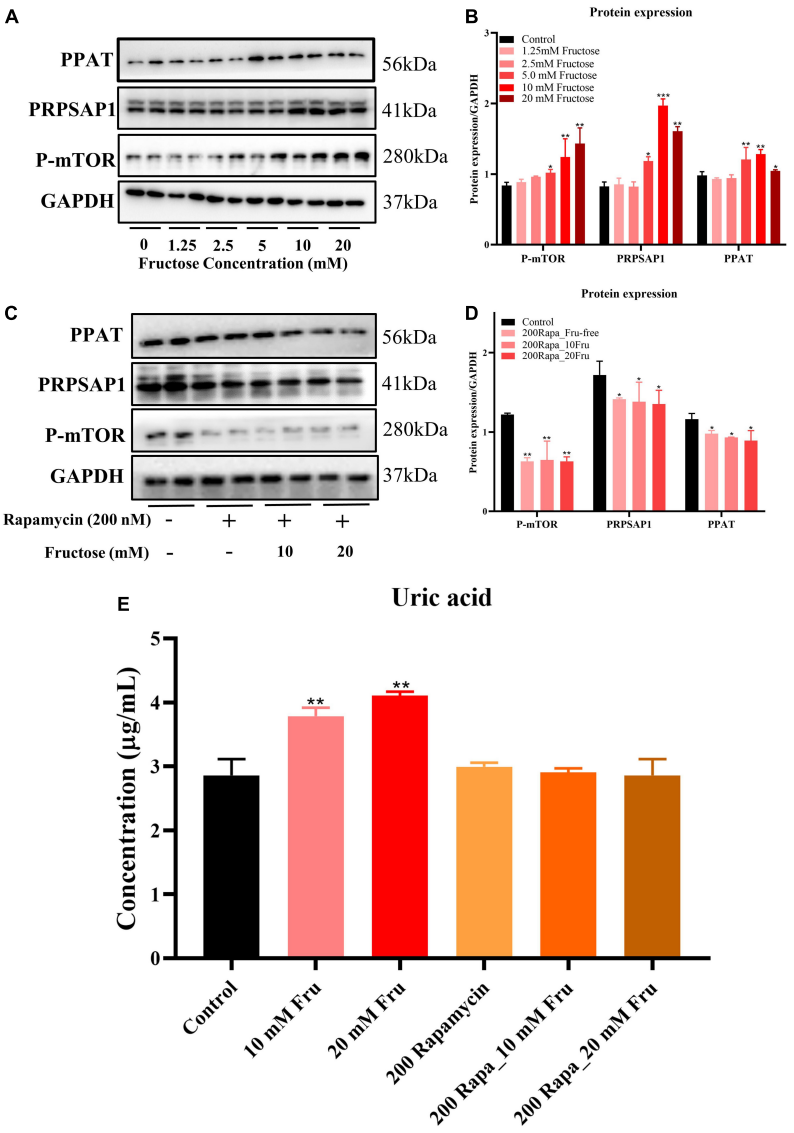

FIGURE 4
Fructose accelerates purine *de novo* synthesis by increasing mammalian target of rapamycin (mTOR) expression. **(A,B)** Immunoblot analysis for P-mTOR, phosphoribosyl pyrophosphate synthase (PRPSAP1), and Glutamine PRPP aminotransferase (PPAT) in fructose-treated NCTC1469 cells. **(C,D)** Immunoblot analysis for P-mTOR, PRPSAP1, and PPAT in the presence of rapamycin. **(E)** The uric acid changes in the supernatant of fructose-treated NCTC 1469 cells. Data are expressed as mean ± SD (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 (*represents the fructose group compared with the control group).

expression (Figure 6A) and protein expression (Figures 6B,C) of uricase significantly. Taken together, these data suggested that fructose consumption increased the serum UA level (P < 0.05) (Figure 5F) and that fructose drives IMP to synthesize AMP and accelerates its catabolism to UA by increasing XOR activity.

Discussion

Conventional thinking dictates that rapid metabolism of fructose can induce ATP consumption to generate AMP, and that AMP accumulation stimulates AMP deaminase, thereby resulting in PNs degradation to UA (9, 10). Furthermore, the possible mechanism related to purine metabolism is: (i) increased synthesis of (*de novo* and salvage) of PNs, (ii) increased breakdown of preformed PNs and PN derivatives, (iii) a combination of (i) and (ii).

Metabolite profiling in mice livers showed an increase in levels of PNs and their derivatives, (AMP, adenine, adenosine, guanine, xanthosine, xanthine, hypoxanthine) (Figure 1E) induced by fructose intake, which suggested that increased breakdown of PNs and their derivatives was operative

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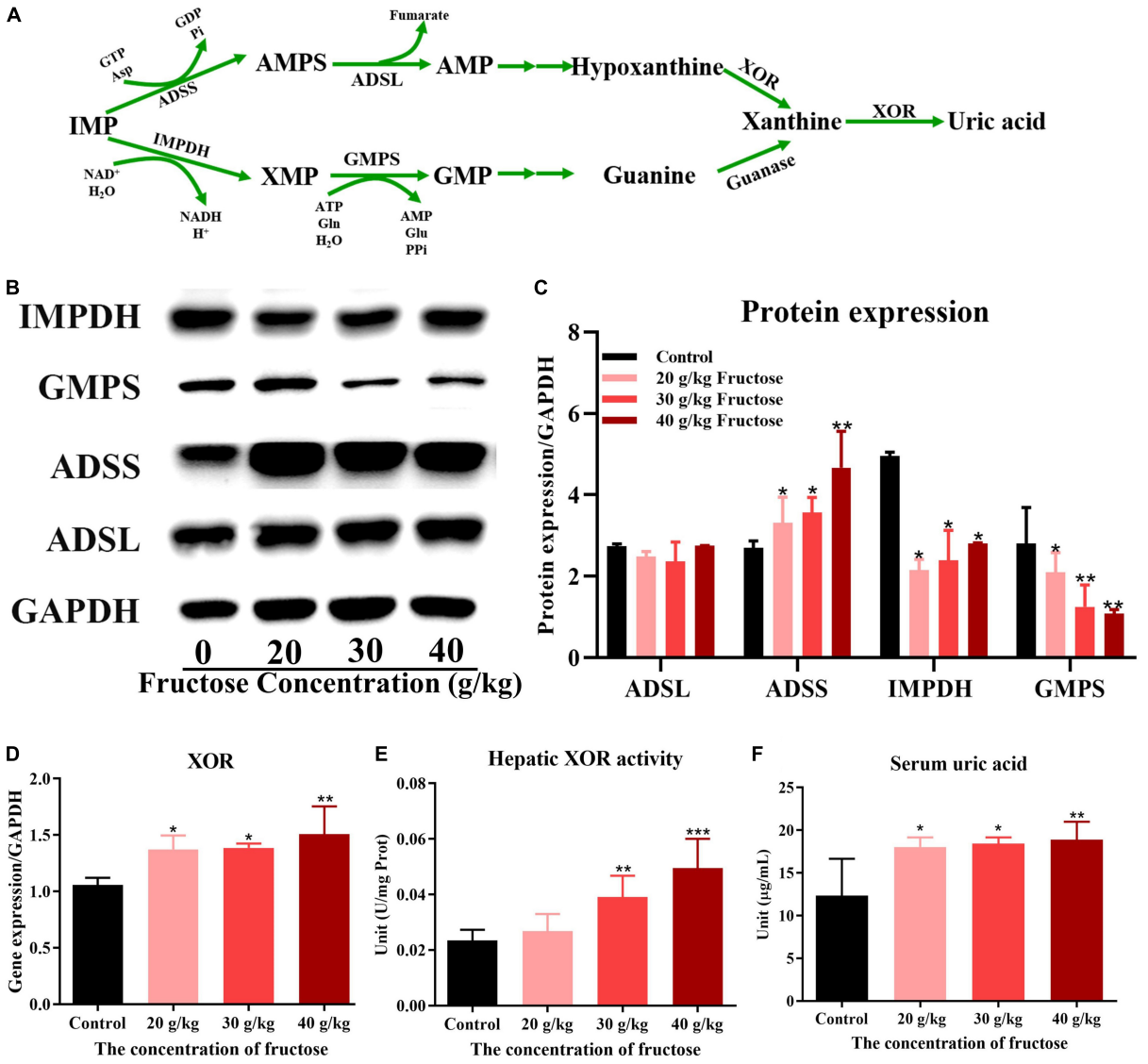

FIGURE 5
Fructose promoted inosine 5′-monophosphate (IMP) to adenosine monophosphate (AMP) conversion and the catabolism of AMP to uric acid (UA). **(A)** The processes of synthesis and catabolism of AMP and guanine monophosphate (GMP). **(B)** Immunoblot analysis for adenyl succinate synthetase isozyme (ADSS), adenyl succinate lyase (ADSL), guanine monophosphate synthetase (GMPS), inosine-5′-monophosphate dehydrogenase 1 (IMPDH) in reference to GAPDH. **(C)** The relative protein expression of adenyl succinate lyase (ADSL), adenyl succinate synthetase (ADSS), inosine-5′-monophosphate dehydrogenase (IMPDH), and guanine monophosphate synthetase (GMPS) in regard to lyceraldehyde 3-phosphate dehydrogenase (GAPDH). **(D)** The gene expression of hepatic xanthine oxidoreductase (XOR) in reference to GAPDH. **(E)** The activity of xanthine oxidoreductase in the liver. **(F)** Changes in serum uric acid level in fructose-fed mice. Data are expressed as mean ± SD (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 (*represents the fructose group compared with the control group).

after fructose administration. Fructose intake can result in stimulation of *de novo* synthesis of purine, so the change in ribose-5-phosphate (R5P) level must be determined first (25). However, the metabolic results failed to show an increase in R5P level in the mice livers during fructose administration. In addition, metabolic intermediates can also serve to control metabolism. AICAR is a critical signaling intermediate within purine *de novo* synthesis pathway, and its expression is increased significantly by fructose intake. Increased AICAR level can promote the ATIC activity, which accelerates the last final two steps in the pathway to convert AICAR to IMP (14).

The non-essential amino acid glycine contributes carbon and nitrogen atoms at positions of C4, C5, and N6 in purine ring at the beginning of purine synthesis (25). Our metabolic data showed a significant decrease in glycine level induced by fructose consumption, so glycine was used rapidly as a supplier of carbon atoms. Combination of metabolic data suggested that fructose administration accelerated purine synthesis as well as

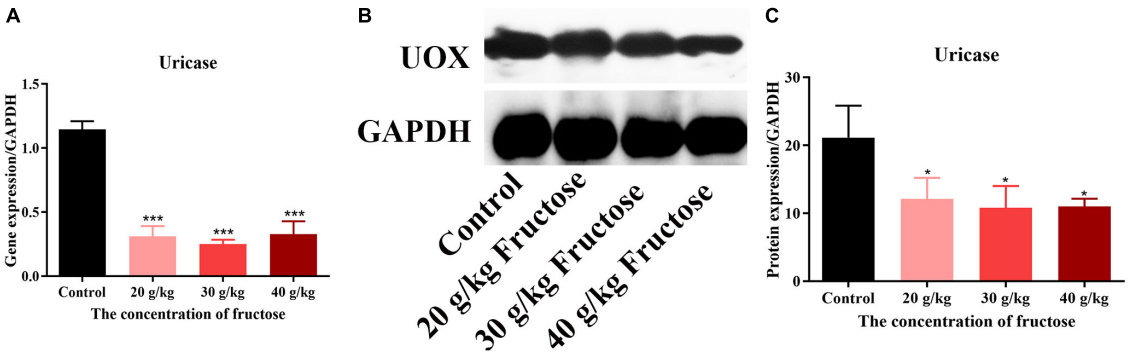

FIGURE 6
Fructose inhibited the gene and protein expression of uricase. **(A)** The gene expression of uricase (UOX) in liver. **(B)** Immunoblot analysis of UOX. **(C)** The relative protein expression of UOX in reference to GAPDH. Data are expressed as mean ± SD (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 (*represents the fructose group compared with the control group).

the breakdown of preformed PNs and their derivatives in the livers of fructose-fed mice.

Under normal physiological conditions, the cellular "purine pool" is derived primarily from recycling of degraded bases *via* the salvage pathway. Nevertheless, the pathway related to purine *de novo* synthesis will be upregulated to meet demand if cells require higher levels of purine (26). Purine *de novo* synthesis occurs in 10 steps, generating IMP from PRPP by sequential orchestration of six enzymes. Transcriptomics analysis in the present study showed that gene expression of PRPS2, PPAT, GART, and ATIC was upregulated upon fructose intake, which can accelerate the pathway for purine *de novo* synthesis. For salvage synthesis, gene expression of APRT and HPRT was not affected significantly by fructose intake. This finding suggested that fructose administration increased purine *de novo* synthesis instead of salvage synthesis in the livers of mice. The significantly increased gene expression of AMPD3, ADA, NT5C, and XOR suggested increased purine catabolism to PNs and their derivatives upon fructose treatment.

To confirm the results of LC-MS and RNA-sequencing, PRPSAP1 and PPAT (rate-limiting enzymes from the first reaction in the pathway purine *de novo* synthesis) were chosen to identify gene and protein expression. Fructose administration increased the gene and protein expression of PRPSAP1 and PPAT in the livers of mice, and the increased fluorescence intensity of PRPSAP1 and PPAT confirmed this finding. Inconsistent mRNA and protein expression of PRPSAP1 and PPAT were observed, but this may have been due to differences between post-translational regulation and modification. With regard to how fructose accelerates purine *de novo* synthesis, mTOR has been shown to promote expression of the genes associated with PRPP synthesis and to regulate co-localization between purinosomes and mitochondria (14). We showed that fructose increased protein expression of P-mTOR, PRPSAP1, and PPAT significantly. In the presence of rapamycin (mTOR inhibitor), fructose did not affect the protein expression of PRPSAP1 or PPAT or the UA level in cell supernatants. The previous study also suggested that inhibition of mTOR expression could reduce fractional co-localization between purinosomes and mitochondria, thereby leading to a marked decrease in the metabolic flux through the purine *de novo* synthesis (14). Thus, fructose appears to accelerate the purine *de novo* synthesis by increasing mTOR expression. Besides, UA is a risk factor that can activate mTOR by inducing phosphorylation of protein kinase B (AKT) and proline-rich AKT substrate-40 (27). We suggest that the increased UA level caused by fructose intake may have a positive feedback effect on mTOR activation and purine *de novo* synthesis.

