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Nutrition and diet practices: Impact on body components and functioning

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Nutrition and diet practices: Impact on body components and functioning

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Editorial: Nutrition and diet practices: impact on body components and functioning

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body composition, obesity, frailty, diet, sarcopenia

Editorial on the Research Topic
Nutrition and diet practices: impact on body components and functioning

Nutritional status is a state of the body resulting from the balance of nutrient intake, absorption, and utilization and the influence of particular physiological and pathological states; it has strong repercussions in clinical practice and public health for being a key risk determinant of adverse health conditions.

To date, a large research window on the role of diet and nutritional state in characterizing body composition as a set of body components and related functional aspects remains open. This Research Topic has contributed to the understanding of the relationship between diet, nutritional status, and body composition features, in order to deepen the comprehension of the role of diet in influencing specific body composition and functioning in the context of well-defined disease states (physical frailty, sarcopenia, obesity, sarcopenic obesity, osteoporosis), or intermediate states.

The collection includes ten articles, mostly original research, and a single systematic review report. A graphical abstract of the research topic has been provided as Figure 1.

A large amount of the research focused on obesity as a target population phenotype. Among these, Zhang and Pu cross-sectionally investigated the level of body mass index (BMI) and waist circumference (WC) to gain the greatest bone mineral density (BMD) benefits in adults, using a representative sample over 50 from the NHANES, a major continuous cross-sectional survey in the United States. Of interest, they found a BMI saturation value, that is, for each unit rise in BMI over 24.3 kg/m2, the femoral neck BMD increases by 0.015 g/cm2. Interestingly, this is partially in contrast with previous literature, suggesting a U-shaped curve in the relationship between BMI and BMD (1). Again, overnutrition status was investigated in the study conducted by Hu et al. Here, some causal genetic relationship was investigated with genome-wide methodology (GWAS) between obesity and skin and soft tissue infections (SSTIs) using two-sample Mendelian randomization. Findings indicated a positive causal effect of obesity on an increased risk of SSTIs. This positive causality persisted after adjusting for the effects of type 2 diabetes (T2D) and peripheral vascular disease.

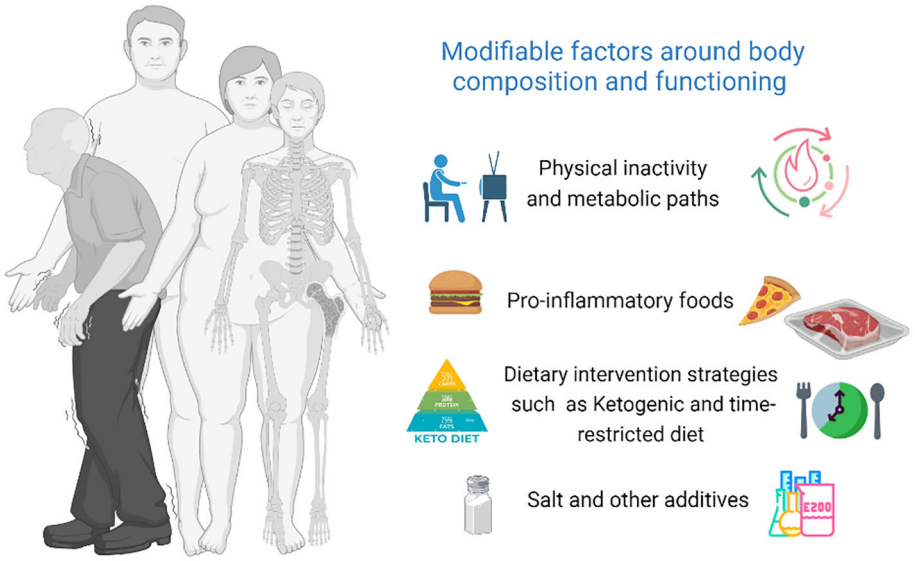

FIGURE 1
Graphical abstract of the Research Topic.

In the contexts of weight-loss strategies, studies by Ernesti et al. reported on predictors of weight loss upon a very-low-energy diet (VLED). A 45-day intervention of VLED, using a so called very-low calorie ketogenic diet (VLCKD) with meal replacement, was performed on a sample of 34 subjects, investigating those molecules involved in energy homeostasis and, more specifically, fibroblast growth factor 21 (FGF21) as an hepatokine of still unclear physiology. Findings indicated men with central obesity and a lower amount of circulating FGF21 to be key factors in reaping greater benefits in terms of weight loss achieved by following a VLCKD diet. Previous research showed that FGF21 is indeed crucial in determining the weight loss effect following a ketogenic diet in mouse models (2), although the physiology of FGF21 in human and murine models seems very different, suggesting that FGF21 is most likely not directly responsible for greater weight loss in human subjects. Moreover, although the effect on weight loss is widely recognized, to date no human studies with adequate and powerful experimental designs have been conducted to definitively understand the impact of ketogenic diet therapy on bone health, according to findings from another Italian research group (Garofalo et al.). Beyond VLCKD, a growing number of studies have explored intervention with time-restricted feeding (TRF). In this regard, Bao et al. conducted the first study to systematically quantify and compare energy balance during intervention with TRF in healthy subjects. Surprisingly, TRF was able to evoke significant fecal energy loss and urinary energy loss tendency without altering energy expenditure, causing a negative energy balance. These findings suggest TRF as an alternative dietary strategy for obesity, although it should be kept in mind that, according to current evidence, TRF seems equivalent to continuous energy restriction (3).

Moving on to other reports on dietary exposure with older target populations, sarcopenia, sarcopenic obesity, and frailty are well-known as important conditions highly prevalent in late life (4, 5). While frailty is a multisystem impairment associated with increased vulnerability to stressors, sarcopenia is the loss of muscle mass and function. As for frailty, this collection reported data on tea consumption in relation to physical frailty (Li et al.), indicating higher tea consumption being associated with lower prevalence of frailty in Chinese men aged 60-79 years, rural residents, and individuals participating in community activities (6).

As for sarcopenia, Chen et al. indicated that both depressive symptoms and the inflammatory potential of diet have direct and indirect effects on low muscle mass, grip strength, and muscle mass, through one and the other, in aging. This provides important insights for integrated nutritional and psychological strategies in the prevention of sarcopenia and loss of muscle in later life (7). This means, as already hypothesized in the literature, that sarcopenia is not only an age-related problem, but also associated with a number of long-term conditions even in early middle age. Comprehensive psychological and behavioral interventions, such as promoting an anti-inflammatory diet and improving mental health, have some potential to prevent sarcopenia early in life (8). Thus, screening is key to stratify risk, and nutritional tools should not be overlooked. the geriatric nutritional risk index has been used as a tool to assess nutrition status in the elderly, and Wang et al. found it to be positively correlated with femoral BMD and negatively correlated with osteoporosis risk in postmenopausal American women.

As for sarcopenic obesity, Kim et al. reported on single food item relationships to sarcopenic obesity outcomes. Here interest shifted to the condition of obesity accompanied by loss of muscle mass and functional impairment, that is, sarcopenic obesity. The target population were aging Koreans living in a rural area, who underwent a comprehensive nutritional and body composition assessment to assess how dietary factors, grip strength, body composition, and prevalence of sarcopenic obesity were associated

with each other in a cross-sectional setting. Findings indicated excessive grain and meat consumption, as well as imbalanced macronutrient intake, to produce significant effects on the risk of prevalence of sarcopenic obesity by increasing the loss of muscle mass and/or body fat in older adults from Korea.

Last, and highly interesting, Liao et al.'s report indicated that in the population over 50, high dietary salt intake is associated with histone methylation in salt-sensitive individuals. This additional piece provides more evidence on how epigenetic mechanisms may influence gene expression and function while mediating crosstalk between genes and environment.

In conclusion, when discussing modifiable factors in contexts of noncommunicable diseases related to body composition and functioning such as obesity, sarcopenia, frailty, and osteoporosis, nutrition is key. Extensive room for research is left on the biological mechanisms underlying dietary strategies, individual food items, nutrition status, and dietary patterns able to shape physical outcome trajectories, especially in the growing aging population. Dietary inflammatory load, positive energy balance, and salt abuse are among the many habits to be monitored. As diet strategies to manage body composition, VLCKD ranks high on the list, but the recent research leaves room for further insights into innovative approaches such as time-restricted feeding.

Author contributions

RZ and MW wrote the first draft of the manuscript; GP and FC read the manuscript and sent additional suggestions. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

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ORIGINAL RESEARCH
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Relationships Between Depressive Symptoms, Dietary Inflammatory Potential, and Sarcopenia: Mediation Analyses

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Background: Sarcopenia is a major public health problem. Depressive symptoms and dietary inflammatory potential play important roles in the development of sarcopenia. We aimed to disentangle the relationships between depressive symptoms, dietary inflammatory potential, and sarcopenia.

Methods: A total of 6,082 participants from the National Health and Nutrition Examination Survey (NHANES) were included in the analyses. Sarcopenia was defined according to the Foundation for the National Institutes for Health (FNIH) criteria. The Depressive symptoms were assessed using the nine-item Patient Health Questionnaire (PHQ-9). Dietary Inflammatory Index (DII) was calculated based on 24-h dietary recall interview. Two sets of mediation models were constructed separately.

Results: Depressive symptoms and DII were associated with sarcopenia, with odds ratios [ORs] (95% CIs) 2.54 (1.27, 5.13) and 1.17 (1.00, 1.37), respectively. DII score mediated the association of depressive symptoms with low muscle mass, explaining a total of 10.53% of the association (indirect effect = 0.004). Depressive symptoms had a significant mediating effects on the association between DII with low muscle mass, explaining a total of 12.50% of the association (indirect effect = 0.001).

Conclusions: Our findings suggested that both depressive symptoms and dietary inflammatory potential had direct effects, and indirect effects on low muscle mass, handgrip strength, muscle mass, through each other. It provides important insights into integrated nutritional and psychological intervention strategies in preventing sarcopenia.

Keywords: mediation analyses, sarcopenia, diet, inflammation, depressive symptoms

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INTRODUCTION

Sarcopenia, a syndrome characterized by a progressive and general loss of muscle mass and strength, is associated with increased adverse outcomes, such as falls, functional decline, frailty, and mortality (1–3). It occurs commonly as an age-related process, with the prevalence of sarcopenia rising in the era of aging (4). Muscle mass gradually decreases from 30 years old and accelerates

gradually as individual ages, accompanied by low muscle strength or poor physical performance (5). A systematic review indicated that sarcopenia often co-occurs with depression (6). Both conditions, separately, are associated with a range of deleterious consequences, whereas the comorbidity can result in particularly worse outcomes (7). Therefore, it is imperative to identify the exact association between depressive symptoms and sarcopenia and explore the potential mechanisms underlying the association.

Existing epidemiological studies have explored the association between depressive symptoms and sarcopenia, with some reporting an increased risk associated with depressive symptoms (8). Regarding the potential mechanisms, depressive symptoms and sarcopenia share common pathophysiological pathways related to neurotrophins, inflammation, and oxidative stress, in which inflammation is one of the most frequently cited mechanisms (9, 10). For example, an increased level of inflammatory cytokines could lead to the neuroinflammation, which contributes to depression (11). Meanwhile, increased inflammatory cytokine levels could trigger the neuroinflammation pathways involved in pathophysiological processes of sarcopenia (12). Evidence is accumulating that both depressive symptoms and inflammation are potentially modifiable status and can be improved through effective strategies (13, 14). Thus, contextualizing chronic inflammation within broader biopsychosocial models of sarcopenia would provide further insights into the development of preventive and therapeutic targets (15).

Levels of inflammation can be modulated by lifestyle behaviors, such as diet. Recent studies have indicated that nutritional assessment and management may have a potential implication in preventing sarcopenia (16). The Dietary Inflammatory Index (DII) was designed to determine the inflammatory potential of the overall diet (17, 18). Moreover, existing studies conducted in older adults have demonstrated that a higher DII score positively correlates with an increased risk of sarcopenia (19). Simultaneously, the inflammatory potential of diet may associate bi-directionally with depressive symptoms. One recent longitudinal study showed that an anti-inflammatory diet is associated with a lower risk of depression (18). In addition, mental health, such as depressive symptoms, has been found to affect the dietary choice or preference, causing more consumption of nutrients related to low diet quality and higher DII (20, 21). Given these findings on the close relationships between depressive symptoms and DII and between DII and sarcopenia, it is reasonable to hypothesize that there may be mediating effects of depressive symptoms and DII on the development of sarcopenia and differential relationships between depressive symptoms and sarcopenia among individuals with different DII level.

Therefore, data from the National Health and Nutrition Examination Survey (NHANES) were used to (1) report the relationships between depressive symptoms, DII, and sarcopenia, (2) explore the mediating effect of DII and depressive symptoms on the development of sarcopenia, and (3) examine the relationships between depressive symptoms and sarcopenia among individuals with different DII level.

METHODS

Study Sample

The NHANES is a nationally representative survey on the US population aimed to assess health and nutrition status. The periodic cross-sectional surveys were conducted every 2 years using a stratified multistage clustered probability sampling approach by the US National Center for Health Statistics (NCHS). Participants completed questionnaires, underwent a medical examination, and provided fasted blood samples. The details of NHANES are available elsewhere (22). The survey was approved by the NCHS Institutional Review Board (Protocol #2011-17). All informed consents had been obtained from participants (23).

Data from two NHANES cycles 2011–2014 were enrolled in the present study. Participants being under 18 years old and over 60 years old (n = 11,586) were excluded. We further excluded participants with missing data on sarcopenia (n = 1,757), depressive symptoms (n = 341), and DII (n = 165). The final analytical sample thus included a total of 6,082 participants. The flow chart of the study sample was shown in **Supplementary Figure 1**.

Assessment of Sarcopenia

Sarcopenia was defined as the presence of weakness and low muscle mass according to the Foundation for the National Institutes for Health (FNIH) criteria (24). The appendicular lean mass (ALM) was assessed by dual-energy x-ray absorptiometry (QDR-4500 Hologic Scanner Bedford, MA, USA). ALM was the sum of lean mass from both arms and legs. Low muscle mass was identified as the ALM adjusted for body mass index (BMI) $<$0.512 for women and 0.789 for men. Handgrip strength was assessed by using a handheld dynamometer. Participants were asked to exert maximum effort three times for each hand, and the highest value measured was used. Relative grip strength was calculated as the highest grip strength divided by BMI. Weakness was defined as the relative grip strength $<$1.00 for men and 0.56 for women.

Assessment of Depressive Symptoms

Depressive symptoms were assessed using the nine-item Patient Health Questionnaire (PHQ-9). The PHQ-9 is composed of items related to symptoms of depression, which has been well-validated in previous studies (25). Each item was scored from "0" (not at all) to "3" (nearly every day), and the total score ranged from 0 to 27, with higher scores indicating higher levels of depressive symptoms. A cutoff score of $\geq$10 was used to define the presence of depressive symptoms.

Assessment of DII

The DII was computed based on the dietary intake data gathered by 24-h dietary recall. We calculated the DII score for 27 food parameters available, such as carbohydrate, energy, protein, fat, fiber, cholesterol, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, β-carotene, vitamins A, B1, B2, B6, B12, C, D, and E, folic acid, iron, magnesium, zinc, selenium,

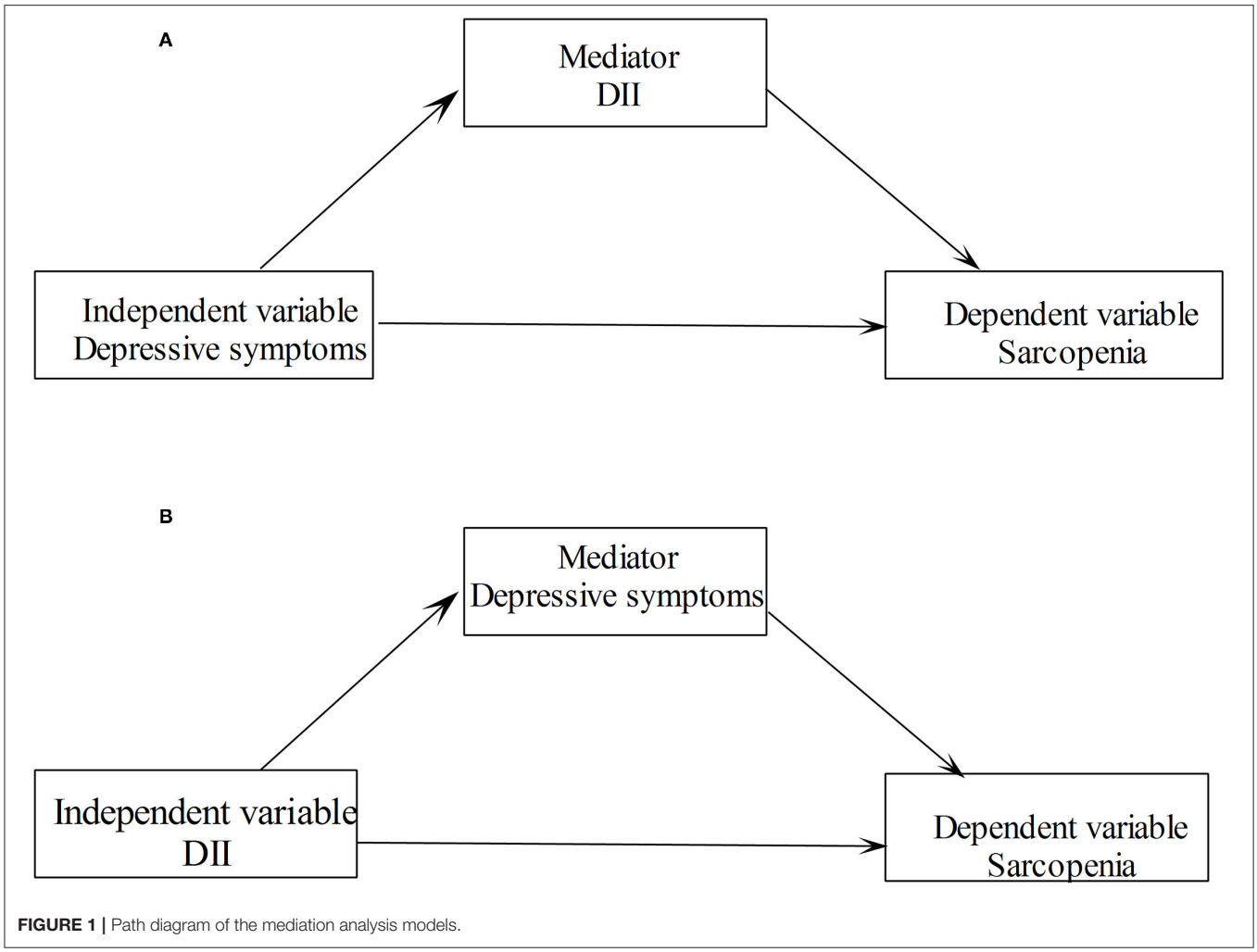

FIGURE 1 | Path diagram of the mediation analysis models.

omega-3, and omega-6 polyunsaturated fatty acids, alcohol, and caffeine. The detail of the DII calculation method developed by Shivappa et al. is available elsewhere (17).

Covariates

The following sociodemographic and health-related characteristics were included in analyses: age, sex, race (non-Hispanic White, Non-Hispanic Black, Mexican American, Other Hispanic, and other race), educational level (below high school, high school and above), marriage status (married/living with partner, widowed/divorced/separated/never married), family poverty income ratio, smoking status (never, current, and former), drinking status (no, yes), physical activity level (low, moderate, and high), BMI status (underweight/normal: $\leq$24.9 kg/m^2, overweight/obese: $>$25 kg/m^2), diabetes, and hypertension. Diabetes was defined as (1) a self-reported previous diagnosis by healthcare professionals, (2) fasting plasma glucose level of 7.0 mmol/L or higher, (3) HbA1c concentration of 6.5% or higher, or (4) use of glucose-lowering medications (insulin or oral hypoglycemic medications). Hypertension was defined as the average systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$90 mmHg, or use of anti-hypertensive medication.

Statistical Analyses

The characteristics of the study population were analyzed using Student's *t*-tests and Chi-square tests for continuous and categorical variables, respectively. The correlations between DII score and depressive symptoms score with the sarcopenia clinical measures were assessed by the Spearman correlation coefficients. Logistic regression models were used to explore the relationships between depressive symptoms and DII with sarcopenia. Linear regression models were used to explore the relationships between depressive symptoms and DII with the measurements of sarcopenia. For dichotomous outcome variables, the models worked out odds ratios (ORs) and 95% CIs, and for continuous variables, the models worked out βs and 95% CIs. Several confounders, such as age, sex, race, educational level, marriage status, family poverty income ratio, smoking status, drinking status, physical activity level, BMI status, diabetes, and hypertension, were controlled in multivariable regression models. Two sets of mediation models

TABLE 1 | Sample characteristics stratified by depressive symptoms.

Characteristics	Total $N = 6,082$	Depressive symptoms $n = 529$	Non-depressive symptoms $n = 5,553$	*P*
Age (years)	37.22 ± 12.52	39.89 ± 12.72	36.96 ± 12.47	<0.01
Sex (%)				<0.01
Male	3,162 (51.99)	197 (37.24)	2,965 (53.39)	
Female	2,920 (48.01)	332 (62.76)	2,588 (46.61)	
Race (%)				<0.01
Non-hispanic white	2,392 (39.33)	247 (46.69)	2,145 (38.63)	
Non-hispanic black	1400 (23.02)	120 (22.68)	1,280 (23.05)	
Mexican-American	779 (12.80)	49 (9.26)	730 (13.14)	
Other hispanic	541 (8.90)	59 (11.15)	482 (8.68)	
Other race	970 (15.95)	54 (10.22)	916 (16.50)	
Educational level (%)				<0.01
Below high school	1,010 (17.10)	145 (27.47)	893 (16.08)	
High school and above	5,042 (82.90)	384 (72.53)	4,660 (83.92)	
Marriage status (%)				<0.01
Married/living with partner	3,487 (57.34)	230 (43.43)	3,260 (58.70)	
Widowed/divorced/separated/never married	2,595 (42.66)	299 (56.57)	2,293 (41.30)	
Family poverty income ratio (%)				<0.01
≤1	1,569 (25.80)	208 (39.24)	1,361 (24.51)	
1–1.84	1,340 (22.03)	156 (29.58)	1,183 (21.31)	
≥1.85	3,173 (52.17)	165 (31.18)	3,009 (54.18)	
Smoking status (%)				<0.01
Never	3,672 (60.38)	210 (39.77)	3,463 (62.38)	
Current	984 (16.18)	95 (17.93)	889 (16.00)	
Former	1,426 (23.44)	224 (42.30)	1,201 (21.62)	
Drinking status (%)				0.07
No	4,556 (74.92)	413 (78.14)	4,143 (74.61)	
Yes	1,526 (25.08)	116 (21.86)	1,410 (25.39)	
Physical activity level (%)				<0.01
Low	2,549 (41.91)	291 (55.13)	2,257 (40.65)	
Moderate	1,905 (31.33)	139 (26.24)	1,767 (31.81)	
High	1,628 (26.76)	99 (18.63)	1,529 (27.54)	
BMI status				<0.01
Underweight/normal	2,044 (33.61)	147 (27.79)	1,897 (34.16)	
Overweight/obese	4,038 (66.39)	382 (72.21)	3,656 (65.84)	
Hypertension (%)	640 (10.53)	73 (13.80)	567 (10.21)	0.01
Diabetes (%)	593 (9.75)	87 (16.45)	506 (9.16)	<0.01
Muscle mass	0.82 ± 0.21	0.74 ± 0.20	0.83 ± 0.21	<0.01
Handgrip strength	1.46 ± 0.49	1.27 ± 0.48	1.48 ± 0.49	<0.01
Sarcopenia (%)	70 (1.15)	16 (3.02)	54 (0.97)	<0.01
Low muscle mass (%)	536 (8.81)	74 (13.99)	462 (8.32)	<0.01
Weakness (%)	133 (2.19)	26 (4.91)	107 (1.93)	<0.01
DII score	0.70 ± 1.89	1.42 ± 1.68	0.63 ± 1.90	<0.01

were constructed and analyzed separately. In a mediation model, we included sarcopenia and its measurements as dependent variables, depressive symptoms as the independent variable, and the DII score as the mediating variable (see **Figure 1A**). We also investigated the mediating effect of depressive symptoms on the association of DII with sarcopenia in another mediation model (see **Figure 1B**). We examined the association between depressive symptoms and sarcopenia stratified by DII categories. All analyses were conducted using Stata 14.0 software (StataCorp LP, College Station, TX, USA).

All values of p were two sides with a statistical significance level of 0.05.

RESULTS

Descriptive and Correlation Analyses

Of the 6,082 participants, 51.99% were men and 48.01% were women, with a mean age of 37.22 years. The prevalence rate of depressive symptoms was 8.70% and sarcopenia 1.15%. **Table 1** shows the characteristics of participants according to depressive symptoms. Participants having depressive symptoms were likely to be older, women, having low education levels, living alone, and low family poverty income ratio. Regarding the health-related variables, lower physical activity, lower handgrip strength, lower muscle mass, sarcopenia, higher DII, and having diabetes and hypertension were significantly associated with depressive symptoms.

Table 2 presents the correlation coefficients between each pair of variables, such as sarcopenia measurements, depressive symptoms scores, and DII. Depressive symptoms scores were negatively correlated with the muscle mass ($r = -0.14$, $p < 0.05$) and handgrip strength ($r = -0.17$, $p < 0.05$). DII was negatively correlated with the muscle mass ($r = -0.23$, $p < 0.05$) and handgrip strength ($r = -0.21$, $p < 0.05$). While, depressive symptom scores were positively correlated with DII ($r = 0.11$, $p < 0.05$).

Relationships Between Depressive Symptoms, DII, and Sarcopenia

After adjustments for potential confounders, depressive symptoms were significantly associated with sarcopenia (OR = 2.54, 95% CI = 1.27, 5.13) and measurements of sarcopenia, such as weakness (OR = 1.89, 95% CI = 1.11, 3.21), low muscle mass (OR = 1.43, 95% CI = 1.05, 1.94), handgrip strength (β = −0.05, 95% CI = −0.08, −0.02), and muscle mass (β = −0.02, 95% CI = −0.03, −0.01). Furthermore, DII was also associated with sarcopenia (OR = 1.17, 95% CI = 1.00, 1.37) and the measurements of sarcopenia. More details are shown in **Table 3**.

Mediation Analyses

In the mediation analyses, the DII score mediated the association between depressive symptoms and low muscle mass, explaining a total of 10.53% of the association (indirect effect = 0.004). Meanwhile, significant mediating effects were established in the handgrip strength and muscle mass. Furthermore, there was a significant mediating effect of depressive symptoms on the association between DII and low muscle mass, explaining a total of 12.50% of the association (indirect effect = 0.001). Meanwhile, significant mediating effects were established for handgrip strength and muscle mass. The results of the mediation analyses are presented in **Table 4**.

Stratified Analyses

In the stratified analyses, depressive symptoms were significantly associated with sarcopenia among participants with the second tertile of DII, with the OR (95% CI) 7.99 (2.50, 25.55). Depressive symptoms were significantly associated with muscle mass, one of the measurements of sarcopenia, among participants with the highest tertile of DII, with the β (95% CI) −0.02 (−0.03, −0.01). The interaction test was not significant ($p > 0.05$). **Supplementary Table 1** shows the association between depressive symptoms and sarcopenia stratified by categories of DII.

TABLE 2 | Correlations between variables.

Variables	Depressive symptoms scores	DII score	Muscle mass	Handgrip strength
Depressive symptoms scores	1			
DII score	0.11*	1		
Muscle mass	−0.14*	−0.23*	1	
Handgrip strength	−0.17*	−0.21*	0.82*	1

*$p < 0.05$.

DISCUSSION

This study disentangled the complex relationships between depressive symptoms, dietary inflammatory potential, and sarcopenia using a nationally representative sample of the US middle-aged adults. Depressive symptoms and dietary inflammatory potential were found to be significantly associated with sarcopenia and its clinical measurements. Of particular importance, there is a novel finding that DII score significantly mediated the association between depressive symptoms and low muscle mass, and there was a significant mediating effect of depressive symptoms on the association between DII and low muscle mass.

Our findings on the relationships between dietary inflammatory potential, depressive symptoms, and sarcopenia supported and extended those from previous studies. Most of the existing studies indicated that depression may be related to sarcopenia, whereas there are also inconsistent results (26–28). Meanwhile, there was a relative scarcity of research on the relationship between depressive symptoms with sarcopenia conducted in middle-aged adults compared to elderly adults previously (26), most of which had limitations related to the assessment of sarcopenia, such as only using one measurement of sarcopenia (29). Notably, the present study not only extended existing evidence but further highlighted the association between depression and sarcopenia in middle-aged adults, thereby further strengthening the possible application of our observation to a wider range of population. With respect to nutritional factors, though the link between diet and sarcopenia has been more widely studied, mainly focusing on nutritional status and specific nutrients (30), little is known about the role of dietary inflammatory potential, presenting a distinct biological mechanism. Our results suggested that the pro-inflammatory

TABLE 3 | Relationships between depressive symptoms, DII, and sarcopenia.

Variables	Crude model		Adjusted model	
	Depressive symptoms	DII score	Depressive symptoms	DII score
Sarcopenia OR (95%CI)	2.94 (1.66,5.21)*	1.11(0.97,1.27)	2.54 (1.27,5.13)*	1.17(1.00,1.37)*
Weakness OR (95%CI)	2.51 (1.62,3.92)*	1.11(0.96,1.17)	1.89 (1.11,3.21)*	1.05(0.94,1.18)
Low muscle mass OR (95%CI)	1.66 (1.27,2.16)*	1.11(1.06,1.17)*	1.43 (1.05,1.94)*	1.11(1.05,1.18)*
Handgrip strength β (95%CI)	−0.17 (−0.22, −0.13)*	−0.05(−0.06, −0.04)*	−0.05 (−0.08, −0.02)*	−0.01(−0.02, −0.004)*
Muscle mass β (95%CI)	−0.07 (−0.09, −0.05)*	−0.02(−0.03, −0.02)*	−0.02 (−0.03, −0.01)*	−0.01(−0.02, −0.005)*

Adjusted model adjusted for age, sex, race, educational level, marriage status, family poverty income ratio, smoking status, drinking status, physical activity level, BMI status, diabetes, and hypertension. $^{*}p < 0.05$. *DII, Dietary Inflammatory Index.*

TABLE 4 | Mediation pathways among depressive symptoms, DII, and sarcopenia.

Independent variables	Mediator	Dependent variable	Exposure to mediator	Mediator to outcome	Direct effect	Indirect effect	Total effect	Proportion Mediated (%)
Depressive symptoms	DII	Sarcopenia	0.492*	0.002	0.015*	0.001	0.016*	6.25
		Weakness	0.492*	0.001	0.019*	0.001	0.020*	5.00
		Low muscle mass	0.492*	0.007*	0.034*	0.004*	0.038*	10.53
		Handgrip strength	0.492*	−0.006*	−0.047*	−0.003*	−0.050*	6.00
		Muscle mass	0.492	−0.006*	−0.016*	−0.003*	−0.019*	15.78
DII	Depressive symptoms	Sarcopenia	0.01*	0.02*	0.002	0.001*	0.003	33.33
		Weakness	0.01*	0.02*	0.002	0.001*	0.003	33.33
		Low muscle mass	0.01*	0.04*	0.007*	0.001*	0.008*	12.50
		Handgrip strength	0.01*	−0.05*	−0.007*	−0.001*	−0.008*	12.50
		Muscle mass	0.01*	−0.02*	−0.006*	−0.001*	−0.007*	14.29

Adjusted for age, sex, race, educational level, marriage status, family poverty income ratio, smoking status, drinking status, physical activity level, BMI status, diabetes, and hypertension $^{*}p < 0.05$. *DII, Dietary Inflammatory Index.*

diet, indicated by a higher DII score, was related to a greater odd of sarcopenia and its component of muscle mass. Diet-related inflammation may affect muscle proteolysis and myocyte apoptosis, in turn leading to muscle loss and dysfunction (31). Overall, our findings provide convincing evidence on the hypothesized complex relationships and the necessity of clarifying the mechanisms underlying the relationships.

Mediation analyses suggested that there were significant mediating effects of depressive symptoms on the association between DII and low muscle mass, muscle mass, and hand grip strength. The finding indicated that a higher DII score was related to the higher risk of depressive symptoms, which in turn, led to sarcopenia independent of the direct effects of DII on sarcopenia. Similarly, our previous meta-analysis showed that a pro-inflammatory diet was associated with an increased risk of common mental health outcomes, such as depressive symptoms, anxiety, distress, and the association presented in a dose-response manner (32). Potential mechanisms underlying the association between DII and depressive symptoms may include oxidant-antioxidant imbalance and modified gut microbiota composition and activity (33, 34). Furthermore, we also observed that DII significantly mediated the association of depressive symptoms with low muscle mass and handgrip strength. Even though inflammation has often been mentioned as a potential mechanism in the relationship between depressive symptoms and musculoskeletal health, the mediating effect is the first time to be quantified. Likewise, our finding also coincides with previous literature suggesting that a chronic pro-inflammatory signaling has been identified as a key pathway from depression to a range of health outcomes (35, 36). Reportedly, psychological health has consistently influenced one's dietary intake (37). For instance, depression is thought to disrupt the hypothalamic-pituitary-adrenal axis system, which elevates cortisol levels, encouraging increased consumption of energy-dense foods, which influences the dietary quality and the overall inflammatory potential (38). In

line with the psycho-neuro-inflammatory theory, the present results improve our understanding the role of dietary and psychological factors in the pathogenesis of sarcopenia (39). In this sense, resolving both factors through efficient nutritional and psychological intervention might have potential to mitigate neuroinflammatory processes and prevent the development of sarcopenia.

Additionally, depressive symptoms were significantly associated with sarcopenia in the subgroup with a higher level of DII score in the stratified analyses. Previous studies suggested the moderating effect of inflammation on the association between depression and adverse health outcomes (40), such as all-cause mortality (41), to some certain degree supporting our findings. Due to the relatively small number of participants with sarcopenia affecting the statistical power, stratified analyses did not demonstrate significant association in individuals with the highest tertile of DII. Further research is necessary to replicate these findings in a larger sample. Although this result warrants further research, the findings suggest that the existence of depressive symptoms, together with a diet-induced inflammatory state, may be associated with higher odds of sarcopenia. Thus, it may be legitimate that integrated utilization of modulating dietary inflammatory potential and addressing depressive symptoms have important public health benefits in the prevention of sarcopenia.

Our study has several important strengths. First, this study included a nationally representative of the general population with a large sample size, which helps to provide convincing support of the hypothesized relationships. Second, the proposed models using mediation and stratified analyses incorporated psychological factors and lifestyle behaviors related to sarcopenia, which enables an insightful understanding of the inter-relationship of these factors with sarcopenia from a comprehensive perspective, thereby facilitating the development of targeted interventions aimed to prevent sarcopenia. Nevertheless, this study has some limitations that need to be mentioned. First, given the cross-sectional design, it is not possible to elucidate the causal associations among dietary inflammatory potential, depressive symptoms, and sarcopenia. Future studies with longitudinal design should seek to clarify the directionality and temporal relationships. Second, although several important confounding variables have been included in the models, there might be unmeasured covariates having a potential confounding effect on the associations. Future studies incorporating these measures are recommended to verify the present conclusion. Third, the mediation effects in our study were generally small. Considering that the etiology of sarcopenia is complex and multifactorial, other biological mechanisms, such as neuroendocrine responses (42, 43) and epigenetic changes (44), might also play a role in the pathophysiology of sarcopenia. It would be important to understand how these different biological processes influence each other. Moreover, future research is needed to incorporate other intermediate mechanisms in the biopsychosocial model of sarcopenia.

CONCLUSIONS

From a practical point of view, the findings of the present study during middle-aged adults may have significant clinical and public health implications. The observed associations provide a useful perspective for prevention and management of sarcopenia as it implies that sarcopenia is not only an age-related problem, but also one associated with a range of long-term conditions even early in mid-life. Comprehensive psychological and behavioral interventions, such as promoting an anti-inflammatory diet and improving mental health, have the potential for prevention and intervention of sarcopenia at earlier stages of life.

In summary, as hypothesized, depressive symptoms, and dietary inflammatory potential were found to be significantly associated with sarcopenia and its individual component low muscle mass. Specifically, both depressive symptoms and dietary inflammatory potential had significant direct effects, and indirect (mediation) effects on low muscle mass, handgrip strength, muscle mass, through each other in the adjusted mediation analyses. Therefore, integrated intervention strategies, such as promoting an anti-inflammatory diet and improving mental health, are suggested in the prevention of sarcopenia.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the National Center for Health Statistics (NCHS) and approved by the NCHS Institutional Review Board (IRB). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YL contributed to study concept, acquisition of data, and participated in critical revision of the manuscript. YL and G-QC participated in the analysis and interpretation of data. G-QC and G-PW contributed to drafting of the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.844917/full#supplementary-material

Supplementary Figure 1 | Flow chart of the study sample.

Supplementary Table 1 | Association between depressive symptoms and sarcopenia stratified by categories of DII.

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Effects of Time-Restricted Feeding on Energy Balance: A Cross-Over Trial in Healthy Subjects

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Time-restricted feeding (TRF) has been recently reported as an effective dietary intervention for losing body weight, implying a negative energy balance, without restricting nutrient intake. However, the detailed energy balance alteration caused by TRF remains unclear. This study was a randomized controlled clinical trial using a within-subject cross-over design. Twelve healthy, normal-weighted volunteers (age: 24 ± 2.3 years; BMI: 21.9 ± 1.71 kg/m^2; 7 females) were studied under a rigorous control for calorie intakes, physical activities as well as sleep-wake cycle to evaluate the energy balance systematically. Each participant consumed an isocaloric diet within either a 5.5-hour TRF or 11-hour control schedule. All energy intake and excretion were traced and collected and accessed by bomb calorimetry. Energy expenditure and substrates oxidation were monitored in a metabolic chamber. TRF compared with control schedule is associated with a 22.7% increase in fecal energy loss (Δ = 32.25 ± 9.33 Kcal, p = 0.005) and a trend in increasing 14.5% urine energy loss (Δ = 6.67 ± 3.14 Kcal, p = 0.058) without change energy expenditure. In total, a negative energy balance (Δ = -45.95 ± 19.00 Kcal, p = 0.034), which was equal to -2.6% of total energy intake, has been observed during TRF interventions. In the meantime, glycemic profiles, heart rate, respiration rate as well as metabolic flexibility were also improved during TRF intervention. Taken together, our findings unravel the mystery of how TRF regulates energy balance, supporting the use of TRF as an alternative dietary strategy for weight loss.

Keywords: time-restricted feeding, energy balance, energy excretion, energy expenditure, blood glucose (BG)

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INTRODUCTION

During the past few decades, the prevalence of obesity and related chronic diseases has increased dramatically (1). The current first line of therapy for overweight/obesity is aggressive lifestyle modifications including reducing calorie intake, improving diet quality, and increasing physical activity (2, 3). However, long-term adherence and maintenance to such strategies are challenging (4). Time-restricted feeding (TRF) is a specific intermittent fasting protocol that has gained

attention as a more acceptable and feasible regimen. Compared to the traditional calories restricted diets, TRF encourages individuals to consume their usual diet within a limited eating window without necessarily altering diet quantity or quality per se (5). Both cross-over and parallel-arms studies in humans have confirmed the modest reductions in body weight and fat mass after TRF intervention (6–13). TRF was also reported to improve glycemic and lipid profiles, reduced plasma triglycerides, inflammatory markers (10, 14, 15) and other metabolic parameters, including blood pressure, oxidative stress, and sleep quality (11, 16, 17).

Body weight changes are fundamentally based on an imbalance between energy intake and energy expenditure over a certain period of time (18, 19). Experiments in rodents have shown that TRF within an 8-hour period increases 24-hour energy expenditure. But this phenomenon has not been validated yet in human beings (20). Some studies speculated that TRF-induced 1-4% weight loss over a short duration (1-16weeks) may be caused by reduced energy intake. During the experiment, participants were allowed for ad libitum intake. Nevertheless, confining the period of eating window might reduce energy intake by ~350–500 kcal/day (8, 9, 11, 21–24). However, the limitation is that food intake measurement in these studies was either based on participants' self-declarations or left untracked (25). Compared with ad libitum intake, several isocaloric intake studies offer different perspectives. In these studies, the subjects consumed an isocaloric diet under rigorous control conditions, but their results are quite different. While both body weight and fat mass were decreased after 8 weeks or 4 weeks of TRF intervention, as reported by (10, 26) respectively, other studies have found no significant body weight change in TRF group compared with the control group (14, 27). The studies to date are limited, there is still controversy about how TRF regulates energy balance and thus affects body weight.