Furthermore, the effect of fructose intake on synthesis of AMP or GMP from IMP is not known. We found that fructose increased protein expression of Adss1 but decreased expression of IMPDH and GMPS significantly. These findings suggested that fructose intake mainly drives IMP to synthesize AMP, not GMP, which could be attributed to rapid depletion of ATP by fructose consumption (28). Fructose intake increased purine *de novo* synthesis as well as the PNs and their derivatives (adenine, xanthine, hypoxanthine). The increased gene expression and activity of XOR in the liver expedited the PNs degradation to UA. Normally, increased UA level can stimulate the expression of uricase, but reduced gene and protein expression of uricase caused by fructose intake was observed (**Figure 6**) in the present study. This phenomenon may be explained by uricase blockage increasing the metabolic rate of fructose and enhancing the ability of fructose to generate fat (29). Taken together, these results implied that fructose could inhibit the oxidation of UA to allantoic acid and increase the serum UA level.

Conclusion

We demonstrated that fructose promoted purine *de novo* synthesis to generate IMP and drive conversion of IMP to AMP

to maintain the rapid depletion of ATP. Fructose increased the breakdown of preformed PNs and their derivatives (adenine, xanthine, and hypoxanthine), accelerated their degradation to UA, and increased the serum UA level. This work revealed that increased purine *de novo* synthesis may be a crucial mechanism in fructose-induced hyperuricemia.

Data availability statement

The data presented in the study are deposited here: https://doi.org/10.6084/m9.figshare.21701060.v1.

Ethics statement

This animal study was reviewed and approved by Animal Care and Use Committee of Guangdong Medical University (Zhanjiang, China).

Author contributions

YZ participated in the literature search, study design, surgery operation, data collection, data analysis, data interpretation, and manuscript writing. CZ carried out the data analysis and provided a critical revision of the manuscript. HS, WM, XC, YL, YZ, HZ, and YD conceived the study and participated in its design and coordination. All authors read and approved the final manuscript as the submitted version.

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Conflict of interest

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

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References

1. Hannou SA, Haslam DE, McKeown NM, Herman MA. Gout. *Nat Rev Dis Primers.* (2019) 5:69. doi: 10.1038/s41572-019-0115-y

2. Mortada I. Hyperuricemia, type 2 diabetes mellitus, and hypertension: an emerging association. *Curr Hypertens Rep.* (2017) 19:69. doi: 10.1007/s11906-017-0770-x

3. Kanbay M, Jensen T, Solak Y, Le M, Roncal-Jimenez C, Rivard C, et al. Uric acid in metabolic syndrome: from an innocent bystander to a central player. *Eur J Intern Med.* (2016) 29:3–8. doi: 10.1016/j.ejim.2015.11.026

4. Lu J, Dalbeth N, Yin H, Li C, Merriman TR, Wei WH. Mouse models for human hyperuricaemia: a critical review. *Nat Rev Rheumatol.* (2019) 15:413–26. doi: 10.1038/s41584-019-0222-x

5. Nakagawa T, Lanaspa MA, Johnson RJ. The effects of fruit consumption in patients with hyperuricaemia or gout. *Rheumatology.* (2019) 58:1133–41. doi: 10.1093/rheumatology/kez128

6. Hannou SA, Haslam DE, McKeown NM, Herman MA. Fructose metabolism and metabolic disease. *J Clin Invest.* (2018) 128:545–55. doi: 10.1172/JCI96702

7. Tappy L. Fructose-containing caloric sweeteners as a cause of obesity and metabolic disorders. *J Exp Biol.* (2018) 221(Pt Suppl. 1):Jeb164202. doi: 10.1242/jeb.164202

8. Liu L, Li T, Liao Y, Wang Y, Gao Y, Hu H, et al. Triose kinase controls the lipogenic potential of fructose and dietary tolerance. *Cell Metab.* (2020) 32:605–18. doi: 10.1016/j.cmet.2020.07.018

9. Choi JWJ, Ford ES, Gao X, Choi HK. Sugar-sweetened soft drinks, diet soft drinks, and serum uric acid level: the third national health and nutrition examination survey. *Arthritis Rheum.* (2008) 59:109–16. doi: 10.1002/art.23245

10. Faller J, Fox IH. Ethanol-induced hyperuricemia: evidence for increased urate production by activation of adenine nucleotide turnover. *N Engl J Med.* (1982) 307:1598–602. doi: 10.1056/NEJM198212233072602

11. Le MT, Frye RF, Rivard CJ, Cheng J, McFann KK, Segal MS, et al. Effects of high-fructose corn syrup and sucrose on the pharmacokinetics of fructose and acute metabolic and hemodynamic responses in healthy subjects. *Metabolism.* (2012) 61:641–51. doi: 10.1016/j.metabol.2011.09.013

12. Raivio KO, Becker A, Meyer LJ, Greene ML, Nuki G, Seegmiller JE. Stimulation of human purine synthesis de novo by fructose infusion. *Metabolism.* (1975) 24:861–9. doi: 10.1016/0026-0495(75)90133-x

13. Sangüesa G, Roglans N, Baena M, Velázquez AM, Laguna JC, Alegret M. mTOR is a key protein involved in the metabolic effects of simple sugars. *Int J Mol Sci.* (2019) 20:1117. doi: 10.3390/ijms20051117

14. Pedley AM, Benkovic SJ. A new view into the regulation of purine metabolism: the purinosome. *Trends Biochem Sci.* (2017) 42:141–54. doi: 10.1016/j.tibs.2016.09.009

15. Li R, Yu C, Li Y, Lam TW, Yiu SM, Kristiansen K, et al. SOAP2: an improved ultrafast tool for short read alignment. *Bioinformatics.* (2009) 25:1966–7. doi: 10.1093/bioinformatics/btp336

16. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods.* (2012) 9:357–9. doi: 10.1038/nmeth.1923

17. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinform.* (2011) 12:323. doi: 10.1186/1471-2105-12-323

18. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* (2014) 15:550. doi: 10.1186/s13059-014-0550-8

19. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc.* (2009) 4:44–57. doi: 10.1038/nprot.2008.211

20. Sangster T, Major H, Plumb R, Wilson AJ, Wilson ID. A pragmatic and readily implemented quality control strategy for HPLC-MS and GC-MS-based metabonomic analysis. *Analyst.* (2006) 131:1075–8. doi: 10.1039/b604498k

21. Zhang P, Li L, Sun H, Zhang Y, Zhang G, Zhang T, et al. Mitochondrial energy-regulating effect of atractyloside inhibits hepatocellular steatosis through the activation of autophagy. *Front Pharmacol.* (2020) 11:575695. doi: 10.3389/fphar.2020.575695

22. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. *Methods.* (2001) 25:402–8. doi: 10.1006/meth.2001.1262

23. Zhao X, Zhu JX, Mo SF, Pan Y, Kong DL. Effects of cassia oil on serum and hepatic uric acid levels in oxonate-induced mice and xanthine dehydrogenase and xanthine oxidase activities in mouse liver. *J Ethnopharmacol.* (2006) 103:357–65. doi: 10.1016/j.jep.2005.08.040

24. Walker JM. The bicinchoninic acid (BCA) assay for protein quantitation. In: Walker JM editor. *[Springer Protocols Handbooks] The Protein Protocols Handbook*. Totowa, NJ: Humana Press (2009). p. 11–5.

25. Lane AN, Fan TWM. Regulation of mammalian nucleotide metabolism and biosynthesis. *Nucleic Acids Res.* (2015) 43:2466–85. doi: 10.1093/nar/gkv047

26. Yamaoka, T, Kondo M, Honda S, Iwahana H, Moritani M, Ii S, et al. Amidophosphoribosyltransferase limits the rate of cell growth-linked de novo purine biosynthesis in the presence of constant capacity of salvage purine biosynthesis. *J Biol Chem.* (1997) 272:17719–25. doi: 10.1074/jbc.272.28.17719

27. Crişan TO, Cleophas MCP, Novakovic B, Erler K, van de Veerdonk FL, Stunnenberg HG, et al. Uric acid priming in human monocytes is driven by the AKT-PRAS40 autophagy pathway. *Proc Natl Acad Sci U.S.A.* (2017) 114:5485–90. doi: 10.1073/pnas.1620910114

28. Jensen T, Abdelmalek MF, Sullivan S, Nadeau KJ, Green M, Roncal C, et al. Fructose and sugar: a major mediator of non-alcoholic fatty liver disease. *J Hepatol.* (2018) 68:1063–75. doi: 10.1016/j.jhep.2018.01.019

29. Johnson RJ, Sánchez-Lozada LG, Andrews P, Lanaspa MA. Perspective: a historical and scientific perspective of sugar and its relation with obesity and diabetes. *Adv Nutr.* (2017) 8:412–22. doi: 10.3945/an.116.014654

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Dietary quality indices and recurrent chronic kidney disease in Taiwanese post-renal transplant recipients

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Background: This study investigated the association between dietary quality indices and recurrent chronic kidney disease (rCKD) in Taiwanese post-renal transplant recipients (RTRs).

Methods: This prospective study recruited RTRs aged >18 years with a functioning allograft and without any acute rejection in the past 3 months from September 2016 to June 2018. Dietary quality indices included the Alternative Healthy Eating Index (AHEI) and AHEI-2010, and the Taiwanese version of the AHEI (AHEI-Taiwan) was calculated using 3-day dietary records, and calculated scores were divided into quartiles. Laboratory data were collected from medical records. rCKD was defined as an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m^2. Logistic regression analysis was performed to analyze the associations.

Results: This study included 102 RTRs. The RTRs with higher AHEI, AHEI-Taiwan, and AHEI-2010 scores were older and had higher eGFRs and lower odds of rCKD. As compared with the lowest quartile, patients with the highest quartiles of the AHEI [odds ratio (OR), 0.10; 95% confidence interval (95% CI): 0.02, 0.49; *p*-trend = 0.004), AHEI-2010 (OR, 0.17; 95% CI: 0.04, 0.72; *p*-trend = 0.016], and AHEI-Taiwan (OR, 0.13; 95% CI: 0.03–0.59; *p*-trend = 0.008) had lower odds of rCKD, respectively. As compared with the lowest quartile, patients who consumed the highest quartiles of red and processed meat had 11.43 times higher odds of rCKD (OR, 11.43; 95% CI: 2.30–56.85; *p* for trend <0.01).

Conclusion: Higher dietary quality indices are associated with lower odds of rCKD in Taiwanese RTRs. Particularly, a positive association between a higher

intake of red meat and processed meat and higher odds of rCKD remained exists after transplantation in Taiwanese RTRs. Further dietary guidelines and individualized dietary education were necessary for RTRs to prevent graft function deterioration.

KEYWORDS

dietary quality, kidney function, chronic kidney disease, renal transplant recipients, Taiwan

1. Introduction

Chronic kidney disease (CKD) was a major global public health problem, and its prevalence is 10–15% worldwide (1) and 11.3% in Taiwan (2). Among renal replacement therapies, renal transplantation was around 2,000 cases in Taiwan (2), which was more favorable compared with dialysis for patients with end-stage renal disease and those requiring dialysis because it had lower medical costs and results in better quality of life and higher survival rates (3). However, the elimination of dietary restrictions and conflict dietary recommendation and habits from dialysis to transplantation may also result in graft function deterioration and cause recurrent chronic kidney disease (rCKD) in renal transplant recipients (RTRs) (4).

Evidence indicates that lifestyle modifications including improved dietary quality can prevent metabolic abnormalities and reduce the risk of CKD (5, 6) and chronic diseases (7). In a previous study, we observed that RTRs had poor adherence to dietary recommendations and the intake of most nutrients was inadequate (8). The National Kidney Foundation (NKF) and National Health and Research Institutes in Taiwan (2, 9) published healthy guideline recommendations as a balance diet for RTRs includes foods from food guides, such as a variety of fresh fruits and vegetables, wholegrains, lean meats, low-fat dairy, and also low salt and high in fiber intake.

The Alternative Healthy Eating Index (AHEI) (10) includes food and nutrient components, such as trans fatty acid, the ratio of polyunsaturated fatty acid and saturated fatty acid (PSR), fruit, vegetables, wholegrains, the ratio of white and red meat, nut and soybean, and vitamin used and alcohol intake and is commonly used for the assessment of dietary quality. Both the AHEI and its updated version, AHEI-2010 (11), are based on the American Dietary Guidelines. AHEI-2010 was according to AHEI and was modified PSR to polyunsaturated fatty acid (PUFA) and n-3 PUFA, meanwhile focusing on red meat, sodium, and sugar intake. The previous study demonstrated that adherence to the AHEI and AHEI-2010 was associated with a lower risk of chronic diseases (12–14). However, Mccullough and Willett (15) reported that the dietary index can be modified according to the national dietary recommendations to be more approached to dietary culture in each country. The Taiwanese version of the AHEI-Taiwan (16) was composed as the original AHEI and modified the cutoff based on Taiwan's dietary recommendations to adapt to Taiwanese dietary pattern.

In addition, several studies had reported that the adherence to the AHEI and AHEI-2010 was associated with decreased kidney function deterioration in CKD populations (6, 17, 18). However, the association between these indices, especially the AHEI-Taiwan, and graft function prevention had not been examined for Taiwanese RTRs. This study aimed to investigate the association between dietary quality indices and graft dysfunction in Taiwanese RTRs. We hypothesized that RTRs with higher AHEI, AHEI-Taiwan, and AHEI-2010 scores would have a lower risk of rCKD. Moreover, we explored the association between the dietary indices food component and the rCKD risk for further analysis.

2. Materials and methods

2.1. Study design and participants

This prospective cross-sectional study was conducted between September 2016 and June 2018 at Linkou Chang Gung Memorial Hospital. Inclusion criteria included that RTRs aged >18 years with a functioning allograft and without any acute rejection reaction in the past 3 months were recruited in this study. Excluded criteria included Patients with an estimated glomerular filtration rate (eGFR) variation of >25% in the past 3 months and other systemic inflammatory diseases.

A total of 106 eligible RTRs were enrolled and referred to qualified registered dietitians in the hospital for face-to-face interviews. Informed consent was obtained from each participant before the interview. The RTRs with considerably low-calorie or high-calorie intake (≤800 or ≥3,000 kcal) were excluded (n = 4). Hence, 102 RTRs were included in the final analysis. The study procedures complied with ethical standards for research with human participants, and the study protocol was reviewed and approved by the Institutional Review Board of Chang Gung Medical Foundation (IRB No. 201600954B0).

TABLE 1 Comparison of the components and scores of the AHEI and AHEI-Taiwan between the lowest and highest quartiles of dietary scores (n = 102)[1].

Item	All	AHEI		AHEI-Taiwan		AHEI-2010	
		Q1: 26.4-37.0	Q4: 49.3-63.2	Q1: 26.7-37.7	Q4: 51.3-68.2	Q1: 37.6-55.7	Q4: 68.3-98.8
	Mean, SD or n,%	Mean, SD or n,%	Mean, SD or n,%	Mean, SD or n,%	Mean, SD or n,%	Mean, SD or n,%	Mean, SD or n,%
Number, n	102	26	25	25	25	26	26
Age, year	48.9 ± 12.8	40.8 ± 11.5	53.1 ± 14.3‡	42.1 ± 10.7	51.7 ± 14.6*	41.0 ± 10.4	52.8 ± 13.7‡
Male, n (%)	59 (57.8)	17 (65.4)	12 (48.0)	18 (72.0)	14 (56.0)	20 (76.9)	14 (53.8)*
Cadaveric, n (%)	83 (81.3)	20 (76.9)	23 (92.0)	19 (76.0)	24 (96.0)	19 (73.1)	22 (84.6)
RT, year	8.5 ± 5.8	6.7 ± 4.2	6.2 ± 4.2	6.8 ± 4.7	5.8 ± 3.6	7.1 ± 4.4	10.4 ± 5.5*
DT, year	6.6 ± 4.9	0.8 ± 0.4	0.9 ± 0.3	0.7 ± 0.5	0.9 ± 0.3	6.6 ± 3.7	5.5 ± 3.9
WC, cm	83.1 ± 9.7	82.8 ± 10.4	81.5 ± 7.9	84.5 ± 10.9	83 ± 8.0	86.8 ± 10.6	83.1 ± 8.8
BH, cm	162 ± 8.6	165.5 ± 8.6	158.7 ± 7.6†	166.7 ± 8.4	159.1 ± 8.1†	166.4 ± 9.0	160.0 ± 8.6*
BW, kg	63.1 ± 13	64.7 ± 15.1	60.3 ± 9.2	67.2 ± 15.7	61.3 ± 9.7	69.5 ± 14.7	64.2 ± 12.2
BMI, kg/m^2	23.9 ± 3.7	23.5 ± 4.5	23.9 ± 2.9	24.1 ± 4.7	24.1 ± 3.0	24.9 ± 4.0	24.9 ± 3.3
FPG, mg/dL	127.6 ± 24.2	121.3 ± 17.9	129.9 ± 22.9	126.5 ± 23.7	132.7 ± 24	126.8 ± 28.8	132 ± 24.2
HOMA	2.3 ± 4.5	1.7 ± 1.3	2.1 ± 1.7	3.7 ± 8.9	2.2 ± 1.7	2.1 ± 1.6	3.8 ± 8.5
TC, mg/dL	205.8 ± 43.9	221.2 ± 40.8	196.7 ± 42.0*	217.5 ± 38.2	195.6 ± 41.4*	213.5 ± 38.5	203.6 ± 45.2
LDL-C, mg/dL	119.8 ± 36.6	135.2 ± 34.3	111.3 ± 38.7*	134.0 ± 32.9	108.8 ± 38.6*	130.3 ± 33.6	116.4 ± 36
HDL-C, mg/dL	52 ± 17.9	55 ± 16.8	52.7 ± 15.2	51.2 ± 16.1	50.4 ± 16.9	53.3 ± 16.8	48.8 ± 16.4
TG, mg/dL	160.2 ± 121.6	142 ± 89.7	135.3 ± 62.8	153.7 ± 98	161.4 ± 112.1	149.5 ± 95.7	164.7 ± 86.2
Alb, g/dL	4.3 ± 0.3	4.4 ± 0.3	4.3 ± 0.3	4.4 ± 0.3	4.3 ± 0.3	4.4 ± 0.3	4.2 ± 0.3†
Cr, mg/dL	1.5 ± 0.9	1.7 ± 1.0	1.1 ± 0.4‡	1.7 ± 1.0	1.2 ± 0.4†	1.8 ± 1.4	1.3 ± 0.7†
eGFR, ml/min/1.73 m^2	54.9 ± 20.9	48.7 ± 15.9	64.9 ± 19.3†	48.5 ± 14.8	64.6 ± 19.7†	50.9 ± 18.4	61.4 ± 23.6*
Hs-CRP, mg/dL	5.1 ± 11.4	4.1 ± 3.9	4.3 ± 5.5	3.6 ± 4.0	4.0 ± 5.5	4.9 ± 5.7	4.3 ± 5.4

[1]Value expressed as mean ± SD and percentages as appropriate. *$p < 0.05$, †$p < 0.01$, and ‡$p < 0.001$ by using Student's t-test or Wilcoxon rank-sum test.

Q, quartile; AHEI, Alternative Health Eating Index; SD, standard deviation; RT, renal transplant time; DT, dialysis time; WC, waist circumference; BH, body height; BW, body weight; BMI, body mass index; FPG, fasting plasma glucose; HOMA, homeostasis model assessment-insulin resistance index; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; eGFR, estimated glomerular filtration rate; Hs-CRP, high-sensitivity C-reactive protein.

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2.2. Characteristics, laboratory data, and rCKD definition

We collected the following patient characteristics and laboratory data from medical records: age, sex, dialysis history, transplant history, years after dialysis or transplantation, body height (without shoes), body weight (two times, tenth of a point taken, no shoes, and wear light clothing), performance of handgrip (measure three times for maximum values), blood pressure (average of three times), fasting plasma glucose, homeostatic model assessment of insulin resistance (HOMA-IR), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), serum albumin, serum creatinine (Cr), estimated glomerular filtration rate, and high sensitive C-reactive protein. rCKD was defined as the deterioration of kidney function to end-stage renal disease (ESRD) after transplantation and was at risk for reverting to ESRD, which eGFR of <60 mL/min/1.73 m^2 based on the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines (9).

3.3. Dietary intake

Dietary intake was determined using self-reported 3-day dietary records (including 2 weekdays and 1 day on the weekend) and evaluated by the qualified registered dietitian one time during the latest clinical follow-up in the study period. Dietary food and nutrient intakes were calculated using nutrition analysis software (CofitPro version 1.0.0. Cofit HealthCare Inc., Taipei, Taiwan), according to Taiwan's Ministry of Health and Welfare Food and Drug Administration database as described previously (19).

3.4. Scoring of dietary quality

A total of three dietary indices based on food and nutrients were used to evaluate dietary quality: the AHEI (10), AHEI-2010 (11), and AHEI-Taiwan (16). The AHEI is based on the 2015–2020 Dietary Guidelines for Americans and includes nine components; the total AHEI score ranges from 0 (unhealthy eating quality) to 87.5 (healthy eating quality). Intermediate intake was proportionally calculated between the range of 0 and 10 points. The AHEI scores are based on the consumption of trans fat, the polyunsaturated-to-saturated fatty acid ratio (PSR) vegetables, fruits, nuts, and soybean, white and red meat, wholegrain fiber, daily multivitamins, and alcohol.

The AHEI-2010 is an updated version of the AHEI and includes 11 components; its total score ranges from 0 (unhealthy eating quality) to 110 (healthy eating quality). Compared with the AHEI, the AHEI-2010 considers the low consumption of sodium (10 points for the lowest decile) and sugar-rich beverages (10 points for <1 serving/day), the ratio of white

TABLE 2 Comparison of the components and scores between the lowest and the highest quartiles of AHEI and AHEI-Taiwan dietary scores[1].

Item	AHEI scores		AHEI-Taiwan scores	
	Q1: 26.4–37.0	Q4: 49.3–63.2	Q1: 26.7–37.7	Q4: 51.3–68.2
Trans fat,% or g[a]	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0
PSR[b]	9.1 ± 1.7	9.9 ± 0.3	9.0 ± 1.8	9.9 ± 0.3
Fruit, servings[c]	1.1 ± 1.6	4.7 ± 2.7‡	1.6 ± 3.0	7.9 ± 2.4‡
Vegetable, servings[d]	4.2 ± 1.7	6.4 ± 2.9*	6.0 ± 2.1	8.9 ± 1.7‡
Wholegrain, g or %[e]	0.8 ± 2.7	8.4 ± 3.6‡	0.5 ± 1.1	5.2 ± 5.1‡
White and red meat, servings[f]	2.0 ± 1.7	4.8 ± 3.6*	1.7 ± 1.6	4.9 ± 3.5†
Nut and soybean, servings[g]	3.3 ± 4.2	9.1 ± 2.4‡	2.5 ± 3.5	7.8 ± 3.4‡
Vitamin used, >5 years[h]	2.5 ± 0.0	2.5 ± 0.0	2.5 ± 0.0	2.5 ± 0.0
Alcohol, equivalent[i]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total score[j]	32.9 ± 2.8	55.8 ± 4.2‡	33.9 ± 2.8	57.1 ± 4.8‡

[1]Value expressed as mean ± SD. *$p < 0.01$, †$p < 0.001$, and ‡$p < 0.0001$ by using Student's *t*-test or Wilcoxon rank-sum test.
Q, quartile; AHEI, Alternative Health Eating Index; PSR, polyunsaturated-to-saturated fatty acid ratio; SD, standard deviation.
[a]*Trans*-fat consumption was calculated in percentage for the AHEI (10 points for ≤0.5% and 0 points for ≥4%) and in grams for the AHEI-Taiwan (10 points for ≤1 g and 0 points for ≥8 g).
[b]PSR consumption was assigned 0–10 points for a ratio <0.1 to ≥1 in both the indices.
[c]Fruit consumption was defined as follows: AHEI (0–10 points for 0–4 servings/day) and AHEI-Taiwan (0–10 points for 0–2 servings/day).
[d]Vegetable consumption was defined as follows: AHEI (0–10 points for 0–5 servings/day) and AHEI-Taiwan (0–10 points for 0–3 servings/day).
[e]Wholegrain consumption was calculated in grams for the AHEI (0–10 points for 0–15 g/day) and percentage for the AHEI-Taiwan (10 points for ≥50% of wholegrain intake).
[f]White-to-red meat ratio was assigned 0–10 points for 0–4 servings/day in both indices.
[g]Nut and soybean consumption was assigned 0–10 points for 0–1 servings/day in both the indices.
[h]Vitamin consumption was assigned 2.5–7.5 points for <5 years to ≥5 years in both the indices.
[i]Alcohol consumption was defined as 0–10 points for 0 or >3.5 equivalent and 0.5–2.5 equivalent in men and 0 or >2.5 equivalent and 0.5–1.5 equivalent in women.
[j]The total score was 2.5–87.5 in both the indices.

meat to red/processed meat (10 points for 0 serving/day and 0 points for ≥1.5 servings/day), PUFA (10 points for ≥10% PUFA consumption), and n-3 PUFA (10 points for 250 mg). Moreover, in the AHEI-2010, the cutoff values were revised for the high consumption of wholegrain fiber (10 points for ≥90 g in men and ≥75 g in women) and the moderate consumption of alcohol (10 points for 0.5–3.5 equivalent in men and 0.5–2.5 equivalent in women).

The AHEI-Taiwan was developed from the AHEI according to Taiwan's dietary recommendations for the convenience of a clinical study and better adaption to the Taiwanese population. Similar to the AHEI, the AHEI-Taiwan includes nine components, and its scores ranged from 0 (unhealthy eating quality) to 87.5 (unhealthy eating quality). In the AHEI-Taiwan, the consumption of trans fat is calculated in grams (10 points for ≥1 g and 0 points for ≤8 g); the measures for the high consumption of vegetables and fruits are revised from 5 and 4 servings/day to 3 and 2 servings/day, respectively; and the consumption of wholegrain cereal was calculated as the total recommended percent intake of cereals in Taiwan. These calculations differ from those in the AHEI.

3.5. Statistical analyses

The characteristics of the RTRs are summarized by the quartile of each dietary index score. Statistical analyses were performed using SAS (version 9.4, SAS Institute, Cary, NC, USA). Descriptive data are presented as the mean, standard deviation, interquartile range, and percentage as appropriate. Logistic regression analysis was performed to analyze associations between dietary quality and rCKD risk. The possible affecting factors of kidney function, such as age, sex, calorie intake, Charlson comorbidity index (CCI), body mass index, geriatric nutrition risk index, handgrip, transplantation time, and dialysis time, were adjusted based on KDOQI guidelines (9). Study data are presented as odds ratio (OR) with 95% confidence interval (95% CI). The significance was set at $P < 0.05$.