In this study, we performed a randomized within-subject cross-over trial to systematically evaluate the energy balance of TRF under rigorous control for calorie intakes, physical activities as well as the sleep-wake cycle. The primary aim of this study was to compare the energy balance of TRF (5.5-hour eating period) and control (11-hour eating period) schedules in healthy subjects. To systematically explain how TRF creates a negative energy balance without reducing calorie intakes. Secondary aims included the acute effects of TRF on blood glucose and physiological parameters.

MATERIALS AND METHODS

Ethical Approval

This study protocol was approved by the ethics committee of Ruijin Hospital affiliated to Shanghai Jiaotong University (Ref. No. 2019-246). Clinical trial registered as "The Impact of Time-Restricted Feeding (TRF) on Human Energy Metabolism" at chictr.org.cn as ChiCTR2000038421. A CONSORT flow diagram outlining the study protocol is displayed in **Figure 1**.

Participants

Fourteen healthy participants were recruited from 159 subjects who were interested in this study and submitted our lifestyle questionnaire online. Finally, twelve participants completed this study. The entry criteria included BMI ≤ 25 kg/m^2, Hb1Ac between 4.7 and 6.4%, fasting glucose between 70 and 110 mg/dl, normal lipid profiles and thyroid parameters, and steady eating habits with breakfast between 7:00 and 9:00, lunch between 11:00 and 14:00, and dinner between 18:00 and 20:00. We excluded subjects with extensive change of body weight (± 3%) and medication use over the past 3 months, diarrhea, diabetes, eating disorder, psychiatric disorder, breastfeeding or pregnant as well as time-shifted works.

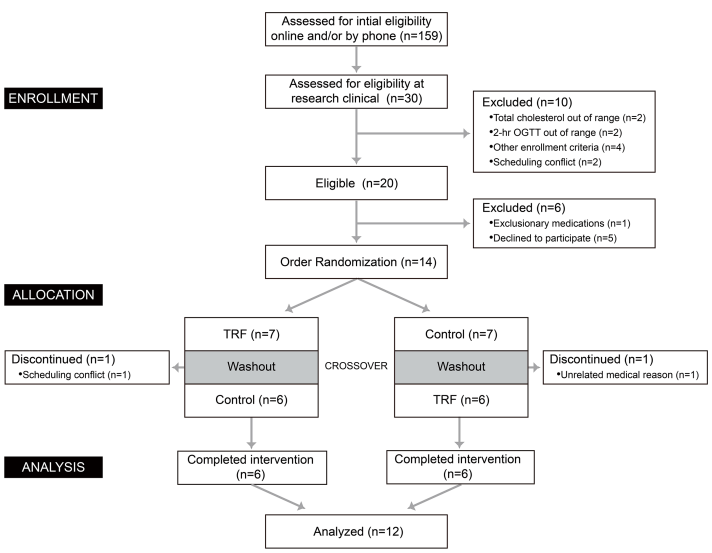

FIGURE 1 | CONSORT flow diagram indicating the design of the trial.

All participants provided written informed consent prior to participation in the trial.

Study Design

The total design of this study was exhibited in **Supplementary Figure 1A**. Each eligible subject underwent a cross-over dietary intervention, in which participants were treated with TRF (eat within 5.5 hours: first meal at 8:00, second meal at 10:45, and third meal at 13:30) and control (eat within 11 hours: first meal at 8:00, second meal at 13:30, and third meal at 19:00) treatment. The order of two dietary schedules for each individual was randomized with a one-week washout period. Each dietary intervention was confined to the metabolic chamber for 3 days, during which calories of each meal and physical activities were rigorously controlled. To exclude the compounding effect of food type, an artificial meal containing 55% carbohydrates, 30% fat, and 15% protein was provided. Participants underwent standardized acclimatization on the first day before the conduction of two diet interventions on the second day. Then all participants were asked to maintain the same lifestyle on the third day until all feces sourced from day 2 intervening food were completely excreted. During the first day and third day, all the food provided was labeled with carmine dye. During the second day, all food was labeled with brilliant green dye to trace and collect the excretion of feces from this day. Individual daily energy requirements were calculated based on body weight, height, and age, using the Harris-Benedict equation with a fixed physical active level (PAL) of 1.2, corresponding to low physical activity (28). Each meal was consumed at a constant rate within 30 minutes during the intervention. Daily fluid intake was provided with the standard of 30ml/kg body weight. Daily activities during the intervention were strictly controlled according to the standardized schedule (**Supplementary Figure 1B**). During the unconstrained hours, participants were only allowed to engage in mild activities including watching videos, using computers, walking in the chamber. To meet the PAL of 1.2, participants were asked to do 15 minutes of exercise on their 40% VO_{2max} level by using an ergometer 3 times every day. During the washout days, participants were asked to keep a stable lifestyle, maintain the diet habit as well as avoid intensive exercise, and the body-weight changes were controlled within 1%. Daytime was defined as 8:00 to 20:00 and nighttime was defined as 20:00 to 8:00 of the next day.

Calorie and Macronutrients in Food, Feces, and Urine

All food and feces labeled with brilliant green dye (used in the intervention) were collected, mixed well, and lyophilized. 24-hour urine from the intervention day was collected and underwent direct lyophilization. Lyophilization was performed at -50°C using an instrument (SJIA-5FE, ShuangJia Instrument, Ningbo, China). Calories were measured by using bomb calorimetry according to the published protocol (29) (Parr 6400 Calorimeter, PARR Instrument Co., Moline, IL). To minimize the error of measurements, all samples were measured repeatedly. If the difference between repeated samples > 50 Cal, measurements were repeated until the difference < 50 cal. We averaged two qualified measurements as the final calories of each sample. Food and fecal protein concentration were determined by the Kjeldahl method, fat concentration was determined by the Soxhlet method, and carbohydrates concentration was calculated by subtracting the protein, fat, water, and ash from the total weight of the sample.

Energy Expenditure and Substrates Oxidation

To measure the substrate oxidation and energy expenditure, participants resided in the metabolic chamber for the whole experiment period. The metabolic chamber is an airtight room with a volume of 30,000L each (Fuji Medical Science Co. Ltd., Chiba, Japan). The chamber is furnished with an adjustable bed, desk, chair, bicycle ergometer, wash basin, and toilet. Air in the chamber was pumped out at a rate of 70 L/min. Room temperature and relative humidity were maintained at 25.0 ± 0.1°C and 50.0 ± 1.5%, respectively. Concentrations of oxygen (O2) and carbon dioxide (CO2) in the sample air were analyzed using an online process mass spectrometer (Prima PRO, Thermo Fisher Scientific, Cheshire, UK) after dehydration. The mass spectrometry was calibrated monthly using standard gas and the accuracy for O2 and CO2 is 0.0013%. O2 consumption (VO2) and CO2 production (VCO2) were calculated by Henning method (30). To minimize the error of the metabolic chamber, we calibrated the accuracy by alcohol combustion. The precision of metabolic chamber was 99.2 ± 0.9% for O2 consumption and 100.0 ± 1.1% for CO2 production during the study. VO2 and VCO2 data produced by Henning's method were obtained at the frequency of 1 min. And then we aggregated these minute-based data into hourly-based for statistical comparison. Macronutrient oxidation and energy expenditure were calculated using the Weir equation with urinary nitrogen excretion (31). To correct the measured RQ for protein oxidation, nonprotein RQ (npRQ) was calculated by using nitrogen excretion in 24-h urine. Twenty-four-hour thermic effect of food (TEF) was determined by plotting energy expenditure against physical activity level, and the intercept of the regression line at the lowest physical activity represents SMR and TEF. All women performed respiratory chamber testing during the follicular or luteal phases of their menstrual cycle.

Continuous Glucose Monitoring

All participants were fitted with a CGM sensor and transmitter (Dexcom G5, Dexcom, San Diego, CA, USA) during the study period. The glucose-oxidase-based electrochemical sensor was inserted into subcutaneous tissue of the abdomen, followed by an initial warm-up period for 2 hours after sensor insertion. Sensor insertion began the day before the first acclimatization day and there was two days of calibration before data collection during the dietary intervention. According to company recommendations, the sensors were calibrated at least once every 12 hours by finger prick (FreeStyle Lite; Abbott Laboratories, Abbott Park, IL).

After a washout period, participants were asked to insert a new sensor the day before the study.

Maximal Oxygen Uptake

Prior to the trial, each participant performed a maximal oxygen uptake (VO_{2max}) test on a cycle ergometer (939E, Monark Ltd, Vansbro, Sweden). Oxygen consumption and carbon dioxide production were measured by a breath-by-breath portable gas analyzer (K5, COSMED, Rome, Italy). The test procedure started with 5 minutes warm-up at a workload of 0 W. Then, the workload was increased by 25W or 20W every minute for men or women, respectively, while participants maintained a pedaling rate of 60 rpm until exhaustion despite verbal encouragement. Participants met at least 2 of the following criteria were considered maximal: respiratory exchange ratio > 1.15, maximal heart rate > 90% of the predicted max (220-age), perceived exertion (RPE) ≥ 18, a plateau in VO2 despite an increasing workload. The exercise intensity was set at 40% VO_{2max} (light intensity) in this study.

Physiological Parameters Measure

Noninvasive blood pressure, 3-lead ECG, and peripheral pulse oximetry SpO2 were continuously monitored by a Cardiac Telemetry System (WEP-5204C, Nihon Kohden Co., Tokyo, Japan). Systolic and diastolic blood pressure were measured every 10 minutes during the study. Heart rate, breath rate, and SpO2 were measured every second during the study. Equivital LifeMonitor system (EQ02 LifeMonitor, Hidalgo Ltd, Cambridge, UK), capable of logging physiological data including respiratory inductance plethysmography, posture, activity, and skin temperature every 15 seconds was used during study. Participants were fitted with a correctly sized chest vest dependent on their chest circumference. Axillary skin temperature was measured by an infrared sensor. Ambulation status was measured by accelerometer sensor and divided into different levels: stationary, moving slowly, and moving fast. Bioelectrical impedance analysis (Inbody 770, Inbody Co. Ltd., Seoul, Korea) was used to measure body composition and weight. Height was measured in centimeters using a height measurement instrument (RGZ-120, DongFang Scales Co. Ltd., Shanghai, China).

Biochemical Analysis

Blood sampling was performed at 0h before and 0.5h, 1h, and 2h after every three meals during the intervention. Samples were collected by using a standard venipuncture. Plasma and serum were centrifugated at 4°C and stored at -80°C until analysis. Insulin was measured by an autoanalyzer (ARCHITECT ci16200 analyzer, Abbott Laboratories, USA).

Appetite and Sleepiness by VAS

Participants rated their hunger, fullness, stomach fullness, desire to eat, capacity to eat, and sleepiness using Visual Analog Scales (VAS; a 0-100 mm scale). The higher the scale, the stronger the sensation. VAS surveys were administered for total fourteen times at pre-meal (0h), 0.5, 1, and 2 hours post meal, 10:00 pm, and the next day morning at 7:00 am.

Statistical Analysis

Based on our preliminary data and a previous report (29), we assumed that the 24-hour calories excretion is 6.7 ± 1.2% of intake during control dietary schedule and 8.6 ± 2.7% of intake during TRF. We calculated that 12 participants in each group would be needed to give this study 82% power to detect 1.9% difference of calories excretion in feces at a two-side alpha value of 0.05.

Participants were randomly allocated in 1:1 ratio to 5.5-hour TRF group or 11-hour control group. After the washout period, crossover was carried out for both groups. The generation of allocation sequence was based on the random-number table.

Statistical analyses were performed using R Studio (version 3.6.0, Boston, Massachusetts). Since the sample size of this study is comparatively small, we exert pairwise t-test with Holm–Bonferroni adjustment to reduce the sampling error. A two-tailed p value of ≤ 0.05 was considered as statistically significant. The Shapiro-Wilk test was used to check the data distribution. For parameters with non-Gaussian distribution, the Mann-Whitney test was used. For parameters with normal Gaussian distribution, a two-tailed pairwise t-test was applied. When applicable, non-parametric Wilcoxon matched-pairs signed ranks test was used. To analysis the time course data, two-way repeated measures ANOVA models were used to evaluated the interaction between time and groups on dependent variable. Baseline data are reported as mean ± standard deviation (SD), and other data are presented as mean ± standard error of the mean (SEM). AUCs were calculated by the trapezoidal rule. To evaluate the contribution of insulin and carbohydrate oxidation in explaining TRF-induced change of postprandial glycemic response, a linear mixed-effects model with incremental glucose AUC as the dependent variable, diet intervention (control as the reference) and pre-prandial glucose as independent variable, participants and meals as random effect was constructed, and pre-prandial insulin and postprandial carbohydrate oxidation were added as independent variable to observed the changes of each effect.

RESULTS

Participants

Fourteen participants were included and twelve (5 men and 7 women) of them completed this study (**Figure 1**). Each participant underwent a crossover trial, in which participants were treated with TRF (eating period: 8:00 AM to 13:30 AM) and control (eating period: 8:00 AM to 19:00 AM) dietary schedules. The order of two dietary schedules for each individual was randomized and the two interventions were separated by a one-week washout period. During the washout period, participants kept their usual lifestyle and the mean body weight upon entering the chamber were same (60.65 ± 2.80 kg versus 60.70 ± 2.77 kg, Δ = 0.05 ± 0.12 kg, p = 0.988). The baseline characteristics of participants were measured from 6:00 to 7:00 in the morning of the intervening day (**Table 1**), during which participants maintained supine position without other activities.

Neither biochemical nor physiological profiles were significantly different between groups.

Energy Intake

Participants were designed to receive an isocaloric, controlled-nutrient food containing 55% carbohydrate, 30% fat, and 15% protein throughout the study. We confirmed the food energy density and macronutrients components by bomb calorimetry and chemical method, respectively. The energy density (Δ = 0.02 ± 0.05 Kcal/g, p = 0.666) and macronutrients are stable and equal except for labeled with brilliant blue or carmine dyes (**Supplementary Table 1**). As shown in **Table 2**, no significant differences in total energy, carbohydrate, fat, and protein intake were observed. Meanwhile, the intake time of three meals also showed no statistical difference. Any beverages containing energy were prohibited, and only mineral water was provided based on 30ml/kg body weight. Fluid intake volumes were same among two groups.

Energy Expenditure

In **Figure 2A** there is an overview of hourly energy expenditure for two groups. There was a statistically different interaction between time and groups on energy expenditure ($F(23,253) = 2.804$, $p<0.001$, **Supplementary Table 2**). Compared with control group, TRF slightly increased energy expenditure after second meal for 2 hours from 11:00 (Δ = 0.182 ± 0.055 Kcal/min, p = 0.007) to 12:00 (Δ = 0.237 ± 0.080 Kcal/min, p = 0.013). In contrast, after the third meal in control group, energy expenditure was also slightly increased for 2 hours from 19:00 (Δ = 0.226 ± 0.037 Kcal/min, p < 0.0001) to 20:00 (Δ = 0.147 ± 0.059 Kcal/min, p = 0.031). These differences were mainly attributed to postprandial TEF. But no significant difference was found in total TEF (Δ = 1.08 ± 3.45 %, p = 0.760) after reciprocal compensation (**Figure 2B**). The 24-hour mean energy expenditure (Δ = 0.005 ± 0.015 Kcal/min, p = 0.763), daytime mean energy expenditure (Δ = 0.040 ± 0.030 Kcal/min, p = 0.211), nighttime mean energy expenditure (Δ = -0.031 ± 0.019 Kcal/min, p = 0.129), basal metabolic rate (BMR) (Δ =0.015 ± 0.034 Kcal/min, p = 0.690), and sleeping metabolic rate (SMR) (Δ = -0.005 ± 0.021 Kcal/min, p = 0.826) were not significantly affected by TRF (**Figure 2C**). Furthermore, the exercise activity thermogenesis (EAT) (p = 0.223) as well as non-exercise activity thermogenesis (NEAT) (p = 0.827) were also not affected (**Figure 2D**).

Energy Balance

As shown in previous results, the energy intake and expenditure showed no difference between groups and then we examined a third part, as an often-neglected factor, fecal energy loss. Surprisingly, after TRF intervention, total fecal wet weight (Δ = 18.14 ± 5.02 g, p = 0.004) was higher than control group while the percentage of water (Δ = 0.58 ± 1.02%, p = 0.579) was unaffected (**Figure 3**). The absolute number of calories lost in TRF group was significantly increased by 22.7% (Δ = 32.25 ± 9.33 Kcal, 174.28 ± 18.04 Kcal versus 142.03 ± 17.33 Kcal, p = 0.005) compared to control group. Macronutrients of feces including carbohydrate, fat, and protein (all p ≤ 0.047) were also increased accordingly. The transit time (Δ = -3.86 ± 4.14 hours, p = 0.371), as interval between the first meal labeled with brilliant blue and the last feces discharging with the same dye marker, was unaffected during TRF intervention. The change in the total volume of urine (Δ = -52.6 ± 91.01 ml, p = 0.575) between TRF and control group was not detected. However, calories in urine showed 14.5% (Δ = 6.67 ± 3.14 Kcal, p = 0.058) elevation in

TABLE 1 | Basal characteristics of participants and pre-intervention parameters.

	Control	TRE	p Value
Participants characteristics			
N	12	12	–
Age (Year)	24 ± 2.3	–	–
BMI (Kg/m^2)	21.9 ± 1.71	–	–
HbA1c (%)	5.11 ± 0.19	–	–
Fasting glucose (mg/dl)	91.44 ± 6.66	–	–
Fasting insulin (μIU/mL)	7.63 ± 3.96	–	–
Triglyceride (mmol/L)	0.81 ± 0.19	–	–
Total cholesterol (mmol/L)	4.86 ± 0.79	–	–
LDL (mmol/L)	2.96 ± 0.76	–	–
HDL (mmol/L)	1.62 ± 0.27	–	–
Pre-intervention parameters			
Blood glucose (mg/dL)	92.97 ± 10.23	91.53 ± 7.76	0.703
Basal metabolic rate (Cal/Kg)	11.94 ± 2.28	11.06 ± 2.29	0.36
Respiratory quotient	0.84 ± 0.06	0.85 ± 0.06	0.724
Resting heart rate (BMP)	61.56 ± 8.79	61.13 ± 7.04	0.896
Resting respiratory rate (RPM)	17.41 ± 1.85	16.82 ± 1.62	0.418
Skin temperature (°C)	36.69 ± 0.85	36.62 ± 0.59	0.834
Systolic BP (mmHg)	102.21 ± 5.75	103.54 ± 7.8	0.639
Diastolic BP (mmHg)	61.83 ± 6.85	63.82 ± 6.07	0.46
Oxygen saturation (%)	98.26 ± 0.7	98.36 ± 0.9	0.765

Data were presented as mean ± SD.

Differences between group were tested by pairwise t-test with Holm–Bonferroni adjustment

Pre-intervention parameters were collected from 6:00 to 7:00 in the morning of intervening day, during which participants maintained supine position without other activities.

TABLE 2 | Energy intake during 24-Hour TRF versus control intervention.

	Control	TRF	Percent change	p Value
Energy intake				
Total energy (Kcal)	1807.07 ± 67.95	1806.05 ± 67.16	0%	0.992
Carbohydrate (Kcal)	1045.61 ± 39.62	1045.01 ± 39.16	0%	0.992
Fat (Kcal)	520.06 ± 19.35	519.76 ± 19.13	0%	0.991
Protein (Kcal)	241.41 ± 8.98	241.27 ± 8.88	0%	0.991
Intake time				
First meal (min)	26.25 ± 1.40	25.83 ± 1.52	-2%	0.842
Second meal (min)	25.58 ± 1.29	26.67 ± 1.59	4%	0.603
Third meal (min)	25.67 ± 1.16	24.50 ± 1.41	-5%	0.530
Fluid intake				
Fluid volume (ml)	2023.09 ± 95.51	2064.50 ± 102.54	2%	0.770

Data were presented as mean ± SEM.

Differences between group were tested by pairwise t-test with Holm–Bonferroni adjustment.

The percentage change was calculated by dividing the differences of mean between TRF and control groups.

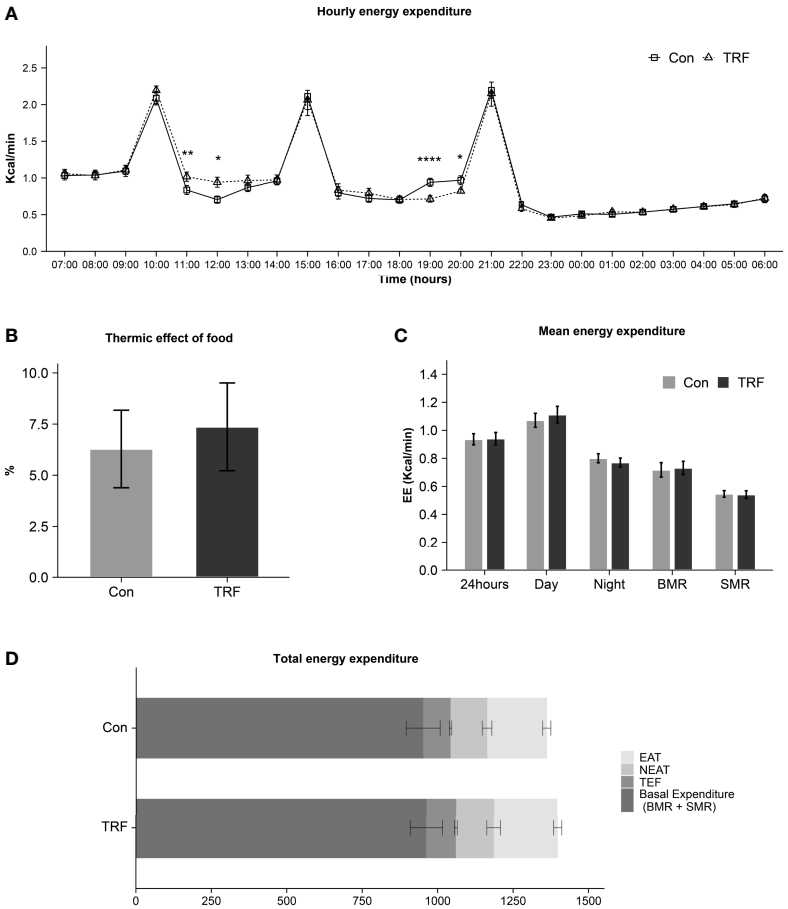

FIGURE 2 | Hourly energy expenditure **(A)**, thermic effect of food **(B)**, mean energy expenditure **(C)**, and total energy expenditure **(D)** between control and TRF groups. Data are mean ± SEM, *P <0.05, **P<0.01, ****P≤ 0.0001.

TRF group that nearly reached statistical significance. In aggregate, **Figure 3J** shows the energy balance status of both groups. Compared with control group, TRF intervention induced a lower energy balance level (226.69 ± 28.56 Kcal versus 272.64 ± 28.16 Kcal, Δ = -45.95 ± 19.00 Kcal, p = 0.034), mainly as a result of increased fecal energy losses.

Substrate Oxidation

An overview of hourly npRQ was shown in **Figure 4A**, there was a statistically different interaction between time and groups on RQ ($F_{(23,253)}$ = 6.603, p<0.001, **Supplementary Table 2**). Compared with control group, TRF increased npRQ nearly continuously across a 7-hour period from 12:00 to 18:00, except for 16:00 (all p ≤ 0.046). In contrast, control group increased npRQ after the third meal for only 2-hour period from 20:00 to 21:00 (all p < 0.001). As shown in **Figures 4B, C**, 24-hour mean npRQ (Δ = 0.018 ± 0.007, p = 0.030) and diurnal mean npRQ (Δ = 0.053 ± 0.008, p < 0.0001) were increased in TRF group. Nocturnal mean npRQ (Δ = -0.017 ± 0.008, p = 0.073) was marginally decreased in TRF group. The day to night transition, as npRQ was higher at daytime and have a lower trend at nighttime in TRF, which can be consider as a marker of metabolic flexibility (Δ = -0.070 ± 0.007, p < 0.001), was significantly increased when compared with control group. The 24-hour net oxidation of carbohydrate (Δ = 16.60 ± 8.32 g/day, p = 0.071) was marginally increased during TRF intervention as compared to control group, and fat oxidation (Δ = -5.87 ± 2.85 g/day, p = 0.064) was tended to decrease in TRF group. No statistical difference was observed in protein oxidation (Δ = -2.77 ± 1.86 g/day, p = 0.165) between groups. After correction for macronutrients intake and lost in feces, carbohydrate balance was significantly decreased in TRF group, as residual carbohydrate was 17.88g ± 8.13 g/day (p = 0.050) less than control group. But no differences were observed in either fat balance (Δ = 4.89 ± 2.92 g/day, p = 0.122) or protein balance (Δ =1.08 ± 1.84 g/day, p = 0.567, **Figures 4D, E**).

Biochemical and Physiological Parameters

CGM data revealed that 24-hour average blood glucose was significantly lower during TRF treatment as compared to control (Δ = -0.27 ± 0.05 mmol/L, p = 0.007, **Figures 5A, B**), which were mainly contributed by a significant reduction of diurnal blood glucose (Δ = -0.33 ± 0.06 mmol/L, p = 0.001), whereas nocturnal glucose level exhibited no statistical difference between groups

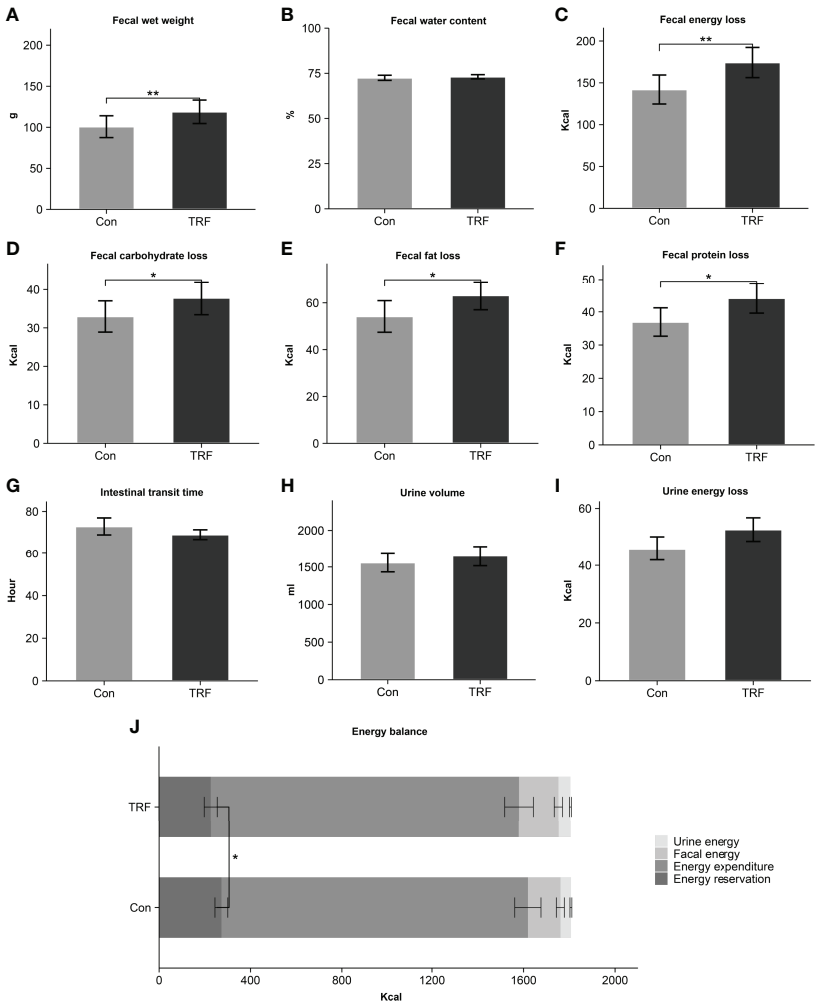

FIGURE 3 | Fecal wet weight **(A)**, fecal water content **(B)**, fecal energy loss **(C)**, fecal carbohydrate loss **(D)**, fecal fat loss **(E)**, fecal protein loss **(F)**, intestinal transit time **(G)**, urine volume **(H)**, urine energy loss **(I)**, and energy balance **(J)** between control and TRF groups. Data are mean ± SEM, *P <0.05, **P<0.01.

(**Supplementary Table 3**). Glycemic variability was significantly blunted during TRF intervention, as reflected by the significantly lower coefficient of variation (CV) (Δ = -0.06 ± 0.01%, p=0.007) and mean amplitude of glycemic excursions (MAGE) (Δ = -1.16 ± 0.08 mmol/L, p=0.014, **Figures 5C–E**). Postprandial glycemic and insulinogenic responses to the two time-shifted meals (the 2nd and 3rd meals) were both significantly attenuated in TRF group as compared to control. The incremental area under curves of postprandial glucose and insulin were paralleling decreased (**Figures 5F, G** and **Supplementary Table 4**). Our mixed-linear model revealed that such extensive reduction of glycemic response to food during TRF could be partially explained by its pre-prandial glucose and insulin level (Pre-prandial glucose: $p < 0.001$; Pre-prandial insulin: $p = 0.015$), but no contribution from carbohydrate oxidation was observed ($P = 0.207$). There is still a large proportion of TRF-induced hypoglycemic effect that could not be explained by current data ($P = 0.002$, **Supplementary Table 5**). Pre- and post-prandial free fatty acid (FFA) were measured simultaneously with insulin and intravenous blood glucose. While no statistical differences of FFA between groups were observed around the 1st meal, both pre- and post-prandial FFA were substantially reduced during TRF (**Figure 5H** and **Supplementary Table 6**). There were a statistically different interactions between time and groups on heart rate ($F(23,230) = 11,109$, $p < 0.001$) and respiratory rate ($F(23, 230) = 1.906$, $p = 0.009$, **Supplementary Table 7**). As compared to control, heart rate was significantly higher for 3-hour period from 11:00 to 13:00 (all $p \leq 0.001$) during TRF intervention. But continuously lower for almost 11-hour period from 19:00 to 05:00 (all $p \leq 0.038$), except for 23:00 and 01:00 (**Figure 6A**). As a result, mean heart rate during the nighttime (Δ = -2.93 ± 0.59 beats/min, $p < 0.001$) and exercise (Δ = -4.07 ± 0.88 beats/min, $p < 0.001$) was significantly lower in TRF (**Figure 6B**). Nocturnal respiration rate (Δ = -0.58 ± 0.26 breaths/min, p=0.042) was slightly reduced in TRF group (**Figures 6G, H**). However, there were no significant interaction between time and group on either blood pressure or

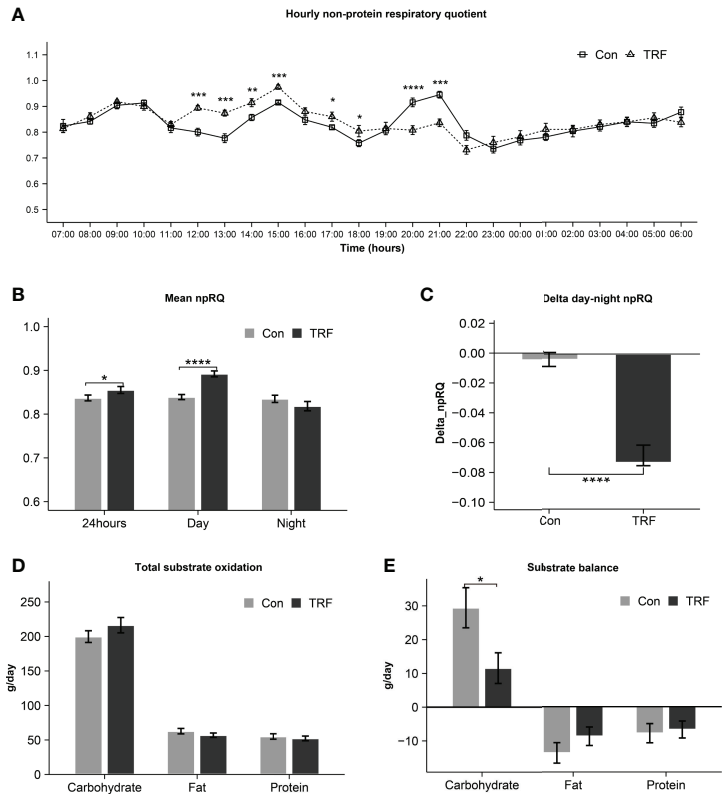

FIGURE 4 | Hourly npRQ **(A)**, mean npRQ **(B)**, delta day-night npRQ **(C)**, total substrate oxidation **(D)**, and substrate balance **(E)** between control and TRF groups. Data are mean ± SEM, *P <0.05, **P<0.01, ***P<0.001, ****P≤0.0001.

skin temperature (**Supplementary Table 7**). Neither hourly nor 24h-average data in blood pressure and skin temperature alternation were observed (**Figures 6C–F, I, J**).

Appetite and Sleepiness Level by VAS

As shown in **Figure 7**, we also evaluated the subjective appetite and sleepiness level by VAS. Significant interactions between time and groups on hunger, fullness was observed in stomach fullness, desire to eat, and capacity to eat were showed in the two-way repeated ANOVA model (**Supplementary Table 8**). While no statistical differences in five appetite indicators were observed at the timepoints of pre-prandial before each meals and 2-hour after the first meal, TRF group exhibited a significant lower score in hunger, desire to eat, and capacity to eat levels (all $p \leq 0.026$). Accordingly, the scores of fullness and stomach fullness levels were significantly higher before the third meal in the TRF group (all $p \leq 0.005$). It is worth noting that, at the timepoint of before sleep, TRF substantially increased the score of hunger ($\Delta = 15.91 \pm 4.66$ mm, p=0.006), desire to eat ($\Delta = 24.45 \pm 3.29$ mm, $p < 0.001$), and capacity to eat ($\Delta = 13.45 \pm 3.49$ mm, p=0.003), as well as reduced the fullness level ($\Delta = -15.18 \pm 4.49$ mm, p=0.007). However, there were not any significant differences in subjective appetite between groups the next day morning. What's more, subjective sleepiness level was unaffected by TRF intervention.

DISCUSSION

An increasing number of studies have demonstrated that TRF intervention can produce 1-4% weight loss over 1 to 16 weeks. Although current available data did not support a stronger effect of TRF on body weight and metabolic improvement as compared to continuous caloric restriction, the mechanistic explanation behind this phenomenon remains raised great interest to researchers. Some controversial issues, including inaccurate recording of food intake, unmonitored energy expenditure and the involvement of physiological adaptation were still need to be determined (8–11, 13, 14, 21–24, 26, 32, 33). Therefore, we conducted the first rigorous trial to systematically quantify and compare the energy balance during TRF intervention in healthy subjects. We strictly controlled and ensured that energy intake, fluid intake, and lifestyle were consistent during the interventions except for eating schedules, while energy expenditure was monitored using metabolic chamber and all energy excretion including feces and urine were measured by bomb calorimeter. Surprisingly, TRF could evoke a significant fecal energy loss and a trend in urine energy loss without energy expenditure alteration, which caused a negative energy balance, while 24-hour blood glucose and heart rate were also improved during TRF. Our findings are consistent with the benefits of

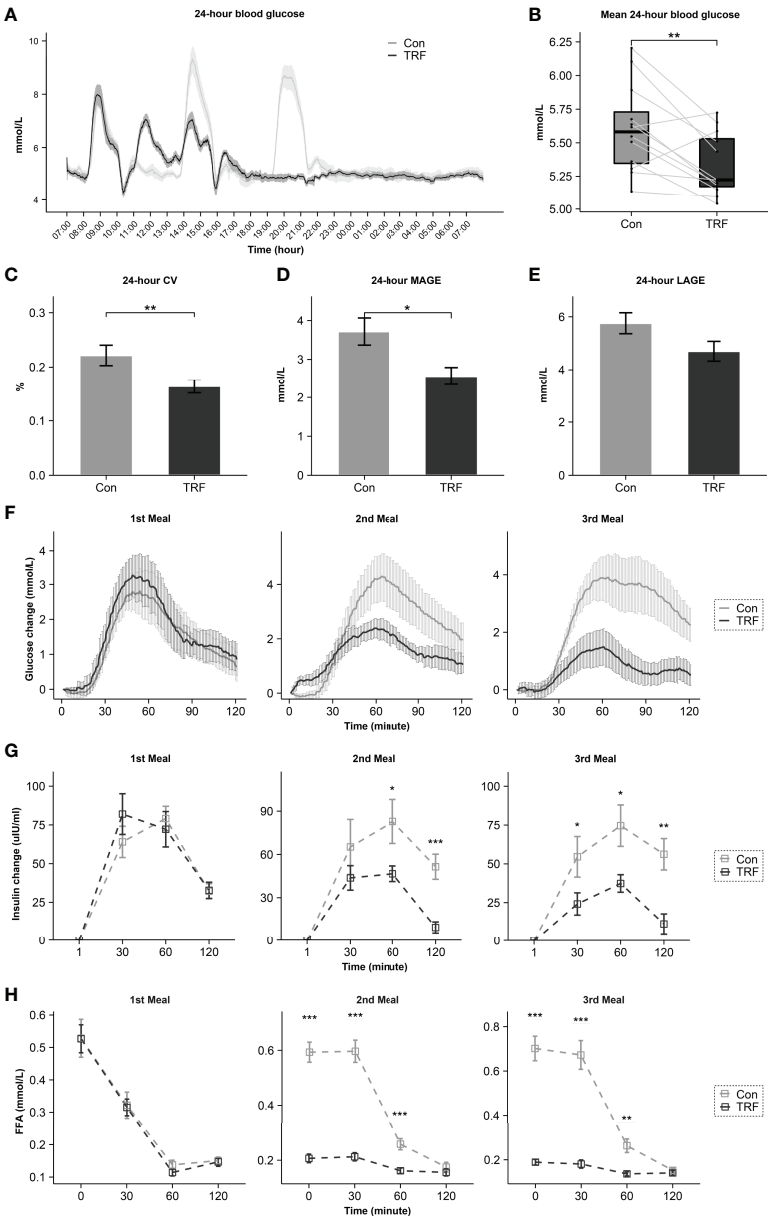

FIGURE 5 | The effect of TRF on 24-hour CGM data **(A)**. Comparison of 24-hour mean glucose **(B)**, CV **(C)**, MAGE **(D)**, LAGE **(E)**, postprandial glycemic **(F)**, postprandial insulin **(G)**, and postprandial FFA **(H)** between control and TRF groups. Data are mean ± SEM, *P <0.05, **P<0.01, ***P<0.001.

long-term TRF intervention reported previously, supporting the use of TRF as an alternative dietary strategy for obesity.

In this study, we found no significant differences in total energy expenditure during the whole intervention period. Despite that some studies suggested that TRF induced adiponectin elevation might contribute to increased energy expenditure (10, 25, 34), the results of the current study are consistent with previous studies: through 24-hour energy monitoring or resting metabolic rate measurements, there is no convincing evidence in support of increased energy expenditure due to TRF (20, 26, 35). But several components of total energy expenditure are inconsistent with a prior study, including higher TEF, increased diurnal energy expenditure, and decreased nocturnal energy expenditure in TRF group (20). These negligible differences might be explained by different food macronutrient compositions and time schedules among two studies, respectively.