4. Results

4.1. Comparison of characteristics between those in the lowest and the highest quartile

The mean scores of AHEI, AHEI-Taiwan, and AHEI-2010 were 43.6 ± 8.8, 44.6 ± 9.0, and 62.1 ± 10.2, respectively. The RTRs in the highest quartile of both the AHEI and AHEI-Taiwan were older (53.1 ± 14.3 vs. 40.8 ± 11.5, $p < 0.001$; 51.7 ± 14.6 vs. 42.1 ± 10.7, $p < 0.05$, respectively), had higher eGFRs (64.9 ± 10.3 vs. 48.7 ± 15.9, $p < 0.01$; 64.6 ± 19.7 vs. 48.5 ± 14.8, $p < 0.01$, respectively), and had lower body height (158.7 ± 7.6 vs. 165.5 ± 8.6, $p < 0.01$; 159.1 ± 8.1 vs. 166.7 ± 8.4, $p < 0.01$, respectively), TC (196.7 ± 42.0 vs. 221.2 ± 40.8, $p < 0.05$; 195.6 ± 41.4 vs. 217.5 ± 38.2, $p < 0.05$, respectively), LDL-C (111.3 ± 38.7 vs. 135.2 ± 34.3, $p < 0.05$; 108.8 ± 38.6 vs. 134.0 ± 32.9, $p < 0.05$, respectively), and Cr (1.1 ± 0.4 vs. 1.7 ± 1.0, $p < 0.001$; 1.2 ± 0.4 vs. 1.7 ± 1.0, $p < 0.01$, respectively) levels. A greater proportion of patients in the highest quartile of the AHEI-2010 were women and older (52.8 ± 13.7 vs. 41.0 ± 10.4, $p < 0.001$) and had higher eGFRs (61.4 ± 23.6 vs. 50.9 ± 18.4, $p < 0.05$), longer transplant time (10.4 ± 5.5 vs. 7.1 ± 4.4, $p < 0.05$), and lower body height (160.0 ± 8.6 vs. 166.4 ± 9.0, $p < 0.05$) and Cr (1.3 ± 0.7 vs. 1.8 ± 1.4, $p < 0.01$) levels. The albumin level was normal in both the lowest and highest quartiles of the AHEI-2010 group (4.2 ± 0.3 vs. 4.4 ± 0.3, $p < 0.01$), but the higher albumin level was significant higher in the highest quartiles of the AHEI-2010 (Table 1).

TABLE 3 Comparison of components and scores between the lowest and the highest quartiles of AHEI-2010 dietary scores[1].

Item	Q1: 37.6–55.7 Score	Q4: 68.3–98.8 Score
Trans fat,%[a]	10.0 ± 0.0	10.0 ± 0.0
n3-PUFA, mg[b]	8.2 ± 2.5	9.5 ± 1.5
PUFA,%[b]	9.2 ± 2.2	9.9 ± 0.2
Fruit, servings[c]	1.1 ± 1.6	4.9 ± 2.5‡
Vegetable, servings[d]	4.0 ± 1.7	6.3 ± 3.1*
Wholegrain, servings[e]	0.6 ± 1.4	4.9 ± 4.2‡
Red meat, servings[f]	0. ± 0.0	2.4 ± 3.1‡
Nut and soybean, servings[g]	4.0 ± 4.5	9.1 ± 2.4†
Alcohol, equivalent[h]	0.0 ± 0.0	0.3 ± 1.4
Na, mg[i]	3.7 ± 3.5	7.9 ± 2.1‡
Sugar, g[j]	9.1 ± 0.7	9.7 ± 0.4*
AHEI-2010[k]	50.0 ± 4.5	74.9 ± 6.9‡

[1]Value expressed as mean ± SD. *$p < 0.01$, †$p < 0.001$, and ‡$p < 0.0001$ by using Student's t-test or Wilcoxon rank-sum test.

Q, quartile; AHEI, Alternative Healthy Eating Index; PSR, polyunsaturated-to-saturated fatty acid ratio; SD, standard deviation; PUFA, polyunsaturated fatty acid.

[a]*Trans*-fat consumption was assigned 0–10 points for ≥4% and ≤0.5%/day.

[b]PSR consumption was assigned 0–10 points for a ratio of <0.1 to ≥1.

[c]Fruit consumption was assigned 0–10 points for 0–4 servings/day.

[d]Vegetable consumption was assigned 0–10 points for 0–5 servings/day.

[e]Wholegrain consumption was assigned 0–10 points for 0–90 g/day in men and 0–75 g/day in women.

[f]Red meat consumption was assigned 0–10 points for 1.5 and 0 servings/day.

[g]Nut and soybean consumption was assigned 0–10 points for 0–1 servings/day.

[h]Alcohol consumption was assigned 0–10 points for 0 or >3.5 equivalent and 0.5–3.5 equivalent in men and 0 or >2.5 equivalent and 0.5–2.5 equivalent in women.

[i]Sodium intake was defined as decile (0–10 points indicated the highest and lowest decile).

[j]Sugar intake was assigned 0–10 points for ≥1 (240 g) and 0 servings/day.

[k]Total score was 0–110 in both the indices.

TABLE 4 Risk of incident chronic kidney disease by the AHEI, AHEI-Taiwan, and AHEI-2010 in the renal transplant recipients[1].

Item	Q1	Q2	Q3	Q4	*P* trend
AHEI					
Crude	1 (reference)	0.23 (0.06–0.87)	0.49 (0.13–1.95)	0.12 (0.03–0.46)	0.002
Model 1	1 (reference)	0.19 (0.05–0.77)	0.38 (0.09–1.62)	0.09 (0.02–0.40)	0.001
Model 2	1 (reference)	0.19 (0.05–0.79)	0.34 (0.08–1.48)	0.09 (0.02–0.39)	0.001
Model 3	1 (reference)	0.15 (0.03–0.79)	0.38 (0.08–1.87)	0.09 (0.02–0.46)	0.003
AHEI-Taiwan					
Crude	1 (reference)	0.52 (0.13–2.05)	0.26 (0.07–0.98)	0.13 (0.03–0.48)	0.003
Model 1	1 (reference)	0.50 (0.12–2.04)	0.22 (0.05–0.89)	0.11 (0.03–0.45)	0.002
Model 2	1 (reference)	0.44 (0.10–1.80)	0.20 (0.05 - 0.82)	0.10 (0.03–0.43)	0.002
Model 3	1 (reference)	0.43 (0.09–2.11)	0.26 (0.06–1.23)	0.12 (0.03–0.59)	0.009
AHEI-2010					
Crude	1 (reference)	0.51 (0.14–1.83)	0.42 (0.12–1.51)	0.18 (0.05–0.61)	0.006
Model 1	1 (reference)	0.50 (0.13–2.00)	0.37 (0.10–1.44)	0.15 (0.04–0.59)	0.006
Model 2	1 (reference)	0.54 (0.13–2.16)	0.32 (0.08–1.29)	0.15 (0.04–0.57)	0.006
Model 3	1 (reference)	0.42 (0.09–2.09)	0.33 (0.07–1.52)	0.17 (0.04–0.73)	0.02

[1]Value expressed as OR (95% CI) by using logistic regression, Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, calorie intake, and CCI. Model 3 was adjusted for age, sex, calorie intake, CCI, BMI, GNRI, handgrip, transplant time, and dialysis time.
Q, quartile; AHEI, Alternative Healthy Eating Index; CCI, Charlson comorbidity index; BMI, body mass index; GNRI, geriatric nutrition risk index.

4.2. Comparison of dietary quality between the lowest and highest quartiles

The RTRs with the highest AHEI and AHEI-Taiwan scores had higher scores for the consumption of fruits (4.7 ± 2.7 vs. 1.1 ± 1.6; 7.9 ± 2.4 vs. 1.6 ± 3.0, $p < 0.001$, respectively), vegetables (6.4 ± 2.9 vs. 4.2 ± 1.7, $p < 0.05$; 8.9 ± 1.7 vs. 6.0 ± 2.1, $p < 0.001$, respectively), wholegrain (8.4 ± 3.6 vs. 0.8 ± 2.7, $p < 0.001$; 5.2 ± 5.1 vs. 0.5 ± 1.1, $p < 0.001$, respectively), white and red meat (4.8 ± 3.6 vs. 2.0 ± 1.7, $p < 0.05$; 4.9 ± 3.5 vs. 1.7 ± 1.6, $p < 0.001$, respectively), and nuts and soybean (9.1 ± 2.4 vs. 3.3 ± 4.2, $p < 0.001$; 7.8 ± 3.4 vs. 2.5 ± 3.5, $p < 0.001$, respectively) as well as higher total dietary scores (Table 2). The RTRs with the highest AHEI-2010 scores had higher scores for the consumption of fruits (4.9 ± 2.5 vs. 1.1 ± 1.6, $p < 0.001$), vegetables (6.3 ± 3.1 vs. 4.0 ± 1.7, $p < 0.01$), wholegrain (4.9 ± 4.2 vs. 0.6 ± 1.4, $p < 0.001$), and nuts and soybean (9.1 ± 2.4 vs. 4.0 ± 4.5, $p < 0.01$) and lower scores for the consumption of red or processed meat (2.4 ± 3.1 vs. 0.0 ± 0.0, $p < 0.001$), sodium (7.9 ± 2. vs. 3.7 ± 3.5, $p < 0.001$), and sugar (9.7 ± 0.4 vs. 9.4 ± 0.7, $p < 0.05$; Table 3).

4.3. Association among dietary quality, component, and rCKD

A total of 65 RTRs (64%) were diagnosed as having rCKD. All the dietary quality scores were associated with lower odds of rCKD. Compared with the lowest quartile, the highest quartile of the AHEI, AHEI-Taiwan, and AHEI-2010 had 88% (OR, 0.12; 95% CI: 0.03–0.46; *p*-trend <0.01), 87% (OR, 0.13; 95% CI: 0.03–0.48; *p*-trend <0.01), and 82% (OR, 0.18; 95% CI: 0.05–0.61; *p*-trend <0.01) lower odds of rCKD, respectively, in the crude model. In model 1, after adjustment for age and sex, the highest quartile of the AHEI, AHEI-Taiwan, and AHEI-2010 had 90% (OR, 0.09; 95% CI: 0.02–0.40; *p*-trend <0.01), 89% (OR, 0.11; 95% CI: 0.03–0.45; *p*-trend <0.01), and 85% (OR, 0.15; 95% CI: 0.04–0.59; *p*-trend <0.01) lower odds of rCKD, respectively. After additional adjustment for calorie intake and CCI, the RTRs in the highest quartile of the AHEI, AHEI-Taiwan, and AHEI-2010 had 90% (OR, 0.09; 95% CI: 0.02–0.39; *p*-trend <0.01), 90% (OR, 0.10; 95% CI: 0.03–0.43; *p*-trend <0.01), and 85% (OR, 0.15; 95% CI: 0.04–0.57; *p*-trend <0.05) lower odds of rCKD, respectively. In model 3, after further adjustment for body mass index (BMI), geriatric nutrition risk index (GNRI), handgrip, transplant time, and dialysis time, the RTRs in the highest quartile of the AHEI, AHEI-Taiwan, and AHEI-2010 had 91% (OR, 0.09; 95% CI: 0.02–0.46; *p*-trend <0.01), 88% (OR, 0.12; 95% CI: 0.03–0.59; *p*-trend <0.01), and 83% (OR, 0.17; 95% CI: 0.04–0.73; *p*-trend = 0.02) lower odds of rCKD, respectively (Table 4). Further analysis revealed that RTRs who consumed high amounts of red/processed meat had 11.43 times higher odds of rCKD (OR, 11.43; 95% CI: 2.30–56.85; *p*-trend <0.01; Table 5).

5. Discussion

The results of this cohort study revealed that the RTRs in the highest quartiles of the AHEI, AHEI-Taiwan, and AHEI-2010 had 91, 88, and 83% lower odds of rCKD, respectively,

TABLE 5 Risk of incident chronic kidney disease by dietary indices in the renal transplant recipients.

Item	Q1	Q2	Q3	Q4	*P* trend
Fruits, servings					
Crude	1 (Reference)	0.77 (0.22–2.73)	0.21 (0.06–0.68)	0.60 (0.17–2.09)	0.42
Model 1	1 (Reference)	0.89 (0.24–3.28)	0.21 (0.06–0.7)	0.63 (0.17–2.29)	0.48
Model 2	1 (Reference)	0.85 (0.23–3.22)	0.18 (0.05–0.64)	0.64 (0.17–2.39)	0.50
Model 3	1 (Reference)	0.68 (0.15–3.14)	0.16 (0.04–0.72)	0.80 (0.17–3.75)	0.77
Vegetable, servings					
Crude	1 (Reference)	0.88 (0.29–2.70)	4.12 (0.98–17.38)	0.50 (0.17–1.51)	0.22
Model 1	1 (Reference)	0.86 (0.28–2.65)	0.93 (0.29–2.97)	1.01 (0.27–3.74)	0.23
Model 2	1 (Reference)	3.97 (0.93–16.86)	3.93 (0.91–17.01)	5.19 (1.08–24.96)	0.21
Model 3	1 (Reference)	0.50 (0.16–1.55)	0.49 (0.16–1.53)	0.57 (0.16–2.11)	0.40
White and red meat ratio					
Crude	1 (Reference)	1.18 (0.33–4.18)	0.58 (0.17–2.05)	0.33 (0.01–1.08)	0.07
Model 1	1 (Reference)	1.22 (0.34–4.36)	1.19 (0.33–4.32)	2.38 (0.55–10.32)	0.08
Model 2	1 (Reference)	0.59 (0.17–2.08)	0.58 (0.16 - 2.11)	1.46 (0.33–6.59)	0.07
Model 3	1 (Reference)	0.33 (0.10–1.12)	0.31 (0.09–1.08)	0.50 (0.13–1.96)	0.32
Red/processed meat, servings					
Crude	1 (Reference)	2.98 (0.93–9.57)	3.89 (1.21–12.55)	8.75 (2.20–34.81)	0.002
Model 1	1 (Reference)	3.08 (0.95–10.05)	3.86 (1.13–13.23)	3.59 (0.94–13.69)	0.003
Model 2	1 (Reference)	4.10 (1.19–14.1)	4.87 (1.34–17.71)	4.06 (0.97–16.97)	0.001
Model 3	1 (Reference)	8.83 (2.13–36.61)	11.54 (2.68–49.77)	11.43 (2.30–56.85)	0.003
Nut and soybeans, servings					
Crude	1 (Reference)	2.00 (0.47–8.59)	0.57 (0.21–1.58)	0.83 (0.28–2.50)	0.75
Model 1	1 (Reference)	2.30 (0.51–10.34)	2.10 (0.46–9.58)	2.86 (0.56–14.74)	0.84
Model 2	1 (Reference)	0.53 (0.19–1.52)	0.52 (0.18–1.49)	0.47 (0.14–1.56)	0.77
Model 3	1 (Reference)	0.89 (0.28–2.79)	0.84 (0.26–2.69)	0.79 (0.21–2.90)	0.72

[1]Value expressed as OR (95% CI) by using logistic regression. Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, calorie intake, and CCI. Model 3 was adjusted for age, sex, calorie intake, CCI, BMI, GNRI, handgrip, transplant time, and dialysis time.
Q, quartile; CCI, Charlson comorbidity index; BMI, body mass index; GNRI, geriatric nutrition risk index.

compared with the RTRs in the lowest quartiles of these indices after adjustment for age, sex, calorie intake, CCI, BMI, GNRI, handgrip, transplant time, and dialysis time. In addition, higher consumption of red/processed meat was associated with 11.4 times higher odds of rCKD.

The results of the present study are consistent with those of a prospective cohort study (20) with a follow-up period of 14.3 years that recruited 4,848 participants and examined their dietary quality by using the Health Eating Index (HEI), AHEI-2010, Dietary Approaches to Stop Hypertension (DASH) diet, and alternate Mediterranean diet (aMED) and indicated that high dietary quality was associated with CKD prevention. Hu et al. (6) included 3,980 patients with CKD with a follow-up period of 24 years and indicated that high HEI, AHEI-2010, and aMED scores were associated with a 13–20% lower risk of incident CKD. Osté et al. (21) reported that the high scores of the DASH diet were associated with lower renal dysfunction and mortality in RTRs. In addition, some studies have demonstrated that the DASH diet and aMED were inversely associated with the risk of CKD and prevented a decrease in the eGFR and an increase in Cr and micro-albuminuria levels (22–25). These findings suggest that high dietary quality is associated with CKD prevention. Notably, the prevention of rCKD is more important for RTRs due to the elimination of dietary restrictions and incorrect dietary habits after transplantation (4). On the contrary, Song et al. (26) demonstrated that a revised version, the DASH-Japan Ube Modified diet Program (DASH-JUMP) and Korean DASH diet (K-DASH) were modified according to Japanese and Korean dietary recommendation, which is consistent with the

present study of AHEI-Taiwan to adapt Taiwanese dietary recommendations.

Regarding the component of dietary indices, vegetable and fruit consumption were not associated with preserving the eGFR in the present study; this finding is in agreement with that of a previous study that enrolled Dutch (27) and American (28) participants. However, Jhee et al. (29) demonstrated that the high consumption of vegetables and fruits was associated with decreased albuminuria and kidney injury. A reason for this finding is that the consumption of vegetables and fruits rich in potassium is associated with lower blood pressure, which possibly prevents kidney function deterioration (30). Another reason for the positive association between vegetable and fruit consumption and lower risk of CKD may be the effect of decreased acid load. The high dietary acid load may increase metabolic acidosis and lead to kidney injury through an increase in the levels of endothelin-1, which stimulates aldosterone production by activating the renin–angiotensin–aldosterone system pathway, increasing the ammonium concentration, and leading to kidney tubular injury, endothelial dysfunction, and inflammation (31–33). Future studies should investigate the effect of vegetable and fruit consumption on rCKD in RTRs.

Previous studies (34) have examined the association between different protein sources such as red/processed meat, nuts, and soybean, and CKD prevalence. Red/processed meat can lead to inflammation, increase sodium load and iron's pro-oxidative effects, and cause DNA damage, thus directly or indirectly affecting kidney function. In addition, animal protein sources increase the acid load, whereas plant protein sources increase alkalosis load; the association between acid load and CKD progression has also been demonstrated (35). No associations between white-to-red meat ratio, nut and soybean consumption, and rCKD were noted. However, O'Keefe et al. (36) demonstrated that the high consumption of soybean was associated with decreased phosphate intake and urinary protein excretion, thus preventing CKD progression. Haring et al. (37) and Mirmiran et al. (38) have reported that replacing one serving of total red/processed meat with one serving of legumes, nuts, wholegrain cereal, low-fat dairy, and fish and seafood was associated with 18–31% and 16–21% lower risk of CKD, respectively. Future studies should evaluate the association between replacing protein sources and rCKD risk in RTRs.

This study has some strengths and limitations. To date, this is the first study to investigate the association between dietary quality and rCKD in Taiwanese RTRs. However, the causality could not be interpreted because of the cross-sectional design of this study. Although the findings of the current study limit the evidence of randomized controlled trials, the results were obtained using 3-day dietary records including 2 weekdays and 1 day on the weekend, and 24-h recall was used to determine dietary quality based on food composition data provided by Taiwan's Ministry of Health and Welfare. Furthermore, a composite definition was used to define rCKD. These methods helped us assess the dietary intake of the RTRs, evaluate nutrition-related problems, and enhance awareness regarding dietary quality and the rCKD in Taiwanese RTRs. The small sample size of this study may reduce the statistical power ($\beta = 0.7$) to detect significant associations. Finally, although many potential confounders were adjusted, the possibility of imperfectly measured or unknown confounders (such as non-immunological and immunological factors) was not excluded in this observational study.

6. Conclusion

This prospective study examined food and nutrient intake, which reflect dietary quality in patients receiving renal transplantation. Overall, higher AHEI, AHEI-Taiwan, and AHEI-2010 scores were associated with lower odds of rCKD in Taiwanese RTRs. Notably, AHEI-Taiwan is based on Taiwan's dietary recommendation, which may be more adaptive to Taiwanese populations. Moreover, further analysis for the dietary component as red/processed meat was positively associated with rCKD. Additional longitudinal and randomized controlled studies are required to verify the association between dietary quality and rCKD.

Data availability statement

The original contributions presented in this study are included in this 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 Institutional Review Board of Chang Gung Medical Foundation (IRB No. 201600954B0). The patients/participants provided their written informed consent to participate in this study.

Author contributions

I-HL, TD, T-CW, and S-HY: conceptualization. I-HL and TD: methodology and analysis and interpretation of data. I-HL, S-WN, and I-HT: software. TD, T-CW, and S-HY: validation and supervision. I-HL, S-WN, H-HW, and Y-JC: investigation. H-HW and Y-JC: resources. I-HL, TD, S-WN, and I-HT: data curation. I-HL: visualization and writing—original draft. I-HL, TD, and S-HY: writing—reviewing and editing. I-HL, S-WN, I-HT, H-HW, and Y-JC: project administration. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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References

1. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: global dimension and perspectives. *Lancet.* (2013) 382:260–72. doi: 10.1016/S0140-6736(13)60687-X

2. National Health and Research Institutes. *Taiwan Chronic Kidney Disease Clinical Guidelines*. Zhunan: National Health and Research Institutes (2015).

3. Reimer J, Franke G, Lütkes P, Kohnle M, Gerken G, Philipp T, et al. Quality of life in patients before and after kidney transplantation. *Psychother Psychosom Med Psychol.* (2002) 52:16–23. doi: 10.1055/s-2002-19662

4. Chan W, Bosch J, Jones D, McTernan P, Phillips A, Borrows R. Obesity in kidney transplantation. *J Ren Nutr.* (2014) 24:1–12. doi: 10.1053/j.jrn.2013.09.002

5. Snelson M, Clarke R, Coughlan M. Stirring the pot: can dietary modification alleviate the burden of CKD? *Nutrients.* (2017) 9:265–93. doi: 10.3390/nu9030265

6. Hu E, Steffen L, Grams M, Crews D, Coresh J, Appel L, et al. Dietary patterns and risk of incident chronic kidney disease: the atherosclerosis risk in communities study. *Am J Clin Nutr.* (2019) 110:713–21. doi: 10.1093/ajcn/nqz146

7. Dubois L, Girard M, Bergeron N. The choice of a diet quality indicator to evaluate the nutritional health of populations. *Public Health Nutr.* (2000) 3:357–65. doi: 10.1017/S1368980000000409

8. Lin IH, Wong TC, Nien SW, Chou YT, Chiang YJ, Wang HH, et al. Dietary compliance among renal transplant recipients: a single-center study in Taiwan. *Transplant Proc.* (2019) 51:1325–30. doi: 10.1016/j.transproceed.2019.02.026

9. Ikizler T, Burrowes J, Byham-Gray L, Campbell K, Carrero J, Chan W, et al. KDOQI clinical practice guideline for nutrition in CKD: 2020 update. *Am J Kidney Dis.* (2020) 76:S1–107. doi: 10.1053/j.ajkd.2020.05.006

10. McCullough M, Feskanich D, Stampfer M, Giovannucci E, Rimm E, Hu F, et al. Diet quality and major chronic disease risk in men and women: moving toward improved dietary guidance. *Am J Clin Nutr.* (2002) 76:1261–71. doi: 10.1093/ajcn/76.6.1261

11. Chiuve S, Fung T, Rimm E, Hu F, McCullough M, Wang M, et al. Alternative dietary indices both strongly predict risk of chronic disease. *J Nutr.* (2012) 142:1009–18. doi: 10.3945/jn.111.157222

12. Ma Y, Li W, Olendzki B, Pagoto S, Merriam P, Chiriboga D, et al. Dietary quality 1 year after diagnosis of coronary heart disease. *J Am Diet Assoc.* (2008) 108:240–6. doi: 10.1016/j.jada.2007.10.047

13. Schwingshackl L, Hoffmann G. Diet quality as assessed by the healthy eating index, the alternate healthy eating index, the dietary approaches to stop hypertension score, and health outcomes: a systematic review and meta-analysis of cohort studies. *J Acad Nutr Diet.* (2015) 115:780–800.

14. Turner-McGrievy G, Barnard N, Cohen J, Jenkins D, Gloede L, Green A. Changes in nutrient intake and dietary quality among participants with type 2 diabetes following a low-fat vegan diet or a conventional diabetes diet for 22 weeks. *J Am Diet Assoc.* (2008) 108:1636–45. doi: 10.1016/j.jand.2014.12.009

15. McCullough M, Willett W. Evaluating adherence to recommended diets in adults: the alternate healthy eating index. *Public Health Nutr.* (2006) 9:152–7. doi: doi.org/10.1079/PHN2005938

16. Lei, WS. *Association Between Alternate Healthy Eating Index for Taiwan (AHEI-T) and Anthropometry, Blood Sugar, Blood Pressure and Serum Lipid Profile Among Type 2 Diabetes Mellitus Patient. Ph.D Thesis.* Taipei: School of Nutrition and Health Sciences Taipei Medical University (2010).

17. Geng T, Jafar T, Neelakantan N, Yuan J, van Dam R, Koh W. Healthful dietary patterns and risk of end-stage kidney disease: the Singapore Chinese health study. *Am J Clin Nutr.* (2021) 113:675–83. doi10.1093/ajcn/nqaa348

18. Hu E, Coresh J, Anderson C, Appel L, Grams M, Crews D, et al. Dietary patterns and risk of chronic kidney disease progression and all-cause mortality: findings from the CRIC study. *Curr Dev Nutr.* (2020) 4:1415.

19. Lin I, Duong T, Wong T, Nien S, Tseng I, Chiang Y, et al. Dietary nutrients and cardiovascular risk factors among renal transplant recipients. *Int J Environ Res Public Health.* (2021) 18:8448–59. doi: 10.3390/ijerph18168448

20. Smyth A, Griffin M, Yusuf S, Mann J, Reddan D, Canavan M, et al. Diet and major renal outcomes: a prospective cohort study. The NIH-AARP diet and health study. *J Ren Nutr.* (2016) 26:288–98. doi: 10.1053/j.jrn.2016.01.016

21. Osté M, Gomes-Neto A, Corpeleijn E, Gans R, de Borst M, van den Berg E, et al. Dietary approach to stop hypertension (DASH) diet and risk of renal function decline and all-cause mortality in renal transplant recipients. *Am J Transplant.* (2018) 18:2523–33. doi: 10.1111/ajt.14707

22. Lin J, Fung T, Hu F, Curhan G. Association of dietary patterns with albuminuria and kidney function decline in older white women: a subgroup analysis from the nurses' health study. *Am J Kidney Dis.* (2011) 57:245–54. doi: 10.1053/j.ajkd.2010.09.027

23. Chang A, Van Horn L, Jacobs D Jr, Liu K, Muntner P, Newsome B, et al. Lifestyle-related factors, obesity, and incident microalbuminuria: the CARDIA (coronary artery risk development in young adults) study. *Am J Kidney Dis.* (2013) 62:267–75. doi: 10.1053/j.ajkd.2013.02.363

24. Rebholz C, Crews D, Grams M, Steffen L, Levey A, Miller IE, et al. DASH (Dietary Approaches to Stop Hypertension) diet and risk of subsequent kidney disease. *Am J Kidney Dis.* (2016) 68:853–61. doi: 10.1053/j.ajkd.2016.05.019

25. Asghari G, Yuzbashian E, Mirmiran P, Azizi F. The association between dietary approaches to stop hypertension and incidence of chronic kidney disease in adults: the Tehran lipid and glucose study. *Nephrol Dial Transplant.* (2017) 32:224–30.