About 2-10% of food consumed ends up being excreted in feces. Even if the nutrients enter the circulation, there is still 1-2% of total energy intake finally being filtered by the kidneys (36). The precise measurement of the third and often neglected factor, energy excretion finally helped reveal that TRF has resulted in a

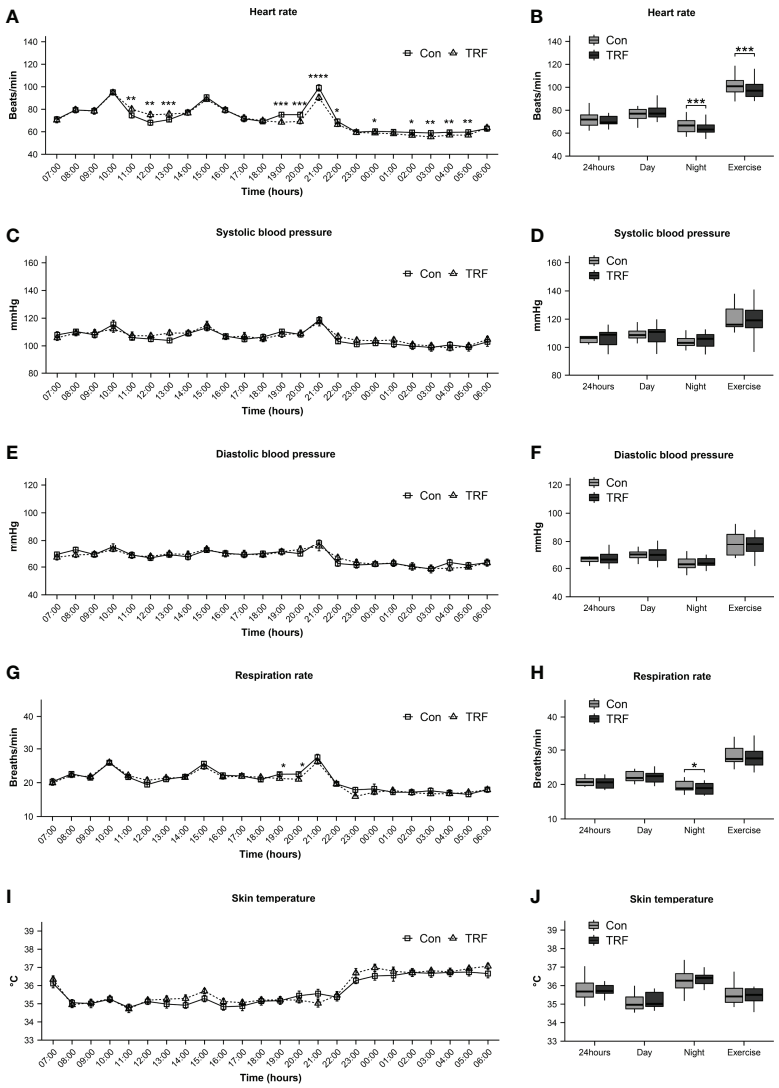

FIGURE 6 | Heart rate **(A, B)**, systolic blood pressure **(C, D)**, diastolic blood pressure **(E, F)**, respiration rate **(G, H)**, and skin temperature **(I, J)** between TRF and control group. Data are mean ± SEM, *P <0.05, **P<0.01, ***P<0.001,****P≤0.0001.

negative energy balance of -46 Kcal, which is equivalent to -2.6% of total energy intake, as a result of increasing fecal (by nearly 22.7%) and urine (by nearly 14.5%) energy loss. Recently, Balso et al. showed that relative fecal energy loss is on average 6% during overfeeding and 9% during underfeeding, further confirming the variability of energy excretion (29). The magnitude of 2.6% energy losing effect could be translated to 0.7 Kg body weight loss in an 83.9 Kg subject over 8 weeks (19). In Moro et al. study, 8-week TRF under isocaloric condition led to average 0.97 Kg weight loss in 17 healthy subjects and our data indicated that TRF induced negative energy balance might explain approximately 72.2% of their weight loss in their long-term intervention (10). In McAllister et al. study, isocaloric treatment in TRF group showed 0.6kg weight loss after 4 weeks of intervention. The –2.6% energy losing effect could be translated to 0.4Kg body weight loss during the same condition, which explained nearly 66.7% of the weight loss (26). In Kahleova et al. study, participants underwent the same calorie restriction amount but with different intervention regimens, either six or two meals a day. After 12 weeks of intervention, body weight decreased (1.4 kg) more in two meals group (breakfast and lunch) than six meals group, although dietary intake, step count, and REE showed no differences. Here, our model could be translated to 0.9kg body weight loss, which explained 64.3% of the total difference (37). Tiny deviations from energy balance, on the order of 1-2% of daily energy intake, can indeed result in considerable long-term changes in body weight (~20 kg) (38). Therefore, the potential long-term implications of such a negative energy balance must not be underestimated. The current energy balance model still cannot explain a small part of weight loss. Therefore, it is undoubtedly necessary to directly evaluate the long-term impact of TRF on energy balance.

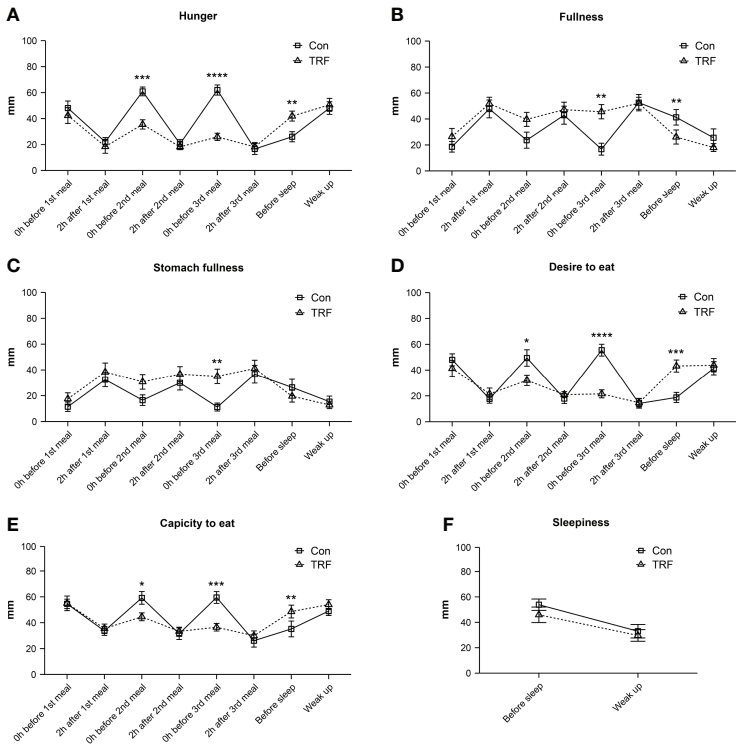

FIGURE 7 | Subjective hunger **(A)**, fullness **(B)**, stomach fullness **(C)**, desire to eat **(D)**, capacity to eat **(E)**, and sleepiness **(F)** responses to VAS from participants during TRF and control intervention. Data are mean ± SEM, *P <0.05, **P<0.01, ***P<0.001,****P≤0.0001.

Metabolic flexibility represents the capacity of body to adapted nutrient challenge from environment. Our results, consistent with the previous study, suggested that TRF intervention significantly enhanced metabolic flexibility (20), but this result should be interpreted with caution, since the dinner in the control group should largely affected the nighttime RQ, such substantial alteration in the delta npRQ might be caused by the imbalance eating schedule, rather than the equal-proportion improvement of adaptation ability (39).Contrary to the previous study, we found that the substrate balance significantly shifted towards carbohydrate in TRF group and npRQ increased accordingly (20). These conflicts may induce by the different experiment schedules. It is also possible that different food compositions and healthy or overweight participants may account for the conflicts. Future investigations including acute and chronic effects of TRF on RQ are encouraged.

Hypoglycemic effect of TRF has also been proved, as in line with previous studies (10, 14, 17). The average glucose over 24-hour TRF intervention was reduced by 5%, which was largely contributed by the extensive attenuation of postprandial glycemic profiles in TRF group. The postprandial insulin secretion was also reduced in TRF group. Therefore, it is unlikely to associate the hypoglycemic effect of TRF with extra insulin secretion. It should be noted that, FFA is a well-known marker of insulin resistance, it could blunt the hypoglycemic effect of insulin by increasing beta-oxidation in the mitochondrial. Thus, as the pre-prandial FFA substantially reduced before 2nd and 3rd meal during TRF, subjects might stay in a more insulin-sensitized status, which explained the simultaneously reduction of postprandial glucose and insulin. Moreover, the FFA levels were constant lower during eating period in TRF group, suggesting a remarkable improvement of lipotoxicity over 24 hours. This might explain the better insulin sensitivity after long-term TRF intervention. Consistently our linear mixed-effects model on postprandial glucose also emphasized the importance of pre-prandial status in explaining the improvement of glycemic profiles during TRF. This model suggested an independent contribution of pre-prandial insulin to TRF-induced glycemic improvement. However, there is still a large part of TRF effect that could not be explained, which deserves to be further studied. Additionally, attenuation of postprandial glycemic responses in TRF group resulted in a remarkable attenuation of glycemic variability over 24 hours, MAGE and glycemic CV were significantly reduced by 26% and 31% respectively over 24-hour TRF intervention. Since glycemic variability has been demonstrated to be closely correlated to oxidative stress and beta-cell function in previous studies (39), such extensive attenuation of glucose fluctuation may help to explain the long-term benefits of TRF for glycemic control (40).

As regarded to the physiological profiles during TRF, we observed a paralleling tendency of energy expenditure, heart rate, and respiratory rate during 11:00 to 13:00 and 19:00 to 21:00, strongly suggested a cardiovascular/respiratory-related thermogenic process, which would be inevitably reflected in the flux of body temperature, but we didn't observe such a corresponding change in

our data. This might due to the infrared sensors in this study, and the responsiveness of the sensor, as time constant, is about 90 minutes. This may largely blunt its sensitivity to skin temperature alternations. The hyperresponsiveness skin temperature and core temperature sensors are needed to capture subtle changes. We didn't observe a decrease in blood pressure and even a temporal increase in systolic blood pressure during postprandial period at TRF 3rd meal, which is inconsistent with previous data (24). Our participants were healthy subjects and only 24-hour TRF intervention could explain this disagreement. Nevertheless, we did observe the TRF significantly reduced heart rate at nocturnal and exercise periods. There is increasing evidence that increased heart rate is associated with hypertension and mortality, while decreased heart rate could help improve the situation (41).

We also investigated the effect of TRF on subjective appetite. Interestingly, although the self-reported appetite scores showed that subjects exhibited higher hunger before bedtime during TRF, such hunger went to equal between groups in the following morning. Since the importance of adherence to dietary recommendations in successful weight loss during long-term (42), this bearable hunger make TRF a more feasible way to control body weight. These improvements of appetite related factors was corroborated by other studies during TRF intervention (16, 20). Despite an overall positive attitude towards TRF eating pattern, this barrier to adopting the TRF eating pattern needs further investigation, as how to overcome the increased desire to eat before bedtime until the next morning.

To the best of our knowledge, we designed the most stringent control study to evaluate the actual effect of TRF on energy balance. Some limitations of the present study should be considered. First, we only observed the acute effect of TRF on energy balance and physiological-biochemical parameters, which may not be long enough to reach a new energy balance or metabolic adaptation. Second, Lowe et al. study found that late TRF, as ate ad libitum from 12:00 PM until 8:00 PM, didn't reduce participants' body weight after 12-week intervention (33). Here, this study only evaluated the effect of early TRF within a fixed time window, thus we were unable to answer whether the energy balance induced by TRF in different time windows shows rhythmic oscillations. Compare with caloric restriction, TRF produces less than 5% weight loss in the trails, which do not meet the threshold of clinically significant weight loss. Whether TRF plus mild caloric restriction produce a 1 + 1>2 effect? Long-term and well-designed trials are needed find out the optimal TRF strategy. Third, the participants recruited in this study were all young healthy adults. Chaix et al. have proved that 9-hour TRF benefits was sex- and age-dependent in mice (43). Thus, whether our finding is equally applicable to other populations including different ages, sexes, and disease states such as obesity and diabetes needs to be further investigated.

In conclusion, our study proved that TRF could induce negative energy balance by increasing fecal and urine energy excretion, and this seemingly negligible energy excretion elevation can explain up to 64.3% - 72.2% of weight loss in previous studies. As a result, the mystery of how TRF regulates energy balance might be unraveled, and hence provides a fundamental explanatory mechanism for weight loss. Our data also indicated an improvement in glycemic profiles, heart rate, respiration rate as well as metabolic flexibility during TRF. However, subjective appetite increased prior to bedtime might hinder the execution of TRF strategy in the long term. In light of these promising results, future research is needed to better elucidate the long-term metabolic adaptation of TRF, including the ketone body concentration and interconversion among macronutrients. On the other hand, more randomize controlled studies with well-designed and diverse population are also needed.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics committee of Ruijin Hospital affiliated to Shanghai Jiaotong University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RB and YS contributed to project management, participants recruitment, data collection, and data analysis. YJ and LY contributed to acquiring ethical approval for this trial. JH and WW contributed to acquiring funding for this trial and oversaw the design, and execution for this study. All authors contributed to the composition and revision of the manuscript and gave final approval of its content.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.870054/full#supplementary-material

Supplementary Figure 1 | – Study Design. **(A)** The overall study design over 3 days. **(B)** The schedule of daily activity during the interventions.

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High Dietary Salt Intake Is Associated With Histone Methylation in Salt-Sensitive Individuals

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Background: High salt diet is one of the important risk factors of hypertension and cardiovascular diseases. Increasingly strong evidence supports epigenetic mechanisms' significant role in hypertension. We aimed to explore associations of epigenetics with high salt diet, salt sensitivity (SS), and SS hypertension.

Methods: We conducted a dietary intervention study of chronic salt loading in 339 subjects from northern China in 2004 and divided the subjects into different salt sensitivity phenotypes. A total of 152 participants were randomly selected from the same cohort for follow-up in 2018 to explore the effect of a high-salt diet on serum monomethylation of H3K4 (H3K4me1), histone methyltransferase Set7, and lysine-specific demethylase 1 (LSD-1).

Results: Among SS individuals, the blood pressure (SBP: 140.8 vs. 132.9 mmHg; MAP: 104.2 vs. 98.7 mmHg) and prevalence of hypertension (58.8 vs. 32.8%) were significantly higher in high salt (HS) diet group than in normal salt (NS) diet group, but not in the salt-resistant (SR) individuals ($P > 0.05$). Serum H3K4me1 level (287.3 vs. 179.7 pg/ml, $P < 0.05$) significantly increased in HS group of SS individuals, but not in SR individuals. We found daily salt intake in SS individuals was positively correlated with serum H3K4me1 ($r = 0.322$, $P = 0.005$) and Set7 ($r = 0.340$, $P = 0.005$) levels after adjusting for age and gender, but not with LSD-1 ($r = -0.137$, $P = 0.251$). In addition, positive correlation between the serum H3K4me1 level and Set7 level ($r = 0.326$, $P = 0.007$) was also found in SS individuals. These correlations were not evident in SR individuals.

Conclusion: Our study indicates that high salt diet increases the serum H3K4me1 and Set7 levels in SS individuals.

Keywords: high salt diet, salt-sensitive hypertension, epigenetic modification, histone methylation, cohort study

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INTRODUCTION

Sodium is an essential nutrient, but excessive sodium intake is one of the important risk factors for hypertension (1). According to survey, dietary sodium intake in China is at a high level worldwide, and high sodium and low potassium are the major dietary feature. Recent data show that the salt intake level in China is still more than double the maximum intake recommended by the World Health Organization (2). Studies have found that individuals in the population have

different blood pressure (BP) responses to salt load or salt restriction and exhibit different salt-sensitivity (SS) phenotypes (3). Studies have reported that SS individuals were not only more likely to develop hypertension than salt-resistant (SR) individuals, but also had higher rates of cardiovascular complications and mortality. High salt diet and salt sensitivity are important risk factors for hypertension and cardiovascular diseases in the population, especially in China (4).

Epigenetic mechanisms can affect gene expression and function without changing the underlying DNA sequence and can mediate crosstalk between genes and the environment (5). Increasingly strong evidence supports epigenetic mechanisms' significant role in the development of essential hypertension (6, 7). Histone methylation and demethylation play an important role in the development of environment-related diseases. Set7, as an important methyltransferase, has been shown to monomethylate H3K4 (8). Monomethylation of histone H3K4 is a signal of gene activation and plays an important role in gene transcriptional activation (9). LSD-1, induces demethylation of H3K4 or H3K9 and thereby alters gene transcription. In animal models and population studies, LSD-1 has been shown to be associated with salt sensitivity of BP (10, 11). However, the associations of histone methylation and demethylation with high salt intake, salt sensitivity, and salt-sensitive hypertension have hardly been explored.

Thus, we aimed to explore the associations of high salt intake and salt sensitive with H3K4me1, Set7, and LSD-1.

METHODS

Study Population and Dietary Intervention

We selected a total of 515 subjects (aged 18–60 years) for epidemiological investigation in seven villages in rural areas of Mei County, Baoji City, Shaanxi Province in 2004. Individuals who had stage 2 hypertension, secondary hypertension, severe cardiovascular disease or diabetes mellitus, liver or renal dysfunction, alcohol abuse, or pregnancy were excluded (12). Eventually, a total of 339 subjects were included in the study and a dietary intervention trial was conducted. The subjects sequentially received daily diet for 3 days, a low-salt (LS) diet (LS: 51.3 mmol/day sodium) for 7 days, a high-salt (HS) diet (307.7 mmol/day sodium) for 7 days, and a high-salt plus potassium diet (307.7 mmol/day sodium + 60 mmol/day potassium) for another 7 days (**Figure 1A**) (12). To ensure the dietary compliance, each participant was required to have their breakfast, lunch, and dinner in research kitchen with professional chefs under the supervision of staff.

BP Measurement and Salt Sensitivity Definition

Subjects' BP was measured at the same time each morning by a professionally trained technician using a randomized-zero sphygmomanometer according to a standard protocol for 3 days during the baseline period and on days 5, 6, and 7 of the meal schedule. Participants were required to avoid coffee/tea, alcohol, cigarette smoking, and strenuous exercise for at least 30 min before BP measurement. Mean arterial pressure (MAP) was calculated as 1/3 systolic blood pressure (SBP) + 2/3 diastolic blood pressure (DBP). The responses of BP to dietary sodium and potassium intervention were calculated based on BP values during the dietary intervention period. Participants whose MAP increased >3 mmHg while changing from a low Na^+ to a high Na^+ diet were defined as SS individuals, and the other participants were defined as SR individuals (13, 14).

Follow-Up Data Collection

A total of 152 participants were randomly selected from the same cohort for follow-up in 2018. In this study, a uniform questionnaire was used to collect information on smoking, alcohol consumption, physical activity, and relevant disease history of the subjects. At the same time, their height, weight, waist circumference and hip circumference were measured by uniformly trained staff using uniform instruments while wearing underwear and removing shoes and hats. Body mass index (BMI) = weight (kg)/height^2 (m^2). Sitting BP was measured in a quiet environment by trained and certified staff according to the procedures recommended by the WHO. BP was measured three times, with an interval of 2 min between each measurement, and the BP level was defined as the mean values of the three BP measurements. We performed vascular function tests on the subjects, including brachial-ankle pulse wave velocity (baPWV), ankle-brachial index (ABI), and carotid intima-media thickness (cIMT). The protocol was approved by the Ethics Committee of First Affiliated Hospital of Xi'an Jiaotong University (code: 2015–128). This study followed the principles of the Helsinki declaration and was clinically registered (NCT02734472). Written informed were obtained from each participant.

Analysis of 24-h Urine

Urine samples were collected from subjects for 24 h to measure urine volume, and appropriate samples were retained and stored at −80°C until use. The 24 h urinary sodium, potassium and urinary microalbumin concentrations were measured in subjects. The 24 h urine sodium (24 h U_{Na}, mmol) and urine potassium (24 h U_K, mmol) were calculated by multiplying the concentration of sodium and potassium, respectively, by the 24 h total urine volume. Daily salt intake was estimated based on 24 h urine sodium excretion: daily salt intake (g) ≈ 24 h urine salt excretion (g) ≈ 24 h urine sodium excretion (mmol) × 0.0585 (g/mmol) (15). The study population were divided into four categories according to quartiles of 24 h salt intake. Individuals in the fourth quartile were defined as the high-salt diet group, while others were assigned to the normal-salt diet group for subsequent analyses.

Serum Biochemical Analyses

Fasting venous blood samples were obtained by experienced nurses in the morning after the participants had fasted for 8–10 h. The serum isolated from the blood samples was centrifuged at a centrifugal radius of 16 cm at 3,000 r/min for 10 min at room temperature and stored at −80°C in aliquots. A Hitachi 7060 automatic biochemical analyzer was used to detect the serum biochemical parameters,

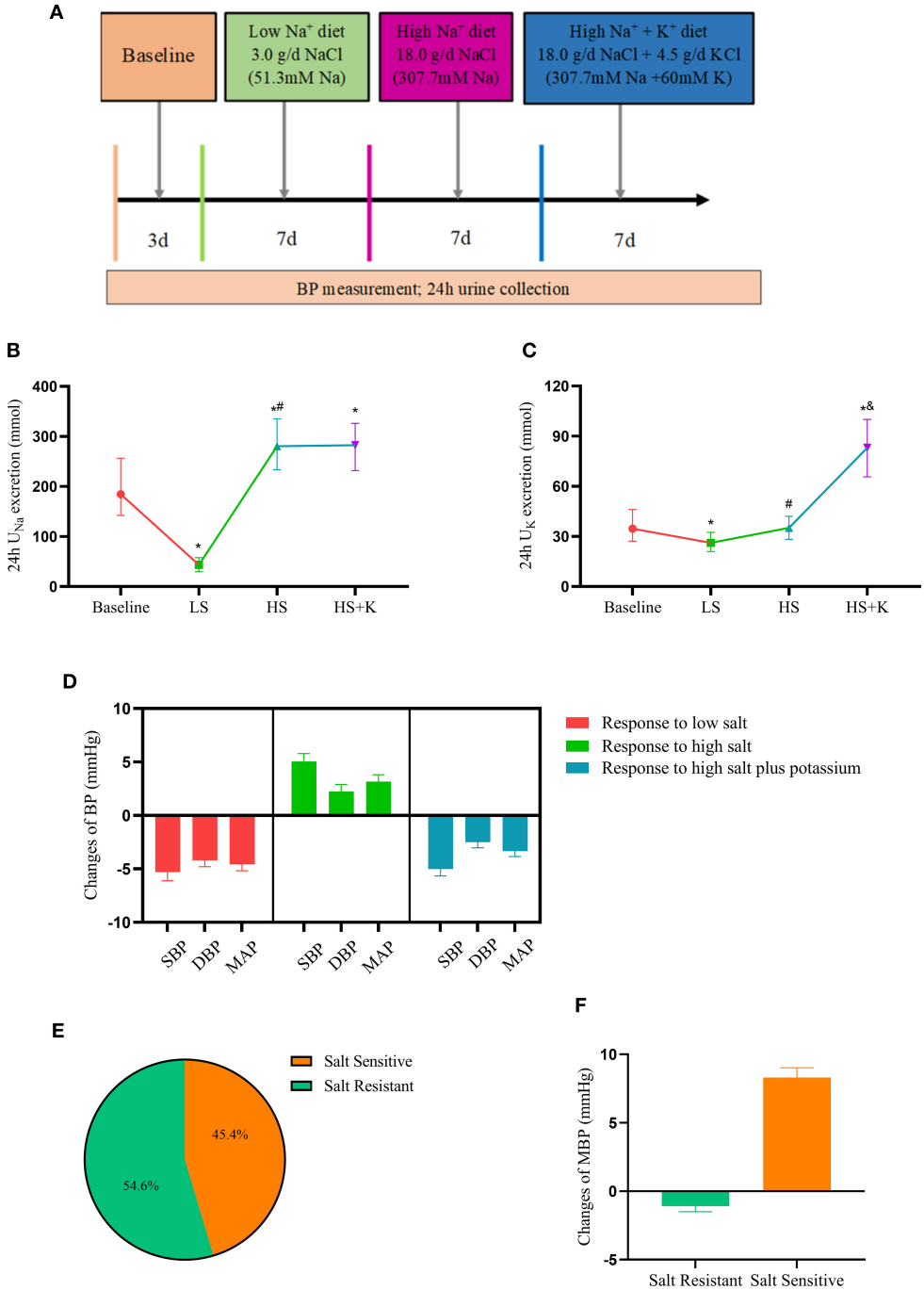

FIGURE 1 | Dietary interventions in subjects-chronic salt loading trial. **(A)** The protocol of dietary intervention; **(B)** The 24 h urinary sodium excretion in participants during the dietary intervention; **(C)** The 24 h urinary potassium excretion in participants during the dietary intervention; **(D)** The responses of blood pressure to dietary intervention; **(E)** The proportion of different salt sensitive phenotypes in the participants; **(F)** The responses of MAP to high salt intervention in salt-sensitive and salt-resistant participants. BP, blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; SBP, systolic blood pressure; HS, high salt intervention; LS, low salt intervention; HS+K, high salt plus potassium intervention. $^{*}P < 0.05$ vs. baseline; $^{\#}P < 0.05$, high salt diet vs. low salt diet; $^{\&}P < 0.05$, high salt plus potassium diet vs. high-salt diet.

including fasting glucose, serum creatinine, uric acid, total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol.

ELISA Detection

The serum H3K4me1, Set7, and LSD-1 concentration of each subject were measured using a commercially available

enzyme-linked immunosorbent assay (ELISA) kit (Xi-Tang., Shanghai, China) and is represented in pg/mL. With regard to specificity, the assay recognized and measured human serum H3K4me1, Set7, and LSD-1 without significant cross reactivity or interference with other cytokines. The sensitivity for H3K4me1, Set7, and LSD-1 were 8, 30, and 9 pg/mL, respectively. For repeatability, the intra- and inter-plate coefficients of variation were <10%.

Statistical Analysis

Continuous variables were shown as mean ± SDs if normally distributed, otherwise, they were reported as medians (25th, 75th percentile ranges). Categorical variables were expressed as numbers and percentages of subjects. Differences between continuous variables were analyzed by the *t*-test for two-group comparisons and one-way ANOVA for three or more groups when the distribution and variance met the conditions; otherwise, the Mann–Whitney *U*-test and Kruskal–Wallis test were used. Partial correlation coefficients were calculated to evaluate the relationships of high salt intake with serum H3K4me1, Set7, and LSD-1 levels after adjusting for age and gender. The linear regression models were used to test the associations of 24 h salt intake with serum H3K4me1, Set7, and LSD-1, after adjustment for age and gender. Statistical analysis was performed using SPSS 25.0 software (SPSS Inc., Chicago, Illinois, USA). A 2-tailed *p*-value < 0.05 was considered to be statistically significant.

RESULTS

The 24 h Urinary Sodium and Potassium Variations During Dietary Intervention

The 24-h urine samples were collected to ensure the compliance of the subjects with the study protocol. Results showed that 24 h U_{Na} excretion significantly decreased with the change from the baseline period to the LS diet (43.5 vs. 184.4 mmol/24 h, $P < 0.001$), but increased with the change from the LS to HS diet (280.5 vs. 43.5 mmol/24 h, $P < 0.001$) (**Figure 1B**). Compared with the HS diet, 24 h U_{Na} excretion increased in the high salt potassium supplement period, but the difference was not statistically significant (282.3 vs. 280.5 mmol/24 h, $P = 0.711$), while 24 h U_K excretion was significantly increased (83.1 vs. 35.2 mmol/24 h, $P < 0.001$) (**Figure 1C**). These results proved the subjects' compliance with the dietary administration protocol.

Responses of BP to Dietary Intervention

We analyzed the responses of the subjects' BP to dietary interventions. In response to LS diet intervention, SBP (−5.78 mmHg), DBP (−3.33 mmHg), and MAP (−3.63 mmHg) levels decreased; in response to HS diet intervention, SBP (+4.00 mmHg), DBP (+1.56 mmHg), and MAP (+2.37 mmHg) levels increased; in response to high-salt potassium diet intervention, SBP (−4.44 mmHg), DBP (−2.22 mmHg), and MAP (−3.04 mmHg) levels decreased again (**Figure 1D**). Furthermore, we divided the subjects into salt sensitive (45.4%) and salt resistant (54.6%) individuals based on their BP responses to dietary intervention (**Figure 1E**). The MAP reactivity was 8.31 mmHg (7.60, 9.02) in SS subjects and −1.09 mmHg (−1.49, −0.68) in SR subjects who switched from LS intervention to HS intervention (**Figure 1F**).

Clinical Characteristics of Participants During Follow-Up

The demographic and clinical characteristics of the study participants by different salt-sensitive phenotypes are shown in **Table 1**. There were 78 SS participants and 74 SR participants. The SS participants were older than SR participants ($P = 0.044$). The heart rate ($P = 0.025$) and rate of drinking ($P = 0.011$) were higher in SS individuals than in SR individuals. There were no significant differences in gender distribution, SBP, DBP, lipid profiles, fast blood glucose, and creatinine between the SS and SR groups (all $P > 0.05$).

Effects of High Salt Diet on BP

The effects of high salt intake on BP level and prevalence of hypertension according to SS phenotypes are shown in **Table 2**. In the total population, although SBP, DBP, MAP levels, and the prevalence of hypertension in the HS diet group were slightly higher than those in the NS group, the differences were not statistically significant ($P > 0.05$). In salt sensitive individuals, SBP (140.8 vs. 132.9 mmHg, $P < 0.05$) level and MAP (104.2 vs. 98.7 mmHg, $P < 0.05$) level as well as the prevalence of hypertension (58.8 vs. 32.8%; $P < 0.05$) were significantly higher in the HS diet group than those in the NS diet group. While these differences were not found in SR individuals ($P > 0.05$). In addition, we found that among the HS diet individuals, the DBP (85.9 vs. 78.9 mmHg, $P < 0.05$) level and the prevalence of hypertension (58.8 vs. 31.3%; $P < 0.05$) were significantly higher in SS individuals than those in SR individuals. However, no differences were found among individuals with NS diets ($P > 0.05$).

Effects of High Salt Diet on Serum H3K4me1, Set7, and LSD-1 Levels

Figure 2 shows effects of high salt diet on serum H3K4me1, Set7, and LSD-1 levels in SS and SR individuals. In the total population, serum H3K4me1 levels (275.9 vs. 198.2 pg/ml, $P < 0.001$) were significantly increased in the HS group compared with the NS group; serum Set7 (685.5 vs. 619.7 pg/ml, $P > 0.05$) and LSD-1 (348.3 vs. 338.9 pg/ml, $P > 0.05$) levels showed an increasing trend in the HS group, but there were no statistically differences compared with the NS group. For SS individuals, serum H3K4me1 (287.3 vs. 179.7 pg/ml, $P < 0.001$) level was significantly higher in HS diet group than NS group, whereas Set7 and LSD-1 did not. For SR individuals, the high salt diet did not affect the levels of H3K4me1, Set7, and LSD-1 ($P > 0.05$).

Correlations of 24 h Salt Intake With Serum H3K4me1, Set7, and LSD-1 Levels

We examined correlations of 24 h salt intake with serum H3K4me1, Set7, and LSD-1 levels by partial correlation analysis.

TABLE 1 | Baseline characteristics of participants by salt sensitivity.

Parameters	Total	Salt sensitive	Salt resistant	*P*-value
N, %	152	78	74	
Gender (M/F)	90 (59.2%)	44 (56.4%)	46 (62.2%)	0.471
Age (y)	54 (50, 59)	54 (50, 60)	53 (48, 57)	0.044
Height (cm)	161.6 ± 8.0	161.7 ± 8.6	161.5 ± 7.4	0.894
Weight (kg)	63.5 (56.6, 72.6)	63.2 (56.3, 72.9)	63.9 (57.2, 72.5)	0.726
BMI (kg/m^2)	24.6 (22.7, 27.0)	24.4 (22.6, 26.8)	24.9 (23.0, 27.1)	0.532
Waist (cm)	84.0 (75.6, 91.9)	85.5 (76.8, 94.0)	82.7 (72.9, 90.2)	0.172
Hips (cm)	93.9 (89.6, 98.9)	93.0 (89.0, 99.1)	95.1 (91.0, 98.0)	0.304
SBP (mmHg)	133.5 ± 16.5	134.6 ± 14.6	132.2 ± 18.3	0.369
DBP (mmHg)	81.2 ± 10.0	82.5 ± 9.3	79.9 ± 10.7	0.108
MAP (mmHg)	98.6 ± 11.5	99.9 ± 10.4	97.3 ± 12.4	0.171
HR (bpm)	73 (67, 81)	76 (69, 82)	72 (67, 79)	0.025
Smoking (%)	56 (71.8%)	30 (38.5%)	26 (35.1%)	0.671
Drinking (%)	24 (30.8%)	18 (23.1%)	6 (8.1%)	0.011
FBG (mg/dL)	90.8 (84.7, 97.2)	90.5 (84.6, 99.0)	91.3 (85.1, 96.9)	0.867
SUA (μmol/L)	334.0 (270.5, 385.0)	347.5 (265.0, 390.1)	325.0 (272.5, 366.8)	0.224
CRE (μmol/L)	60.0 (50.0, 69.0)	59.5 (50.8, 68.0)	62.0 (48.3, 70.0)	0.958
TGs (mg/dl)	126.5 (93.7, 200.3)	146.9 (98.6, 204.2)	116.7 (87.0, 171.2)	0.087
TC (mg/dl)	172.6 (151.5, 195.4)	176.4 (157.5, 199.0)	167.4 (146.6, 192.0)	0.070
HDL-C (mg/dl)	49.1 (42.3, 59.3)	47.6 (40.6, 58.8)	49.8 (43.0, 60.5)	0.510
LDL-C (mg/dl)	88.3 (70.6, 111.8)	92.4 (71.3, 113.1)	81.0 (70.5, 107.2)	0.277
baPWV (cm/s)	1,508.0 (1,322.0, 1,777.3)	1,559.0 (1,360.0, 1,818.3)	1,473.0 (1,305.0, 1,724.0)	0.088
ABI	1.12 (1.08, 1.16)	1.12 (1.09, 1.15)	1.12 (1.07, 1.17)	0.994
CIMT (mm)	0.89 (0.83, 1.00)	0.91 (0.86, 1.00)	0.88 (0.81, 1.00)	0.081
24 h U_{Na} (mmol)	142.1 (97.2, 190.7)	137.8 (91.9, 176.1)	150.9 (103.4, 200.2)	0.262
24 h U_K (mmol)	29.4 (20.3, 39.0)	29.7 (20.5, 38.7)	28.6 (20.0, 39.5)	0.971
24 h Salt intake (g)	8.3 (5.7, 11.2)	8.1 (5.4, 10.3)	8.8 (6.0, 11.7)	0.262
24 h uALB (mg)	3.04 (0.00, 8.41)	4.10 (0.00, 10.40)	0.00 (0.00, 7.33)	0.165
H3K4me1(pg/mL)	200.0 (142.6, 276.8)	195.1 (142.0, 273.7)	206.8 (148.6, 281.3)	0.806
Set7 (pg/ml)	656.6 (530.1, 902.5)	693.7 (531.1, 953.7)	647.4 (527.3, 888.8)	0.643
LSD-1 (pg/mL)	340.4 (313.1, 363.9)	335.8 (311.2, 363.9)	344.1 (314.0, 365.0)	0.510

ABI, ankle-brachial index; BMI, body mass index; baPWV, brachial-ankle pulse wave velocity; CIMT, carotid intima-media thickness; CRE, serum creatinine; DBP, diastolic blood pressure; FBG, fasting glucose; HR, heart rate; H3K4me1, monomethylation of H3K4; HDL-C, high density lipoprotein cholesterol; LSD-1, lysine-specific demethylase 1; MAP, mean arterial pressure; SBP, systolic blood pressure; SUA, serum uric acid;TGs, serum triglycerides; TC, total cholesterol; uALB, urine microalbumin; 24 h UNa, 24-hour urine sodium; 24 h UK, 24-hour urine potassium.

As shown in **Figure 3**, we found that 24 h salt intake in SS individuals was positively correlated with serum H3K4me1 ($r = 0.322$, $P = 0.005$) and Set7 ($r = 0.340$, $P = 0.005$) levels after adjusting for age and gender, but not with LSD-1 ($r = -0.137$, $P = 0.251$). In addition, positive correlation ($r = 0.326$, $P = 0.007$) between serum H3K4me1 level and Set7 level was also found in SS individuals. These correlations were not evident in SR individuals ($P > 0.05$).

Furthermore, we analyzed associations of 24 h salt intake with serum H3K4me1, Set7, and LSD-1 levels in individuals with different salt sensitive phenotypes using linear regression models (**Table 3**). The 24 h salt intake in SS individuals was significantly associated with serum H3K4me1 ($\beta = 0.348$, $P = 0.005$) and Set7 ($\beta = 0.366$, $P = 0.005$) levels after adjusting for age and gender, but not with LSD-1. These associations were not found in SR individuals ($P > 0.05$).

DISCUSSION

In the present study, we analyzed for the first time the effect of a high salt diet on serum histone methylation as well as methylation-related enzymes in SS individuals using a population cohort in Mei County, Shaanxi Province. We found that a high salt diet increased BP levels and the prevalence of hypertension in SS individuals but not in SR individuals. More importantly, we found significantly positive correlations of 24 h salt intake with serum H3K4me1 and Set7 levels except for LSD-1 in SS individuals, whereas these associations were not found in SR individuals.

Studies have found that individuals in the population have different BP responses to salt load or salt restriction and exhibit different salt sensitivity phenotypes. We conducted a dietary intervention trial with subjects in the order of baseline period,

TABLE 2 | The effect of high salt intake on blood pressure level and prevalence of hypertension according to salt sensitivity.

	Total		Salt sensitive		Salt resistant	
	NS	HS	NS	HS	NS	HS
SBP (mmHg)	132.3 ± 16.1	137 ± 17.1	132.9 ± 14.5	140.8 ± 13.3*	131.6 ± 17.9	133.9 ± 19.5
DBP (mmHg)	81.0 ± 9.8	82.0 ± 10.7	81.6 ± 8.9	85.9 ± 10.0#	80.3 ± 10.9	78.9 ± 10.5
MAP (mmHg)	98.1 ± 11.2	100.3 ± 12.1	98.7 ± 10.1	104.2 ± 10.7*	97.4 ± 12.4	97.2 ± 12.6
Hypertension (%)	34 (29.8%)	16 (42.1%)	20 (32.8%)	11 (58.8%)*#	14 (26.4%)	5 (31.3%)

*DBP, diastolic blood pressure; MAP, mean arterial pressure; SBP, systolic blood pressure; NS, normal salt diet group; HS, high salt diet group. *P < 0.05, HS vs. NS in each group; #P < 0.05, Salt sensitive vs. Salt resistant in NS group and HS group.*

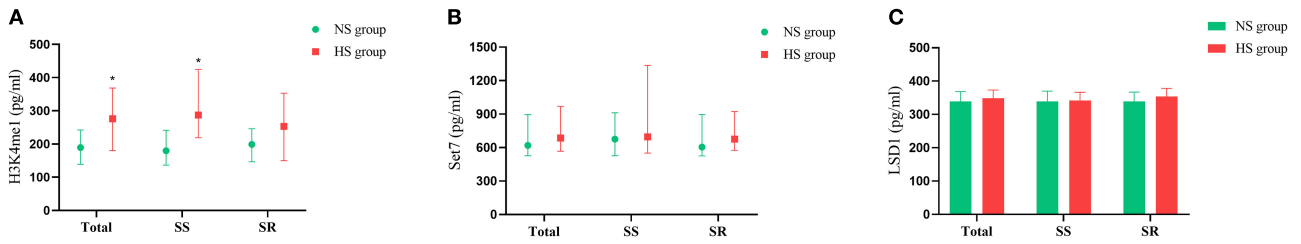

FIGURE 2 | Effects of high salt diet on serum H3K4me1 **(A)**, Set7 **(B)**, and LSD-1 **(C)** in SS and SR participants. SS, salt sensitive participants; SR, salt resistant participants; NS, normal salt group; HS, high salt group. *$P < 0.05$, high salt group vs. normal salt group.

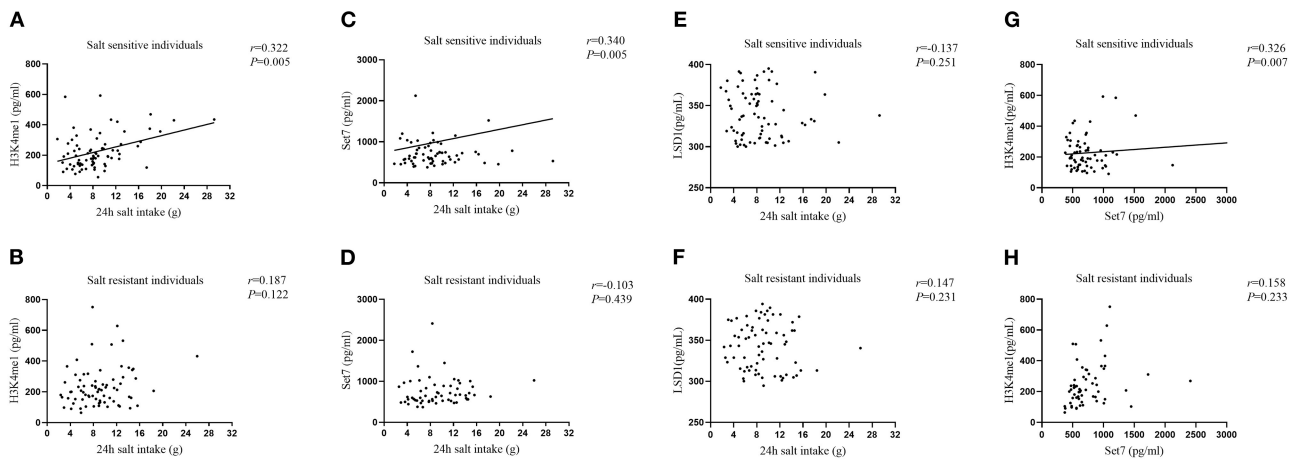

FIGURE 3 | Correlations of 24 h salt intake with serum H3K4me1 in SS individuals **(A)**, H3K4me1 in SR individuals **(B)**, Set7 in SS individuals **(C)**, Set7 in SR individuals **(D)**, LSD-1 in SS individuals **(E)**, and LSD-1 in SR individuals **(F)** after adjusting age and gender. The correlation between serum H3K4me1 and Set7 in SS individuals **(G)** and in SR individuals **(H)** after adjusting for age and gender.

low salt intervention, high salt intervention and high salt plus potassium intervention. The study found that subjects had higher urinary sodium excretion and lower urinary potassium excretion at baseline, which reflecting the dietary characteristics of high sodium and low potassium in this region. This is also in line with the reported dietary habits of rural residents in northern China (16). In addition, we found that the BP level of the subjects in the high salt intervention period was not significantly different from those at baseline. However, the subjects' BP response to dietary sodium-potassium intervention was normal, which again proved the high salt characteristics of the subjects' daily diet. These findings are similar to the result of a meta-analysis of 70 studies in 2019. The study reported that the average sodium and potassium excretion of individuals over 16 years of age in China were 189.07 mmol/24 h and 36.35 mmol/24 h, respectively, and was significantly higher in the north than in the south (2). Therefore, it is of great significance to conduct an epidemiological study of high salt diet and explore its impact on hypertension and related diseases, especially for residents in northern China.