26. Song Y, Lobene A, Wang Y, Hill Gallant K. The DASH diet and cardiometabolic health and chronic kidney disease: a narrative review of the evidence in East Asian countries. *Nutrients.* (2021) 13:984–99. doi: 10.3390/nu13030984

27. Herber-Gast G, Boersma M, Verschuren W, Stehouwer C, Gansevoort R, Bakker S, et al. Consumption of whole grains, fruit and vegetables is not associated with indices of renal function in the population-based longitudinal doetinchem study. *Br J Nutr.* (2017) 118:375–82.

28. Foster M, Hwang S, Massaro J, Jacques P, Fox C, Chu A. Lifestyle factors and indices of kidney function in the Framingham heart study. *Am J Nephrol.* (2015) 41:267–74.

29. Jhee J, Kee Y, Park J, Chang T, Kang E, Yoo T, et al. A diet rich in vegetables and fruit and incident CKD: a community-based prospective cohort study. *Am J Kidney Dis.* (2019) 74:491–500. doi: 10.1053/j.ajkd.2019.02.023

30. Aburto N, Hanson S, Gutierrez H, Hooper L, Elliott P, Cappuccio F. Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. *BMJ.* (2013) 346:f1378.

31. Khanna A, Simoni J, Hacker C, Duran M, Wesson D. Increased endothelin activity mediates augmented distal nephron acidification induced by dietary protein. *J Am Soc Nephrol.* (2004) 15:2266–75.

32. Wesson D, Simoni J, Broglio K, Sheather S. Acid retention accompanies reduced GFR in humans and increases plasma levels of endothelin and aldosterone. *Am J Physiol Renal Physiol.* (2011) 300:F830–7. doi: 10.1152/ajprenal.00587.2010

33. Scialla J. The balance of the evidence on acid–base homeostasis and progression of chronic kidney disease. *Kidney Int.* (2015) 88:9–11.

34. Mafra D, Borges N, de Franca Cardozo L, Anjos J, Black A, Moraes C, et al. Red meat intake in chronic kidney disease patients: two sides of the coin. *Nutrition.* (2018) 46:26–32. doi: 10.1016/j.nut.2017.08.015

35. Gaggl M, Sliber C, Sunder-Plassmann G. Effect of oral alkali supplementation on progression of chronic kidney disease. *Curr Hypertens Rev.* (2014) 10:112–20.

36. O'Keefe S, Bianchi L, Sharman J. Soybean nutrition. *SM J Nutr Metab.* (2015) 1:1006–14.

37. Haring B, Selvin E, Liang M, Coresh J, Grams M, Petruski-Ivleva N, et al. Dietary protein sources and risk for incident chronic kidney disease: results from the atherosclerosis risk in communities (ARIC) study. *J Ren Nutr.* (2017) 27:233–42. doi: 10.1053/j.jrn.2016.11.004

38. Mirmiran P, Yuzbashian E, Aghayan M, Mahdavi M, Asghari G, Azizi F. A prospective study of dietary meat intake and risk of incident chronic kidney disease. *J Ren Nutr.* (2020) 30:111–8.

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Association of macronutrients intake distribution with osteoarthritis risk among adults in NHANES, 2013–2016

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The association between dietary macronutrient distribution and the risk of OA remains unknown. We aimed to evaluate how dietary macronutrient distribution was correlated with the risk of OA in US adults. We performed a cross-sectional study consisting of 7,725 participants from National Health and Nutrition Examination Survey (NHANES) 2013–2016. Dietary macronutrient intake and OA status were assessed by using dietary recall method and self-reported questionnaire, respectively. We evaluated the association between dietary macronutrient distribution and the risk of OA using multivariate regression models. We conducted the isocaloric substitution analysis using the multivariate nutrient density method. Higher percentage of energy intake from fat was associated with higher risk of OA [OR = 1.05 (95% CI, 1.00, 1.09); P = 0.034]. No significant correlation was observed between the percentage of energy intake from carbohydrate or protein and risk of OA. Isocaloric substitution analysis revealed that only the substitution between fat and carbohydrate was significantly associated with the risk of OA [OR = 1.05 (95% CI, 1.003 to 1.09); P = 0.037]. Our findings suggested that a diet with low percentage of energy intake from fat may be beneficial in the prevention of OA. Further prospective cohort studies are needed to assess our results.

KEYWORDS

osteoarthritis, macronutrients, NHANES, dietary pattern, cross-sectional study

Introduction

Osteoarthritis (OA) is a high incidence joint disease characterized by degeneration in joint tissue structure, which often causes chronic pain and joint dysfunction among the patients (1). Globally, more than 360 million people are currently suffering from this disease, and the prevalence of OA keeps increasing yearly (2). Approximately 27 million US adults have clinical OA in 2005, an increase from 21 million in 1995 (3). OA has a huge impact economically, in addition to its effect on health. In the United States, the annual cost of joint replacement for OA was estimated at $22.6 billion, and the job-related OA cost was approximately $13.2 billion (4, 5).

The pathogenesis of OA is multifactorial, involving inflammatory, mechanical, and metabolic factors, which can ultimately lead to synovial inflammation and structural destruction of the joint (6). Accumulating evidence has shown that nutrition intake is involved in the development or progression of OA (7–9). Dietary patterns has also been studied in the occurrence and prevention of OA. A large prospective study with 2,842 participants found that higher adherence to western dietary pattern was associated with higher risk of knee OA (10). A cross-sectional study revealed that healthy dietary patterns were related to reduced joint symptoms but dietary patterns were not related to joint structural change in OA patients (11). In a 4-years longitudinal follow-up cohort study, researchers demonstrated that participants with a higher adherence to Mediterranean diet had a lower risk of pain worsening and symptomatic knee OA (12).

Recently, the importance of the proportions of macronutrients intake is also emphasized in the development of chronic diseases (13–15). However, there are limited data on the association between the proportions of macronutrient intake with the risk of OA. High intake of total fat and saturated fatty acids (SFA) may be related to increased progression of structural knee OA, whereas higher intake of mono- and poly-unsaturated fatty may be related to reduced radiographic progression (16). However, only fat intake in macronutrient was analyzed, and other nutrients (carbohydrate and protein) were not adjusted into the model, which would make the interpretation of the results become difficult because the estimated effect of fat may depend on other nutrients (carbohydrate and protein) it replaces. More studies are needed to better investigate the relationship between dietary macronutrient distribution and risk of OA.

To fill the aforementioned knowledge gaps, we aimed to explore the association between dietary macronutrient distribution and risk of OA in US adults using data from the National Health and Nutrition Examination Survey (NHANES) database.

Materials and methods

Study population

The NHANES database is an ongoing population-based national survey focusing on the nutrition and health of the American population. The NHANES database is available publicly at www.cdc.gov/nchs/nhanes. Data from 2013 to 2016 in NHANES were combined in this study. We investigated the links between macronutrient distribution with risk of OA in adult participants, including 12,105 participants aged over 20 years. Participants with missing value for arthritis status information (n = 2,309), dietary recall and other covariates (n = 1,882), were excluded. After the exclusion of 189 participants with unusually low or high total energy intake (< 500 kcal/day or > 5,000 kcal/day), 7,725 participants were enrolled, including 1,039 OA patients (**Figure 1**).

Assessment of OA status

Osteoarthritis (OA) status was determined by a questionnaire survey (17). Participants were asked: "Has a doctor or other health professional ever told you that you have arthritis?" Those who answered "no" were defined as without OA. If the answer is "yes," the patients will be further to answer a follow-up question, "Which type of arthritis was it?" Those who self-reported "osteoarthritis" were defined as with OA.

Assessment of macronutrients intake distribution

Nutrient intake information was collected through two non-consecutive 24-h dietary recalls (18). The "automated multiple pass method" was used to improve the precision of food recall, the steps of this methods are as follows: finishing a self-reported food list, probing for forgotten foods, collecting details of foods, and final probing for any other foods. To avoid the difference in dietary intake between weekdays and weekends, only recalls of weekdays were chosen. If both recalls were recorded on weekdays, the first recall would be chosen. A standardized measuring guide was also used to quantify the amount of food items. Total consumption of protein, carbohydrate, and fat were calculated according to the recorded food items. Daily total energy intake was generated by summing the calories from protein, carbohydrate, and fat (1 g protein = 4 kcal, 1 g carbohydrate = 4 kcal, 1 g fat = 9 kcal) (19). Macronutrients distribution was further calculated as follows:

$$\text{Carbohdrate intake}(\%) = \frac{4\ \text{kcal/g} \times \text{daily carbohydrate intake}}{\text{daily total energy intake (kcal)}} \times 100\% \quad (1)$$

$$\text{Protein intake}(\%) = \frac{4\ \text{kcal/g} \times \text{daily protein intake}}{\text{daily total energy intake (kcal)}} \times 100\% \quad (2)$$

$$\text{Fat intake}(\%) = \frac{9\ \text{kcal/g} \times \text{daily fat intake}}{\text{daily total energy intake (kcal)}} \times 100\% \quad (3)$$

Covariates

To assess physical activity, weekly metabolic equivalent (MET) minute aggregated scores were calculated for each participant (20). Referring to the recommended method of NHANES, weekly MET-minutes were calculated as follows: [4.0 MET scores × (weekly minutes of moderate work-related activity + weekly minutes walking or bicycling for transportation) + weekly minutes of moderate leisure-time physical activity] + [8.0 MET scores × (weekly minutes of vigorous work-related activity + weekly minutes of vigorous leisure-time physical activity)]. Using the calculated MET-minutes, participants were categorized into inactive (< 600 MET-minute/week), moderately active (600–3,000 MET-minute/week), and highly active (> 3,000 MET-minute/week). Dietary fiber intake were collected based on dietary recall and supplement use recall. Diabetes status was determined based on a questionnaire, in which the patient answers the question "Have you ever been told by a doctor or health

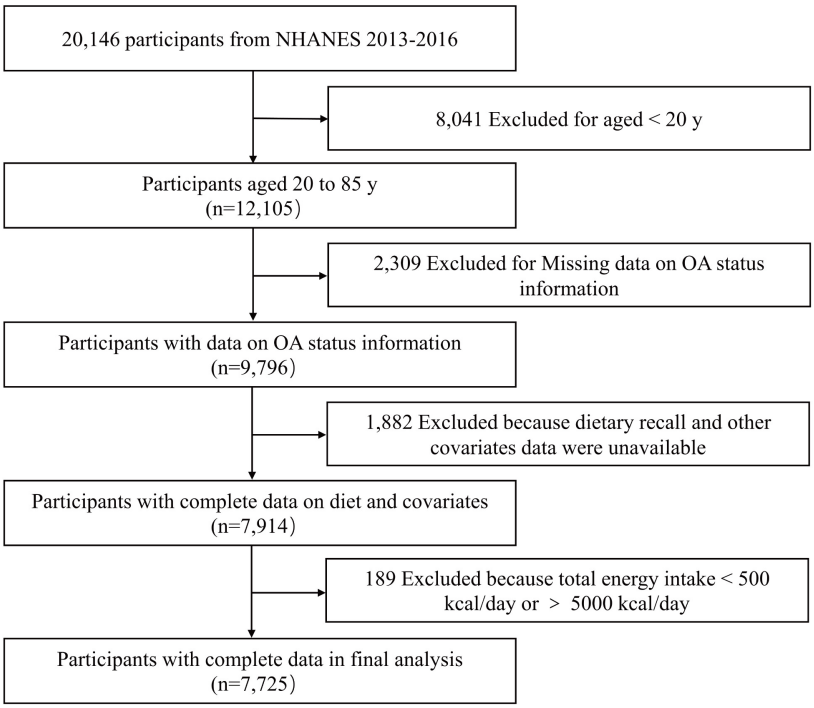

FIGURE 1
Flowchart of the participants selection.

professional that you have diabetes or sugar diabetes?" Those who answered "yes" were defined as with self-report diabetes status. Those who answered "no" or "borderline" were defined as without self-report diabetes status. The other covariates included age, race/ethnicity, education, body mass index, total protein, total cholesterol, serum calcium, and serum 25-hydroxyvitamin D. The examination parts related to clinical and laboratory evaluations were all carried out by well-trained medical experts. Information on each variable and acquisition process are publicly available at www.cdc.gov/nchs/nhanes.

Statistical analysis

Continuous variables were presented as medians and inter quartile ranges and categorical variables as percentages. We used the Kruskal-Wallis test for continuous variables and the $\chi 2$ test for categorical variables to assess the characteristics of the participants by self-reported OA status. Multivariate logistic regression analyses were performed to evaluate the relationship between macronutrient distribution with OA risk with odds ratio (OR) and corresponding 95% confidence interval (CI). Three models were constructed, as follows: crude model, no adjustment for covariates; model 1, adjusted for age, gender, and race/ethnicity; model 2, additionally adjusted for education, self-reported diabetes, body mass index, total protein, total cholesterol, serum calcium, serum 25-hydroxyvitamin D, metabolic equivalent task minutes, dietary fiber intake and total energy intake. Sensitivity analysis was conducted by stratifying participants according to different intake levels of macronutrients with reference to the acceptable macronutrient distribution ranges (21). The trend test was calculated by treating the intake of each category of macronutrients as a continuous variable in multivariable models.