Salt sensitivity is an intermediate phenotype of hypertension that results from the interaction of genetic and environmental factors (17). Studies have suggested that SS individuals were more

TABLE 3 | Associations of 24 h salt intake with serum H3K4me1, Set7, and LSD1 according to salt sensitivity.

	B-value	*β*-value	*t*-value	95% CI	*P*-value
Salt sensitive					
H3K4me1 (pg/mL)	9.158	0.348	2.868	2.79–15.53	0.005
Set7 (pg/mL)	93.816	0.366	2.913	29.50–158.13	0.005
LSD1 (pg/mL)	−1.025	−0.148	−1.159	−2.79–0.74	0.251
Salt resistant					
H3K4me1 (pg/mL)	5.558	0.184	1.566	−1.52–12.64	0.122
Set7 (pg/mL)	−13.525	−0.102	−0.779	−48.28–21.23	0.439
LSD1 (pg/mL)	0.974	0.144	1.209	−0.64–2.58	0.231

The linear regression models were used to test the associations of 24 h salt intake with serum H3K4me1, Set7, and LSD1, after adjustment for age, sex.

likely to develop hypertension and ultimately cardiovascular diseases than SR individuals (4, 18). Our study found that the BP level and prevalence of hypertension were significantly higher in the high salt diet group than in the normal salt diet group among SS individuals, but not in SR individuals. In addition, SS individuals had higher BP levels and higher prevalence of hypertension than SR individuals in the high salt diet, but no difference in the normal salt diet. These results suggest that SS individuals have a higher risk of hypertension in the presence of a high salt diet. Therefore, in SS individuals, reducing sodium intake is of great significance for reducing the risk of hypertension.

Histones are important proteins responsible for maintaining the structure of chromatin and play a role in the dynamic and long-term regulation of genes. The N-terminal tail of histone 3, one of the five histones found in eukaryotic nuclei, is subject to methylation or acetylation of lysine and arginine residues as well as phosphorylation of serine and threonine residues (19). Monomethylation of histone H3K4 is a signal of gene activation and plays an important role in environmentally induced diseases. Our study found that 24 h salt intake in SS individuals was positively correlated with serum H3K4me1 and Set7 levels. In addition, positive correlation between the serum H3K4me1 level and Set7 level was also found in SS individuals. These correlations were not evident in SR individuals. Various findings suggest that epigenetic mechanisms have an important role in obesity-induced hypertension. Obesity-induced hypertension is associated with increased levels of histone deacetylase 1 and acetylated histone H3 in mice fed a high-fat diet (20). In addition, previous study had found the epigenetic modulation of WNK4 transcription in the development of salt sensitive hypertension (21). Histone deacetylation has also been found to play an important role in angiotensin II-induced hypertension (22). The result of this study suggest that a high-salt diet increases the expression of the lysine methyltransferase Set7 and increases the monomethylation of lysine at position 4 of histone H3 in salt sensitive individuals. And these changes may lead to a series of related diseases. Then, exploring histone methylation may be important for the prevention and treatment of salt sensitive hypertension.

LSD-1 is an epigenetic regulator of gene transcription, and it has been demonstrated to be associated with salt sensitivity of BP. Luminita et al. found that LSD-1 deficiency is associated with increased BP and vascular reactivity in mice during liberal salt intake, suggesting that salt intake has an epigenetic effect (10). Similar to LSD-$1^{+/-}$ mice, African-American minor allele carriers of two LSD-1 SNPs displayed greater change in SBP in response to change from low to liberal salt diet (11). Differently, in our study, there was no association between high salt diet and serum LSD-1, neither in SS individuals or SR individuals. There may be two reasons for this, one may be ethnic differences, and the other may be that our study did not explore SNPs but only analyzed protein levels.

Epigenetic modifications alter gene function without altering gene sequence, with heritable and sustainable properties. This property may also explain the results of some studies. For example, the well-known THOP study has demonstrated that sodium reduction can lower BP (23), and also reduce long-term risk of cardiovascular events. People with prehypertension assigned to a sodium reduction intervention experienced a 25–30% lower risk of cardiovascular outcomes in the 10–15 years after the trial. Furthermore, some scholars have studied and explored this phenomenon (24). Oguchi et al. (25) showed that transient high salt intake during early phases in the development of hypertension induced sustained elevation of BP in hypertensive model rats and first named this phenomenon salt memory. More and more researches show that epigenetics play an important role in the pathogenesis of metabolic memory (26, 27). In this study, we found an association between high salt intake and histone methylation in salt sensitive individuals. Whether epigenetic changes are also involved in the formation and regulation of salt memory effect deserves further exploration.

The greatest strength of this study is the first exploration of the effects of a high-salt diet on serum histone methylation and related enzymes in a population with different salt sensitive phenotypes. This finding is important because it provides evidence for dietary salt affecting epigenetic modification. There are, of course, some limitations to this study. Firstly, the recruited population in this study was restricted to northern Chinese individuals and relatively small. Therefore, we could not generalize the results of this study to other races. Multiethnic clinical trials were required to determine whether our results could be generalized to populations with multiple backgrounds. Secondly, we focused on changes in histone methylation

and related enzymes at the protein expression level under a high-salt diet. Next, we can deeply explore the changes in SNPs of these proteins. Finally, further studies are warranted to elucidate the genes modified by methylation and the exact mechanisms.

CONCLUSION

In conclusion, the present study indicated that a high-salt diet was associated with serum H3K4me1 and Set7 levels in salt sensitive individuals but not in salt resistant individuals. This finding provides evidence for high salt diet affecting epigenetic modification and may have potential clinical and public health implications.

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 protocol was approved by the Ethics Committee of First Affiliated Hospital of Xi'an Jiaotong University (code: 2015–128). This study followed the principles of the Helsinki declaration and was clinically registered (NCT02734472). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JM conceived, designed the study, and secured funding. YL analyzed the data. YL and CC wrote the first draft of the manuscript. QM, KG, YS, JH, and WZ supervised the data collection. YY and DW provided technical direction and writing assistance in the preparation of this manuscript. All authors critically revised the manuscript and approved the final version to be published.

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The Saturation Effect of Obesity on Bone Mineral Density for Older People: The NHANES 2017–2020

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Introduction: Previous studies have shown that obesity has a positive effect on bone mineral density (BMD). However, excessive obesity is harmful to health, especially in older adults. In addition, it is unclear what body mass index (BMI) and waist circumference (WC) to maintain for the most beneficial BMD in older adults.

Methods: Multivariate logistic regression models were used to investigate the association between BMI, WC, and femoral neck BMD using the most recent data from the 2017–2020 National Health and Nutrition Examination Survey (NHANES). Fitting smoothing curves and saturation effects analysis were also used to determine the association of nonlinear relationships between BMI, WC, and femoral neck BMD.

Results: The analysis included a total of 2,903 adults. We discovered that BMD and WC were positively linked to femoral neck BMD. The favorable associations of BMI and WC with femoral neck BMD were maintained in all subgroup analyses stratified by sex and race, except among Mexican Americans. Furthermore, smoothing curve fitting revealed that the link between BMI and BMD was not only a linear connection, and that there was a saturation point. The BMI saturation value in the femoral neck BMD was 24.3 (kg/m^2), according to the saturation effect analysis.

Conclusions: In persons over the age of 50, our research found a positive relationship between obesity and BMD, and we also found a saturation value between BMI and BMD. According to this study, maintaining BMI at a moderate level (about 24.3 kg/m^2) would result in an optimal balance between BMI and BMD in adults over 50 years of age.

Keywords: bone mineral density, osteoporosis, NHANES, obese, body mass index

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INTRODUCTION

Osteoporosis is a long-term disorder marked by reduced bone mineral density (BMD) that affects a huge number of people (1). According to the International Osteoporosis Foundation, more than 30% of women and more than 20% of men over the age of 50 have osteoporosis or osteopenia, putting them at risk for osteoporotic fractures (2). Simultaneously, the prevalence of osteoporosis

Abbreviations: BMD, bone mineral density; NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; WC, waist circumference; NHANES, National Health and Nutrition Examination Survey; MEC, mobile examination center.

continues to climb as the population ages and expands (3). Apart from genetics, age, and gender, other variables that affect bone metabolisms, such as food intake and lifestyle, have lately received a lot of attention (4–6). Meanwhile, scientists are working to discover novel ways to prevent and treat osteoporosis.

Obesity is a huge medical problem that affects people all over the world (7). Body mass index (BMI) is commonly used to assess overall obesity, while waist circumference (WC) is used to assess central obesity (8, 9). According to most of the previous studies, obesity and BMD have a strong favorable relationship in older adults (10–12). However, excessive BMI and WC may be associated with other systemic diseases and comorbidities such as atherosclerosis (13), non-alcoholic fatty liver disease (NAFLD), type 2 diabetes (14), and obstructive sleep apnea (15). Therefore, it is crucial to strike a balance between BMI, WC, and BMD. Existing studies are inconclusive on how much BMI and WC is most beneficial for BMD while minimizing the risk of other obesity-related problems. Therefore, we assessed the connection of BMI and WC with BMD in older adults in this study using a comprehensive fraction of individuals aged above 50 years from the National Health and Nutrition Examination Survey (NHANES). We hypothesized that BMI and WC had a saturation point, respectively, and that maintaining BMI and WC at this point would result in the best balance between obesity and BMD.

MATERIALS AND METHODS

Data Source and Study Population

The NHANES is a major, continuing cross-sectional survey in the United States that aims to give objective statistics on health issues and address emerging public health concerns among the general public. The NHANES datasets were utilized for this investigation from 2017 to 2020. The participants in the research had to be between the ages of 50 and 80. Among the 15,560 individuals, we excluded 10,543 individuals younger than 50 years, 3,545 individuals with missing BMD, 19 with missing BMI data, 29 with missing WC data, and 594 individuals with cancer diagnoses. Finally, 2,903 people were enrolled in the study (**Figure 1**).

Ethics Statement

The National Center for Health Statistics Research Ethics Review Board authorized the protocols for the NHANES and obtained signed informed consent. After anonymization, the NHANES data are available to the public. This enables academics to transform data into a study-able format. We agree to follow the study's data usage guidelines to guarantee that data are only utilized for statistical analysis and that all experiments are carried out in compliance with applicable standards and regulations.

Study Variables

In the mobile examination facility, certified health workers collected anthropometric data (MEC). Individuals were measured while standing with their arms crossed across their chest. The iliac crests were palpated bilaterally and a horizontal line was drawn just above the uppermost lateral border of the right ilium. After that, the right midaxillary line was drawn. At the point where the two lines crossed, the measuring tape was positioned in the horizontal plane. At the end of the individual's normal expiration, his or her WC was measured. The BMI was calculated by multiplying the weight by the squared height. Dual-energy x-ray absorptiometry was performed using a Hologic QDR 4500A device and Apex software version 3.2 by qualified radiology technologists to assess femoral neck BMD. Covariates in multivariate models may cause the correlations between BMI, WC, and femoral neck BMD to be muddled. Age, gender, race, education level, activities status, diabetes status, NAFLD status (controlled attenuation parameter values≥274 dB/m were considered suggestive of NAFLD) (16), smoking status, ALT, ALP, AST, total calcium, total cholesterol, direct HDL cholesterol, LDL cholesterol, triglyceride, and serum phosphorus were all covariates in this study. The NHANES website (https://www.cdc.gov/nchs/nhanes/) has a thorough explanation of how these variables are calculated.

Statistical Analysis

We used R software (version: 4.0.3, Vienna, Austria: R Foundation for Statistical Computing, 2016) and EmpowerStats (version: 2.0, X&Y Solutions, Inc., Boston, MA; http://www.empowerstats.com) for all statistical analyses, with statistical significance set at $p < 0.05$. p-values for continuous variables in the population baseline table were calculated by weighted linear regression models, and p-values for categorical variables were calculated by weighted chi-square tests. The relationship between exposure factors and BMD was calculated using a weighted multiple regression model. The trend of the effect values is illustrated by calculating p for trend from the linear trend test in the regression model. Model 1 did not adjust for variables; Model 2 adjusted for age, sex, and race; and Model 3 adjusted for all covariates listed in **Table 1**. In addition, we performed subgroup analyses, stratifying the variables according to gender, race, and categorization based on the distribution of exposure factors.

RESULTS

Baseline Characteristics

The demographic and laboratory data of 1,537 men and 1,366 women are presented in **Table 1**. The evaluated BMI, WC, and femoral neck BMD for men were 28.773 ± 5.508 kg/m^2, 103.307 ± 14.256 cm, and 0.820 ± 0.145 g/cm^2, respectively. In women, the respective values were 29.530 ± 6.722 kg/m^2, 99.069 ± 14.739 cm, and 0.725 ± 0.139 g/cm^2, respectively. The percentage of people with abdominal obesity in each group according to gender and race is shown in **Supplementary Table S1**. Compared to female participants, male participants are more likely to suffer from diabetes and NAFLD. Male participants had significantly higher WC and smoking status, and significantly higher levels of ALT, AST, triglyceride, and femoral neck BMD, while education level,

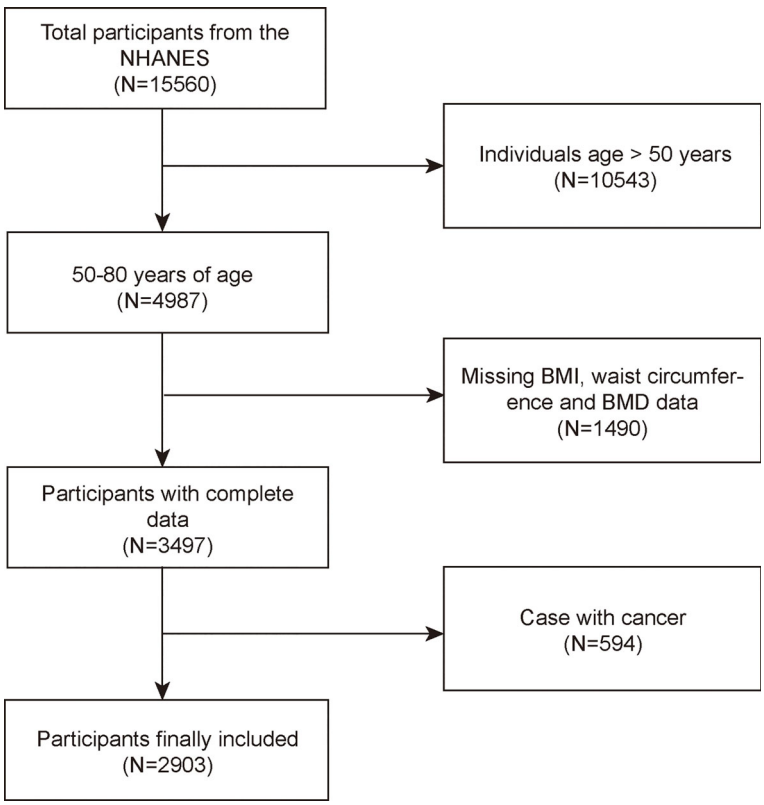

FIGURE 1 | Flowchart of participant selection. NHANES, National Health and Nutrition Examination Survey; BMD, bone mineral density; BMI, body mass index; WC, waist circumference.

BMI, HDL-cholesterol, LDL, total calcium, total cholesterol, and serum phosphorus were lower than those in female individuals.

Relationship Between BMI and Femoral Neck BMD

The findings of the multivariate regression analysis are shown in **Table 2**. BMI was strongly positively linked with femoral neck BMD in the unadjusted model [0.009 (0.008, 0.010)]. In addition, this correlation remained significant after adjusting for the covariates in Model 2 [0.008 (0.007, 0.009)] and Model 3 [0.008 (0.007, 0.010)].

On a subgroup analysis stratified by gender, BMI was positively associated with femoral neck BMD in men [0.008 (0.006, 0.010)] and women [0.008 (0.006, 0.010)] in the fully adjusted model with same effect values and confidence intervals. On subgroup analysis stratified by race, the positive relationship between BMI and femoral neck BMD was retained in whites [0.008 (0.006, 0.011)], blacks [0.009 (0.007, 0.011)], and other race [0.008 (0.007, 0.010)] in the fully adjusted model. However, this association became insignificant (p = 0.30963) after adjusting all other covariates in Mexican Americans [0.002 (−0.002, 0.007)]. On a subgroup analysis stratified by BMI (underweight <18.5 kg/m^2; normal, 18.5–24.9 kg/m^2; overweight, 25–29.9 kg/m^2; and obese, ≥30 kg/m^2) (17), using the normal group as the reference group, the overweight group [0.010 (0.004, 0.016)] and obese group [0.006 (0.005, 0.008)] all maintained a significant positive relationship in Model 1, and the relationship between BMI and BMD was not statistically significant in the underweight group, although they had the highest beta values. In the overweight group, BMD increased by 0.010 g/cm^2 per unit increase in BMI, while in the obese group, BMD increased by 0.006 g/cm^2 per unit increase in BMI.

We discovered a saturation effect value between BMI and BMD when we performed smoothing curve fitting in the amended model (**Figure 2**). We employed the saturation effect analysis model to look into the BMI turning point and discovered that the saturation effect value in the femoral neck BMD was 24.3 kg/m^2 (**Table 4**). For each unit rise in BMI over 24.3 kg/m^2, the femoral neck BMD rose by 0.015 g/cm^2. When BMI exceeded 24.3 kg/m^2, however, the femoral neck BMD increased only by 0.007 g/cm^2 per unit rise in BMI.

Relationship Between Waist Circumference and Femoral Neck BMD

Table 3 represents the association between WC and femoral neck BMD for the three linear regression models. WC was significant positively linked with femoral neck BMD in the unadjusted model [0.004 (0.003, 0.004)], Model 2 [0.003 (0.003, 0.003)], and Model 3 [0.003 (0.003, 0.004)].

TABLE 1 | Characteristics of the participants.

Characteristics	Male (*n* = 1,537)	Female (*n* = 1,366)	*p*-value
Age (years)	63.462 ± 8.662	63.446 ± 8.703	0.960
Race (%)			0.200
Non-Hispanic White	33.637	34.919	
Non-Hispanic Black	28.562	25.915	
Mexican American	10.475	9.370	
Other race	27.326	29.795	
Education level (%)			0.006
Less than high school	22.837	17.570	
High school	25.569	26.061	
More than high school	51.594	56.369	
Moderate activities (%)			0.456
Yes	39.493	38.141	
No	60.507	61.859	
Diabetes status (%)			<0.001
Yes	25.374	18.448	
No	70.397	78.331	
NAFLD status (%)			<0.001
Yes	51.997	42.113	
No	48.003	57.887	
Smoked at least 100 cigarettes in life (%)			<0.001
Yes	57.189	33.602	
No	42.811	66.398	
Borderline	4.229	3.221	
ALT (U/L)	24.143 ± 23.565	19.703 ± 13.894	<0.001
AST (U/L)	23.343 ± 17.798	21.444 ± 10.898	<0.001
ALP (U/L)	78.728 ± 24.659	85.055 ± 27.148	<0.001
Total calcium (mmol/L)	2.318 ± 0.095	2.337 ± 0.099	<0.001
Total cholesterol (mmol/L)	4.702 ± 1.079	5.202 ± 1.062	<0.001
Direct HDL cholesterol (mmol/L)	1.281 ± 0.354	1.568 ± 0.438	<0.001
LDL cholesterol (mmol/L)	2.756 ± 0.928	3.032 ± 1.000	<0.001
Triglyceride (mg/dl)	1.370 ± 1.587	1.213 ± 0.654	0.019
Serum phosphorus (mmol/L)	1.106 ± 0.175	1.187 ± 0.156	<0.001
Waist circumference (cm)	103.307 ± 14.256	99.069 ± 14.739	<0.001
Body mass index (kg/m²)	28.773 ± 5.508	29.530 ± 6.722	<0.001
Femoral neck bone mineral density (g/cm^2)	0.820 ± 0.145	0.725 ± 0.139	<0.001

Mean ± SD for continuous variables: p-value was calculated by weighted linear regression model.
% for categorical variables: p-value was calculated by weighted chi-square test.
NAFLD, non-alcoholic fatty liver disease.

On a subgroup analysis according to sex, WC was positively linked to femoral neck BMD in both men and women in the fully adjusted model [0.003 (0.002, 0.004)] with same effect values and confidence intervals. On subgroup analysis according to race, the relationship was the same as in BMI and femoral neck BMD, and the positive association was retained in whites [0.003 (0.002, 0.004)], blacks [0.004 (0.003, 0.005)], and other race [0.003 (0.003, 0.004)] in the fully adjusted model. However, this association became insignificant (p = 0.92756) after adjusting all other covariates in Mexican Americans [0.000 (−0.002, 0.002)]. On a subgroup analysis stratified by WC quartiles, group Q1 served as the reference group and both group Q2 [0.007 (0.003, 0.011)] and group Q4 [0.003 (0.001, 0.004)] maintained a significant positive correlation in Model 1. The positive correlation between WC and BMD in group Q3 [0.001 (−0.002, 0.005)] was weak and insignificant. **Figure 3** depicted smooth curve fits and generalized additive models that were utilized to define the nonlinear connection between WC and femoral neck BMD. The saturation effect analysis of WC and BMD was not significant with LRT test = 0.245 (**Table 4**).

DISCUSSION

In this study of individuals aged over 50 years, we demonstrated the positive association between BMI, WC and BMD. Of note, a BMI saturation value (24.3 kg/m^2) was discovered in the femoral neck BMD in all subjects. At BMI levels <24.3 (kg/m^2), the increase in BMD was greater as BMI increased, whereas at BMI levels above 24.3 (kg/m^2), the increase was small, which is important for maintaining optimal BMD.

Osteoporosis and obesity have become serious health concerns across the world. The high frequency of these two disorders necessitates a deeper understanding of their link. Evidence on the link between WC and BMD has remained disputed to this point. In a recent cross-sectional study of 5,084 adults, Hua et al. found an inverse relationship between WC and BMD in middle-aged men with obesity (BMI ≥ 30 kg/m^2) and overweight women (BMI < 25 kg/m^2) (18). This conclusion is also supported by three cross-sectional studies from Asia (19–21). By comparison, in a cross-sectional study done in Turkey, WC was found to have a substantial positive

TABLE 2 | Association between body mass index (kg/m²) and femoral neck bone Mineral density (g/cm²).

	Model 1β (95% CI) p-value	Model 2β (95% CI) p-value	Model 3β (95% CI) p-value
Body mass index (kg/m²)	0.009 (0.008, 0.010) <0.00001	0.008 (0.007, 0.009) <0.00001	0.008 (0.007, 0.010) <0.00001
<18.5 kg/m²	0.038 (–0.002, 0.077) 0.07327	0.024 (–0.008, 0.056) 0.14941	0.021 (–0.006, 0.051) 0.26542
18.5–25 kg/m²	Reference	Reference	Reference
25–29.9 kg/m²	0.010 (0.004, 0.016) 0.00062	0.009 (0.004, 0.014) 0.00035	0.004 (–0.004, 0.012) 0.30468
≥30 kg/m²	0.006 (0.005, 0.008) <0.00001	0.006 (0.005, 0.008) <0.00001	0.007 (0.004, 0.009) <0.00001
p for trend	<0.001	<0.001	<0.001
Subgroup analysis stratified by gender			
Male	0.009 (0.007, 0.010)<0.00001	0.008 (0.007, 0.009) <0.00001	0.008 (0.006, 0.010) <0.00001
Female	0.010 (0.009, 0.011)<0.00001	0.008 (0.007, 0.009) <0.00001	0.008 (0.006, 0.010) <0.00001
Subgroup analysis stratified by race			
Non-Hispanic White	0.008 (0.006, 0.009) <0.00001	0.007 (0.006, 0.009) <0.00001	0.008 (0.006, 0.011) <0.00001
Non- Hispanic Black	0.008 (0.007, 0.010) <0.00001	0.009 (0.008, 0.011) <0.00001	0.009 (0.007, 0.011) <0.00001
Mexican American	0.007 (0.004, 0.009) <0.00001	0.006 (0.003, 0.009) <0.00001	0.002 (–0.002, 0.007) 0.30963
Other race	0.009 (0.007, 0.010) <0.00001	0.008 (0.007, 0.009) <0.00001	0.008 (0.007, 0.010) <0.00001

Model 1: No covariates were adjusted.
Model 2: Age, gender, and race were adjusted.
Model 3: Age, gender, race, education level, activities status, diabetes status, NAFLD status, smoking status, ALT, ALP, AST, total calcium, total cholesterol, direct HDL cholesterol, LDL cholesterol, triglyceride, and serum phosphorus were adjusted.
In the subgroup analysis stratified by gender or race, the model is not adjusted for the stratification variable itself.

relationship with total hip BMD, but a negative relationship with non-weight-bearing locations (22). These differences could be due to differences in study design, population, BMD quantification method, measurement site, or covariates. The WC has been used to assess abdominal obesity, whereas the BMI has been used to assess overall obesity. A cross-sectional research of the American population found a link between BMI and BMD (23). Another study by Wiacek et al. indicated that among Polish women aged 40–79 years, there was a significant positive correlation between BMI and BMD (24). Morin et al. noted that low BMI not only predicted the development of osteoporosis leading to osteoporosis, but also predicted an increased risk of fracture (25). A recent meta-analysis showed that adults with high BMI have higher lumbar BMD and femoral neck BMD compared to healthy weight individuals (26). BMI and WC are often congruent. However, people with normal BMI but large WC have a higher risk of developing metabolic diseases (27). A cohort study based on 44,366 women and men (mean age 70 years) in Sweden comprehensively assessed the relationship between body composition and fracture risk. The study found that fat distribution has a very strong effect on BMD and fracture risk and suggests that for optimal bone loss and fracture prevention, both men and women should avoid low BMI while having a high degree of central obesity (abdominal obesity).

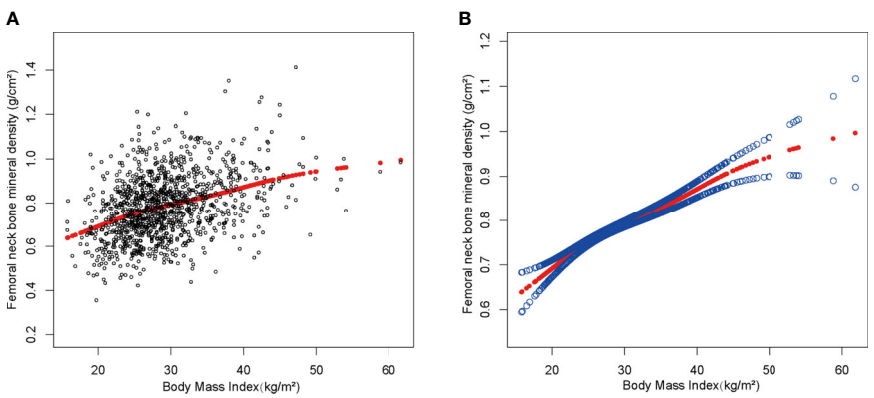

FIGURE 2 | The association between body mass index and femoral neck bone mineral density. **(A)** Each black point represents a sample. **(B)** The solid red line represents the smooth curve fit between variables. Blue bands represent the 95% confidence interval from the fit. Age, gender, race, education level, activities status, diabetes status, NAFLD status, smoking status, ALT, ALP, AST, total calcium, total cholesterol, direct HDL cholesterol, LDL cholesterol, triglyceride, and serum phosphorus were adjusted.

TABLE 3 | Association between waist circumference (cm) and femoral neck bone Mineral density (g/cm^2).

	Model 1β (95% CI) *p*-value	Model 2β (95% CI) *p*-value	Model 3β (95% CI) *p*-value
Waist circumference (cm)	0.004 (0.003, 0.004) <0.00001	0.003 (0.003, 0.003) <0.00001	0.003 (0.003, 0.004) <0.00001
Q1	Reference	Reference	Reference
(<91.3), *n* = 725			
Q2	0.007 (0.003, 0.011)	0.005 (0.001, 0.008)	–0.000 (–0.006, 0.006)
(91.4–99.9), *n* = 715	0.00151	0.01232	0.99360
Q3	0.001 (–0.002, 0.005) 0.42547	0.001 (–0.002, 0.004) 0.42149	0.000 (–0.005, 0.005)0.92797
(100.0–110.1), *n* = 730			
Q4	0.003 (0.001, 0.004) 0.00003	0.002 (0.001, 0.003) 0.00045	0.003 (0.001, 0.004)0.00598
(>110.2), *n* = 733			
p for trend	<0.001	<0.001	<0.001
Subgroup analysis stratified by gender			
Male	0.003 (0.002, 0.003) <0.00001	0.003 (0.002, 0.003) <0.00001	0.003 (0.002, 0.004) <0.00001
Female	0.004 (0.003, 0.004) <0.00001	0.003 (0.003, 0.004) <0.00001	0.003 (0.002, 0.004) <0.00001
Subgroup analysis stratified by race			
Non-Hispanic White	0.003 (0.003, 0.004) <0.00001	0.003 (0.002, 0.003) <0.00001	0.003 (0.002, 0.004) <0.00001
Non- Hispanic Black	0.004 (0.003, 0.004) <0.00001	0.004 (0.003, 0.004) <0.00001	0.004 (0.003, 0.005) <0.00001
Mexican American	0.002 (0.001, 0.003) 0.00022	0.002 (0.001, 0.003) 0.00090	0.000 (–0.002, 0.002) 0.92756
Other race	0.004 (0.003, 0.005) <0.00001	0.003 (0.003, 0.003) <0.00001	0.003 (0.003, 0.004) <0.00001

Model 1: No covariates were adjusted.

Model 2: Age, gender, and race were adjusted.

Model 3: Age, gender, race, education level, activities status, diabetes status, NAFLD status, smoking status, ALT, ALP, AST, total calcium, total cholesterol, direct HDL cholesterol, LDL cholesterol, triglyceride, and serum phosphorus were adjusted.

In the subgroup analysis stratified by gender or race, the model is not adjusted for the stratification variable itself.

In past studies, obesity has not only been shown to be associated with BMD, but has also been found to correlate with bone quality and fracture risk. Shen et al. studied 3,067 men in a cross-sectional research to determine the association between BMI and hip QCT measures, as well as to perform finite element analysis of hip QCT scans to offer a measure of hip strength during simulated falls. Men with obesity have better hip strength, but they also have a higher impact-to-strength ratio, which means that despite having stronger bones, they have a higher chance of hip fracture (28). The incidence of fracture declined with rising BMI and plateaued in men with obesity, according to a cohort study of 43,000 individuals aged 60 to 79 years from Norway. After adjusting for BMI and other possible confounders, larger WC and waist-to-hip ratio were linked to an increased risk of hip fracture. In fact, compared to men in the lowest tertile of WC, those in the highest tertile had a 100% increased risk of hip fracture. When a low BMI is combined with abdominal fat, the risk of hip fracture skyrockets (29). In 2005, a meta-analysis of 12 prospective population-based cohorts was released (approximately 60,000 individuals with a mean age of 62.2 years). Men and women with a low BMI had a greater age-corrected risk of any type of fracture, whereas those with a higher BMI had a lower risk. The increased risk, on the other hand, was not linear, and the gradient seemed steeper at lower BMI levels (30). This point is similar to the relationship between BMI, WC, and BMD in our study. The decreased bone mass and increased risk of fragility fractures associated with obesity also suggest that we need to keep BMI and WC in a reasonable range.

TABLE 4 | Saturation effect analysis of BMI (kg/m^2) and waist circumference (cm) on femoral neck BMD (g/cm^2) of all participants.

Femoral neck bone mineral density	Model: Saturation effect analysis
BMI turning point (K), kg/m^2	24.3
<K, effect 1	0.015 (0.009, 0.021) <0.0001
>K, effect 2	0.007 (0.006, 0.009) <0.0001
Effect 2 – 1	–0.008 (–0.014, –0.001) 0.0165
LRT test	0.015
Waist circumference (K), cm	95
<K, effect 1	0.004 (0.003, 0.005) <0.0001
>K, effect 2	0.003 (0.002, 0.004) <0.0001
Effect 2 – 1	–0.001 (–0.003, 0.001) 0.2499
LRT test	0.245

Age, gender, race, education level, activities status, diabetes status, NAFLD status, smoking status, ALT, ALP, AST, total calcium, total cholesterol, direct HDL cholesterol, LDL cholesterol, triglyceride, and serum phosphorus were adjusted.

The mechanisms behind the relationship between obesity and BMD are unclear. Increased static mechanical compliance due to excessive fat accumulation is one of the hypothesized reasons. Increased static mechanical stresses on the skeleton are caused by excessive fat accumulation and body weight, and when bone tissue recognizes the mechanical forces imposed by the body, it undergoes a series of changes (31, 32). Another probable cause is the replacement of osteoblasts in the bone marrow by adipocytes. Because both osteoblasts and adipocytes are formed from mesenchymal stem cells in the bone marrow, enhanced lipogenic differentiation reduces osteogenic differentiation (33). In addition to the aforementioned two possibilities, another possibility is that obesity-induced hypermetabolism caused by increased insulin signaling causes bone marrow stromal stem cells to age more quickly (34). Furthermore, the more body fat a patient with obesity has, the higher the levels of different hormones such as estrogen (35) and insulin (36), which are helpful to BMD by blocking bone resorption and boosting bone remodeling (37, 38).

Nevertheless, when BMI exceeded a particular threshold of 24.3 (kg/m^2), the femoral neck BMD increased by just 0.007

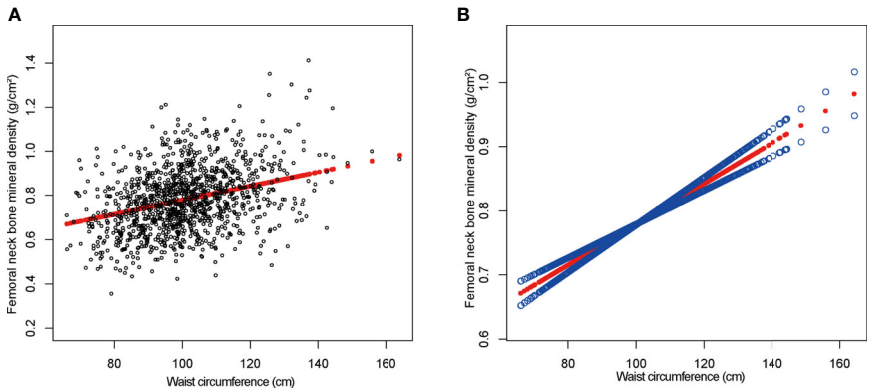

FIGURE 3 | The association between waist circumference and femoral neck bone mineral density. **(A)** Each black point represents a sample. **(B)** The solid red line represents the smooth curve fit between variables. Blue bands represent the 95% confidence interval from the fit. Age, gender, race, education level, activities status, diabetes status, NAFLD status, smoking status, ALT, ALP, AST, total calcium, total cholesterol, direct HDL cholesterol, LDL cholesterol, triglyceride, and serum phosphorus were adjusted.

(g/cm^2) per unit of BMI. The reasons for the saturating effect of BMI on BMD remain to be fully understood. Early in infancy, bone development trajectories and peak bone mass are defined, which might explain why adult BMD does not rise after a period of restricted growth (39, 40). Another cause for the occurrence of BMI saturation effects is a distinct bone–fat axis that exists *in vivo* between adipose and bone tissue (41), connected by various bioactive chemicals and maintaining bone homeostasis. According to existing research, bone and adipocytes are both derived from the same stem cell ancestor and are competitive, with an increase in excess fat leading to bone loss (42). BMD diminishes with growing obesity in animals with obesity, according to experiments in animal models caused by high-fat diets (43, 44).

Our findings are extremely relevant to the entire population since we used a nationally representative sample. We were also able to undertake subgroup analyses of BMI, WC, and femoral neck BMD across gender and ethnicity, and find the saturation effect value of obesity on BMD because of our large sample size. However, it is crucial to acknowledge the study's limitations. The fundamental weakness of the study is its cross-sectional design. The causal relationship between BMI, WC, and femoral neck BMD could not be determined. To understand the specific mechanism of the relationship between obesity and BMD, further fundamental mechanistic research and large sample prospective studies are required. Second, due to database limitations, we were unable to identify participants with visceral fat, participants with fractures, or participants with osteoarthritis, as well as participants' lumbar spine BMD data, so our findings should be viewed with caution. Third, our study lacked data on participants' medications, such as calcium supplements and lipid-lowering drugs, which may also account for the differences in calcium and cholesterol levels in the baseline tables. Fourth, due to the limitations of the NHANES database for race-based classification criteria, we are unable to provide the percentage of abdominally obese individuals in each group for all race-specific thresholds. Finally, we excluded participants with cancer and our findings cannot be applied to a specific group.

CONCLUSION

In conclusion, we discovered not only a significant positive connection between BMI, WC, and BMD, but also a BMI saturation value for femoral neck BMD. According to this study, maintaining BMI at a moderate level (about 24.3 kg/m^2) would result in an optimal balance between BMI and BMD in adults over 50 years of age.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

YZ and JP designed the research. YZ collected and analyzed the data, and drafted the manuscript. YZ and JP revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.883862/full#supplementary-material

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Associations of the Geriatric Nutritional Risk Index With Femur Bone Mineral Density and Osteoporosis in American Postmenopausal Women: Data From the National Health and Nutrition Examination Survey

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Background: The geriatric nutritional risk index (GNRI) has been used as a significant tool to access the nutritional status of the elderly. However, the relationship between the GNRI and femur bone mineral density (BMD) and the risk of osteoporosis remains unclear in American postmenopausal women.

Objectives: We aimed to explore associations between the GNRI with femur BMD and the risk of osteoporosis in American postmenopausal women.

Methods: We merged the continuous National Health and Nutrition Examination Survey (NHANES) 2005–2006, 2007–2008, 2009–2010, 2013–2014, and 2017–2018 to ensure a large and representative sample, including 3,152 participants. The linear relationship between the GNRI and femur BMD was assessed *via* a weighted multivariate linear regression model. The odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association between the GNRI and the risk of osteoporosis were assessed by a weighted logistic regression model. Moreover, the nonlinear relationship was also characterized by smooth curve fitting (SCF) and a weighted generalized additive model (GAM).

Results: After adjusting for all covariates, the weighted multivariable linear regression models demonstrated that the GNRI was positively correlated with femur BMD. The weighted logistic regression models demonstrated that each unit of increased GNRI value was associated with a decreased risk of osteoporosis of 4.13%. When categorizing GNRI based on quartiles, ORs between the risk of osteoporosis and the GNRI across quintiles 2, 3, and 4 compared with quintile 1 were 0.5565 (95% CI: 0.4791, 0.6463; $P < 0.000001$), 0.5580 (95% CI: 0.4600, 0.6769; $P < 0.000001$), and 0.3475 (95% CI: 0.2681, 0.4505; $P < 0.000001$). The trends similar to the above were also observed in SCF and GAM.

Conclusion: This study indicated that nutritional status, represented by the GNRI, was positively associated with femur BMD and negatively associated with the risk of osteoporosis in American postmenopausal women. The GNRI may be a good tool to identify American postmenopausal women who need further bone health nutritional support.

Keywords: geriatric nutrition risk index, nutrition, bone mineral density, osteoporosis, American postmenopausal women

INTRODUCTION

Osteoporosis is a kind of skeletal disease with degradation of bone tissue microstructure and low bone mineral density (BMD). It usually results in increased bone fragility and an increased risk of fractures (1). In the United States, there are 1.5 million fractures caused by osteoporosis each year, most of which occur in postmenopausal women (2). This can lead to a poor quality of life, a dependent living situation, increased fracture-related mortality, and medical care costs. Especially in elderly women, hip fractures can be devastating (2). Therefore, the prevention and management of low femur BMD and osteoporosis in postmenopausal women are of great significance (3, 4). In addition, identifying the disease-related risk factors is a clinical priority.

Several studies have reported that nutrients, including some micronutrients such as calcium, magnesium, phosphorous, and vitamin D, could influence BMD (5, 6). Besides, an easily neglected fact is that proteins are also crucial for bone health. Adequate protein intake contributes to bone development and bone maintenance (7). As key constituents of the bone mineral matrix, proteins regulate bone metabolism by providing building blocks and performing specific regulatory functions (8). In recent years, the geriatric nutritional risk index (GNRI) has been used as a significant tool to access the nutritional status of the elderly. It reflects the level of serum albumin and also includes height and body weight for the overall evaluation (9). Some studies have shown that the GNRI is associated with nutritional-related complications in hospitalized elderly patients (10), patients with hemodialysis (11), patients with chronic kidney disease (12), patients with heart failure (13), and patients with obstructive pulmonary disease (14).

However, the relationship between the GNRI and femur BMD and the risk of osteoporosis has not been adequately investigated. To the best of our knowledge, no studies have evaluated this relationship in American postmenopausal women (15, 16). The aim of this study was to explore associations of the GNRI with femur BMD and the risk of osteoporosis in this specific population.

MATERIALS AND METHODS

Study Population

Data used in this study were extracted from the National Health and Nutrition Examination Survey (NHANES). NHANES data were collected from a nationally representative sample of American civilians using a multistage probability design. All participants provided written informed consent, and NHANES was approved by the National Center for Health Statistics Ethics Review Board. For the study, we merged the continuous NHANES 2005–2006, 2007–2008, 2009–2010, 2013–2014, and 2017–2018 to ensure a large and representative sample. The inclusion criteria were as follows: (1) participants with available femur BMD and GNRI data, and (2) postmenopausal women and women over the age of 55. The exclusion criteria were participants with incomplete data on race/ethnicity, educational level, marital status, poverty income ratio (PIR), body mass index (BMI), who smoked at least 100 cigarettes, with hypertension status, with diabetes status, who ever used prednisone or cortisone daily, who ever used female hormones, who had a hysterectomy, with moderate or vigorous activity, and with a postmenopausal period (**Figure 1**).

BMD and Definition of Osteoporosis

Bone mineral density was evaluated using dual-energy x-ray absorptiometry scans with Hologic QDR-4500A fan-beam densitometers (Hologic, Inc., Bedford, MA, United States). The assessed femoral regions included total femur, femur neck, trochanter, and intertrochanter. According to the World Health Organization classification criteria, a BMD value in any femoral region lower than –2.5 standard deviations of the young adult reference group can be defined as osteoporosis. The specific thresholds were 0.68 g/cm^2, 0.59 g/cm^2, 0.49 g/cm^2, and 0.78 g/cm^2 for total femur, femur neck, trochanter, and intertrochanter, respectively (17).

Geriatric Nutritional Risk Index

According to the parameters of serum albumin (g/L), ideal body weight (WL0; kg), and actual body weight (kg), the GNRI was calculated as follows (18):

$$\text{GNRI} = (1.489 \times \text{albumin}) + (41.7 \times \frac{\text{weight}}{\text{WL0}})$$

The WL0 can be calculated using the parameter of height (H; cm) as follows:

$$\text{Women}: \text{WL0} = \text{H} - 100 - (\frac{[\text{H} - 150]}{2.5})$$

Covariates

Based on the previous literature and clinical experience, the selected covariates were obtained as follows:

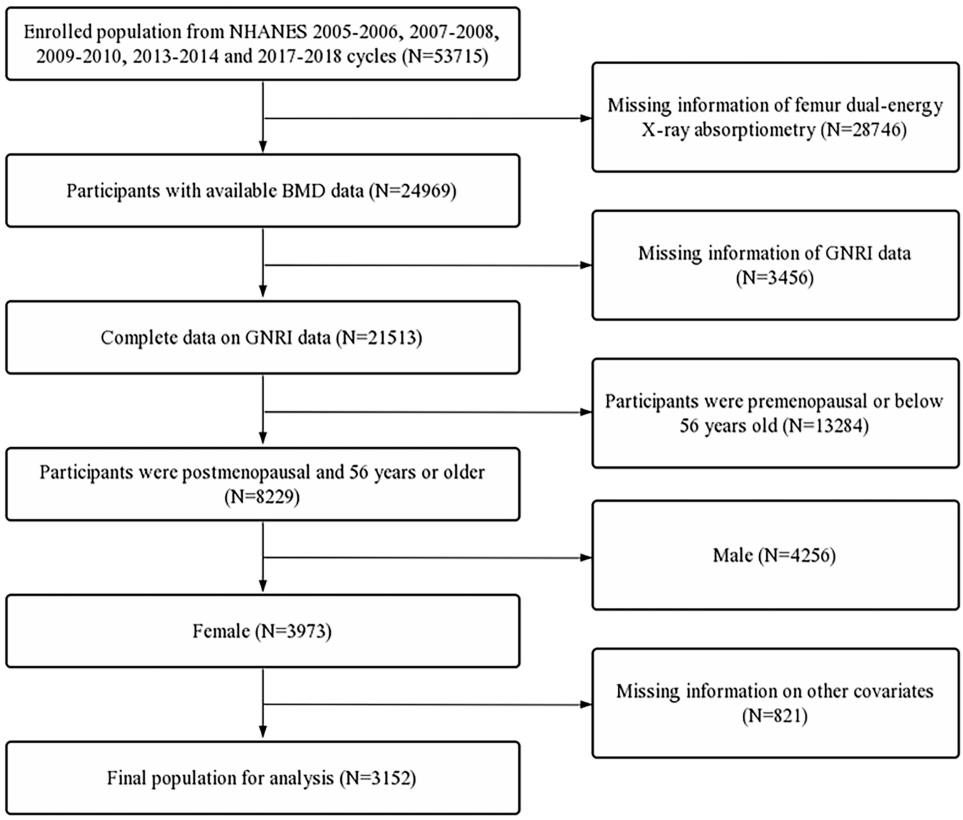

FIGURE 1 | Flow diagram of the inclusion criteria and exclusion criteria. NHANES, National Health and Nutrition Examination Survey; GNRI, geriatric nutritional risk index; BMD, bone mineral density.

1. Demographic data: age (66–70 years, ≥70 years), race/ethnicity (Mexican Americans, other Hispanic, non-Hispanic white, non-Hispanic black, and other races), educational level (less than 9th grade, 9–11th grade, high school, some college, and college graduate), marital status (married, widowed, divorced, separated, never married, and living with a partner), and PIR (≤ 1, 1–3, and > 3).
2. Examination data: BMI (< 25, 25–30, and > 30).
3. Questionnaire data: smoked at least 100 cigarettes (yes or no), hypertension status (yes or no), diabetes status (yes, no, or borderline), ever used prednisone or cortisone daily (yes or no), ever used female hormones (yes or no), had a hysterectomy (yes or no), moderate or vigorous activity (yes or no), which was defined by the criterion that usually does moderate or vigorous activities for at least 10 min that cause a moderate or vigorous increase in breathing or heart rate, and postmenopausal period, which was calculated by the age when taking the questionnaire minus age at last menstrual period.

Statistical Analysis

Based on the weight selection criteria of NHANES, the weight value used in the study was one-fifth of the full sample two-year mobile examination center of exam weight. Data from continuous and categorical variables were described by the mean and proportion, respectively. The Chi-squared test was used to compare the differences in categorical variables between the osteoporosis and non-osteoporosis groups and for continuous variables, a Student's *t*-test was used. The linear relationship between the GNRI and femur BMD was assessed using a weighted multivariate linear regression model. The odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association between the GNRI and the risk of osteoporosis were assessed using a weighted logistic regression model. Model 1 was adjusted for no covariates. Model 2 was adjusted for age, race, and BMI. Model 3 was adjusted for all the covariates. Moreover, we performed subgroup analyses using weighted stratified line regression models based on age. The nonlinear relationship in this study was also characterized by smooth curve fitting (SCF) and a weighted generalized additive model (GAM). Furthermore, the following analyses were performed to ensure the robustness of the data analysis. First, the values of GNRI were categorized based on quartiles, and tests for linear trends were performed. All the steps described above were also performed to evaluate the relationship between the categorized GNRI and femur BMD and the risk of osteoporosis.

All analyses were performed using R software (4.0.3) and EmpowerStats (2.0). A two-sided p-value < 0.05 was considered to have statistical significance.

TABLE 1 | Weighted characteristics of the study population.

	Non-osteoporosis (*N* = 2484, 78.807%)	Osteoporosis (*N* = 668, 21.200%)	*P* value
GNRI (mean ± SD)	125.122 ± 12.739	116.510 ± 11.406	< 0.00001
Age (%)			< 0.00001
<70	69.628	46.018	
≥70	30.372	53.982	
Race (%)			0.00004
Mexican Americans	4.076	2.84	
Other Hispanic	2.941	3.577	
Non-Hispanic White	79.618	82.341	
Non-Hispanic Black	8.648	3.893	
Other race	4.716	7.349	
BMI (%)			< 0.00001
<25	24.751	50.814	
≥25, <30	34.068	32.603	
≥30	41.181	16.583	
PIR (%)			< 0.00001
<1	7.914	9.48	
≥1, <3	37.851	49.126	
≥3	54.235	41.393	
Educational level (%)			< 0.00001
Less than 9th grade	4.502	7.255	
9–11th grade	9.366	13.209	
High school	27.746	29.845	
Some college	29.79	29.606	
College graduate	28.596	20.085	
Marital status (%)			< 0.00001
Married	58.725	45.263	
Widowed	18.15	33.207	
Divorced	16.354	16.737	
Separated	1.373	0.913	
Never married	3.638	2.776	
Living with partner	1.761	1.105	
Diabetes status (%)			0.17031
Yes	14.696	12.555	
No	82.035	85.04	
Borderline	3.27	2.405	
Hypertension status (%)			0.4737
Yes	55.32	53.765	
No	44.68	46.235	
Ever use prednisone or cortisone daily (%)			0.73002
Yes	8.013	8.423	
No	91.987	91.577	
Smoked at least 100 cigarettes (%)			0.0036
Yes	39.548	45.789	
No	60.452	54.211	
Had a hysterectomy (%)			0.00245
Yes	40.603	47.124	
No	59.397	52.876	
Ever use female hormones (%)			0.00006
Yes	49.633	40.903	
No	50.367	59.097	
Postmenopausal period (years, mean ± SD)	18.882 ± 10.954	24.791 ± 11.544	< 0.00001
Moderate or vigorous activity (%)			0.00165
Yes	51.054	44.194	
No	48.946	55.806	
Total femur BMD (g/cm^2, mean ± SD)	0.882 ± 0.111	0.669 ± 0.076	< 0.00001
Femur neck BMD (g/cm^2, mean ± SD)	0.741 ± 0.103	0.550 ± 0.070	< 0.00001
Trochanter BMD (g/cm^2, mean ± SD)	0.666 ± 0.097	0.508 ± 0.079	< 0.00001
Intertrochanter BMD (g/cm^2, mean ± SD)	1.051 ± 0.139	0.800 ± 0.106	< 0.00001

BMD, bone mineral density; GNRI, geriatric nutritional risk index; PIR, poverty income ratio; BMI, body mass index; SD standard deviation; %, weighted percentage.

TABLE 2 | Associations of the GNRI with femur BMD and the risk of osteoporosis.

	Model 1 β (95% CI) *P* value	Model 2 β (95% CI) *P* value	Model 3 β (95% CI) *P* value
Total femur BMD (g/cm^2)	0.0048 (0.0044, 0.0051) < 0.000001	0.0026 (0.0021, 0.0032) < 0.000001	0.0024 (0.0019, 0.0029) < 0.000001
Femur neck BMD (g/cm^2)	0.0037 (0.0034, 0.0040) < 0.000001	0.0019 (0.0014, 0.0024) < 0.000001	0.0017 (0.0012, 0.0023) < 0.000001
Trochanter BMD (g/cm^2)	0.0037 (0.0034, 0.0039) < 0.000001	0.0020 (0.0016, 0.0025) < 0.000001	0.0019 (0.0014, 0.0024) < 0.000001
Intertrochanter BMD (g/cm^2)	0.0056 (0.0052, 0.0060) < 0.000001	0.0029 (0.0022, 0.0036) < 0.000001	0.0026 (0.0020, 0.0033) < 0.000001
Osteoporosis	0.9381 (0.9340, 0.9421) < 0.000001	0.9529 (0.9458, 0.9600) < 0.000001	0.9587 (0.9514, 0.9660) < 0.000001

Model 1: no covariates were adjusted.
Model 2: age, race/ethnicity, and BMI were adjusted.
Model 3: age, race/ethnicity, BMI, educational level, marital status, PIR, smoked at least 100 cigarettes, hypertension status, diabetes status, ever used prednisone or cortisone daily, ever used female hormones, had a hysterectomy, moderate or vigorous activity, and postmenopausal period were adjusted.
BMD, bone mineral density; GNRI, geriatric nutritional risk index; PIR, poverty income ratio; BMI, body mass index.

TABLE 3 | Associations of the GNRI.Q4 with femur BMD and the risk of osteoporosis.

	Model 1 β (95% CI) *P* value	Model 2 β (95% CI) *P* value	Model 3 β (95% CI) *P* value
Total femur BMD (g/cm^2)			
GNRI.Q4			
Q1 (77.5361–114.9444)	References	References	References
Q2 (114.9520–122.5324)	0.0522 (0.0402, 0.0641) < 0.000001	0.0300 (0.0153, 0.0447) 0.000066	0.0234 (0.0090, 0.0378) 0.001463
Q3 (122.5404–130.8391)	0.1008 (0.0887, 0.1129) < 0.000001	0.0443 (0.0263, 0.0624) 0.000002	0.0365 (0.0188, 0.0542) 0.000055
Q4 (130.8491–195.4885)	0.1560 (0.1440, 0.1680) < 0.000001	0.0643 (0.0427, 0.0859) < 0.000001	0.0574 (0.0362, 0.0786)
P for trend	< 0.001	<0.001	< 0.001
Femur neck BMD (g/cm^2)			
GNRI.Q4			
Q1 (77.5361–114.9444)	References	References	References
Q2 (114.9520–122.5324)	0.0414 (0.0302, 0.0526) < 0.000001	0.0253 (0.0115, 0.0390) 0.000326	0.0212 (0.0076, 0.0348) 0.002323
Q3 (122.5404–130.8391)	0.0778 (0.0664, 0.0892) < 0.000001	0.0371 (0.0202, 0.0540) 0.000018	0.0312 (0.0145, 0.0480) 0.000254
Q4 (130.8491–195.4885)	0.1230 (0.1118, 0.1342) < 0.000001	0.0567 (0.0365, 0.0769) < 0.000001	0.0506 (0.0305, 0.0706)
P for trend	< 0.001	<0.001	< 0.001
Trochanter BMD (g/cm^2)			
GNRI.Q4			
Q1 (77.5361–114.9444)	References	References	References
Q2 (114.9520–122.5324)	0.0440 (0.0340, 0.0541) < 0.000001	0.0286 (0.0160, 0.0411) 0.000009	0.0233 (0.0110, 0.0356) 0.000211
Q3 (122.5404–130.8391)	0.0796 (0.0694, 0.0899) < 0.000001	0.0387 (0.0233, 0.0542) < 0.000001	0.0321 (0.0170, 0.0473) 0.000032
Q4 (130.8491–195.4885)	0.1219 (0.1118, 0.1321) < 0.000001	0.0547 (0.0362, 0.0732) < 0.000001	0.0495 (0.0314, 0.0676)
P for trend	< 0.001	<0.001	< 0.001
Intertrochanter BMD (g/cm^2)			
GNRI.Q4			
Q1 (77.5361–114.9444)	References	References	References
Q2 (114.9520–122.5324)	0.0608 (0.0460, 0.0757) < 0.000001	0.0327 (0.0143, 0.0511) 0.000502	0.0243 (0.0062, 0.0424) 0.008461
Q3 (122.5404–130.8391)	0.1207 (0.1056, 0.1358) < 0.000001	0.0506 (0.0280, 0.0731) 0.000012	0.0408 (0.0186, 0.0630) 0.000325
Q4 (130.8491–195.4885)	0.1822 (0.1673, 0.1971) < 0.000001	0.0695 (0.0425, 0.0965) < 0.000001	0.0606 (0.0340, 0.0872) 0.000008
P for trend	< 0.001	<0.001	< 0.001
Osteoporosis			
GNRI.Q4			
Q1 (77.5361–114.9444)	1	1	1
Q2 (114.9520–122.5324)	0.4358 (0.3896, 0.4875) < 0.000001	0.5140 (0.4448, 0.5939) < 0.000001	0.5565 (0.4791, 0.6463)
Q3 (122.5404–130.8391)	0.3089 (0.2733, 0.3492) < 0.000001	0.4839 (0.4017, 0.5830) < 0.000001	0.5580 (0.4600, 0.6769)
Q4 (130.8491–195.4885)	0.1455 (0.1255, 0.1688) < 0.000001	0.3196 (0.2483, 0.4114) < 0.000001	0.3475 (0.2681, 0.4505)
P for trend	< 0.001	<0.001	< 0.001

Model 1: no covariates were adjusted.
Model 2: age, race/ethnicity, and BMI were adjusted.
Model 3: age, race/ethnicity, BMI, educational level, marital status, PIR, smoked at least 100 cigarettes, hypertension status, diabetes status, ever used prednisone or cortisone daily, ever used female hormones, had a hysterectomy, moderate or vigorous activity, and postmenopausal period were adjusted.
BMD, bone mineral density; GNRI.Q4, geriatric nutritional risk index quartile; PIR, poverty income ratio; BMI, body mass index.

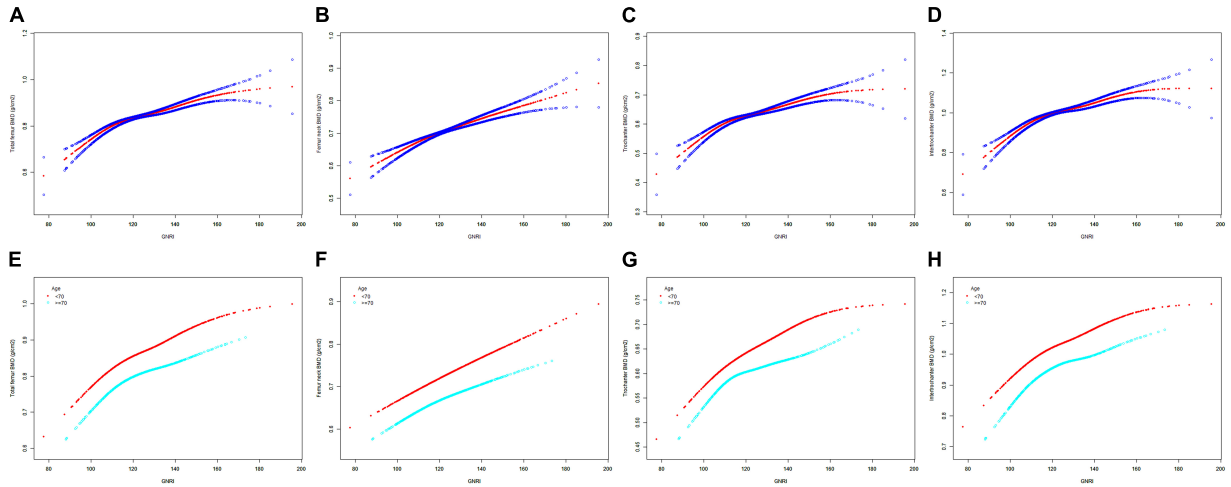

FIGURE 2 | The SCF for the association between GNRI and femur BMD. Age [in panels **(A–D)**], race/ethnicity, BMI, educational level, marital status, PIR, smoked at least 100 cigarettes, hypertension status, diabetes status, ever used prednisone or cortisone daily, ever used female hormones, had a hysterectomy, moderate or vigorous activity, and postmenopausal period were adjusted. **(A,E)** Total femur BMD; **(B,F)** Femur neck BMD; **(C,G)** Trochanter BMD; **(D,H)** Intertrochanter BMD. SCF, smooth curve fit; BMD, bone mineral density; GNRI, geriatric nutritional risk index; PIR, poverty income ratio; BMI, body mass index.

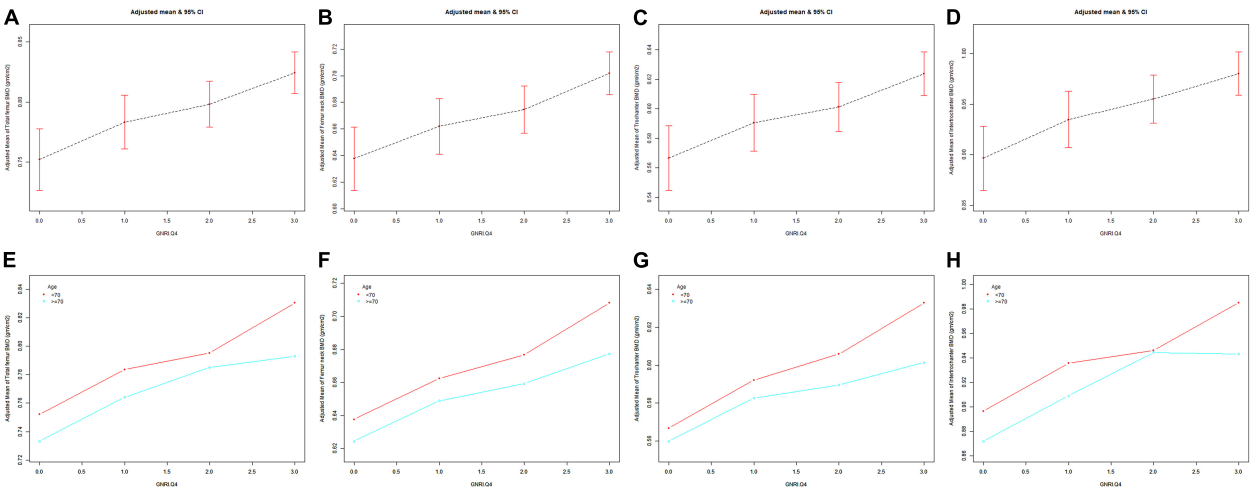

FIGURE 3 | The association between GNRI.Q4 and femur BMD. Age [in panels **(A–D)**], race/ethnicity, BMI, educational level, marital status, PIR, smoked at least 100 cigarettes, hypertension status, diabetes status, ever used prednisone or cortisone daily, ever used female hormones, had a hysterectomy, moderate or vigorous activity, and postmenopausal period were adjusted. **(A,E)** Total femur BMD; **(B,F)** Femur neck BMD; **(C,G)** Trochanter BMD; **(D,H)** Intertrochanter BMD. BMD, bone mineral density; GNRI.Q4, geriatric nutritional risk index quartiles; PIR, poverty income ratio; BMI, body mass index.

RESULTS

Baseline Characteristics of Participants

First, 53,715 participants were selected from the NHANES 2005–2006, 2007–2008, 2009–2010, 2013–2014, and 2017–2018. In these datasets, participants with missing femur BMD data (n = 28,746) and incomplete GNRI data (n = 3,456) were excluded. Furthermore, participants aged below 56 years (n = 13,284), male participants (n = 4,256), and participants with missing data on other covariates (n = 821) were also excluded. A total of 3,152 participants were included in the final analysis (**Figure 1**).

The baseline characteristics of selected participants were compared between the osteoporosis and non-osteoporosis groups (**Table 1**). According to the diagnosis criteria for osteoporosis (17), the prevalence of osteoporosis was 21.200% (668/3,152) in this study. Compared with patients with osteoporosis, participants without osteoporosis were more likely to have higher values of GNRI (125.122 ± 12.739 vs. 116.510 ± 11.406, $P < 0.00001$) but shorter postmenopausal years (18.882 ± 10.954 vs. 24.791 ± 11.544, $P < 0.00001$). Moreover, participants in the osteoporosis group tended to be older, more emaciated, widowed, smoked more cigarettes, poorer, have less activity, and have lower educational levels. Besides, the

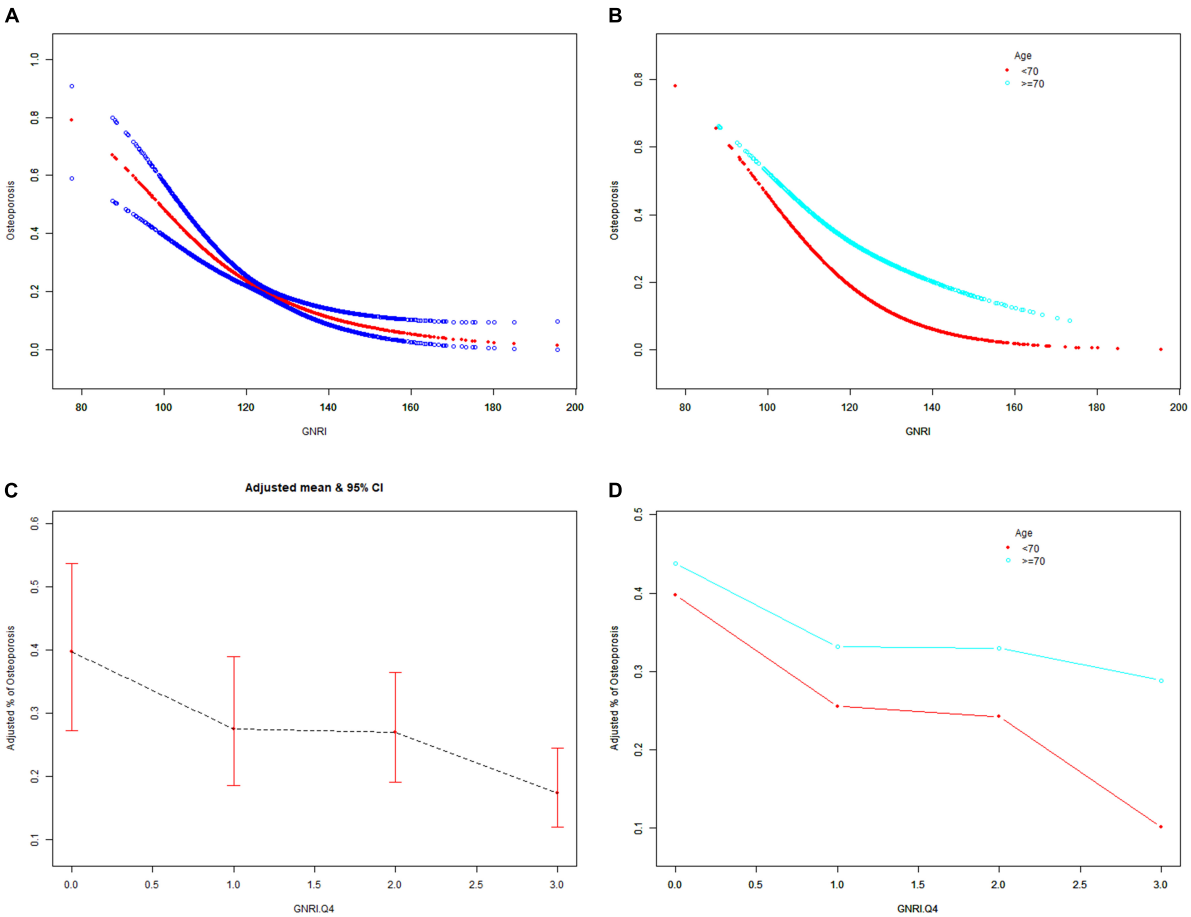

FIGURE 4 | The associations of GNRI and GNRI.Q4 with the risk of osteoporosis. Age [**(A,C)** were applicable, **(B,D)** were not applicable], race/ethnicity, BMI, educational level, marital status, PIR, smoked at least 100 cigarettes, hypertension status, diabetes status, ever use prednisone or cortisone daily, ever use female hormones, had a hysterectomy, moderate or vigorous activity, and postmenopausal period were adjusted. BMD, bone mineral density; GNRI.Q4, geriatric nutritional risk index quartiles; PIR, poverty income ratio; BMI, body mass index.

percentage of participants who ever used female hormones and had a hysterectomy was significantly higher in the osteoporosis group ($P < 0.05$, **Table 1**).

Associations of GNRI With Femur BMD and Osteoporosis

The GNRI values showed a positive association with femur BMD and a negative association with the risk of osteoporosis in Model 1. After adjusting for confounding factors in Model 2 (age, race/ethnicity, and BMI) and Model 3 (age, race/ethnicity, BMI, educational level, marital status, PIR, smoked at least 100 cigarettes, hypertension status, diabetes status, ever used prednisone or cortisone daily, ever used female hormones, had a hysterectomy, moderate or vigorous activity, and postmenopausal period), the relationship between exposed variables and outcomes was still stable. When adjusting for all covariates, each unit of increased GNRI value was associated with a decreased risk of osteoporosis of 4.13% (**Table 2**). After categorizing GNRI based on quartiles, ORs between the risk of osteoporosis and GNRI values across quintiles 2, 3, and 4 compared with quintile 1 were 0.5565 (95% CI: 0.4791, 0.6463; $P < 0.000001$), 0.5580 (95% CI: 0.4600, 0.6769; $P < 0.000001$), and 0.3475 (95% CI: 0.2681, 0.4505; $P < 0.000001$), respectively, in Model 3. The trend test also showed that, with the increase of GNRI quartile groups, the risk of osteoporosis decreased (for trend, $P < 0.001$) (**Table 3**). Moreover, the trends similar to the above were also observed in SCF and GAM (**Figures 2A–D**, **3A–D**, **4A,C**).

Subgroup Analyses

After stratification by age, the results presented a similar trend to the above. Whether or not the participants were older than 70 years, the GNRI values presented a positive association with femur BMD and a negative association with the risk of osteoporosis in Model 1, Model 2, and Model 3. When adjusting for all covariates except age, each unit of increased GNRI value was associated with 2.74 and 4.64% decreased risk of osteoporosis in people aged below 70 years and those aged 70 years or older, respectively (**Table 4**). Moreover, in people aged below 70 years, the ORs between the risk of osteoporosis and GNRI across

TABLE 4 | Associations of the GNRI with femur BMD and the risk of osteoporosis stratified by age.

	Age < 70 β (95% CI) *P* value	Age ≥ 70 β (95% CI) *P* value
Total femur BMD (g/cm^2)		
Model 1	0.0044 (0.0040, 0.0048) < 0.000001	0.0048 (0.0042, 0.0053) < 0.000001
Model 2	0.0021 (0.0015, 0.0028) < 0.000001	0.0035 (0.0026, 0.0045) < 0.000001
Model 3	0.0019 (0.0012, 0.0026) < 0.000001	0.0032 (0.0023, 0.0042) < 0.000001
Femur neck BMD (g/cm^2)		
Model 1	0.0035 (0.0031, 0.0038) < 0.000001	0.0034 (0.0029, 0.0040) < 0.000001
Model 2	0.0014 (0.0007, 0.0020) 0.000035	0.0029 (0.0020, 0.0037) < 0.000001
Model 3	0.0012 (0.0006, 0.0019) 0.000262	0.0025 (0.0017, 0.0034) < 0.000001
Trochanter BMD (g/cm^2)		
Model 1	0.0035 (0.0032, 0.0039) < 0.000001	0.0034 (0.0029, 0.0039) < 0.000001
Model 2	0.0018 (0.0012, 0.0024) < 0.000001	0.0025 (0.0016, 0.0033) < 0.000001
Model 3	0.0016 (0.0011, 0.0022) < 0.000001	0.0022 (0.0014, 0.0031) < 0.000001
Intertrochanter BMD (g/cm^2)		
Model 1	0.0050 (0.0045, 0.0055) < 0.000001	0.0059 (0.0052, 0.0066) < 0.000001
Model 2	0.0022 (0.0014, 0.0031) < 0.000001	0.0042 (0.0030, 0.0054) < 0.000001
Model 3	0.0020 (0.0011, 0.0028) 0.000004	0.0038 (0.0026, 0.0050) < 0.000001
Osteoporosis		
Model 1	0.9286 (0.9228, 0.9345) < 0.000001	0.9529 (0.9470, 0.9589) < 0.000001
Model 2	0.9582 (0.9480, 0.9686) < 0.000001	0.9516 (0.9418, 0.9616) < 0.000001
Model 3	0.9726 (0.9620, 0.9834) < 0.000001	0.9536 (0.9434, 0.9640) < 0.000001

Model 1: no covariates were adjusted.
Model 2: race/ethnicity and BMI were adjusted.
Model 3: race/ethnicity, BMI, educational level, marital status, PIR, smoked at least 100 cigarettes, hypertension status, diabetes status, ever used prednisone or cortisone daily, ever used female hormones, had a hysterectomy, moderate or vigorous activity, and postmenopausal period were adjusted.
BMD, bone mineral density; GNRI, geriatric nutritional risk index; PIR, poverty income ratio; BMI, body mass index.

quintiles 2, 3, and 4 compared with quintile 1 were 0.6400 (95% CI: 0.5193, 0.7887; $P = 0.000028$), 0.7007 (95% CI: 0.5321, 0.9229; $P = 0.011359$), and 0.4918 (95% CI: 0.3274, 0.7387; $P = 0.000628$), respectively. In people who were 70 years or older, the ORs were 0.5055 (95% CI: 0.4074, 0.6273; $P < 0.000001$), 0.4877 (95% CI: 0.3702, 0.6426; $P < 0.000001$), and 0.3550 (95% CI: 0.2497, 0.5049; $P < 0.000001$), respectively (**Table 5**). In addition, the results of stratified analyses were also supported by the trends presented in SCF and GAM (**Figures 2E–H**, **3E–H**, **4B,D**).

DISCUSSION

Based on the representative sample of American postmenopausal women in NHANES (2005–2006, 2007–2010, 2013–2014, and 2017–2018), this study found that the GNRI value was positively correlated to the femur BMD and negatively correlated to the risk of osteoporosis in this population. In addition, we demonstrated that the above associations were stable and not affected by age subgroups. To the best of our knowledge, this study is the first to explore the associations of the GNRI with femur BMD and the risk of osteoporosis in American postmenopausal women.

Previous studies have explored the relationship between protein intake and the risk of osteoporosis. For example, Looker et al. found that optimal protein intake could help to enhance BMD and prevent osteoporosis and fractures in postmenopausal women (19). Rizzoli et al. have systematically summarized a series of reviews and meta-analyses about the impact of protein intake on bone health, highlighting the key message that optimal maintenance of bone health in adults requires adequate supplies

TABLE 5 | Associations of the GNRI.Q4 with femur BMD and the risk of osteoporosis stratified by age.

	Age < 70 **β (95% CI) *P* value**	**Age ≥ 70** **β (95% CI) *P* value**
Total femur BMD (g/cm^2)		
GNRI.Q4		
Q1 (77.5361–114.9444)	References	References
Q2 (114.9520–122.5324)	0.0104 (−0.0084, 0.0291) 0.279484	0.0428 (0.0202, 0.0653) 0.000214
Q3 (122.5404–130.8391)	0.0183 (−0.0048, 0.0414) 0.119757	0.0629 (0.0350, 0.0908) 0.000011
Q4 (130.8491–195.4885)	0.0409 (0.0134, 0.0683) 0.003552	0.0779 (0.0436, 0.1123) 0.000010
P trend	0.004	< 0.001
Femur neck BMD (g/cm^2)		
GNRI.Q4		
Q1 (77.5361–114.9444)	References	References
Q2 (114.9520–122.5324)	0.0149 (−0.0033, 0.0332) 0.108267	0.0307 (0.0103, 0.0512) 0.003230
Q3 (122.5404-130.8391)	0.0227 (0.0003, 0.0451) 0.047516	0.0432 (0.0180, 0.0685) 0.000815
Q4 (130.8491–195.4885)	0.0346 (0.0079, 0.0613) 0.011097	0.0679 (0.0368, 0.0990) 0.000020
P trend	0.013	< 0.001
Trochanter BMD (g/cm^2)		
GNRI.Q4		
Q1 (77.5361–114.9444)	References	References
Q2 (114.9520–122.5324)	0.0147 (−0.0014, 0.0308) 0.073631	0.0359 (0.0167, 0.0552) 0.000266
Q3 (122.5404–130.8391)	0.0252 (0.0054, 0.0449) 0.012837	0.0412 (0.0174, 0.0650) 0.000717
Q4 (130.8491–195.4885)	0.0432 (0.0196, 0.0667) 0.000334	0.0549 (0.0256, 0.0842) 0.000252
P trend	< 0.001	<0.001
Intertrochanter BMD (g/cm^2)		
GNRI.Q4		
Q1 (77.5361–114.9444)	References	References
Q2 (114.9520–122.5324)	0.0077 (−0.0157, 0.0311) 0.517915	0.0488 (0.0204, 0.0772) 0.000790
Q3 (122.5404–130.8391)	0.0147 (−0.0141, 0.0435) 0.317872	0.0803 (0.0452, 0.1155) 0.000008
Q4 (130.8491–195.4885)	0.0403 (0.0060, 0.0746) 0.021238	0.0883 (0.0451, 0.1315) 0.000067
P trend	0.021	< 0.001
Osteoporosis		
GNRI.Q4		
Q1 (77.5361–114.9444)	References	References
Q2 (114.9520–122.5324)	0.6400 (0.5193, 0.7887) 0.000028	0.5055 (0.4074, 0.6273) < 0.000001
Q3 (122.5404–130.8391)	0.7007 (0.5321, 0.9229) 0.011359	0.4877 (0.3702, 0.6426) < 0.000001
Q4 (130.8491–195.4885)	0.4918 (0.3274, 0.7387) 0.000628	0.3550 (0.2497, 0.5049) < 0.000001
P trend	< 0.001	<0.001

Model 1: no covariates were adjusted.
Model 2: race/ethnicity and BMI were adjusted.
Model 3: race/ethnicity, BMI, educational level, marital status, PIR, smoked at least 100 cigarettes, hypertension status, diabetes status, ever used prednisone or cortisone daily, ever used female hormones, had a hysterectomy, moderate or vigorous activity, and postmenopausal period were adjusted.
BMD, bone mineral density; GNRI.Q4, geriatric nutritional risk index quartile; PIR, poverty income ratio; BMI, body mass index.

of dietary proteins (20). As a reflection of nutritional status with regards to protein, serum albumin can be associated with BMD. In a large cross-sectional observation of 21,121 patients, Afshinnia et al. reported an independent association of osteoporosis with low levels of serum albumin and long-term hypoalbuminemia (21) that supported the view of Coin et al. (22). Moreover, the association between BMI and BMD has also been shown in many studies. Tomlinson et al. hold the view that bones could benefit from combining high BMI with moderate-to-vigorous activities and an optimal diet (23). The study of Lloyd et al. found that higher BMI was conducive to increasing BMD (24). The views of Wang et al. (25) and Wu et al. (26) also supported this result. Compared with the individual variables of serum albumin or BMI, the GNRI combines serum albumin with body weight and height, which can be more comprehensive and effective for evaluating systemic nutritional status. Besides, in a receiver operating characteristic analysis for predicting osteoporosis, compared with serum albumin, BMI, and age, the GNRI had the largest area under the curve, indicating that the GNRI was a powerful indicator to improve the accuracy of diagnosis (27).

The specific mechanisms leading to the positive correlation between the GNRI and BMD may be multiple. On the one hand, dietary protein supplements can increase insulin-like growth factor I (IGF-I) (20, 28, 29) and decrease parathyroid hormone (PTH) (20, 30) and further reduce age-related BMD loss. On the other hand, optimal protein intake can help to resist loss of muscle and prevent sarcopenia in the elderly (31–33). Previous studies have demonstrated that, although many potential confounding factors were adjusted, the risk of BMD loss was still higher in the sarcopenic population (34–38). As is known to all, muscles can influence bones through secreting bone factors and exerting physical forces (39). Some molecules secreted by skeletal muscle, such as IGF-I, interleukin-6 (IL-6), IL-15, basic fibroblast growth factor, myostatin, and osteoglycin, have impacts on bone metabolism (40). Physical forces are usually produced by gravity, locomotion, or external devices (41). In this respect, the positive effect of high BMI on BMD has been recognized as a result of increased mechanical loading exerted on the skeleton (42). In short, the mechanism of the significant associations between GNRI and BMD and the risk of osteoporosis may be explained by an increase in IGF-I, a decrease in PTH, and resistance to muscle loss.

There are several strengths in this study. First, we used a large, nationally representative database, which was collected using standardized protocols to minimize possible bias. Second, we adequately controlled for confounders and assessed the difference in the association of the GNRI with femur BMD and the risk of osteoporosis in diverse populations by stratifying age. Third, we also categorized the GNRI by quartiles and performed tests for linear trends to ensure the robustness and accuracy of the data analyses. In addition, this study also has some potential limitations. First, because this study was a cross-sectional analysis, the evidence for a causal relationship may be insufficient. Second, the data collected from questionnaires and interviews may result in recall bias. Third, although we have adjusted some covariates, other unmeasured confounding factors may also lead to potential bias. In the future, more prospective studies need to be performed to confirm the results of this study.

CONCLUSION

Our results indicated that nutritional status, represented by the GNRI, was positively associated with femur BMD and negatively associated with the risk of osteoporosis in American postmenopausal women. The GNRI may be a good tool to identify American postmenopausal women who need further bone health nutritional support.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

JW, FX, and NS: conceptualization and investigation. JW: methodology, analysis, and writing the original draft. JW, FX, NS, and ZX: writing—review and editing. All authors contributed to the development of this manuscript, and they read and approved the final version.