We performed isocaloric substitution analysis to assess whether substituting certain type of macronutrient (as 5% of energy) with another is associated with OA risk using multivariate nutrient density method (e.g., replacing 5% of the energy intake of carbohydrate with fat intake while leaving protein intake unchanged) (22). Subgroup analyses were also conducted stratified by different age, gender, BMI, self-reported diabetes, total cholesterol, and physical activity level. A two-sided P-value < 0.05 was considered statistically significant. Statistical analyses were done with the EmpowerStats ([1]X&Y Solutions, Inc., Boston, MA, USA) and statistical software packages R ([2]The R Foundation).

Results

Characteristics of participants

The characteristics of participants were presented in **Table 1**. Among the 7,725 participants, 1,039 were diagnosed with OA. Compared with the non-OA group, the OA group was older, and had a higher proportion of women than men (64.58 versus 49.28%). Participants with OA or non-OA were similar in education level and serum calcium, while race/ethnicity, self-reported diabetic status, BMI, total protein, serum total cholesterol, serum 25-hydroxyvitamin D, and MET minutes were significantly different

1 http://www.empowerstats.com
2 http://www.R-project.org

TABLE 1 Characteristics of participants stratified by self-reported osteoarthritis (OA) status (*N* = 7,725).

Characteristics	Total	OA	Non-OA	*P*-value
N	7,725	1,039	6,686	
Age (years)	45 (32–61)	65 (55–73)	42 (31–57)	<0.001
Gender, n (%)				<0.001
Male	3759 (48.66)	368 (35.42)	3391 (50.72)	
Female	3966 (51.34)	671 (64.58)	3295 (49.28)	
Race/Ethnicity, n (%)				<0.001
Non-hispanic White	3019 (39.08)	637 (61.31)	2382 (35.63)	
Non-hispanic Black	1458 (18.87)	147 (14.15)	1311 (19.61)	
Non-hispanic Asian	907 (11.74)	46 (4.43)	861 (12.88)	
Mexican American	1224 (15.84)	94 (9.05)	1130 (16.90)	
Other hispanic	860 (11.13)	77 (7.41)	783 (11.71)	
Other	257 (3.33)	38 (3.66)	219 (3.28)	
Education, n (%)				0.336
Lower than high school	1541 (26.97)	186 (17.9)	1355 (20.27)	
High school or equivalent	1692 (21.90)	216 (20.79)	1476 (22.08)	
More than high school	4492 (51.13)	637 (61.31)	3855 (57.65)	
Self-reported diabetes, n (%)				<0.001
Yes	933 (12.08)	249 (23.97)	684 (10.23)	
No	6792 (87.92)	790 (76.03)	6002 (89.77)	
BMI (kg/m^2)	27.8 (24.2–32.4)	30.4 (25.9–35.6)	27.5 (23.9–31.9)	<0.001
Total protein (g/dL)	71 (68–74)	70 (67–73)	72 (69–75)	<0.001
Total cholesterol (mg/dL)	186 (161–214)	190 (162–220)	186 (161–213)	0.019
Serum calcium (mg/dL)	9.4 (9.2–9.6)	9.37 (9.2–9.6)	9.4 (9.22–9.6)	0.077
Serum 25-hydroxyvitamin D (nmol/L)	61.6 (44.8–79.8)	75.3 (56.3–95.75)	59.85 (43.6–77.3)	<0.001
MET minutes per week	400 (60–1,200)	180 (60–720)	480 (80–1,200)	<0.001
Total energy intake (kcal/d)	1953 (1,463–2,568)	1767 (1,309–2,357.5)	1978 (1,484–2,602)	<0.001
Protein intake (%)	15.2 (12.4–18.7)	15.15 (12.35–18.25)	15.25 (12.45–18.85)	0.212
Carbohydrate intake (%)	48.25 (41–55.5)	48.25 (41.2–55.25)	48.3 (40.9–55.5)	0.987
Fat intake (%)	34.3 (28.2–40.1)	34.9 (29.2–40.55)	34.15 (28.05–40)	0.004
Dietary fiber intake (g/d)	15 (9.8–21.9)	14.70 (9.5–21.35)	15 (9.8–22)	0.066

Median (inter quartile range) for continuous variables and *P*-value was calculated by Kruskal-Wallis test. N (%) for categorical variables and *P*-value was calculated by weighted chi-square test. BMI, body mass index; MET, metabolic equivalent task.

between these two groups. For dietary intake, total energy, protein, carbohydrate, and dietary fiber intake were all similar between OA and non-OA groups. The percentage of energy intake from fat was significantly higher in the OA group.

Macronutrients distribution and OA risk

Table 2 showed the association between macronutrients intake distribution and risk of OA. A negative association between fat intake with risk of OA was found in the crude model [OR = 0.95 (95% CI, 0.92, 0.99); $P = 0.010$]. However, higher fat intake was associated with higher risk of OA [OR = 1.05 (95% CI, 1.00, 1.09); $P = 0.034$] after full adjustment. Fat intake level above reference range (> 35% energy) was highly correlated with higher risk of OA [OR = 1.36 (0.96, 1.92); $P = 0.042$]. Linear trend was shown across the intake levels of fat. Intake of carbohydrate was not significantly associated with OA risk [OR = 0.98 (0.95, 1.02); $P = 0.261$]. Meanwhile, no significant association was observed between protein intake and the risk of OA [OR = 1.03 (0.95, 1.011); $P = 0.489$].

Isocaloric substitution analysis

Table 3 presented the analysis of isocaloric substitution of macronutrients. Isocaloric substitution of carbohydrate by fat was associated with higher risk of OA [OR = 1.05 (95% CI, 1.003 to

TABLE 2 Association between macronutrients distribution and the risk of osteoarthritis (OA) among 7,725 participants from 2013 to 2016 National Health and Nutrition Examination Survey (NHANES).

Nutrients distribution	OA OR (95% CI)		
	Crude	Model 1	Model 2
Carbohydrate intake (5% increase)	0.99 (0.97, 1.02)	1.003 (0.97, 1.04)	0.98 (0.95, 1.02)
Carbohydrate intake levels			
Below reference (<45%)	[Ref]	[Ref]	[Ref]
Reference intake (45–65%)	0.98 (0.85, 1.12)	1.05 (0.90, 1.22)	0.98 (0.83, 1.15)
Above reference (>65%)	0.92 (0.70, 1.21)	0.88 (0.65, 1.20)	0.81 (0.59, 1.12)
P for trend	0.579	0.934	0.366
Protein intake (5% increase)	1.05 (0.99, 1.11)	1.02 (0.96, 1.10)	1.03 (0.95, 1.11)
Protein intake levels			
Below reference (<10%)	[Ref]	[Ref]	[Ref]
Reference intake (10–35%)	1.17 (0.95, 1.45)	1.33 (1.04, 1.68)	1.40 (1.08, 1.80)
Above reference (>35%)	1.20 (0.60, 2.41)	1.09 (0.50, 2.39)	1.11 (0.49, 2.48)
P for trend	0.150	0.042	0.024
Fat intake (5% increase)	0.95 (0.92, 0.99)	1.001 (0.96, 1.04)	1.05 (1.00, 1.09)
Fat intake levels			
Below reference (<20%)	[Ref]	[Ref]	[Ref]
Reference intake (20–35%)	0.90 (0.67, 1.21)	1.07 (0.77, 1.48)	1.15 (0.82, 1.61)
Above reference (>35%)	0.79 (0.59, 1.06)	1.09 (0.78, 1.51)	1.36 (0.96, 1.92)
P for trend	0.026	0.649	0.015

Crude model: No covariate was adjusted. Model 1: Age, gender, race/ethnicity were adjusted. Model 2: Additionally adjusted for education, self-reported diabetes, body mass index, total protein, total cholesterol, serum calcium, serum 25-hydroxyvitamin D, metabolic equivalent task minutes, dietary fiber intake, and total energy intake. CI, confidence interval; OR, odds ratio.

TABLE 3 Association between isocaloric substitution of macronutrients and the risk of osteoarthritis (OA) among 7,725 participants from 2013 to 2016 National Health and Nutrition Examination Survey (NHANES).

Isocaloric substitution (5% energy)	OA OR (95% CI)		
	Crude	Model 1	Model 2
Protein substituting carbohydrate	1.05 (0.99, 1.11)	1.02 (0.96, 1.10)	1.02 (0.95, 1.10)
Fat substituting carbohydrate	0.95 (0.92, 0.99)	1.001 (0.96, 1.04)	1.05 (1.003, 1.09)
Fat substituting protein	0.95 (0.92, 0.99)	1.003 (0.95, 1.06)	1.06 (0.99, 1.12)

Crude model: No covariate was adjusted. Model 1: Age, gender, race/ethnicity were adjusted. Macronutrient intakes also entered multivariate regression models apart from the substituted one. Model 2: Additionally adjusted for education, self-reported diabetes, body mass index, total protein, total cholesterol, serum calcium, serum 25-hydroxyvitamin D, metabolic equivalent task minutes, dietary fiber intake, and total energy intake. CI, confidence interval; OR, odds ratio.

1.09); $P = 0.037$], whereas replacement of protein with fat was not significantly associated with risk of OA [OR = 1.06 (95% CI, 1.00 to 1.12); $P = 0.062$]. No significant association between carbohydrate and protein substitution with OA risk was detected.

Subgroup analysis was conducted to examine whether the association between isocaloric fat-carbohydrate substitution and the risk of OA were consistent among different population groups (**Figure 2**). When stratified by age, gender, BMI, diabetes, total cholesterol, or physical activity level, no statistically significant difference was observed.

Discussion

In the current study, we found that higher percentage of energy intake from fat was associated with higher risk of OA. No significant correlation was observed between the percentage of energy intake from carbohydrate or protein and the risk of OA. Isocaloric substitution analysis indicated that only the substitution between fat and carbohydrate was significantly correlated with the incidence of OA. The replacement of carbohydrate with fat for every 5% of energy intake was correlated with 5% higher risk of OA. The association between fat-carbohydrate isocaloric substitution and the risk of OA remained consistent in subgroup analysis, indicating the correlation was not modified by age, gender, BMI, diabetes, total cholesterol, and physical activity levels.

Osteoarthritis (OA) is recognized as a multifactorial inflammatory disease, including obesity, synovitis, and systemic inflammatory mediators (10). Numerous studies have suggested that diet nutrients could related to inflammation markers (23–25), which may lead to OA progression. Western dietary pattern has been demonstrated to be associated with chronic inflammatory process that was involved in many chronic degenerative diseases (26). A systematic review reported that adherence to a western dietary pattern was associated with higher levels of pro-inflammatory biomarkers such as interleukin (IL)-6, c-reactive protein (CRP), and tumor necrosis factor-alpha (TNF-α) (27). Western diet can induced gut-derived inflammation, which disrupts mechanisms for maintaining energy homeostasis and lead to obesity and subsequent metabolic disease (28). In addition, two studies investigated the data from the Osteoarthritis Initiative (OAI) suggested that adopting western dietary pattern was associated with increased risk and radiographic progression of knee OA (10, 29). In general, western dietary pattern is correlated with higher risk of OA.

Western dietary pattern is characterized by high-fat dairy products, refined grains, and large consumption of red meat. Some findings revealed that lipids can interact with chondrocytes and articular cartilage, leading to inflammation and cartilage degradation (30). With diet influencing systemic lipid levels (31), dietary fat may play a role in the development and progression of OA. A Multicenter Osteoarthritis Study (MOST) detected a positive association between the n-6 polyunsaturated fatty acid (PUFA) with synovitis in OA but an inverse relationship between total plasma n-3 PUFA (32). Western dietary pattern contain a higher levels of n-6 PUFAs than n-3 PUFAs, which predisposes to inflammation (33). A prospective cohort study reported that higher intake of total fat and saturated fatty acids (SFA) may be related to increased

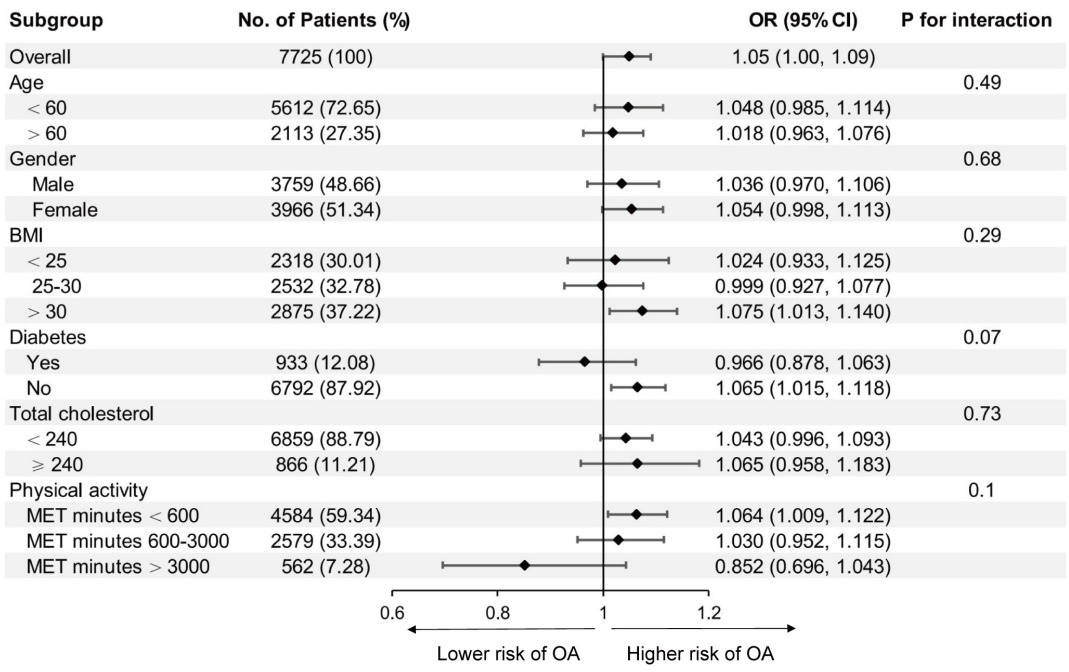

FIGURE 2
Association between isocaloric substitution of fat for carbohydrate intake with osteoarthritis (OA) risk in different subgroups. BMI, body mass index; MET, metabolic equivalent task. Age, gender, race/ethnicity, education, diabetes, body mass index, total protein, total cholesterol, serum calcium, serum 25-hydroxyvitamin D, metabolic equivalent task minutes, dietary fiber intake, total energy intake, and protein intake were adjusted (the stratified variable was omitted from the model).

progression of structural knee OA (16), which is consistent with our findings.