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Association of Dietary Factors With Grip Strength, Body Fat, and Prevalence of Sarcopenic Obesity in Rural Korean Elderly With Cardiometabolic Multimorbidity

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Background and Aims: Aging accompanied by cardiometabolic multimorbidity (CM) promotes chronic low-grade inflammation, increased oxidative stress, and insulin resistance (IR), which result in loss of muscle mass and functional impairment. Better quality diets have been directly associated with muscle health and decreased risk of all-cause mortality. However, no study has investigated the relationship of dietary factors with grip strength, body composition, and prevalence of sarcopenic obesity (SO) in Korean rural residents according to their CM pattern. Therefore, we aimed to examine this association among this population.

Materials and Methods: This cross-sectional study utilized data from 932 rural residents aged $\geq$ 65 years. An exploratory tetrachoric factor analysis revealed four multimorbidity patterns: CM, inflammatory disease, respiratory disease, and cancer and other diseases. All participants were categorized into the CM and non-CM groups. Skeletal muscle mass and the prevalence of sarcopenia were estimated using bioelectrical impedance analysis (BIA). Dietary assessment was analyzed using a validated 106-item food frequency questionnaire. Adjusted multiple linear regression and multivariate logistic regression were employed to examine the association of dietary factors with muscle strength, quality, and SO prevalence ratio in elderly participants.

Results: The mean age of the participants was 71.8 $\pm$ 0.1 years (65.8% women). Dietary fat and protein intake were positively correlated with handgrip strength in women with CM, after adjusting for covariates (p = 0.001). Similarly, protein intake (g/kg) was positively associated with appendicular skeletal muscle mass (ASM; kg/m^2) and ASM (%) in both sexes in the CM and non-CM groups. Regarding the tertiles of wheat intake (g/d), 2.1-fold increase in SO prevalence ratios [prevalence ratio (PR): 2.149, confidence intervals (CIs): 1.134–4.071] was observed in the highest tertile (T3: 269.1 g/d), compared to the lowest tertile (Q1: 8.6 g/d) in the CM group. Higher tertile

of meat intake (T2: 34.8 g/d, T3: 99.5 g/d) had a 2-fold increase in SO (PR: 1.932, CIs: 1.066–3.500) compared to the lowest tertile (T1: 9.2 g/d) in the CM group.

Conclusion: Overconsumption of wheat and meat negatively impacted the development of SO, while protein intake was positively associated with grip strength and skeletal muscle mass in elderly Koreans with CM.

Keywords: diet, elderly, macronutrients, sarcopenic obesity, cardiometabolic multimorbidity

INTRODUCTION

Sarcopenic obesity (SO) is related to not only aging but also cardiovascular diseases (CVDs) and metabolic diseases such as insulin resistance (IR), type 2 diabetes (T2DM), and obesity (1). A recent review reported that more than one in 10 older adults face a health crisis due to SO (2).

Cardiometabolic multimorbidity (CM), i.e., the presence of two or more chronic diseases such as T2DM and CVDs, requires comprehensive care to prevent functional incapacity and worsening in the elderly population (3, 4). Compared to patients with single diseases, those with multimorbidity have cumulative interactions between diseases (5). Aging increases susceptibility to multimorbidity due to multisystem homeostatic dysregulation (3). A report from 18 countries identified a 4–8 times greater risk of all-cause mortality in participants with more than three chronic diseases when compared to those with a single disease (6). Physiological, hormonal, and other lifestyle factors may act as mediators between CM and SO (7–9). In geriatrics, multimorbidity is a more common clinical problem that affects several pathways (3). Therefore, multidisciplinary integrated care approaches are required so as to not only focus on single diseases or risk factors.

A recent study pointed out the requirement of muscle functional evaluation in the sarcopenia research field (10), particularly, related to the coexistence of sarcopenia and obesity, due to accelerated loss of muscle mass, strength, and quality in the elderly (11). Handgrip strength has been reported as a strong predictor of all-cause and CVD mortality in people with diverse economic and sociocultural backgrounds in a large longitudinal prospective study (12). Decreased handgrip strength has also been reported in adults (aged > 50 years) with multimorbidity compared to those without chronic diseases (13).

Adequate nutrition is an essential contributor to maintaining health and decreasing the risk of all-cause mortality, CVDs, and T2DM in older adults (14–16). Macronutrients (carbohydrates, fat, and proteins) play an important role in preventing muscle loss and IR in aging muscle biology (17–19). A healthy and balanced diet rich in vegetables, fruits, legumes, and fish is associated with a lower 10-year risk of incidence of CVDs than the typical white rice and grain-based Korean diet in elderly people (20). The recent Korean National Health and Nutrition Examinations Survey (KNHANES) reported that excessive carbohydrate, low fruit, and imbalanced food and nutrient intakes contribute to cardiometabolic abnormalities in rural residents (21). However, no study has investigated that associations between nutritional risk factors and grip strength, body composition, and prevalence of SO by considering regional specificity in Korean rural residents. We hypothesized that elderly with CM would have an inadequate dietary intake compared to the non-cardiometabolic multimorbidity pattern (CMP) group. Thus, this study aimed to identify CM patterns in elderly Koreans living in a rural area and examine how the dietary factors, grip strength, body composition, and prevalence of SO among this population may relate to their CM pattern.

Abbreviations: AWGS, Asian Working Group for Sarcopenia; ASM, appendicular skeletal muscle mass; BIA, bioelectrical impedance analysis; BMI, body mass index; BP, blood pressure; CI, confidence interval; CM cardiometabolic multimorbidity; CVD, cardiovascular disease; FA, fatty acid; HDL, high-density lipoprotein; IR, insulin resistance; KDRIs, Korean Dietary Reference Intakes; KM, Korean Medicine; KNHANES, Korean National Health and Nutrition Examinations Survey; MetS, metabolic diseases; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; PR, prevalence ratio; SD, standard deviation; SFA, saturated fatty acid; SO, sarcopenic obesity; T2DM, type 2 diabetes; TG, triglycerides.

MATERIALS AND METHODS

Design, Setting, and Participants

Data were collected from secondary research conducted in February 2014 to assess health status (22, 23), Korean Medicine (KM) constitutional type (24), and health-related clinical outcomes of the general population according to sub-health status. Baseline survey data were obtained after the participants provided written informed consent. Each participant was asked to visit the local health examination center for a health survey and examination. To use well-curated validation datasets for the present analysis, approvals were obtained from the web-based Korean Medicine Data Center (KDC) electronic data capture system by the KDC of the Korean Institute of Oriental Medicine (25).

Anthropometric measurements [height, weight, waist circumference, systolic blood pressure (BP), diastolic BP, and handgrip strength] and laboratory data [triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and fasting plasma glucose after overnight fasting] were recorded. Of the 1,890 participants in the survey data, 932 rural residents in Gyeongju, South Korea, aged ≥65 years, were included in the present analysis.

Definition of Cardiometabolic Multimorbidity Pattern

We defined multimorbidity (3, 4) as the co-occurrence of any of the following 27 chronic diseases: stroke, transient

ischemic attack, angina (or myocardial infarction), hypertension, dyslipidemia, pulmonary tuberculosis, thyroid disorder (other than thyroid cancer), chronic gastritis, ulcer (gastric/duodenal), diabetes, intestinal polyposis syndrome, acute hepatic disease, fatty liver, chronic hepatitis (or hepatic cirrhosis), cholelithiasis (or cholecystitis), chronic bronchitis, asthma, allergy, arthritis, cystitis, cataract, glaucoma, depression, Parkinson's disease, osteoporosis, prostatic hyperplasia, and cancer. A combination of methods is used to determine the presence of multimorbidity, including physician diagnosis and/or current use of medications for a particular disease (25–27).

Four multimorbidity patterns were identified among the participants: isolated CM [23.8% (n = 222)], respiratory diseases [12.4% (n = 116)], inflammatory diseases [12.3% (n = 115)], and cancer and other diseases [19.6% (n = 183)] (data not shown). A total of 4.7% of the participants had all four patterns, whereas 26.2% (n = 244) had none of the four patterns. CM pattern included diabetes, dyslipidemia, hypertension, and angina (or myocardial infarction). Respiratory disease pattern included pulmonary tuberculosis, chronic bronchitis, asthma, and allergies. Inflammatory diseases pattern included chronic gastritis, arthritis, cystitis, cataracts, and osteoporosis. Finally, cancer and other diseases pattern included acute hepatic disease, fatty liver, chronic hepatitis, glaucoma, depression, and cancers. Participants with cardiometabolic multimorbidity, such as diabetes, dyslipidemia, hypertension, and angina (or myocardial infarction), were extracted and further categorized into a CM pattern only group (50.0%, n = 466; if not, non-CMP).

Definition of Sarcopenic Obesity

Sarcopenic obesity is generally defined as the coexistence of sarcopenia and obesity (11). Recently, broad diagnostic criteria have been employed to identify both sarcopenia and obesity, such as sex-specific weak handgrip strength, body mass index (BMI), or total body fat percentage (%BF) (26). We used the cross-validated equation (27) for the estimation of appendicular skeletal muscle mass (ASM) of the four limbs using bioelectrical impedance analysis (BIA; InBody 720, Seoul, Korea). Muscle strength was assessed by handgrip strength (kg) on either or both hands using a grip strength dynamometer (TANITA 6103, Tokyo, Japan); the maximum reading of two trials was used.

Sarcopenia

Muscle Mass (Kg, %)

We defined sarcopenia as having low ASM in kilograms (kg), as reflected by:

A. ASM (kg/m^2): Skeletal muscle mass index was calculated as the sum of the ASM divided by the square of height (kg/m^2). Sarcopenia was defined as a skeletal muscle mass index $<$7.0kg/m^2 for men and $<$5.7 kg/m^2 for women using BIA, based on the Asian Working Group for Sarcopenia (AWGS) 2019 criteria (28).

B. ASM (%): ASM was calculated as a percentage of body weight according to Janssen's formula (ASM/weight [kg] $\times$ 100 [%]) and as one standard deviation (SD) below the value of a young reference group (men = 51, women = 128, aged 20–40 years) (29).

Muscle Strength (Kg)

Following the AWGS 2019 recommendation, a handgrip strength $<$ 28.0 kg for men and $<$ 18.0 kg for women or the lowest quintile of muscle strength among the study participants indicated low muscle strength (28).

Obesity

Obesity was defined as BMI $\geq$ 25 kg/m^2 (30) or the upper two quintiles for%BF using BIA for each sex. For women, %BF quintiles were Q1: 28.9, Q2: 33.2–36.4, Q3: 36.5–39.3, Q4: 39.4–42.0, and Q5: 42.1–51.7, and for men, these values were Q1: 18.8, Q2: 22.1–26.0, Q3: 26.1–29.1, Q4: 29.2–32.8, and Q5: 39.4–42.0.

Using these cut-offs, we identified two subgroups: SO**A** and SO**B**.

Dietary Assessment

Dietary factors were assessed using a validated quantitative food frequency questionnaire, which contained 106 food items with serving sizes based on the Korean Genome and Epidemiology Study (31). Daily food amount in grams and frequency were calculated from the energy (kcal/d), protein, calcium, phosphorus, iron, vitamin B1, vitamin B2, niacin, vitamin C, zinc, vitamin B6, potassium, sodium, vitamin E, and fiber based on the recommended nutritional intake from the Dietary Reference Intakes for Koreans (KDRIs) [Korean Nutrients Society (32)] to evaluate the nutrient adequacy of the individual diet among the elderly participants. Foods were classified into carbohydrate-rich (white rice, mixed rice, noodles, and wheat) or protein-rich (beans, nuts, tofu, fish, meat, and poultry eggs) group based on the Korean Nutrient Database.

Outcome Variables

The primary outcome variable was the association of macronutrient intake with handgrip strength and body composition of the participants by CM pattern. The secondary outcome variable was the association between food sources and the prevalence of SO in the elderly participants by CM pattern.

Covariates

Participants' age, sex, BMI, health-related behaviors (smoking status, alcohol consumption, and physical activity level), working hours, KM type, and energy intake (kcal/day) were used as covariates in the statistical analysis. Previously, we confirmed high-risk associations with cardiometabolic outcomes (33) and inflammatory status (34) based on the two KM types in Korean adults. Age, BMI, and energy intake were used as continuous variables, and sex (male vs. female), smoking (no vs. yes), alcohol consumption (no vs. yes), physical activity (no vs. yes), working hours ($\leq$8 h/day or $\leq$20 h/week vs. $>$8 h/day or $>$20 h/week), and KM type (Taeeum vs. Non-Taeeum) were used as categorical variables. Active daily working hours and KM type were also included as strong predictors to build better adjustment for baseline data. Both

variable types showed considerable regional (rural area) or national features.

Ethics Statement

This study was approved by the Korean Institute of Oriental Medicine Ethics Committee (No. I-1401/001-001-01) and the Ethics Committee of the Institute of Medicine at the Seoul National University (IRB No. 1310-060-528). Written informed consent was obtained from all participants.

Statistical Analysis

We followed a previously published method to identify non-random multimorbid groups. Multimorbidity patterns were analyzed using exploratory factor analysis (35), which identifies the tendencies of diseases to co-occur, by selecting sets of variables with potentially common underlying causal factors. A tetrachoric correlation matrix will lead to more valid results for the assessment of the correlation structure between the variables to account for binary morbidity data (36). The number of factors extracted by the scree plot was utilized, in which the eigenvalues of the correlation matrix were represented in descending order to produce the inflection point of the curve, an eigenvalue of 1.0. To facilitate interpretation, the factors were rotated using the oblique rotation (oblimin) method. The Kaiser–Meyer–Olkin method was implemented to determine the adequacy of the sample in the factor analysis. To determine the most appropriate multimorbidity pattern, we selected variables with factor loadings ≥ 0.30 (37, 38) and classified them into patterns based on common disease features (**Supplementary Table 1**).

Frequencies and percentages were used for categorical variables in the descriptive analysis. Chi-square (χ^2) test was used to compare general and health-related characteristics (sex, smoking, drinking, physical activity, working hours, and KM type). All data on continuous variables related to muscle strength (handgrip strength and weight-adjusted handgrip strength), body composition, sarcopenia (ASM, kg, kg/m^2, and %), and cardiometabolic profiles are presented in **Table 1** and nutrients and food groups in **Table 2**. *P* values from the multiple comparisons were obtained using a Bonferroni corrected one-way analysis of variance and analysis of covariance (ANCOVA). Multiple linear regression models were employed to determine the best risk predictors between handgrip strength (muscle strength, kg), weight-adjusted handgrip strength (%), body fat mass (kg, %), ASM (kg/m^2), and ASM (%). Dietary food sources (g), handgrip strength (kg), and body composition [body fat mass (%), and ASM (kg/m^2, %)] variables were assessed for normality using the Shapiro–Wilk test. Correlations of dietary food sources (g) with handgrip strength (kg) and body composition (kg/m^2, %) were determined using Pearson correlation coefficient. Multivariable logistic regression was used to evaluate the association between macronutrient consumption (%) and prevalence of SO according to the CM pattern; adjusted prevalence ratios (PRs) and 95% confidence intervals (CIs) were also estimated. All analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, United States). All statistical tests were two-tailed, and *p*-values < 0.05 were considered statistically significant.

TABLE 1 | General characteristics, handgrip strength, body composition and cardiometabolic profiles of the participants according to their CM pattern[1].

Multimorbidity pattern	All (*n* = 932)	Non-CM (*n* = 466)	CM (*n* = 466)	*P-value*
Characteristics				
Age, years (mean ± SE)	71.8 ± 0.1	71.0 ± 0.2	72.0 ± 0.2	0.062
Sex (*n*, %)				
Men	319(34.2)	172(36.9)	147(31.6)	0.084
Women	613(65.8)	294(63.1)	319(68.5)	
Smoking (%)				
No	657(70.5)	319(68.5)	338(72.5)	0.172
Yes	275(29.5)	147(31.5)	121(27.5)	
Drinking (%)				
No	413(44.3)	215(46.1)	198(42.5)	0.262
Yes	519(55.7)	251(53.9)	268(57.5)	
Physical activity (%)				
No	770(82.6)	391(83.9)	379(81.3)	0.300
Yes	162(17.4)	75(16.1)	87(18.7)	
Working hours (%)				
≤8 h/day or ≤ 20 h/week	324(34.8)	128(27.5)	196(42.1)	<0.0001
>8 h/day or > 20 h/week	608(65.2)	338(72.5)	270(57.9)	
KM type[2] (%)				
Non-Taeeum	617(66.2)	347(74.5)	270(57.9)	**<0.0001**
Taeeum	315(33.8)	119(25.5)	196(42.1)	
Hand grip strength (kg)				
Men		27.8 ± 0.7	28.9 ± 0.8	0.296
Women		15.3 ± 0.3	15.6 ± 0.3	0.493
HGSWR[3] (%)				
Men		43.9 ± 1.1	41.6 ± 1.2	0.151
Women		27.9 ± 0.6	26.7 ± 0.6	0.153
Body composition (mean ± SE)				
BMI, kg/m^2		24.0 ± 0.1	**26.0 ± 0.1**	**<0.0001**
Body fat mass, kg		18.7 ± 0.3	**22.9 ± 0.3**	**<0.0001**
Body fat mass, %		32.0 ± 0.3	**36.2 ± 0.3**	**<0.0001**
ASM[4] (kg/m^2)				
Men		7.3 ± 0.0	**7.4 ± 0.0**	**<0.0001**
Women		6.4 ± 0.0	**6.5 ± 0.0**	**<0.0001**
ASM[5] (%)				
Men		**31.4 ± 0.2**	29.1 ± 0.2	**<0.0001**
Women		**26.7 ± 0.1**	25.2 ± 0.1	**<0.0001**
Sarcopenic Obesity[6] (%)				
height-adjusted[4] *(women only)*	70(7.5)	14(3.0)	56(12.0)	**<0.0001**
weight-adjusted[5]	155(33.3)	31(6.6)	124(30.5)	**<0.0001**
Cardio-metabolic profiles (mean ± SE)		1.8 ± 0.1	**2.2 ± 0.1**	**<0.0001**
WC, cm		80.4 ± 0.4	**82.2 ± 0.4**	**0.000**
Systolic BP, mmHg		136.1 ± 0.6	**139.2 ± 0.7**	**0.001**
Diastolic BP, mmHg		76.5 ± 0.4	**77.8 ± 0.4**	**0.048**
Fasting plasma glucose, mg/dL		95.1 ± 1.1	**106.3 ± 1.1**	**<0.0001**
HDL-C, mg/dL		**53.1 ± 0.6**	51.1 ± 0.6	**0.018**

(Continued)

TABLE 1 | (Continued)

Multimorbidity pattern	All (*n* = 932)	Non-CM (*n* = 466)	CM (*n* = 466)	*P-value*
TG, mg/dL		123.8 ± 3.5	**149.9 ± 3.5**	**<0.0001**

[1]Cardio-metabolic multimorbidity (CM) pattern included diabetes, dyslipidemia, and hypertension, and angina (or myocardial infarction).
[2]Korean medicine (KM) type was diagnosed into Taeeum or non-Taeeum type according to participants' personal and physiological character by KM doctors.
[3]Hand grip strength weight ratio was calculated by hand grip strength / weight x 100.
[4]ASM, kg/m^2 by Asian Working Group for Sarcopenia (AWGS) 2019 criteria.
[5]ASM, % by Janssen's formula [ASM /weight (kg) × 100 (%)].
[6]Sarcopenic Obesity (SO) is generally defined as the coexistence of sarcopenia and obesity.
P-values were obtained from Rao-Scott chi-square tests for categorical variables and Bonferroni multiple comparison of one-way analysis of variance and analysis of covariance (ANCOVA).
Least-square means ± SE adjusted for age and sex. Bold letters represent a significant value.

RESULTS

General characteristics, handgrip strength, body composition, and cardiometabolic profiles of the participants according to the CM pattern are presented in **Table 1**. The mean age of participants was 71.8 ± 0.1 years, and 65.8% (n = 613) were women. No significant health-related behaviors were observed in the CM and non-CM groups. In total, 72.5% of those in the non-CM group worked for longer durations (>8 h/day or > 20 h/week) compared to those in the CM group ($p < 0.0001$). Regarding the KM type, 17.6% more Taeeum-type individuals were observed in the CM group than in the non-CM group ($p < 0.0001$).

Regarding handgrip strength (kg) and weight-adjusted handgrip strength (%), no significant differences were found between the CM and non-CM groups. Body composition showed that BMI (kg/m^2) and body fat mass in kilogram (kg) and in percentage (%; non-CM: 32.0 ± 0.3 vs. CM: 36.2 ± 0.3) were significantly higher in the CM group than in the non-CM group ($p < 0.0001$). Both men (non-CM: 7.3 ± 0.0 vs. CM: 7.4 ± 0.0, $p < 0.0001$) and women (non-CM: 6.4 ± 0.0 vs. CM: 6.5 ± 0.0, $p < 0.0001$) had higher height-adjusted ASM (kg/m^2) in the CM group than in the non-CM group. However, weight-adjusted ASM (%) in both sexes was significantly lower in the CM group than in the non-CM group ($p < 0.0001$). The prevalence of SO was 7.5% [height-adjusted, n = 70 (women only)] and 33.3% (weight-adjusted, n = 155), respectively. Height-adjusted SO was observed only in women. Significant group differences in SO prevalence were observed by CM pattern. Regarding the cardiometabolic profiles, higher waist circumference (cm), systolic and diastolic BP (mmHg), fasting plasma glucose (mg/dl), and TG (mg/dl) and lower HDL-C (mg/dl) were observed in the CM group than in the non-CM group ($p < 0.05$; **Table 1**).

Table 2 shows the daily energy (kcal/d), nutrient, and food groups (g/d) consumed by the participants according to their CM patterns. Overall, there were no significant differences in the daily energy intake of the participants (both sexes) between the groups. Regarding macronutrient intake, protein intake (g) was higher (non-CM: 46.1 ± 0.4 vs. CM: 47.6 ± 0.4, $p = 0.009$) and protein distribution (g/kg) was lower (non-CM: 0.81 ± 0.0 vs. CM: 0.76 ± 0.0, $p < 0.0001$) in the CM group than in the non-CM group. Regarding micronutrient intake, the vitamin B1, B2, potassium, phosphorus, and iron levels were higher in the CM group than in the non-CM group ($p < 0.05$). No significant differences were observed in the food groups between the two groups (**Table 2**).

The association between macronutrient intake, handgrip strength, and body composition of the participants according to their CM patterns is presented in **Table 3**. Regarding

TABLE 2 | Daily energy and nutrients intakes and food groups of the participants according to their CM pattern.

Dietary factors	Non-CM (*n* = 466)	CM (*n* = 466)	*P*-value
Energy (kcal/d)[1]			
% RDA	85.1 ± 1.1	84.5 ± 1.0	0.708
Men	1625.6 ± 31.7	1674.0 ± 34.3	0.302
Women	1433.3 ± 23.1	1399.5 ± 22.1	0.291
Nutrients[2]			
Carbohydrates (g)	278.7 ± 1.0	279.0 ± 1.0	0.826
Fat (g)	18.4 ± 0.3	18.0 ± 0.3	0.356
Protein (g)	46.7 ± 0.4	47.2 ± 0.4	0.361
Protein [g, per body weight (kg)]	**0.81 ± 0.0**	0.76 ± 0.0	**<0.0001**
C:F: P (%)	74.8: 10.8: 12.4	75.1: 10.4: 12.5	N/S
Vitamin A, μgRAE	306.5 ± 8.8	317.4 ± 8.8	0.384
Vitamin B1, mg	0.7 ± 0.0	0.7 ± 0.0	0.978
Vitamin B2, mg	0.6 ± 0.0	0.6 ± 0.0	0.734
Niacin	11.5 ± 0.1	11.5 ± 0.1	0.912
Vitamin C, mg	69.9 ± 1.8	72.0 ± 1.8	0.419
Vitamin E, mg	5.6 ± 0.1	5.6 ± 0.1	0.908
Vitamin B6, mg	1.2 ± 0.0	1.2 ± 0.0	0.856
Potassium, mg	1714.4 ± 25.5	1746.2 ± 25.5	0.378
Calcium, mg	321.7 ± 6.8	328.6 ± 6.8	0.477
Phosphorus, mg	735.6 ± 6.1	747.7 ± 6.1	0.161
Iron, mg	7.7 ± 0.1	7.9 ± 0.1	0.137
Fiber, g	4.6 ± 0.1	4.8 ± 0.1	0.065
Sodium, mg	2420.9 ± 57.4	2450.3 ± 57.4	0.717
Food groups (g/d)[2, 3]			
Carbohydrates-rich			
White rice	648.7 ± 10.0	635.3 ± 13.0	0.416
Mixed rice	607.8 ± 7.9	613.8 ± 6.9	0.574
Noodles	32.0 ± 1.7	30.0 ± 1.7	0.403
Wheats[4]	46.0 ± 1.9	43.4 ± 1.9	0.345
Protein-rich			
Beans-nuts-tofu	38.3 ± 2.3	43.1 ± 2.3	0.141
Fish	23.3 ± 1.0	25.1 ± 1.0	0.237
Meats	24.0 ± 1.0	21.5 ± 1.0	0.072
Poultry-eggs	9.2 ± 0.5	9.4 ± 0.6	0.819

[1]Adjusted age only.
[2]Least-square means ± SE adjusted for age, sex and energy intake (kcal).
[3]Food groups were surveyed using short-form of the food frequency questionnaires (FFQ) which includes grains (white rice, mixed rice, noodles and wheats, potatoes and sweet potatoes).
[4]Wheats: wheat and other types of refined grains based extra food intakes for snacks; cereals, breads, ricecakes.
P-values were obtained from Bonferroni multiple comparison of one-way analysis of variance and analysis of covariance (ANCOVA). Bold letters represent a significant value.

TABLE 3 | The association between macronutrients intake, handgrip strength, and body composition of the participants according to their CM pattern.

Macronutrients intake	Carbohydrates (g)				Fat (g)				Protein (g)				Protein [g, per body weight (kg)]			
	Non-CM		CM		Non-CM		CM		Non-CM		CM		Non-CM		CM	
Variables	*adjusted beta*	*P-value*	*adjusted beta*	*P-value*	*adjusted beta*	*P-value*	*adjusted beta*	*P-value*	*adjusted beta*	*P-value*	*adjusted beta*	*P-value*	*adjusted beta*	*P-value*	*adjusted beta*	*P-value*
Hand grip strength (kg)																
Men	−0.019	0.623	0.008	0.743	0.040	0.707	−0.005	0.941	0.032	0.724	−0.009	0.885	−2.242	0.689	−4.147	0.327
Women	0.025	0.216	−0.050	**0.001*** **	−0.094	0.100	0.157	**0.001*** **	−0.035	0.496	0.120	**0.001*** **	−2.092	0.446	3.811	**0.039***
HGSWR (%)																
Men	−0.026	0.654	0.018	0.631	0.052	0.746	−0.012	0.911	0.035	0.802	−0.042	0.672	3.125	0.714	−0.342	0.957
Women	0.058	0.111	−0.088	**0.001*** **	−0.212	**0.040***	0.281	**0.001*** **	−0.083	0.366	0.212	**0.001*** **	2.585	0.602	10.577	**0.001*** **
Body composition																
Body fat mass, kg																
Men	−0.006	0.574	−0.016	0.096	0.012	0.577	0.038	0.177	0.021	0.412	0.039	0.121	−1.519	0.326	0.061	0.971
Women	−0.007	0.380	−0.012	**0.046***	0.038	0.177	0.030	0.088	0.015	0.442	0.023	0.100	−2.469	**0.017***	−1.443	**0.04***
Body fat mass, %																
Men	−0.011	0.461	−0.024	0.069	0.023	0.563	0.061	0.115	0.023	0.497	0.055	0.112	1.399	0.503	5.174	0.053
Women	0.010	0.424	−0.020	**0.017***	−0.034	0.327	0.054	**0.031***	−0.028	0.358	0.032	0.103	0.216	0.897	1.881	0.059
ASM, kg/m^{2a}																
Men	0.000	0.734	0.000	0.799	0.000	0.763	0.000	0.975	0.000	0.819	−0.001	0.511	0.291	**<0.0001*** **	0.282	**<0.0001*** **
Women	0.001	**0.026***	0.000	0.941	−0.001	**0.037***	0.000	0.912	−0.001	**0.040***	0.000	0.839	0.157	**<0.0001*** **	0.116	**<0.0001*** **
ASM, %^{b}																
Men	−0.003	0.364	0.001	0.479	0.008	0.230	0.006	0.432	0.001	0.858	−0.007	0.166	1.611	**<0.0001*** **	0.901	**0.009** *
Women	0.002	0.324	0.002	0.241	−0.005	0.290	−0.005	0.314	−0.004	0.412	−0.002	0.522	1.083	**<0.0001*** **	0.580	**0.001*** **

Multiple linear regression was used to estimate after adjusting age, sex, BMI, energy intake(kcal), smoking, alcohol consumption, physical activity, working hours, and KM type.

[a]ASM, kg/m^2 by Asian Working Group for Sarcopenia (AWGS) 2019 criteria.

[b]ASM, % by Janssen's formula [ASM/weight (kg) × 100 (%)].

*Statistical significance was accepted at *P < 0.05, **P < 0.01, and ***P < 0.001. Bold letters represent a significant value.*

TABLE 4 | Adjusted prevalence ratios (PRs) in multivariate logistic regression between daily food sources and Sarcopenic Obesity.

Daily food sources, (min.-max.)	Sarcopenic Obesity[a] (*n* = 70)	Sarcopenic Obesity[b] (*n* = 155)
	PRs (95% CIs)	
Wheats[c] (g/day)	ref. T1: 20.0 (0.0–51.7)	
T2: 101.2 (51.8–150.5)	0.784 (0.397–1.549)	0.994 (0.561–1.758)
T3: 269.1 (150.9–874.6)	1.793 (0.868–3.707)	**2.149 (1.134–4.071)**
P for trend	0.118	**0.016**
	PRs (95% CIs)	
Meats (g/day)	ref. T1: 9.2 (0.0–25.1)	
T2: 34.8 (25.2–45.0)	1.421 (0.709–2.850)	**2.554 (1.380–4.726)**
T3: 99.5 (45.2–717.4)	1.438 (0.712–2.904)	**1.932 (1.066–3.500)**
P for trend	0.323	**0.047**

[a]*ASM, kg/m^2 by Asian Working Group for Sarcopenia (AWGS) 2019 criteria.*
[b]*ASM, % by Janssen's formula [ASM/weight (kg) × 100 (%)].*
[c]*Wheats included wheat and other types of refined grains based extra food intakes for snacks; cereals, breads, ricecakes.*
Multivariate logistic regression was used to estimate PRs (95% CIs) after adjusting covariates; age, sex, BMI, energy intake (kcal), smoking, alcohol consumption, physical activity, working hours, KM type, and CM pattern.
P for trend was tested across three levels (tertiles) of the food sources (g/day) by including the median score as a continuous measure in the regression model after adjusting all covariates. Bold letters represent a significant value.

carbohydrate intake (g), there was a negative relationship between handgrip strength (kg: beta = −0.05, p = 0.001 and $^{weight-adjusted}$%: −0.09, p = 0.001, women only) and body fat mass (kg: beta = −0.01, p = 0.046 and %: beta = −0.02, p = 0.017, women only) in the CM group. Meanwhile, fat intake was positively associated with body fat mass (% only) in women in the CM group. Dietary fat and protein intake (g) [g per body weight (g/kg)] were positively related to handgrip strength (both kg and $^{weight-adjusted}$%) in women in the CM group after adjusting for covariates (p = 0.001). Similarly, protein intake (g/kg) showed significantly positive associations with ASM (kg/m^2) and ASM (%) in both sexes in the CM and non-CM groups (**Table 3**).

The correlations between dietary food sources (g), handgrip strength, and body composition of the participants are shown in **Figure 1**. As shown in *panel A*, handgrip strength positively correlated with dietary fat (non-CM: r = 0.28, CM: r = 0.24), protein (non-CM: r = 0.24, CM: r = 0.23), and meat (non-CM: r = 0.22, CM: r = 0.19) in the CM and non-CM groups (p < 0.001). In contrast, macronutrient intake was inversely related to body fat mass (%) in the CM group only (p < 0.001; *panel B*). Similar to the handgrip strength (*panel A*), *panel C* reveals positive correlations between ASM (kg/m^2) and macronutrients, including carbohydrate-rich wheat, meat, and fish intake, in the non-CM and CM groups (p < 0.001). However, ASM (kg/m^2) showed a strong positive correlation with wheat, meat, and fish in the CM group and a positive correlation with meat and fish in the non-CM group (p < 0.001). In *panel D*, ASM (%) positively correlated with fat and meat intake in the CM group only (p < 0.001; **Figure 1**).

The number of participants with SO is presented in **Table 4** by the two criteria; Sarcopenic Obesity[a]: (n = 70) and Sarcopenic Obesity[b]: (n = 155). Multivariate logistic regression was performed and presented between daily food sources and SO prevalence (**Table 4**). According to the tertiles of wheat intake (g/d), 2.1-fold increase in the risk of prevalence of SO (PR: 2.149, CIs: 1.134–4.071) was reported in the highest tertile (T3: 269.1 g/d) compared to that in the lowest tertile (T1: 20.0 g/d) in the CM group. Regarding meat intake (g/d), higher tertile (T2: 34.8 g/d, T3: 99.5 g/d) showed about 2-fold increase in the risk of prevalence of SO (PR: 1.932, CIs: 1.066–3.500) compared to the lowest tertile (T1: 9.2 g/d; **Table 4**).

DISCUSSION

This study was performed to identify CM patterns in elderly Koreans living in a rural area, and examine the interrelationships between dietary factors, grip strength, body composition, and prevalence of SO with respect to CM pattern. The results indicated that overconsumption of wheat and meat, with imbalanced macronutrient intake, interacted with CM to produce significant effects on the risk of prevalence of SO by increasing muscle loss and/or body fat in elderly with CM.

Sarcopenia, Obesity, and Cardiometabolic Diseases in Elderly

A recent large cohort study reported significantly higher prevalence of non-alcoholic fatty liver disease (NAFLD) in men with both T2DM and sarcopenia than in those without sarcopenia (39). A previous cross-sectional study found that coexistent sarcopenia and obesity had greater risks than did the single components or when associated with the multimorbidity prevalence in Korean adults (40). Elderly people are a vulnerable population as they are likely to have co-existing risk factors (41). In a Dutch Lifelines cohort study, the prevalence of SO increased with age, and SO participants with > 3 comorbidities had 2.7 times higher risk than those with no morbidities (42). Similarly, our results showed higher risk factors for body composition, cardiometabolic profiles, and prevalence of SO in the CM group than in the non-CM group. Multifactorial relationships have been hypothesized between SO and cardiometabolic diseases in elderly participants.

Lifestyle Risk Factors of Sarcopenia, Obesity, Muscle Strength, Function, and Quality in Elderly With Cardiometabolic Multimorbidity

From an etiopathogenesis viewpoint, age- and sex-related physiological, hormonal, and body compositional changes, lifestyle factors such as diet, physical activity, and activities of daily living, and other unidentified factors might be mediators between CM and SO (7–9). Poor nutrition and physical inactivity are key contributors to sarcopenia and its risk (43, 44). A beneficial effect was reported in elderly sarcopenic adults who gained 1.7 kg of fat-free mass after participating in a resistance training program with dietary protein and vitamin D supplementation (45). However, excessive calorie intake from

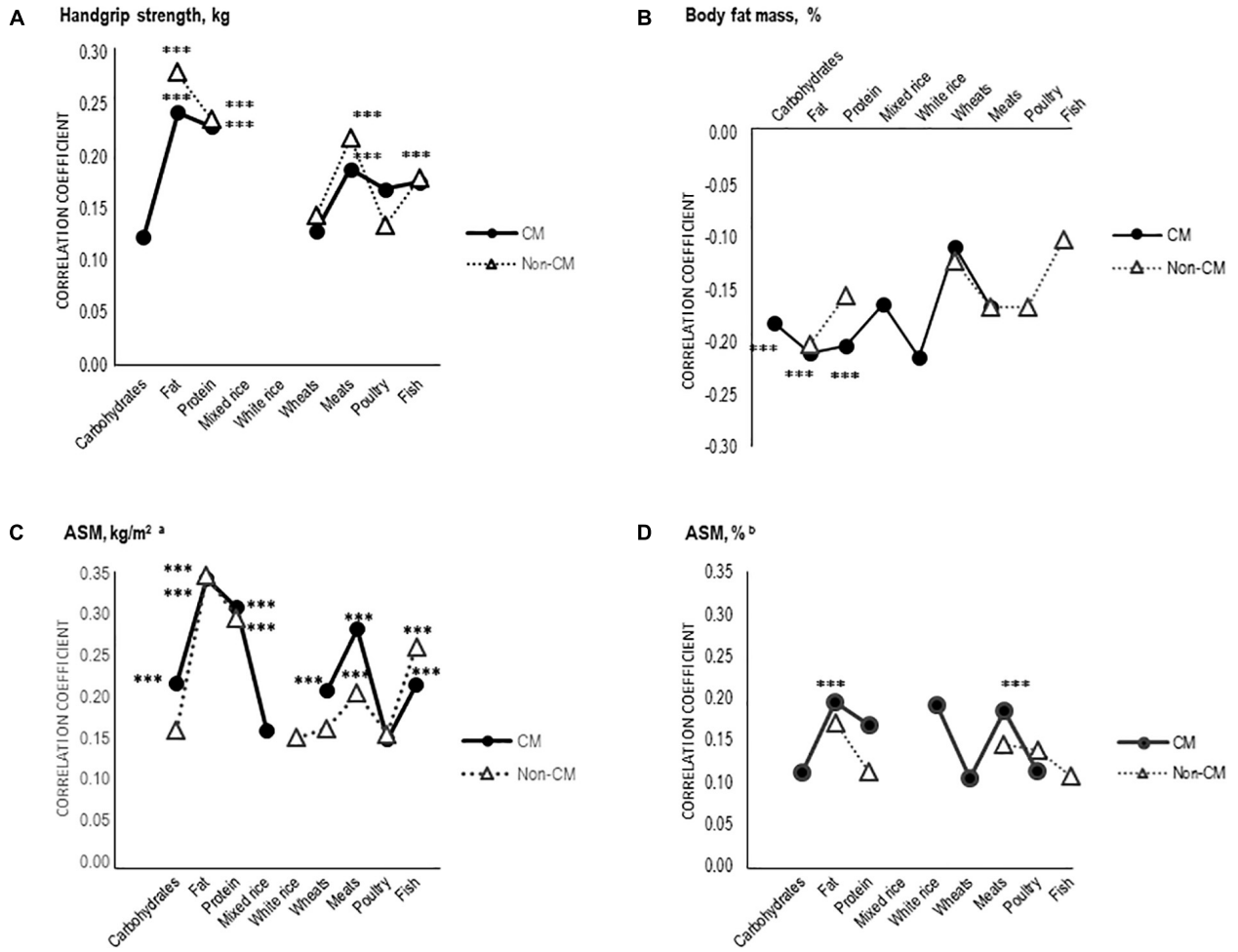

FIGURE 1 | Relationship between handgrip strength, body fat mass, appendicular skeletal muscle mass (ASM), and dietary food sources (g) of the participants by CMP. Partial Pearson correlation coefficients controlling for individual energy intake were used for the correlation analysis. Statistical significance was accepted at $^{***}P < 0.001$. C. ASM, kg/m^2 by Asian Working Group for Sarcopenia (AWGS) 2019 criteria. D. ASM, % by Janssen's formula (ASM/weight [kg] × 100 [%]).

proteins might lead to altered body composition with higher body fat mass, lower muscle mass, and metabolic quality in older men with obesity (46). Consistently, elderly participants with CM patterns were more sedentary, more obese, had higher body fat mass, worsened cardiometabolic profiles, and high-risk sarcopenia or higher prevalence of SO than those without CM. Regarding the KM type, more participants in the CM group had Taeeum-type, with a predisposing metabolic risk (33, 34) than those in the non-CM group. Our ongoing cohort study showed a higher prevalence of pre-metabolic diseases (MetS), lower nutritional status, higher high-sensitivity C-reactive protein (hs-CRP) level, and lower vegetable consumption in Taeeum-type middle-aged Korean adults (33, 34). These results showed that individual personality or obesity-related physiological characteristics, which were associated to the KM type, might be negatively related to the occurrence of SO by uncontrolled diet and progressive reduction of physical activity in elderly participants with CM patterns.

Inappropriate Macronutrient Intake Impacts Muscle Strength and Body Composition in Elderly With Cardiometabolic Multimorbidity

Different proportions of macronutrients in the diet have different effects on carbohydrate and fat storage in the human body (47). While overconsumption of carbohydrates promotes hepatic *de novo* "lipogenesis," fat accumulation in adipose tissue promotes increased "lipolysis," which are the main pathways that contribute to intrahepatic TG (48). Therefore, macronutrient composition in one's diet acts as an important mediator. Repeated overconsumption of carbohydrate leads to development of MetS, which increases hepatic fat (47), serum TG levels, and reduces HDL-C (49). Consistently, a positive association between carbohydrate intake and the prevalence of MetS was reported in a previous Korean adult population-based study (17). The highest carbohydrate intake in men and refined grain intake in women

were associated with elevated TG and blood glucose levels in combination with reduced HDL-C levels (17).

In aging muscle biology, dietary fatty acids (FAs) play an important role as activators by increasing levels of pro-inflammatory cytokines, which are implicated in muscle wasting (18). Saturated fatty acids (SFAs) influence IR and CVD by increasing serum low-density lipoprotein cholesterol (LDL) levels (50). Previous clinical research showed significant changes in higher fasting serum insulin levels after the consumption of an SFA-rich diet than of other unsaturated FA or carbohydrates (48). WHO reported that results from the regression analysis showed a more favorable effect on the serum lipoprotein profile of reducing dietary SFA intake by replacing with polyunsaturated or monounsaturated FA than with carbohydrate mixtures (51). A significantly higher inverse association between the omega-3 FA ratio and prevalence of SO has been reported in elderly women in the recent KNHANES data (52).

We found imbalanced macronutrient ratios, higher carbohydrate levels, and lower total fat intake in elderly participants compared to the KDRIs. Lower total protein adequacy (g/kg), excessive carbohydrate-induced muscle weakness, and fat intake related to a higher percentage of body fat mass in women were observed in the CM group. In addition, overconsumption of wheat and meat increased the risk of prevalence of SO about 2-folds in the CM group. We also identified protein adequacy as a contributor to improving muscle mass in elderly of both genders. These results suggest that elderly with CM make inappropriate food choices, such as the consumption of high-carbohydrate diets or high animal-fat with low protein foods. Therefore, making right food choices and adequate intake of carbohydrates and protein are recommended to improve IR and mitigate MetS, maintain muscle strength and quality, and prevent SO in elderly with CM.

Nutritional Recommendation for Elderly With Cardiometabolic Multimorbidity to Improve Clinical and Nutritional Status

Saturated fatty acids are found mostly in animal products, as well as in some high-fat plant foods, such as palms, coconuts, avocados, olives, nuts, and seeds [Food Composition Table. 9.1 version. National Institute of Agricultural Sciences, Rural Development Administration of Korea (53)]. The American College of Cardiology/American Heart Association (ACC/AHA) guidelines recommends a reduced SFA intake of $< 7\%$ of total calorie intake to reduce the risk of CVDs (54). It is also recommended that SFAs should be replaced with polyunsaturated fatty acids (PUFAs) which lower TG (55) and cholesterol (56). Omega-6 and omega-3 PUFAs are the only essential dietary fats (57). The ideal ratio of omega-3/6 PUFAs is 1:1 to 1:4; however, an imbalance in the ratio of omega-3/omega-6 PUFAs ratio due to westernized diets, leads to increased synthesis of arachidonic acid from linoleic acid (55), accelerating the prevalence of atherosclerosis, chronic diseases such as obesity, and diabetes (56, 58).

Protein is a good dietary source for preserving muscle mass during weight loss (59). Research based on nationally representative data showed that optimal protein intake (at least 0.8 g/kg per day) reduced MetS risk (42%) and its components such as abdominal obesity (44%), lower HDL-C (47%), and elevated TG (45%) in 1,567 elderly participants (60). Another KNHANES report revealed protein-derived body composition changes and a higher prevalence of SO in 1,433 participants aged > 60 years (19).

Plant-based proteins, such as soy products, have high-quality sustainable proteins for optimal muscle protein synthesis, to prevent muscle loss, and manage weight by reducing hunger (61). Nuts, seeds, and legumes are other beneficial dietary sources of protein, magnesium, and fiber. However, they should be taken in small portions without added salt or sugar (54). Mediterranean diets (62) and dietary approaches to stop hypertension (DASH) (63) diets are well-known nutritional therapies high in vegetables and fruits, low-fat dairy products, fish, and legumes and low in red-meat, SFA, and sugars. Adherence to these healthy dietary patterns has favorable effects on CVD mortality and improves cardiometabolic markers (64) and intrahepatic fat (65) in older adults. A plant-based diet is also encouraged as an ideal diet for the elderly population as it lowers the risk of cardiovascular disease, provides protective effects through multiple beneficial nutrients, and includes a wide range of antioxidants (56).

In Asian or Korean culture, carbohydrate-based diet (rice-based meals) with various vegetables and low-fat protein sources (fish and seafood, legumes, and bean/tofu-based side dishes) was the usual diet of those who lived in rural/coastal areas for their whole life. Both CM and non-CM participants adhere to traditional rural lifestyle, which consists of the consumption of high-carbohydrate diets and spending most of their time working. However, age-related body composition changes, obesity-related chronic multi-disease status, and imbalanced macronutrients might reduce protein synthesis in chronic catabolic states, leading to worsening muscle strength and quality in Korean elderly people with CM.

The WHO recommends an intake of > 400 g/d of non-starch fruits and vegetables to prevent chronic diseases (66). Replacing plant-based protein intake and n-3 FAs (vegetable oils, soybean, rapeseed oil, walnuts, and fish oils) might enhance muscle mass and strength in women according to individual differences in age, sex, muscle capability and function, exercise, and menopause. Lastly, active daily living and physical activity could improve protein metabolism and counteract anabolic stimulus in muscle loss in the elderly population with CM.

Strength and Limitation

To the best of our knowledge, this study is the first study to investigate the relationship between dietary factors, grip strength, body composition, and prevalence of SO in Korean rural residents according to their CM pattern. We employed multiple representative definition criteria for the diagnosis of SO by adapting the AWGS 2019 criteria and Janssen's formula.

This study has some limitations. Being a cross-sectional study, it was difficult to make causal inferences due to limited evidence. Second, we did not consider other chronic diseases, multimorbidity pattern effects, or periods of disease in the present study. Lastly, we evaluated only residents of a rural area in Korea;

therefore, careful insights are required when interpreting the present data for the sake of generalizability.

CONCLUSION

This study highlights that overconsumption of wheat and meat negatively impacts the prevalence of SO in Korean elderly with CM. Furthermore, nutritional management is strongly required to improve cardiometabolic profiles and macronutrient imbalance, protein adequacy, and food choices (avoiding high-carbohydrates/fat-based snacks or meals). A patient-centered integrated multisectoral team-based approach is required to manage systematic guidelines for diet and health in older adults with multiple chronic diseases.

DATA AVAILABILITY STATEMENT

The datasets are not available due to confidentiality and security of ethical issues; further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Korean Institute of Oriental Medicine Ethics Committee (No. I-1401/001-001-01) and the Ethics Committee of the Institute of Medicine at the Seoul National University (IRB No. 1310-060-528). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JK: conceptualization, analysis and interpretation of data, and original manuscript. KJ: data curation. YB and SL: review and editing. SL: funding acquisition and project administration. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Association between tea consumption and frailty among Chinese older adults: A cross-sectional study

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Background: Chronic inflammation is considered one of the main mechanisms leading to frailty. It has been demonstrated that tea consumption reduces chronic inflammation. Few epidemiological studies have investigated the association between tea consumption and frailty.

Objective: This study aimed to analyze the association between tea consumption and frailty in Chinese older adults.

Methods: Between March and May 2021, we enrolled 2,144 older adults aged ≥60 years in Jinan City, Shandong Province, China, using multi-stage stratified cluster sampling. We assessed tea consumption and frailty in older adults using the Tilburg Frailty Indicator (TFI) and the frequency of tea consumption, respectively. We applied multiple logistic regression analysis to examine the association between tea consumption and frailty, controlling for a set of potential covariates.

Results: The prevalence of frailty among older Chinese adults was 38.3% (821/2,144). Tea consumption was categorized as daily (30.4%), occasionally (20.9%), and rarely or never (48.7%). As indicated by the fully adjusted model, daily tea consumption was associated with a lower prevalence of frailty (OR = 0.73, 95%CI = 0.57–0.94). However, this association only applied to men, younger older adults aged 60–79 years, rural residents, and regular participants in community activities. In addition, we observed a linear relationship between tea consumption and the prevalence of frailty (*P* for trend = 0.017).

Conclusions: Higher tea consumption was associated with a lower prevalence of frailty in older adults, especially those men, older adults aged 60–79, rural residents, and individuals who regularly participated in community activities. Further longitudinal and experimental studies are needed to determine the causation between tea consumption and frailty.

KEYWORDS

tea consumption, frailty, older adults, China, Tilburg Frailty Indicator

Introduction

Frailty is a common geriatric syndrome, that refers to a non-specific condition in which the physiological reserves of an older individual are diminished for a variety of reasons, leading to increased vulnerability and a decreased capacity to withstand stress (1, 2). Currently, there are two main perspectives in assessing frailty (3). One regards frailty as a biological concept mainly evaluated from a single physical function dimension (4). The other believes that frailty has multiple dimensions, including psychological and social fields in addition to the physical field (5). In recent years, this view of multidimensional frailty has been increasingly recognized (6). Meta-analyses have shown that frailty is associated with multiple adverse health outcomes such as premature mortality, disability, and cognitive impairment (7, 8). Moreover, frailty has become a global concern in geriatric health. A meta-analysis of 62 countries and territories showed that the prevalence of frailty and prefrailty was 12% and 46%, respectively, among older adults (9). Data from the China Health and Retirement Longitudinal Study (CHARLS) showed that the prevalence of frailty among older adults has been on the rise, which increased from 18.7% to 28.4% between 2011 and 2015 (10). In addition, CHARLS showed that the incidence of frailty was 60.6/1,000 person-years during an average of 2.1 years of follow-up in 4,939 community-dwelling older adults (11). Given the adverse effects of frailty and its high prevalence and incidence, a growing body of research is exploring factors associated with frailty to provide a scientific basis for developing interventions.

Studying frailty-associated factors from the perspective of pathogenesis is the primary focus of current research. Existing studies have suggested that inflammation may contribute to frailty directly or indirectly through pathophysiological processes such as inhibiting growth factors and by interfering with homeostatic signaling (12–15). Accordingly, studies have explored the relationship between an anti-inflammatory diet (such as the Mediterranean diet) and frailty (16, 17). It has been suggested that these lifestyles may reduce age-related oxidative damage and inflammation, thereby reducing the risk of frailty. In recent years, attention has been drawn to the potential health benefits of tea consumption through its anti-inflammatory qualities. Multiple meta-analyses of observational studies have shown that tea consumption is associated with decreased risks of chronic inflammatory diseases, including cardiovascular disease (18), cancer (19), and depression (20). Existing research has demonstrated that tea contains many polyphenols, especially catechins and their derivatives, which have powerful antioxidant and anti-inflammatory effects *in vivo* (21). Meanwhile, a previous study found that consuming polyphenol plants may slowdown aging and progression of related diseases (22). Moreover, a systematic review found that tea seemed to improve oral microbiota, thus promoting oral health (23) while existing research suggests that oral health may be one of the aspects concerning frailty (24). These findings indicated that tea consumption might be associated with frailty. However, epidemiological studies evaluating the relationship between tea consumption and frailty are limited. To the best of our knowledge, only a few studies have explored the association between green tea consumption and frailty in the older population (25–27). Tea originated in China, and some Chinese people have a habit of drinking tea (28). It is unclear, however, whether tea consumption is associated with a reduced risk of frailty in older adults in China.

Therefore, this study aimed to analyze the association between tea consumption and frailty in older Chinese adults to provide research evidence for potential frailty prevention in the Chinese population. In addition, this study performed a stratified analysis of sex, age, residence, and community activity participation to understand the heterogeneity of the association between tea consumption and frailty.

Materials and methods

Participants

We first used the epidemiological sample size estimation formula (29), $n = \frac{Z_{\frac{\alpha}{2}}{}^2 P(1-P)}{\delta^2}$ to estimate the expected sample size. A previous cross-sectional study conducted in Jinan City, China, showed that the prevalence of frailty among community-dwelling older adults was 34.2% (30). Therefore, in this study using, $P = 0.342$, $\alpha = 0.05$, $\mu_{\alpha/2} = 1.96$, and $\delta = 0.03$ resulted in a minimum sample size of 961.

We conducted this cross-sectional survey from March to May 2021 using multi-stage stratified cluster random sampling in Jinan City, Shandong Province. In the first stage, we randomly selected six districts/counties from the 12 districts/counties of Jinan City as the survey area. In the second stage, we randomly selected two townships or streets from the selected six districts/counties. In the third stage, we randomly selected all the older adults in two communities from the selected two townships or streets. The inclusion criteria were as follows: age $\geq$60 years, living in Jinan for more than 6 months, with no hearing or language impairment, and voluntary participation in the survey. The exclusion criteria were severe diseases, such as dementia (as reported by family members) and complete disability. We recruited 2,557 participants; of these, 356 were excluded because they did not meet the criteria. A total of 2,201 older adults participated in the survey. Among them, 57 participants failed to complete the questionnaire due to reasons such as leaving the survey site. Finally, we received 2,144 completed questionnaires, and the effective survey rate was 97.41%. Figure 1 shows the sampling procedure. All surveys were completed by trained investigators using face-to-face interviews. Written informed consent was obtained from all the participants before the investigation. This study

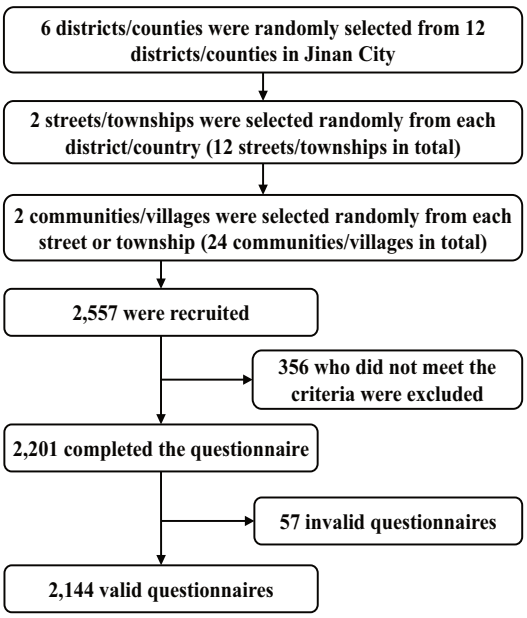

FIGURE 1
The sampling procedure.

was approved by the Medical Ethics Committee of Xiangya School of Public Health, Central South University (identification code: XYGW-2020-101).

Measures

Tea consumption

We measured tea consumption by asking participants how often they drank tea (approximately 200 ml in each serving) in the past year, and the options included four categories: almost daily, 1–2 times a week, 1–2 times a month, less than once a month or never. Since "1–2 times a month" accounted for a small proportion of our population (<5%), we combined it and "less than once a month or never" in the data analysis. Finally, according to their answers, the frequency of tea consumption was divided into three categories: rarely or never ("1–2 times a month" and "less than once a month or never"), occasionally (1–2 times a week), and daily (almost daily).

Tilburg Frailty Indicator

We used the Chinese version of the Tilburg Frailty Indicator (TFI) (31) to evaluate frailty in older adults. The scale has 15 items, including three dimensions: physical frailty, psychological frailty, and social frailty. Each item was converted into two categories: yes (1) and no (0). A total score of 0 to 15 points was derived from the sum of all items. Frailty is defined as a total score greater than or equal to five. In this study, the Cronbach's alpha for the Chinese version of the TFI was 0.778.

Covariates

According to the previous studies (32), we controlled for two sets of covariates, namely sociodemographic characteristics/health status and lifestyle.

Sociodemographic characteristics and health status included age, sex (male vs. female), residence (urban vs. rural), marital status (married vs. unmarried), education level (primary school and below, junior high school, or high school and above), average monthly personal income (<2,000, 2,000–3,000, or >3,000 yuan), chronic diseases (yes vs. no), body mass index (BMI), and disability. The BMI was calculated using self-reported height and weight. Disability was assessed using the Activities of Daily Living Scale (ADLS). The 14-item scale consists of two subscales: the Physical Self-Maintenance Scale (6 items) and the Instrumental Activities of Daily Living Scale (8 items) (33). Each item was used for a self-assessed four-point Likert-type rating scale (from 1 = no difficulties to 4 = I am not able to do that). The sum of all items gave the total score, ranging from 14 to 56. The higher the score, the more compromised the respondent's ability to perform activities of daily living. A score of 15 or higher was assigned the ADL disability status. In this study, Cronbach's alpha of the ADLS was 0.939.

Lifestyle factors included smoking (yes vs. no), drinking (yes vs. no), vegetable consumption (regular intake vs. occasional or seldom intake), fruit consumption (regular intake vs. occasional or seldom intake), physical activity (regular vs. occasional or seldom participation), and community activity (regular vs. occasional or seldom participation).

Statistical methods

The Kolmogorov–Smirnov test was used to assess the normal distribution of continuous variables (34). Continuous variables such as age, TFI score, and BMI were described as means ± standard deviation (SD). Categorical variables such as sex and tea consumption were described by frequency and composition ratios. Student's *t*-tests, one-way analyses of variance (ANOVA), the Wilcoxon rank sum test, and the Kruskal–Wallis *H*-test were used to test the differences in age, TFI score, and BMI between categories of tea consumption and frailty, respectively. The chi-squared test was used to test the differences in categorical covariates between tea consumption and frailty categories. We tested the association between tea consumption and frailty using a multiple logistic regression model. Three models were constructed. Model 1 was the raw model. In Model 2, the covariates such as sociodemographic characteristics and health status were included. In Model 3, we included lifestyle factors, including smoking, drinking, vegetable consumption, fruit consumption, physical activity, and community activity. In addition, we performed a stratified analysis to examine the disparity in the association between tea consumption and frailty according to sex, age, and residence.

TABLE 1 Descriptive statistics of participants' characteristics.

Variables	Total sample	Tea consumption				Frailty		
		Rarely or never	Occasional	Daily	*P*-value	Non-frailty	Frailty	*P*-value
Total sample, *n* (%)	2,144 (100.0)	1,044 (48.7)	448 (20.9)	652 (30.4)		1,323 (61.7)	821 (38.3)	
TFI								
Total score, Mean ± SD	4.23 ± 2.91	4.60 ± 3.07	4.10 ± 2.83	3.73 ± 2.58	<0.001	2.33 ± 1.16	7.29 ± 2.16	<0.001
Physical frailty, Mean ± SD	1.83 ± 2.04	2.05 ± 2.15	1.76 ± 2.00	1.53 ± 1.84	<0.001	0.62 ± 0.81	3.78 ± 1.91	<0.001
Psychological frailty, Mean ± SD	1.27 ± 1.16	1.38 ± 1.18	1.25 ± 1.17	1.10 ± 1.11	<0.001	0.69 ± 0.87	2.20 ± 0.95	<0.001
Social frailty, Mean ± SD	1.13 ± 0.47	1.17 ± 0.49	1.09 ± 0.44	1.10 ± 0.46	0.002	1.03 ± 0.40	1.31 ± 0.52	<0.001
≥5, *n* (%)	821 (38.3)	454 (43.5)	162 (36.2)	205 (31.4)	<0.001	0 (0.0)	821 (100.0)	<0.001
Age, years, Mean ± SD	72.01 ± 6.96	72.27 ± 7.19	71.84 ± 6.82	71.71 ± 6.68	0.224	70.87 ± 6.37	73.85 ± 7.47	<0.001
Age group, years, *n* (%)					0.209			<0.001
60–79	1,822 (85.0)	875 (83.8)	380 (84.8)	567 (87.0)		1,198 (65.8)	624 (34.2)	
Male, *n* (%)	1,075 (50.1)	395 (37.8)	269 (60.0)	411 (63.0)	<0.001	721 (67.1)	354 (32.9)	<0.001
Rural residents, *n* (%)	1,315 (61.3)	664 (63.6)	272 (60.7)	379 (58.1)	0.076	740 (56.3)	575 (43.7)	<0.001
Married	1,646 (76.8)	745 (71.4)	378 (84.4)	523 (80.2)	<0.001	1,111 (67.5)	535 (32.5)	<0.001
Educational level, *n* (%)					<0.001			<0.001
Primary school and below	1,329 (62.0)	707 (67.7)	252 (56.3)	370 (56.7)		743 (55.9)	586 (44.1)	
Junior high school	494 (23.0)	203 (19.4)	119 (26.6)	172 (26.4)		336 (68.0)	158 (32.0)	
High school and above	321 (15.0)	134 (12.8)	77 (17.2)	110 (16.9)		244 (76.0)	77 (24.0)	
Average monthly personal income (RMB), *n* (%)					<0.001			<0.001
<2,000	1,053 (49.1)	579 (55.5)	182 (40.6)	292 (44.8)		524 (49.8)	529 (50.2)	
2,000–3,000	720 (33.6)	338 (32.4)	173 (38.6)	209 (32.1)		512 (71.1)	208 (28.9)	
>3,000	371 (17.3)	127 (12.2)	93 (20.8)	151 (23.2)		287 (77.4)	84 (22.6)	
Chronic disease, *n* (%)	1,509 (70.4)	744 (71.3)	299 (66.7)	466 (71.5)	0.164	830 (55.0)	679 (45.0)	<0.001
ADL disability, *n* (%)	951 (44.4)	514 (49.2)	180 (40.2)	257 (39.4)	<0.001	383 (40.3)	568 (59.7)	<0.001
Body mass index, Mean ± SD	23.19 ± 3.43	22.96 ± 3.51	23.27 ± 3.37	23.49 ± 3.33	0.006	23.28 ± 3.19	23.04 ± 3.78	0.123
Smoking, *n* (%)	636 (29.7)	311 (29.8)	124 (27.7)	201 (30.8)	0.528	320 (50.3)	316 (49.7)	<0.001
Drinking, *n* (%)	356 (16.6)	169 (16.2)	69 (15.4)	118 (18.1)	0.438	194 (54.5)	162 (45.5)	0.002
Vegetable consumption, *n* (%)	1,912 (89.2)	919 (88.0)	408 (91.1)	585 (89.7)	0.192	1,218 (63.7)	694 (36.3)	<0.001
Fruit consumption, *n* (%)	1,167 (54.4)	525 (50.3)	251 (56.0)	391 (60.0)	0.001	778 (66.7)	389 (33.3)	<0.001
Physical activity, *n* (%)	1,599 (74.6)	750 (71.8)	343 (76.6)	506 (77.6)	0.016	1,108 (69.3)	491 (30.7)	<0.001
Community activity, *n* (%)	1,703 (79.4)	813 (77.9)	356 (79.5)	534 (81.9)	0.136	1,130 (66.4)	573 (33.6)	<0.001

TFI, Tilburg Frailty Indicator; SD, standard deviation; RMB, renminbi; ADL, activity of daily living. *P*-values are calculated with chi-square test (*n*, %) or students' t-test or ANOVA.

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the association between tea consumption and frailty. All statistical analyses were performed using STATA version 16.0 (Stata Corp, College Station, TX, USA).

Results

Descriptive statistics

The mean age of the 2,144 older adults enrolled was 72.0 ± 7.0, ranging from 60 to 99 years. A total of 48.7% never or rarely drank tea, 20.9% occasionally drank tea, and 30.4% consumed it daily. The mean TFI score was 4.23 ± 2.91, and the prevalence of frailty was 38.3% (821/2,144). In addition, 1,075 men (50.1%) and 61.3% of the population lived in rural areas, 62.0% had primary education or less, 76.8% were married, and 48.8% had an average monthly income of 2,000 yuan or less. Moreover, compared with participants in the rarely or never drinking tea group, the ones in the daily tea consumption group had significantly lower TFI scores, lower prevalence of frailty, were more likely to live in urban areas, be married, have higher education and monthly income, and had a lower ADL disability rate. In addition, those who consumed tea daily had higher BMIs and were more likely to consume fruits and engage in physical activity. Detailed information regarding the participants' general characteristics is summarized in Table 1.

TABLE 2 Associations between tea consumption and frailty among whole sample.

Variables	Model 1		Model 2		Model 3	
	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
Daily consumption of tea	0.60 (0.49–0.73)	<0.001	0.71 (0.56–0.90)	0.005	0.73 (0.57–0.94)	0.012
Occasionally consumption of tea	0.74 (0.59–0.93)	0.009	1.00 (0.77–1.30)	1.000	1.03 (0.79–1.35)	0.838
Age			1.03 (1.01–1.04)	<0.001	1.03 (1.01–1.04)	0.001
Male			0.88 (0.71–1.09)	0.245	0.85 (0.68–1.06)	0.145
Rural residents			1.23 (0.97–1.55)	0.089	1.24 (0.97–1.58)	0.081
Married			0.51 (0.40–0.65)	<0.001	0.52 (0.40–0.66)	<0.001
Junior high school			0.74 (0.52–1.04)	0.086	0.83 (0.58–1.19)	0.318
High school and above			1.03 (0.79–1.33)	0.843	1.04 (0.80–1.35)	0.786
Income >3,000			0.64 (0.44–0.91)	0.014	0.67 (0.46–0.97)	0.032
Income 2,000–3,000			0.62 (0.49–0.78)	<0.001	0.65 (0.51–0.83)	<0.001
Chronic disease			2.38 (1.88–3.01)	<0.001	2.10 (1.64–2.69)	<0.001
ADL disability			3.73 (3.03–4.58)	<0.001	3.15 (2.55–3.90)	<0.001
Body mass index			1.00 (0.97–1.03)	0.917	1.00 (0.97–1.03)	0.935
Smoking					1.56 (1.24–1.95)	<0.001
Drinking					1.33 (1.02–1.73)	0.037
Vegetable consumption					0.70 (0.50–0.97)	0.030
Fruit consumption					0.77 (0.62–0.95)	0.013
Physical activity					0.56 (0.44–0.71)	<0.001
Community activity					0.60 (0.47–0.77)	<0.001
R^2	0.009		0.181		0.214	

OR, odds ration; CI, confidence interval.

Association between tea consumption and frailty

Table 2 shows the logistic regression results for the association between tea consumption and frailty. The results of model 1 indicated that occasional consumption of tea (OR = 0.74, 95%CI = 0.59–0.93, $P < 0.01$) and daily consumption of tea (OR = 0.60, 95%CI = 0.49–0.73, $P < 0.001$) were both associated with a lower prevalence of frailty. After controlling for sociodemographic characteristics and health status, the association was weakened, and only daily tea consumption (OR = 0.71, 95%CI = 0.56–0.90, $P < 0.01$) was associated with a lower prevalence of frailty. After further controlling for lifestyle factors, the association of tea consumption with frailty weakened, but the daily consumption of tea (OR = 0.73, 95%CI = 0.57–0.94, $P < 0.05$) was still associated with a lower prevalence of frailty. Furthermore, the study also found a linear relationship between tea consumption and frailty, indicating that higher tea consumption was associated with a lower prevalence of frailty (P for trend = 0.017).

Stratified analysis

Table 3 shows the results of a subgroup analysis of the association between tea consumption and frailty according to sex, age group, residence, and participation in community activities. In particular, daily consumption of tea was associated with a lower prevalence of frailty in men (OR = 0.69, 95%CI = 0.48–0.98, $P < 0.05$) but not in women (OR = 0.76, 95%CI = 0.54–1.08, $P > 0.05$). Meanwhile, the association between daily tea drinking and frailty was only statistically significant in older adults aged 60–79 years (OR = 0.70, 95%CI = 0.53–0.91, $P < 0.01$), but not in older adults aged ≥80 years. In addition, daily consumption of tea was significantly associated with a lower prevalence of frailty in rural residents (OR = 0.67, 95%CI = 0.50–0.91, $P < 0.05$), but not in urban residents (OR = 0.90, 95%CI = 0.59–1.37, $P > 0.05$). Moreover, this association was applicable only to older adults who regularly participated in community activities (OR = 0.76, 95%CI = 0.58–1.00, $P = 0.05$), and not to those who participated occasionally or rarely (OR = 0.73, 95%CI = 0.41–1.29, $P > 0.05$).

Discussion

We found that daily tea consumption was associated with a reduced prevalence of frailty in older adults. The results of this study confirm the findings of previous observational studies on the association between tea consumption and frailty in older adults (25–27). However, it should be noted that this association was heterogeneous regarding age, sex, residence, and participation in community activities. In particular, the

TABLE 3 Subgroup analyses of associations between tea consumption and frailty.

Subpopulation	Occasionally consumption of tea		Daily consumption of tea	
	OR (95%CI)	*P*	OR (95%CI)	*P*
By sex				
Male	1.07 (0.72–1.57)	0.750	0.69 (0.48–0.98)	0.037
Female	0.97 (0.65–1.43)	0.871	0.76 (0.54–1.08)	0.129
By age group				
60–79 years	0.96 (0.71–1.28)	0.769	0.70 (0.53–0.91)	0.008
≥80 years	1.58 (0.74–3.40)	0.240	0.96 (0.49–1.88)	0.909
By residence				
Urban residents	1.03 (0.65–1.63)	0.901	0.90 (0.59–1.37)	0.624
Rural residents	1.01 (0.72–1.41)	0.968	0.67 (0.50–0.91)	0.010
By community activity				
Regular participate	1.12 (0.83–1.50)	0.450	0.76 (0.58–1.00)	0.050
Occasional or seldom participate	0.74 (0.40–1.36)	0.328	0.73 (0.41–1.29)	0.278

association between tea consumption and frailty was significant among men, older adults aged 60–79, rural residents, and individuals who regularly participated in community activities.

We divided tea consumption into three categories to examine its association with frailty in this study. Only daily tea consumption was associated with a reduced prevalence of frailty, while occasional tea drinking was not. According to a study conducted in Japan, only consumption of high green tea was associated with a reduced risk of frailty (25). Two possible explanations can be offered for this association: physiological and psychosocial mechanisms. It is essential to first understand the pathophysiological pathways of frailty and its biomarkers before explaining the possible physiological mechanisms. Inflammation has previously been considered as one of the biological determinants of frailty, and biomarkers of frailty have been divided into four categories: inflammatory markers, oxidative stress, muscle protein turnover, and physical inactivity (35). It has been demonstrated that catechins, especially epigallocatechin gallate (EGCG), possess antioxidant, anti-inflammatory, and neuroprotective properties that can improve redox status at the tissue level, possibly preventing systemic structural damage (36, 37). In animal experiments, EGCG stimulates myogenic differentiation (38), inhibits aging-induced cardiac hypertrophy, fibrosis, and apoptosis (39), and reduces osteoclastogenesis (40). In addition, previous human intervention studies have also found that tea consumption protects against increased oxidative stress in older adults (41). These results support the beneficial biological effects of tea consumption on frailty. Moreover, in addition to its anti-inflammatory aspect, tea has long been associated with mood and performance enhancements. Previous study suggested that tea enhanced cognitive performance and psychological well-being (42). Tea consumption has increased during the COVID-19 pandemic, confirming the psychological effects of tea consumption as it may help relieve the higher levels of stress and disorder caused by the epidemic (43). In China and other Asian countries, consuming tea is an important way to reduce psychological stress, interact with others and participate in social activities (44, 45). A study of Chinese older adults indicated that tea drinking was associated with a lower risk of depression (46). Therefore, older adults who drink tea daily may be more likely to relieve stress and have a reduced risk of developing negative emotions. This may help reduce the risk of psychological frailty. In addition, people who drink tea regularly engage in more social activities and have wider social networks, reducing the risk of social frailty.

In this study, we further explored the heterogeneity in age, gender, residence, and participation in community activities on the association between tea consumption and frailty. The results showed that the negative relationship between daily consumption of tea and frailty was only observed in men, younger older adults, rural residents, and participants who regularly participated in community activities. These results are similar to those of previous studies that found a negative association between tea consumption and depressive symptoms, health status, and mortality in Chinese older adults (45, 47). Among those aged <80 years, daily tea consumption was associated with a lower prevalence of frailty compared to those aged >80 years. A possible explanation is that frailty is an age-related disease; the oldest-old are more prone to frailty; therefore, the benefits of drinking tea may be difficult to highlight in the oldest-old. Previous research has also suggested that the health benefits of tea drinking may play a role in the early stages (under the age of 80) of health deterioration (45). In addition, we also found that the benefits of tea drinking on frailty were more pronounced in men and rural residents. This may be related to the high frequency of tea consumption among participants of these categories. Using univariate analysis, we found that men and rural residents consume more tea on a daily basis than women and urban residents; therefore, those who consume tea more frequently may experience greater health benefits. Finally, we found that tea consumption was negatively associated with frailty in older adults who regularly participated in community activities, but not in individuals who participated occasionally or rarely. As mentioned above, tea drinking was seen as one of the indicators of the social participation of older adults in China (48). Therefore, older adults who regularly participate in community activities are more likely to drink tea, which promotes social interactions and mental health; therefore, the association between tea drinking and frailty is more apparent. Since we did not find significant interactions between tea consumption and four of the factors on frailty, further research is warranted to explore the heterogeneities

of these associations and their biological, psychological, and social mechanisms.

This study had several advantages. First, to our knowledge, this study is the first to explore the association between tea consumption and frailty among older adults in mainland China. In particular, we comprehensively evaluated the frailty of older adults from three levels: physical, psychological, and social, rather than focusing solely on physical frailty. Second, we controlled for a set of covariates in the regression to make the association between tea consumption and frailty more robust. In short, this study provides research evidence from China to explore the association between tea drinking and frailty, thus further enriching the literature on the health benefits of drinking tea. Considering the availability of tea, this study suggests that promoting tea consumption in older adults may be an effective measure to help reduce the prevalence of frailty. However, it must be noted that the results of this study should be interpreted with caution, as the underlying mechanisms of action of tea on health, remain unclear. The statistical associations in this study did not demonstrate a clinical effect of tea drinking on decrease of frailty in older adults.

This study also had several limitations. First, the cross-sectional study design made it impossible to infer a causal relationship between tea consumption and frailty. In the future, it will be necessary to use longitudinal studies to examine the association between the two and to use interventional studies to establish a causal relationship. Second, tea consumption in this study was self-reported, and the exact amount consumed could not be determined due to a lack of data, which may introduce both recall and measurement bias. In addition, we did not analyze the association between different types of tea and frailty. Further research should be conducted on the association of different tea types with frailty. Green tea and fermented tea contain different levels of tea polyphenols; thus, their associations with frailty may also differ. Third, when explaining the association between tea consumption and frailty, we mainly demonstrated the aspect of inflammation. However, we did not collect inflammatory biomarkers in the survey. Future studies should further focus on the levels of inflammation in the investigated individuals and a rather healthy and active lifestyle that may be associated with tea drinking. This may help further clarify the mechanism of the association between healthy lifestyles, including tea consumption and frailty, to provide a higher level of scientific evidence for the prevention of frailty.

Conclusions

Tea consumption is associated with a lower prevalence of frailty among older adults, especially those men, older adults aged 60–79, rural residents, and individuals who regularly participated in community activities. Further longitudinal and experimental studies are needed to determine the causal relationship between tea consumption and frailty.

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 Medical Ethics Committee of Xiangya School of Public Health, Central South University (identification code: XYGW-2020-101). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SL, FL, and YYa contributed to the study design. SL analyzed the data and drafted the manuscript. GC and YYi conducted data collection and gave comments on the draft. FL and YYa revised the draft. All authors read and approved the final manuscript.

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Conflict of interest

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

Publisher's note

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The causal relationship between obesity and skin and soft tissue infections: A two-sample Mendelian randomization study

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Objective: Many observational studies have shown that obesity strongly affects skin and soft tissue infections (SSTIs). However, whether a causal genetic relationship exists between obesity and SSTIs is unclear.

Methods: A two-sample Mendelian randomization (MR) study was used to explore whether obesity is causally associated with SSTIs using a publicly released genome-wide association study (GWAS). An inverse-variance weighted (IVW) analysis was used as the primary analysis, and the results are reported as the odds ratios (ORs). Heterogeneity was tested using Cochran's Q test and the I^2 statistic, and horizontal pleiotropy was tested using the MR–Egger intercept and MR pleiotropy residual sum and outlier (MR-PRESSO).

Results: The results of the MR analysis showed a positive effect of BMI on SSTIs (OR 1.544, 95% CI 1.399-1.704, $P = 5.86 \times 10^{-18}$). After adjusting for the effect of type 2 diabetes (T2D) and peripheral vascular disease (PVD), the positive effect still existed. Then, we further assessed the effect of BMI on different types of SSTIs. The results showed that BMI caused an increased risk of impetigo, cutaneous abscess, furuncle and carbuncle, cellulitis, pilonidal cyst, and other local infections of skin and subcutaneous tissues, except for acute lymphadenitis. However, the associations disappeared after adjusting for the effect of T2D and PVD, and the associations between BMI and impetigo or cellulitis disappeared. Finally, we assessed the effects of several obesity-related characteristics on SSTIs. Waist circumference, hip circumference, body fat percentage, and whole-body fat mass, excluding waist-to-hip ratio, had a causal effect on an increased risk of SSTIs. However, the associations disappeared after adjusting for the effect of BMI.

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Conclusion: This study found that obesity had a positive causal effect on SSTIs. Reasonable weight control is a possible way to reduce the occurrence of SSTIs, especially in patients undergoing surgery.

KEYWORDS

obesity, skin and soft tissue infections, genome-wide association study, Mendelian randomization, causal relationship

Introduction

Obesity is an excess of body fat that is detrimental to health and is often assessed clinically by the body mass index (BMI) (1). Obesity is a major public health problem, and in 2016, the World Health Organization (WHO) estimated that more than 1.9 billion people aged 18 years and older were overweight. More than 650 million of them are obese. In addition, more than 340 million children and adolescents aged 5-19 years are overweight or obese (2). Obesity reduces the health-related quality of life and longevity, and increases the risk of type 2 diabetes, coronary artery disease, gallbladder disease, and hyperlipidemia (3). Skin and soft tissue infections (SSTIs) are pathogenic bacteria that invade the epidermis, dermis, and subcutaneous tissues and induce a host response, and the diagnosis is based primarily on clinical features (4–6). The clinical features include sclerosis, erythema, fever, and pain or induration. Local manifestations may be accompanied by systemic signs and symptoms, such as fever, chills, and sometimes hemodynamic instability (5). SSTIs constitute the most common infectious disease in all age groups, and their incidence is increasing every year with the increase in surgical procedures, the use of immunosuppressive drugs, and cancer (7). SSTIs often require inpatient treatment and place a great burden on the health care system (8–10).

A cohort study involving 171,322 adults from 2011 to 2016 found that increased BMI was associated with an increased risk of cellulitis and hospitalization for cellulitis, and that obesity was an independent risk factor for cellulitis, after adjusting for confounders (11). Another case–control study noted that obese patients were 1.76 times more likely to have a surgical site infection than nonobese individuals (12). However, recently, it has been found that adipogenesis of the skin is an important source of resistance to infection and antimicrobial peptides (13, 14), which contradicts many observations regarding the increased risk of bacterial skin infections in obese individuals (15). Based on the results of these studies, the causal relationship between obesity and SSTIs is unclear as some confounding factors may have biased the results, such as the strong association between obesity and type 2 diabetes (T2D) and peripheral vascular disease (PVD) (5, 16). Therefore, the causal relationship between obesity and SSTIs has not been established.

Genetic epidemiology is used to elucidate the determinants of disease because the inheritance of genetic variants is random and cannot be confounded by additional risk factors (17, 18). Mendelian randomization is a method used to assess whether a causal relationship exists between exposure factors and outcomes using genetic variants as instrumental variables that are equally, randomly, and independently distributed during the split (19–21). Therefore, we used two-sample MR to assess the causal relationship between study obesity and SSTIs.

Methods

Study design

A two-sample MR design were used in this study, and genome-wide association study (GWAS) data were used to determine the causal relationship between obesity and SSTIs. Single nucleotide polymorphisms (SNPs) were instrumental variables for obesity. The BMI is often used to measure and assess obesity in the clinical practice (22). In addition, several other obesity-related characteristics were used in the assessment, including waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR), body fat percentage (BF), and whole body fat mass (FM) (23–25). The main assumptions of this method include the following: 1) SNPs are associated with exposure factors, 2) SNPs are independent of confounding factors, and 3) SNPs affect outcomes only *via* exposure factors (Figure 1).

Genome-wide association study summary data

The two-sample MR analysis in this study was performed based on data from a GWAS. BMI, obesity-related characteristics, and SSTIs (SSTIs as defined by the International Classification of Diseases- Tenth Revision code L00-L08) were obtained from the

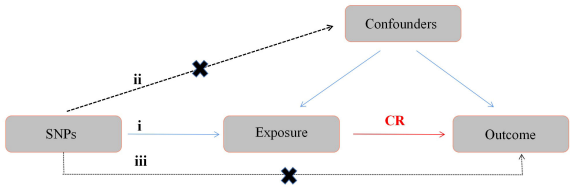

FIGURE 1
Diagram of the MR study design. The following three assumptions should be satisfied: 1) SNPs should be closely associated with exposure; 2) the selected SNPs should be independent of confounding factors; and 3) the SNPs affect the results only through the exposure factor. MR, Mendelian randomization; SNPs, single nucleotide polymorphisms; CR, causal relationship.