Based on our results and previous studies, higher fat consumption may contribute to the development of OA. Several animal studies have shown a high link between dietary fat intake and OA. In rabbit model, increased dietary fat was associated with changes in cartilage and appears to be a risk factor for the development of OA (34). A high fat diet seems to induce or exacerbate the progression of OA in mice by causing metabolic changes and systemic inflammation (35). In another mice study, a very high fat diet increased knee OA scores and the levels of serum leptin, adiponectin, IL-8, and IL-1α (36).

Limited study available regarding the association between carbohydrate and protein intake with the prevalence of OA. In our study, no significant correlation was observed between the percentage of energy intake from carbohydrate or protein with the risk of OA. Interestingly, we found that isocaloric replacement of carbohydrate with fat was associated with the incidence of OA, which may indicated that diet with high percentage of carbohydrate intake coupled with low percentage of fat intake would be beneficial in the prevention of OA. More clinical and basic experiments are needed to prove it.

The strength of this study is that the NHANES database contains representative samples of the multi-ethnic population. In addition, the large sample size allows us to better conduct subgroup analyses. In terms of limitations, first, the nature of the cross-sectional design makes it difficult to determine the causal link between macronutrients intake and risk of OA. Second, the intake of each macronutrient was obtained according to one weekday 24 h dietary recall, which may bias the estimation of usual dietary intake. Third, we used self-reported disease status, making our data susceptible to recall and information biases. In addition, the association of specific types of nutrients with OA has not been studied in the current study. For example, we did not assess the percentage intake of saturated fat or unsaturated fat, because individual types of fat were calculated different from total consumption and the sum of all types of fat was not equal to total consumption. Finally, though we adjusted for several potential confounding variables associated with dietary intake and OA, residual confounding is possible.

Conclusion

In summary, we found that higher percentage of energy intake from fat was associated with higher risk of OA, while the consumption of carbohydrates and protein were not significantly associated with OA. Isocaloric substitution analysis further indicated that only the substitution between fat and carbohydrate was significantly associated with OA risk. Our findings suggested that a diet with low percentage of energy intake from fat may be beneficial in the prevention of OA. Further prospective cohort studies are needed to assess our results.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by board of the National Center for Health Statistics. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

PP and SG conceived the idea of this study. PP and FX wrote the manuscript. WF and MH collected the data and performed the statistical analysis. YZ and QW reviewed the data and revised the manuscript. All authors contributed toward data analysis, drafting and critically revising the manuscript, agreed to be accountable for all aspects of the work, read, and approved the final manuscript.

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Conflict of interest

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

Publisher's note

References

1. Mandl L. Osteoarthritis year in review 2018: clinical. *Osteoarthritis Cartilage.* (2019) 27:359–64. doi: 10.1016/j.joca.2018.11.001

2. Gu Y, Chen J, Meng Z, Ge W, Bian Y, Cheng S, et al. Research progress on osteoarthritis treatment mechanisms. *Biomed Pharmacother.* (2017) 93:1246–52. doi: 10.1016/j.biopha.2017.07.034

3. Lawrence R, Felson D, Helmick C, Arnold L, Choi H, Deyo R, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. *Arthritis Rheum.* (2008) 58:26–35. doi: 10.1002/art.23176

4. Chen A, Gupte C, Akhtar K, Smith P, Cobb J. The global economic cost of osteoarthritis: how the UK compares. *Arthritis.* (2012) 2012:698709. doi: 10.1155/2012/698709

5. Buckwalter J, Saltzman C, Brown T. The impact of osteoarthritis: implications for research. *Clin Orthop Relat Res.* (2004) 427:S6–15. doi: 10.1097/01.blo.0000143938.30681.9d

6. Hunter D, Bierma-Zeinstra S. Osteoarthritis. *Lancet.* (2019) 393:1745–59. doi: 10.1016/S0140-6736(19)30417-9

7. Maly M, Marriott K, Chopp-Hurley J. Osteoarthritis year in review 2019: rehabilitation and outcomes. *Osteoarthritis Cartilage.* (2020) 28:249–66. doi: 10.1016/j.joca.2019.11.008

8. Joseph G, McCulloch C, Nevitt M, Neumann J, Lynch J, Lane N, et al. Associations between vitamins c and d intake and cartilage composition and knee joint morphology over 4 years: data from the osteoarthritis initiative. *Arthritis Care Res.* (2020) 72:1239–47. doi: 10.1002/acr.24021

9. Dai Z, Niu J, Zhang Y, Jacques P, Felson D. Dietary intake of fibre and risk of knee osteoarthritis in two US prospective cohorts. *Ann Rheum Dis.* (2017) 76:1411–9. doi: 10.1136/annrheumdis-2016-210810

10. Xu C, Liu T, Driban J, McAlindon T, Eaton C, Lu B. Dietary patterns and risk of developing knee osteoarthritis: data from the osteoarthritis initiative. *Osteoarthritis Cartilage.* (2021) 29:834–40. doi: 10.1016/j.joca.2021.02.571

11. Zheng S, Wu F, Winzenberg T, Cicuttini F, Wluka A, Antony B, et al. The cross-sectional and longitudinal associations of dietary patterns with knee symptoms and MRI detected structure in patients with knee osteoarthritis. *Osteoarthritis Cartilage.* (2021) 29:527–35. doi: 10.1016/j.joca.2020.12.023

12. Veronese N, Koyanagi A, Stubbs B, Cooper C, Guglielmi G, Rizzoli R, et al. Mediterranean diet and knee osteoarthritis outcomes: a longitudinal cohort study. *Clin Nutr.* (2019) 38:2735–9. doi: 10.1016/j.clnu.2018.11.032

13. Blanck H, Gillespie C, Serdula M, Khan L, Galusk D, Ainsworth B. Use of low-carbohydrate, high-protein diets among Americans: correlates, duration, and weight loss. *MedGenMed.* (2006) 8:5.

14. Larsen T, Dalskov S, van Baak M, Jebb S, Papadaki A, Pfeiffer A, et al. Diets with high or low protein content and glycemic index for weight-loss maintenance. *N Engl J Med.* (2010) 363:2102–13. doi: 10.1056/NEJMoa1007137

15. de Koning L, Fung T, Liao X, Chiuve S, Rimm E, Willett W, et al. Low-carbohydrate diet scores and risk of type 2 diabetes in men. *Am J Clin Nutr.* (2011) 93:844–50. doi: 10.3945/ajcn.110.004333

16. Lu B, Driban J, Xu C, Lapane K, McAlindon T, Eaton C. Dietary fat intake and radiographic progression of knee osteoarthritis: data from the osteoarthritis initiative. *Arthritis Care Res.* (2017) 69:368–75. doi: 10.1002/acr.22952

17. Li Y, Zhu J, Fan J, Cai S, Fan C, Zhong Y, et al. Associations of urinary levels of phenols and parabens with osteoarthritis among US adults in NHANES 2005-2014. *Ecotoxicol Environ Saf.* (2020) 192:110293. doi: 10.1016/j.ecoenv.2020.110293

18. Beaton G, Milner J, Corey P, McGuire V, Cousins M, Stewart E, et al. Sources of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. *Am J Clin Nutr.* (1979) 32:2546–59. doi: 10.1093/ajcn/32.12.2546

19. Kwon Y, Lee H, Park J, Lee J. Associating intake proportion of carbohydrate, fat, and protein with all-cause mortality in Korean adults. *Nutrients.* (2020) 12:3208. doi: 10.3390/nu12103208

20. Kyu H, Bachman V, Alexander L, Mumford J, Afshin A, Estep K, et al. Physical activity and risk of breast cancer, colon cancer, diabetes, ischemic heart disease, and ischemic stroke events: systematic review and dose-response meta-analysis for the Global Burden of Disease Study 2013. *BMJ.* (2016) 354:i3857. doi: 10.1136/bmj.i3857

21. Institute of Medicine. *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, protein and amino acids*. Washington, DC: The National Academies Press (2005).

22. Dehghan M, Mente A, Zhang X, Swaminathan S, Li W, Mohan V, et al. Associations of fats and carbohydrate intake with cardiovascular disease and mortality

in 18 countries from five continents (PURE): a prospective cohort study. *Lancet.* (2017) 390:2050–62. doi: 10.1016/S0140-6736(17)32252-3

23. Telle-Hansen V, Holven K, Ulven S. Impact of a healthy dietary pattern on gut microbiota and systemic inflammation in humans. *Nutrients.* (2018) 10:1783. doi: 10.3390/nu10111783

24. Bonaccio M, Pounis G, Cerletti C, Donati M, Iacoviello L, de Gaetano G, et al. Mediterranean diet, dietary polyphenols and low grade inflammation: results from the MOLI-SANI study. *Br J Clin Pharmacol.* (2017) 83:107–13. doi: 10.1111/bcp.12924

25. Shivappa N, Bonaccio M, Hebert J, Di Castelnuovo A, Costanzo S, Ruggiero E, et al. Association of proinflammatory diet with low-grade inflammation: results from the Moli-sani study. *Nutrition.* (2018) 54:182–8. doi: 10.1016/j.nut.2018.04.004

26. Barbaresko J, Koch M, Schulze M, Nöthlings U. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. *Nutr Rev.* (2013) 71:511–27. doi: 10.1111/nure.12035

27. Bujtor M, Turner A, Torres S, Esteban-Gonzalo L, Pariante C, Borsini A. Associations of dietary intake on biological markers of inflammation in children and adolescents: a systematic review. *Nutrients.* (2021) 13:356. doi: 10.3390/nu13020356

28. Zinöcker M, Lindseth I. The western diet-microbiome-host interaction and its role in metabolic disease. *Nutrients.* (2018) 10:365. doi: 10.3390/nu10030365

29. Xu C, Marchand N, Driban J, McAlindon T, Eaton C, Lu B. Dietary patterns and progression of knee osteoarthritis: data from the osteoarthritis initiative. *Am J Clin Nutr.* (2020) 111:667–76. doi: 10.1093/ajcn/nqz333

30. Masuko K, Murata M, Suematsu N, Okamoto K, Yudoh K, Nakamura H, et al. A metabolic aspect of osteoarthritis: lipid as a possible contributor to the pathogenesis of cartilage degradation. *Clin Exp Rheumatol.* (2009) 27:347–53.

31. Clifton P. Diet, exercise and weight loss and dyslipidaemia. *Pathology.* (2019) 51:222–6. doi: 10.1016/j.pathol.2018.10.013

32. Baker K, Matthan N, Lichtenstein A, Niu J, Guermazi A, Roemer F, et al. Association of plasma n-6 and n-3 polyunsaturated fatty acids with synovitis in the knee: the MOST study. *Osteoarthritis Cartilage.* (2012) 20:382–7. doi: 10.1016/j.joca.2012.01.021

33. Simopoulos A. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med.* (2008) 233:674–88. doi: 10.3181/0711-MR-311

34. Brunner A, Henn C, Drewniak E, Lesieur-Brooks A, Machan J, Crisco J, et al. High dietary fat and the development of osteoarthritis in a rabbit model. *Osteoarthritis Cartilage.* (2012) 20:584–92. doi: 10.1016/j.joca.2012.02.007

35. Sansone V, Applefield R, De Luca P, Pecoraro V, Gianola S, Pascale W, et al. Does a high-fat diet affect the development and progression of osteoarthritis in mice?: a systematic review. *Bone Joint Res.* (2020) 8:582–92. doi: 10.1302/2046-3758.812.BJR-2019-0038.R1

36. Griffin T, Huebner J, Kraus V, Yan Z, Guilak F. Induction of osteoarthritis and metabolic inflammation by a very high-fat diet in mice: effects of short-term exercise. *Arthritis Rheum.* (2012) 64:443–53. doi: 10.1002/art.33332

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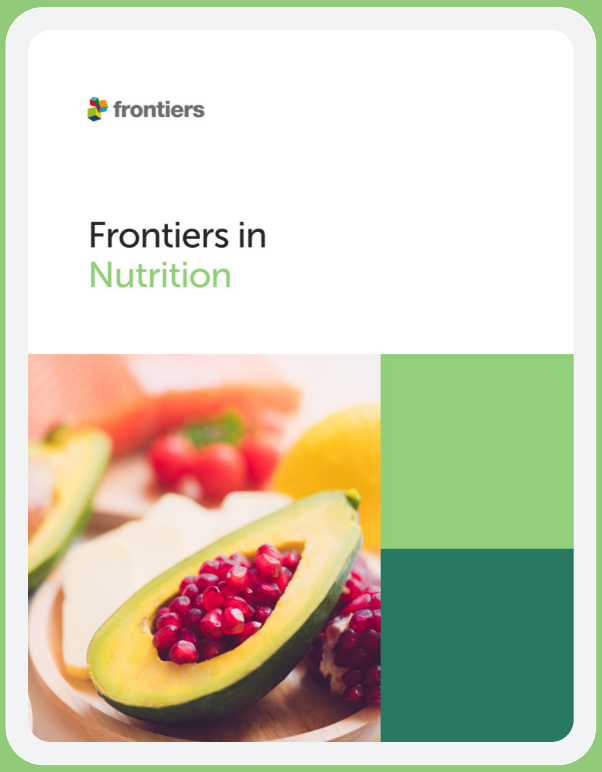

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