MRC IEU OpenGWAS database (https://gwas.mrcieu.ac.uk/), which consists mainly of GWAS data that are currently publicly available for use in Mendelian randomization analyses (26, 27). All GWAS were tested using imputed genotype data from the UK Biobank study. All GWASs were tested using imputed genotype data from the UK Biobank study. The GWAS summary of BMI was based on 461460 individuals of European ancestry, and the GWAS summary statistics of SSTIs were based on 218792 individuals in the European population (10343 SSTI cases and 208449 controls).

We analyzed the causal relationship between BMI and the risk of SSTIs using univariate MR. Next, we reassessed the effect of BMI on SSTIs after adjusting for the effect of T2D and PVD on BMI. Then, we assessed the effect of SSTI on BMI. We performed a stratified analysis of the different types of SSTIs to further assess the effect of BMI on different types of SSTIs. Finally, we assessed the causal effects of several obesity-related characteristics. The details of the data sources are provided in Supplementary Table S1.

Instrumental variable selection

To select eligible genetic IVs that met the MR assumptions, we established a quality control technique. First, we selected independent SNPs that were strongly associated with the exposure factors with *p values* < 5×10^{-8}. In assessing the effect of SSTIs on BMI, we expanded the *p* value to < 1×10^{-5} to select suitable genetic instruments considering that few SNPs were associated with SSTIs at the *p* value < 5×10^{-8} level. Then, to exclude SNPs with strong linkage disequilibrium, we performed an aggregation process (R2<0.001) (28). Finally, to ensure that the effect alleles belonged to the same allele, we adjusted the exposure and outcome datasets to eliminate ambiguous SNPs with inconsistent alleles and SNPs with intermediate allele frequencies (29).

We calculated the *F* statistic of each SNP as follows: F=R2×(N-2)/(1-R2) (30). R2 denotes the variance of exposure explained by each IV according to the calculation method used in Papadimitriou et al. (30). IVs with F-statistic values less than 10 are considered weak instruments and were excluded from the MR analysis (31).

Statistical analysis

The inverse variance weighted (IVW) method was used as the main method to analyze the causal relationship between obesity and SSTIs. The causal effect of each SNP on the outcome was assessed by calculating the Wald ratio of each SNP, and the inverse variance of the SNP was used as the weight in a meta-analysis to evaluate the combined causal effect. In addition, we used MR–Egger, weighted median, sample mode, and weighted mode to assess the causal relationship between BMI and SSTIs. MR–Egger has low statistical power; thus, the focus is more on direction and effect (32, 33). The weighted median provides a reliable Mendelian evaluation when 50% of the instrument variables are not valid (34). The odds ratio (OR) and 95% confidence interval (CI) were used to assess the relative risk.

We used the Cochran's Q value and I^2 statistic to assess heterogeneity among the SNPs and the MR–Egger method to test for horizontal pleiotropy. In addition, the MR pleiotropy residual sum and outlier (MR-PRESSO) method was used to detect outliers in the analysis and assess the adjusted causal effects after excluding the outliers (35). A sensitivity analysis was performed using the leave-one-out sensitivity test to assess the validity and stability of the MR results. We used MR-Steiger filtering to remove SNPs that implied reverse causal direction (36).

The data were analyzed using R (version 4.1.2) software with the R packages "two-sample MR" and "MRPRESSO". $P< 0.05$ was considered statistically significant. The data used in this study were publicly available, and therefore, the study did not require ethical approval.

Results

Mendelian randomization to assess the causal relationship between obesity and SSTIs

The effect of BMI on the risk of an SSTI was first assessed using the univariate MR method (Figure 2 and Supplementary Figure S1). The results using the IVW method showed that with a 1-SD increase in the BMI level, the OR of SSTIs was 1.544 (95% CI 1.399-1.704, $P= 5.86 \times 10^{-18}$). The MR–Egger method (OR=1.322, 95% CI 1.013-1.726, $P= 4.09 \times10^{-2}$) and weighted median (OR=1.458, 95% CI 1.246-1.706, $P=2.64 \times 10^{-6}$) also showed consistent results. In the above analysis, Cochran's Q and I^2 tests did not detect heterogeneity, and the MR–Egger test did not detect horizontal pleiotropy (Supplementary Figure S2 and Supplementary Table S2). The MR-PESSO method did not detect outliers; thus, the association between BMI and SSTIs and

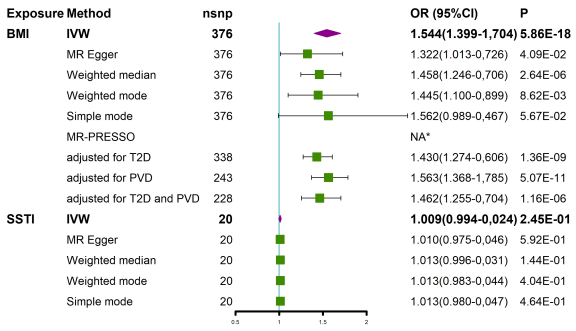

FIGURE 2
MR analysis showed the causal relationship between BMI and SSTIs. *No outliers were detected. BMI, body mass index; IVW, inverse variance weighted; nsnp, number of single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; MR-PRESSO, MR Pleiotropy REsidual Sum and Outlier; T2D, type 2 diabetes; PVD, peripheral vascular disease; SSTI, skin and soft tissue infection.

its significance remained unchanged (Figure 2). To assess whether these results were affected by a single SNP, we performed the leave-one-out sensitivity test, which showed that the causal effect of BMI on SSTIs did not significantly fluctuate in the absence of any single SNP (Supplementary Figure S3). We performed MR-Steiger filtering of the SNPs of BMI and did not find SNPs with reverse causality.

Next, we assessed the effect of an adjusted BMI on SSTIs after excluding T2D and PVD, which often coexist with obesity (Figure 2). After adjusting for T2D, BMI was still causally associated with an increased risk of SSTIs (OR=1.430, 95% CI 1.274-1.606, P=1.36×10^{-9}). After adjusting for PVD, BMI was also causally associated with an increased risk of SSTIs (OR=1.563, 95% CI 1.368-1.785, P=5.07×10^{-11}). Finally, after we adjusted for T2D and PVD, an increase in BMI was still positively associated with an increased risk of SSTIs (OR=1.462, 95% CI 1.255-1.704, P=1.16×10^{-06}).

Finally, we also explored whether SSTIs had a causal effect on the BMI. The results of the IVW method showed no effect of SSTIs on the BMI. Due to the presence of heterogeneity (Supplementary Table S2), the results of the weighted median analysis were used according to Nazarzadeh et al. (37). The results of the weighted median method showed no effect of SSTIs on BMI (Figure 2).

Causal effects of BMI on different types of SSTIs

We performed a stratified analysis of different types of SSTIs (according to ICD-Tenth Revision) to further assess the effect of BMI on different types of SSTIs (Figure 3). SNPs associated with staphylococcal scalded skin syndrome (SSSS) were not available in the GWAS; thus, SSSS was not included in the type stratification of SSTIs. The results of the univariate MR analysis showed that an increase in BMI caused an increased risk of impetigo, cutaneous abscess, furuncle and carbuncle (CA-F-C), cellulitis, pilonidal cyst (PC), and other local infections of skin and subcutaneous tissues, except for acute lymphadenitis (AL) (Figure 3). Next, we adjusted for the effects of T2D and PVD. The results of the multivariate MR study showed that after adjusting for both T2D and PVD, an increase in BMI resulted in an increased risk of CA-F-C, PC, and other local infections of skin and subcutaneous tissues (Figure 3). In addition, no outliers of BMI were detected by the MR-PESSO method (Figure 3).

Causal effect of obesity-related characteristics on SSTIs

We investigated whether obesity-related characteristics (WC, HC, WHR, BF, and FM) had a causal effect on SSTIs. The univariate MR analysis showed that the obesity-related characteristics of WC, HC, BF, and FM, excluding WHR, had a causal effect on the increased risk of SSTIs (Figure 4). The

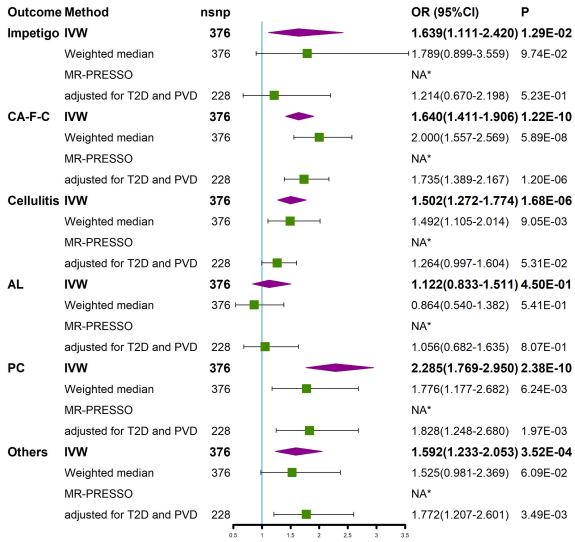

FIGURE 3
Univariable MR and multivariable MR analyses showed the causal relationship between BMI and different types of SSTIs. Univariable MR was performed using the IVW, weighted median, and MR-PRESSO methods. Multivariable MR was performed to assess the effect of BMI on different types of SSTIs after adjusting for T2D and PVD. *No outliers were detected. BMI, body mass index; IVW, inverse variance weighted; nsnp, number of single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; MR-PRESSO, MR Pleiotropy REsidual Sum and Outlier; T2D, type 2 diabetes; PVD, peripheral vascular disease; CA-F-C, cutaneous abscess, furuncle and carbuncle; AL, acute lymphadenitis; PC, pilonidal cyst; Others, other local infections of skin and subcutaneous tissues; SSTIs, skin and soft tissue infections.

correlations were consistent in all sensitivity analyses, although the MR–Egger regression analysis detected pleiotropy in HC and BF. After removing outliers using the MR-PRESSO method, these factors remained significantly associated with SSTIs (Figure 4). Multivariate MR analyses adjusting for BMI were performed because these obesity-related characteristics were highly correlated with BMI. The results of the study showed that none of these obesity-related features were significantly associated with SSTIs after adjusting for BMI (Figure 4).

Discussion

Many previous clinical studies have noted that obesity is a risk factor for a variety of diseases, including SSTIs. However, due to the limitations of previous studies, these findings may be influenced by confounding factors and reverse causality. Our study was based on MR to explore the relationship between obesity and SSTIs. The results of the study found a positive causal effect of obesity on an increased risk of SSTIs. This positive causality persisted after adjusting for the effects of T2D and PVD.

Our findings suggest that obesity increases the risk of SSTIs, which is similar to the results of previous studies (15). This finding may be related to the metabolic effects of obesity and

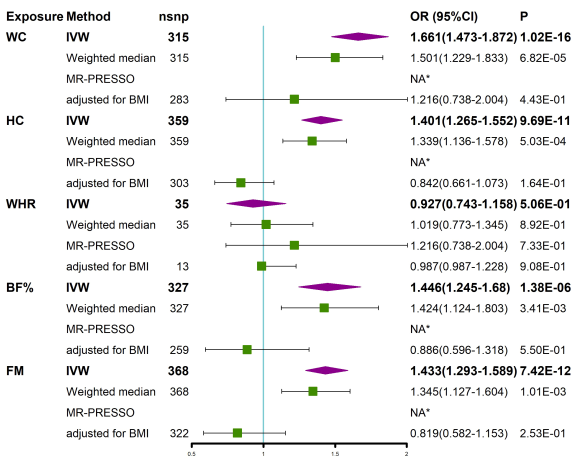

FIGURE 4
MR analyses showed a causal relationship between obesity-related characteristics and SSTIs. Univariable MR was performed using the IVW, weighted median, and MR-PRESSO methods. Multivariable MR was performed to assess the effect of obesity-related characteristics on SSTIs after adjusting for BMI. *No outliers were detected. BMI, body mass index; IVW, inverse variance weighted; nsnp, number of single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; MR-PRESSO, MR Pleiotropy REsidual Sum and Outlier; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; BF, body fat percentage, FM, whole body fat mass; SSTIs, skin and soft tissue infections.

inadequate blood supply to adipose tissue (AT), which leads to poor host immune recruitment and thus causes skin defenses to be diminished (9, 38). Healthy AT acts as a defense barrier against microorganisms. Adipocytes are able to produce antimicrobial peptides (AMPs) in response to skin infections, thereby strengthening the innate immune defenses of the skin (39). Several findings confirm that host defense is mediated through adipocyte production of AMPs and that the inhibition of adipocytes reduces the expression of AMPs (13, 14). Recently, an animal study (40) found that obesity in mice leads to a loss of dermal preadipocytes (pADs) and inhibits the ability to initiate responsive adipose expression of antimicrobial peptides, and obesity leads to a loss of dermal fibroblasts (dFBs), which have the ability to polarize into mature adipocytes capable of expressing antimicrobial peptides, further promoting the development of infection. Obesity causes hypertrophy and proliferation in adipocytes, leading to a rapid increase in adipose tissue, and when angiogenesis cannot match the expanded AT, local hypoxia in AT causes an increase in pro-inflammatory leptin and a decrease in anti-inflammatory adiponectin (41–44). In addition, hypoxia progresses to induce endoplasmic reticulum stress, which combined with excess extracellular matrix (ECM) deposition, causes AT fibrosis (41, 45). All these changes promote the release of inflammatory factors and intensify the local inflammatory response, ultimately leading to adipocyte dysfunction and metabolic changes and causing decreased immunity and insulin resistance (41, 43–46). Furthermore, hypoxia in AT causes adipocytes to undergo anaerobic glycolysis, promoting the increased production and release of lactate, which further promotes the inflammatory pathway of macrophages, resulting in chronic inflammation (47, 48).

In chronic inflammation, immune cells are constantly stimulated by cytokines or chemokines and are activated, eventually causing immune cell depletion (49). In addition, chronic inflammation decreases the formation of memory T cells, reducing the ability of T cells to protect the host against infection (50). Recently, it has been found that long-term stimulation by chronic inflammation causes a significant decrease in the proliferative capacity of hematopoietic stem cells, the origin of immune cells, resulting in accumulative and irreversible functional damage and long-term suppressive effects on hematopoiesis, further contributing to immune cell failure and decreased immune function (51).

Obesity also affects the function of immune cells, which leads to decreased immune function and promotes the development of infections. Macrophages are key mediators of inflammation in adipose tissue and are the most abundant immune cells (52). In the obese state, macrophages mainly accumulate around adipocytes, forming crown-like structures and proliferating to clear dysfunctional and necrotic adipocytes, and inflammation cannot be cleared due to persistent obesity; then, inflammation changes from local inflammation to a systemic chronic inflammatory state

(41, 53). Other research has found that macrophages exhibit an anti-inflammatory M2 phenotype in thin mice, whereas in diet-induced obese (DIO) mice, macrophages switch from an anti-inflammatory M2 phenotype to a proinflammatory M1 phenotype, leading to chronic inflammation (54, 55). Additionally, these macrophages show decreased phagocytosis and a decreased ability to clear bacteria (44). In addition, obesity also inhibits T-lymphocyte receptor diversity, leading to abnormalities in the antigen presentation process, decreasing the efficiency of T-lymphocyte responses and increasing susceptibility to infection in obese individuals (44, 56). Neutrophils play a role in the inflammatory response induced by obesity, and neutrophils in AT produce chemokines and cytokines that promote macrophage infiltration, contribute to chronic low-grade inflammation, and induce insulin resistance (57). Furthermore, the imbalance of neutrophil elastase (NE) and its inhibitor α1-antiprotease in obese individuals causes chronic inflammation and insulin resistance (58). Moreover, obesity causes the downregulation of neutrophil function, which reduces bacterial clearance (44, 59). Furthermore, the humoral immune response is defective in obese individuals, and B lymphocytes in obese individuals express a proinflammatory phenotype that reduces their ability to optimally respond to infection (44).

Obesity or high-fat diets modify the composition of the gut microbiota and increase intestinal permeability, leading to the passage of bacteria and bacterial products (e.g., lipopolysaccharides, LPS) across the intestinal barrier into the circulation, which can activate innate immune cells and immune signaling pathways and contribute to inflammation formation (2, 44, 60–63). In addition, a high-fat diet increases the proportion of gram-negative bacteria, further promoting the absorption of LPS across the intestinal barrier (60, 64). These factors all lead to a low-grade systemic inflammatory response (65–67). As obesity advances, the eventual mild low-grade inflammation becomes chronic inflammation that further causes systemic inflammation (68–70).

Overall, obesity leads to immune system dysregulation, a reduced cell-mediated immune response, and modified gut microbiota, representing possible reasons for the increased risk of infection associated with obesity (16, 38, 71–73). Similar to the findings reported by Conway et al. (74), the present study also showed that the genetic prediction of BMI was positively causally associated with an increased risk of SSTIs. Therefore, the risk of infection should be noted in obese patients, especially those who undergo surgery. Preoperatively, obese patients may be advised to lose weight to reduce systemic inflammation and the risk of infection (75).

We performed a stratified analysis of the types of SSTIs, and the findings showed that an increased BMI was positively associated with an increased risk of impetigo, CA-F-C, cellulitis, PC, and other local infections of skin and subcutaneous tissues. However, after adjusting for T2D and PVD, an increased BMI was not significantly associated with the development of cellulitis, which may be due to obesity-induced elevated blood glucose due to insulin resistance and PVD to further mediate the development of cellulitis (76, 77). In addition, although we stratified the degree of severity and site of SSTIs, the GWAS lacked data of the different degrees or different sites of SSTIs; thus, an MR analysis with stratification of different degrees or sites of SSTIs is not available.

In addition, this study evaluated the relationship between several other obesity-related characteristics and the risk of SSTIs. Previous studies have found that obesity characteristics, such as WC and BF, are predictors of infection (78, 79). In the univariate MR analysis, we also found a positive association between waist circumference, hip circumference, body fat percentage and whole-body fat mass and the risk of SSTIs. However, this association may be mediated through the effect of BMI as we did not observe this significant effect after adjusting for BMI. Furthermore, the OR of HC, BF and FM on SSTIs was less than 1 after adjusting for BMI, although there was no significance, possibly implying that increased HC, BF, and FM are protective factors against SSTIs, similar to a recent cohort study that found a lower risk of death in people with a small WC and large HC (80). However, our findings are only based on the MR method, and large clinical studies are still needed to confirm whether such a relationship exists. In view of our findings, we recommend using BMI rather than other obesity characteristics in the risk assessment of SSTIs in the clinic.

To the best of our knowledge, this study is the first to use a two-sample MR analysis to assess the causal relationship between obesity and SSTIs. In addition, the genetic variants used as IVs were extracted from the largest GWAS. However, there are some limitations to this study. 1). The MR analysis conducted in this study was based on a European cohort, and whether there is a demographic effect on the results is unclear. 2). The genetic data of exposure and outcome were pooled data from a GWAS, and relevant data, such as disease prevalence and age specificity, are lacking. 3). This study focuses on the causal relationship between obesity and SSTIs, but whether a causal relationship exists between weight loss and SSTIs is unclear.

Conclusion

This study shows that a positive causal relationship exists between a genetically predicted increase in BMI and an increased risk of SSTIs, further deepening our understanding of obesity causing SSTIs and providing guidance for prevention and treatment.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and

accession number(s) can be found below: https://gwas.mrcieu.ac.uk/datasets/.

Author contributions

HH and ML performed the study and wrote the manuscript. XW and HL revised the manuscript. JM wrote sections of the manuscript and performed the statistical analysis. GC designed the study. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.996863/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Plots of MR estimates of the causal relationship between SSTIs and BMI. **(A)** Scatter plot of SNPs showing the causal effect of BMI on SSTIs. The log odds ratio of risk is demonstrated, and five different methods were used. **(B)** Forest plot of SNPs associated with BMI on SSTIs. MR, Mendelian randomization; SSTI, skin and soft tissue infection; BMI, body mass index; SNP, single nucleotide polymorphism.

SUPPLEMENTARY FIGURE 2
Funnel plot showed there were no significant heterogeneity among SNPs. SNPs, single nucleotide polymorphisms.

SUPPLEMENTARY FIGURE 3
Leave-one-out analysis plots for BMI on SSTIs. MR, Mendelian randomization; SSTI, skin and soft tissue infection; BMI, body mass index.

SUPPLEMENTARY TABLE 1
Data source information. BMI, body mass index; T2D, type 2 diabetes; SSTI, skin and soft tissue infection.

SUPPLEMENTARY TABLE 2
Heterogeneity tests and directional horizontal pleiotropy test.

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Predictors of weight loss in patients with obesity treated with a Very Low-Calorie Ketogenic Diet

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Introduction: The Very Low-Calorie Ketogenic Diet (VLCKD) has emerged as a safe and effective intervention for the management of metabolic disease. Studies examining weight loss predictors are scarce and none has investigated such factors upon VLCKD treatment. Among the molecules involved in energy homeostasis and, more specifically, in metabolic changes induced by ketogenic diets, Fibroblast Growth Factor 21 (FGF21) is a hepatokine with physiology that is still unclear.

Methods: We evaluated the impact of a VLCKD on weight loss and metabolic parameters and assessed weight loss predictors, including FGF21. VLCKD is a severely restricted diet (< 800 Kcal/die), characterized by a very low carbohydrate intake (< 50 g/day), 1.2–1.5 g protein/kg of ideal body weight and 15–30 g of fat/day. We treated 34 patients with obesity with a VLCKD for 45 days. Anthropometric parameters, body composition, and blood and urine chemistry were measured before and after treatment.

Results: We found a significant improvement in body weight and composition and most metabolic parameters. Circulating FGF21 decreased significantly after the VLCKD [194.0 (137.6–284.6) to 167.8 (90.9–281.5) $p < 0.001$] and greater weight loss was predicted by lower baseline FGF21 (Beta = −0.410; $p = 0.012$), male sex (Beta = 0.472; $p = 0.011$), and central obesity (Beta = 0.481; $p = 0.005$).

Discussion: VLCKD is a safe and effective treatment for obesity and obesity related metabolic derangements. Men with central obesity and lower circulating FGF21 may benefit more than others in terms of weight loss obtained following this diet. Further studies investigating whether this is specific to this diet or to any caloric restriction are warranted.

KEYWORDS

fibroblast growth factor 21, insulin resistance, body composition, low carbohydrate diet (LCD), very low energy diet, protein sparing modified fasting

1. Introduction

In the last decades there has been a growing interest for the Very Low-Calorie Ketogenic Diet (VLCKD) as a feasible nutritional intervention for the management of obesity providing significant weight loss and improvement in obesity-related diseases. This diet is characterized by a very low carbohydrate intake (< 50 g/day), 1.2–1.5 g protein/kg of ideal body weight, 15–30 g of fat/day, in the context of a very low-calorie intake (approximately 800 kcal/day) (1). The restriction of carbohydrates induces the lipolysis of fat depots and leads to nutritional ketosis, modulating the gut microbiome (2) and inducing a metabolic effect that stabilizes glucose levels and minimizes insulin release (3). Circulating levels of ketone bodies, especially B-hydroxybutyrate (BHB), promote an anorexigenic effect, reducing appetite and food intake (4, 5), which is one of the mechanisms accounting for the tolerability and high adherence to such a restrictive diet (6). Recently, a meta-analysis was conducted to assess the efficacy of the VLCKD in subjects with overweight and obesity (7). The main results reported a significant weight loss in the short, intermediate, and long term and improvements in body composition parameters (reducing waist circumference and fat mass without inducing lean mass loss) as well as glucose and lipid profile (8). In particular, the VLCKD is associated with a larger reduction in fasting glucose, the homeostasis model of assessment-IR (HOMA-IR) index, total cholesterol and triglycerides levels, compared to other weight loss programs such as balanced low-calorie diets (7). Emerging evidence suggests that ketogenic diets may have several applications in the treatment of metabolic disease, including NAFLD and type 2 diabetes (9–11), as well as pre (12) and post bariatric surgery (13). More generally speaking, carbohydrate restriction even in the form of high fat ketogenic diets was proven to be anti-inflammatory and effective in improving several metabolic parameters compared to control (14–16), suggesting that a low carbohydrate approach may be more beneficial compared to isocaloric diets with higher carbohydrate content. However, it should be pointed out that evidence on this regard is still limited, and further studies comparing strictly isocaloric diets in a residential setting should be conducted in order to confirm this. Interestingly, emerging evidence also suggests that exogenous ketone bodies may prove beneficial, but more studies are needed to further elucidate their effects (17). However, the extent of weight loss with VLCKD highly varies among patients and some factors, including the presence of specific genetic variants, have been supposed to cause this variability (18). Beside this evidence, while the metabolic predictors of weight reduction were investigated for other lifestyle therapeutic approaches (19, 20) no study investigated metabolic predictors of weight loss after VLCKD treatment.

Among the many molecules involved in energy homeostasis and, more specifically, in metabolic changes induced by ketogenic diets, Fibroblast Growth Factor 21 (FGF21) is a hormone predominantly secreted by the liver that exerts endocrine and paracrine effects, although its physiology is not fully understood yet (21, 22). The association between FGF21 and obesity in humans seems more complex and controversial than that observed in primate and murine models (23). Mice fed a ketogenic diet express increased FGF21 levels (24, 25), whereas our group and others have demonstrated that ketogenic diets result in decreased FGF21 levels in human subjects (26, 27). Moreover, the positive correlation with obesity, insulin resistance (IR) and metabolic syndrome (MetsS) has been confirmed *in vivo* (21), which, together with the known *in vitro* effects of this hepatokine, makes it reasonable to hypothesize that FGF21 may play a role in the response to weight loss interventions.

With the prevalence of obesity steadily increasing in most countries (28, 29), it is of utmost importance to find effective treatments, and nutrition strategy personalization is key. Identifying predictors of weight loss prior to specific treatment initiation may help choosing the right treatment for each patient, likely enhancing the success rate. In fact, the first cause of diet discontinuation is the poor response to the diet (30). In addition, recognizing weight loss predictors among novel molecules may help generating hypotheses regarding the physiology of weight loss, possibly paving the way for further mechanistic studies.

In this preliminary report, we aimed to assess baseline predictors of greater weight loss in patients with obesity undergoing a VLCKD focusing on the predictive role of FGF21.

2. Materials and methods

2.1. Study design and population

In this single-center, observational prospective before-after study, we evaluated baseline predictors of weight loss amount after 45 VLCKD diet. Variables collected at baseline were: demographic data, anthropometric data, glycol-metabolic data, liver function tests, kidney function test, C-reactive protein and FGF21.

The primary study outcome was to investigate the predictive role of FGF21 on weight loss amount after short-term VLCKD diet. Secondary outcomes were to investigate which of the routinary clinical, anthropometrical, or biochemical baseline characteristics predict a larger weight loss in these patients.

Weight loss amount was defined as before-after weight delta.

Patients were enrolled in the Center of High Specialization for the Care of Obesity (CASCO), Rome, Italy. The inclusion criteria were: BMI over 30 kg/m^2, 18–60 years of age; stable body weight in the preceding 3 months. No gender ratio was set upon enrolment. Exclusion criteria were: contraindications to a VLCKD, as severe organ failure, insulin dependent diabetes, current pregnancy or breastfeeding, any allergy to meal replacements components impossible to be avoided, no signed informed consent, psychiatric diseases possibly hindering compliance (31).

2.2. Intervention

All patients followed a VLCKD with meal replacements (New Penta s.r.l., Cuneo, Italy) for 45 days at home. Participants were clinically evaluated at baseline and every 2 weeks up to the end of the study. All patients could contact the dieticians directly by phone or sms whenever needed in order to improve adherence. They were encouraged to reduce their sedentary lifestyle, although no formal exercise program was provided. The nutritional intervention was ∼800 kcal/day, consisting of 4 or 5 meal replacements daily which were provided to the patient at each follow up visit, and one serving of vegetables with a low glycemic index at lunch and dinner, which the patients were required to purchase and prepare autonomously

at home. The composition was as follows: carbohydrates 26 g, protein 1.2–1.5 g/Kg of ideal body weight (32), fat 35 g. The protein source in the meal replacements mainly came from whey, egg, and soy, and fats were from extra virgin olive oil. All patients were encouraged to drink at least 2 L of water daily and to take daily multimineral and vitamin supplements which were provided as per current recommendations (7). The patients were also provided with urine test strips for acetoacetate and were asked to self-test the first morning urine weekly. The same strips were used at each visit to confirm compliance to the VLCKD.

2.3. Measurements

All subjects were evaluated before and right after the end of the dietary intervention. The same stadiometer and calibrated scale were used to measure height and body weight. Waist circumference (WC) was measured midway between the lower rib and the iliac crest, at the end of a normal expiration. Hip circumference (HC) was measured at the level of the widest circumference over the great trochanters to the closest 1.0 cm. The waist-to-hip (W/H) ratio was calculated as WC divided by HC. An automated device was used to measure at each visit.

2.4. Body composition evaluation

All subjects had their body composition assessed before and right after the end of the dietary intervention through dual-energy-X-ray absorptiometry (DXA) (Hologic 4500, Bedford, MA, USA) as previously described (33).

2.5. Laboratory assay

All patients' blood samples were drawn in the morning following an overnight fast at baseline and at the end of the treatment. The parameters measured at the hospital laboratory following the local standards of practice were complete blood count, total, HDL and LDL cholesterol, triglycerides, electrolytes, glucose and insulin, albumin, C-reactive protein (CRP), creatinine and estimated glomerular filtration rate (eGFR), alanine transaminase (ALT), aspartate transaminase (AST), uric acid. FGF21 serum levels were measured after an overnight fast using a commercial assay (R&D Systems, Inc., Minneapolis, MN, USA). Metabolic Syndrome (MetS) was defined by the modified ATP-III criteria (34). Urinary acetoacetate was self-measured in the first morning urine at baseline and weekly until the end of the study (Ketur-Test, Accu-Chek, Roche Diagnostics, Rome, Italy), to monitor dietary adherence. Patient reporting negative urinary tests more than once were to be excluded. IR was determined through HOMA-IR calculation as (35):

$$\mathrm{HOMA-IR} = \frac{\text{fasting serum insulin } (\mathrm{mIU/ml}) \times \text{fasting plasma glucose } (\mathrm{mg/dL})}{405}$$

2.6. Statistical analysis

Data are expressed as mean and standard deviation for normally distributed variables and median and interquartile range for non-normally distributed ones. Group comparisons were performed by unpaired Student's *t*-test and ANOVA test or by Mann–Whitney and Kruskal–Wallis test as appropriate. Proportions and categorical variables were tested by the Chi square test. Data before and after intervention were compared with paired *t*-test or Wilcoxon rank test. A Spearman correlation method was used to analyze the correlation between continuous variables. To test independent predictors of weight loss, a first multivariate analysis was performed including demographic variables (gender and age) as clinically relevant factors and variable significantly correlated to weight loss at univariate analysis (baseline weight, W/H ratio, FGF21), after excluding collinear variables (lean mass, waist circumference). Non-normally distributed variables were log-transformed. Further multivariate analyses were performed including either sex or W/H ratio. Analyses were performed using computer software packages (SPSS-27.0, SPSS Inc., Armonk, NY, USA: IBM Corp.).

The mean ± SD body weight we observed in the population accessing our clinical center was 105 ± 21. Twenty-nine patients were identified as an appropriate sample size to detect a clinically relevant reduction of 10% in body weight with a power of 0.80 and alpha 0.05. Foreseeing up to 20% drop-out rate, 34 patients were then enrolled.

2.7. Ethical approval

The study was carried out in accordance with the code of ethics of the World Medical Association for human studies (Declaration of Helsinki, 2001). All patients signed an informed consent form to voluntarily participate in this study. The research protocol was approved by the Ethical Committee of Sapienza University of Rome (rif. 5475, date of approval 24-10-2019).

3. Results

3.1. Anthropometric and biochemical changes

A total of 34 patients [14 male (41%), 20 female (59%)] were enrolled in this study. The mean age was 54 ± 12 years, the mean BMI was 36.3 ± 4.1 kg/m^2. The baseline characteristics of our population are reported together with variations after VCLKD treatment in **Table 1**. All patients had detectable urinary acetoacetate reflecting ketosis until the end of the diet. No patient dropped out during the study due to extreme hunger or intolerable physical symptoms. No significant adverse event was recorded. The most common minor adverse events recorded were bloating, constipation, headache and self-limiting palpitations. All symptoms were deemed as bearable by all patients and most were controlled by increasing water intake or adjusting the quality or quantity of consumed vegetables. All patients reported an improvement in their sedentary lifestyle, however no one started light or moderate-intensity aerobic physical activity during the VLCKD treatment.

TABLE 1 Participants characteristics at baseline (T0), and after the very low-calorie ketogenic diet (VLCKD) (T45).

	T0 $n = 34$	T45 $n = 34$	p
Age (years)	54 ± 12	54 ± 12	
Gender (% female)	59	59	
Weight (Kg)	102.144 ± 13.1	93.8 ± 13.3	< 0.001
BMI (kg/m^2)	36.3 ± 4.1	33.29 ± 4.0	< 0.001
WC (cm)	108.6 ± 8.9	102.2 ± 8.8	< 0.001
W/H ratio	0.8 ± 0.1	0.9 ± 0.1	< 0.001
FGF21 (ng/ml)	194.0 (137.6–284.6)	167.8 (90.9–281.5)	< 0.001
Glucose (mg/dL)	102.5 (94–108.5)	95.5 (88–106)	0.004
Insulin (μUI/ml)	17 (13.4–22.6)	7 (5.12–12.5)	< 0.001
HOMA IR	4.3 (3.1–5.9)	1.9 (1.1–3.2)	< 0.001
Triglycerides (mg/dL)	133.3 ± 53.9	96.9 ± 45.9	< 0.001
Total cholesterol (mg/dL)	215.74 ± 36.9	177.1 ± 37.1	< 0.001
LDL cholesterol (mg/dL)	137.1 ± 32.8	103.7 ± 36.3	< 0.001
HDL cholesterol (mg/dL)	52.1 ± 13.6	49.4 ± 12.4	0.112
Albumin (g/dL)	44.3 ± 2.6	43.9 ± 2.8	0.350
AST (U/L)	20 (16–23)	19.5 (17–23)	0.903
ALT (U/L)	23.5 (18–35.5)	20.5 (18–30.75)	0.174
Creatinine (mg/dL)	0.8 (0.7–0.9)	0.8 (0.7–0.9)	0.969
CRP (μg/dL)	3200 (1900–8100)	2750 (905–5900)	0.340
Lean Mass (kg)	61.03 ± 9.4	59.3 ± 9.7	0.058
Fat Mass (kg)	38.0 ± 9.1	33.3 ± 8.8	< 0.001
Fat mass (%)	37.1 ± 7.3	34.9 ± 7.6	< 0.001

Variables with normal distribution are expressed as mean ± SD, those with non-normal distribution as median (interquartile range). BMI, body mass index; WC, waist circumference; W/H ratio, waist-to-hip circumference ratio; FGF21, fibroblast growth factor 21; HOMA-IR, homeostasis model assessment-insulin resistance; AST, aspartate transaminase; ALT, alanine transaminase; CRP, C-reactive protein. p is from a mixed-effects analysis.

Body weight (102.1 kg ± 13.1 to 93.8 kg ± 13.3, $p < 0.001$), BMI (36.3 ± 4.1 kg/m^2 to 33.3 kg/m^2 ± 4.0, $p < 0.001$), WC (108.6 cm ± 8.9 to 102.2 cm ± 8.8, $p < 0.001$), and W/H ratio (0.8 ± 0.1 to 0.9 ± 0.1, $p < 0.001$) were significantly improved at the end of the diet (Table 1). Regarding body composition, the VLCKD induced a significant decrease in fat mass (38.0 kg ± 9.1 to 33.3 kg ± 8.8, $p < 0.001$), and fat mass percentage (37.1% ± 7.3 to 34.9% ± 7.6, $p < 0.001$), No difference was observed in absolute lean mass.

Metabolic parameters achieved a significant change, including fasting glucose [102.5 mg/dL (94.0–108.5) to 95.5 mg/dL (88.0–106.0), $p < 0.004$] and insulin [17.0 μUI/ml (13.4–22.6) to 7.0 μUI/ml (5.1–12.5), $p < 0.001$], triglycerides (133.3 mg/dL ± 53.9 to 96.9 mg/dL ± 45.9, $p < 0.001$), total cholesterol (215.7 mg/dL ± 36.9 to 177.1 mg/dL ± 37.1, $p < 0.001$), LDL cholesterol (137.1 mg/dL ± 32.8 to 103.7 mg/dL ± 36.3 $p < 0.001$), and HOMA-IR [4.3 (3.1–5.9) to 1.0 (1.1–3.2), $p < 0.001$]. No differences were observed in AST, ALT, creatinine or in albumin levels. Circulating FGF21 level decreased significantly from baseline to the end of the treatment [194.0 ng/ml (137.6–284.6) to 167.8 ng/ml (90.9–281.5) $p < 0.001$]. CRP was unchanged.

3.2. Predictors of weight loss

In order to assess whether parameters included in the evaluation conducted at baseline could predict the weight loss obtained with a VLCKD, the study population was stratified according to weight loss tertiles, reported in kg, after VLCKD treatment. Baseline metabolic markers, anthropometric parameters and body composition are reported according to weight loss tertiles in Table 2. Patients in the highest weight loss tertile showed higher baseline values of W/H ratio and MetS prevalence; this sub-group of patients also showed lower baseline circulating FGF21 levels. No significant difference among groups was observed regarding baseline BMI, BW, WC, albumin, glucose, insulin, HOMA-IR, AST, ALT, CRP, lean mass, and fat mass.

To further investigate whether weight loss was associated to any collected baseline parameter, so to identify possible predictors of weight loss following a VLCKD, we performed a univariate analysis. Weight loss positively correlated with baseline body weight (rS = 0.399; $p = 0.020$), waist circumference (rS = 0.359; $p = 0.037$), W/H ratio (rS = 0.406; $p = 0.017$) and was negatively correlated with baseline circulating FGF21 levels (rS = –0.381; $p = 0.026$). Regarding body composition, weight loss positively correlated with baseline lean mass (rS = 0.473; $p = 0.005$) (Figure 1) but not with baseline fat mass (rS = –0.132; $p = 0.456$) or HOMA-IR (rS = 0.129; 0.466).

We therefore proceeded to test independent predictors of weight loss. To do so, we included in a first multivariate analysis demographic variables (gender and age) as clinically relevant factors and variables which were found to be significantly correlated to weight loss at univariate analysis (baseline weight, W/H ratio, FGF21). After correction for age, sex, W/H ratio and baseline weight, weight loss correlated with FGF21 (Beta = –0.410; $p = 0.012$) (Table 3, panel A). In further analyses, including alternatively sex (Table 3, panel B), or W/H ratio (Table 3, panel C) we observed that, in addition to FGF21, male sex (Beta = 0.472; $p = 0.001$) and W/H ratio (Beta = 0.481; $p = 0.005$) correlated with weight loss as well. No difference in FGF21 level was found between male and female.

4. Discussion

We report a significant reduction of fat mass and weight with improvement of metabolic parameters in patients with obesity following a VLCKD for 45 days, with no dropouts or significant adverse events. These results are in line with previous studies reporting that the VLCKD is both safe and effective in subjects with obesity, since it promotes satiety, rapid weight loss preserving lean mass and metabolic improvement (7, 36, 37). The extremely high adherence was likely due to the fact that all patients could contact their dietician directly by phone or sms whenever needed. Remote monitoring is a well-established means of achieving good compliance, especially in nutritional studies (38).

A higher W/H ratio predicted greater weight loss in this population, in line with previous studies (39). Central obesity, reflecting increased visceral fat, is typically unhealthy, and it comes with increased cardiovascular disease risk and metabolic derangements. It was previously reported that visceral fat depots retain higher lipolytic activity compared to peripheral depots (40), and that diet-induced weight loss is associated with a greater visceral fat loss compared to peripheral fat loss (41). This may explain the finding that those with a prevalence of fat more likely to be decreased

TABLE 2 Baseline clinical, biochemical characteristics and body composition of patients according to weight loss, stratified into tertiles.

	I tertile (n = 11)	II tertile (n = 12)	III tertile (n = 11)	P among groups	P I vs. III tertile
Age (years)	52.7 ± 5.7	55.6 ± 7.9	55.6 ± 10.1	0.630	1.00
Female sex	72.7%	50.0%	54.5%	0.510	
Weight (kg)	101.9 ± 7.1	105.1 ± 14.6	99.2 ± 16.3	0.572	1.00
BMI (kg/m^2)	36.7 ± 4.6	37.1 ± 4.3	35.1 ± 3.7	0.507	1.00
WC (cm)	106.3 ± 9.3	111.2 ± 9.9	108.2 ± 7.5	0.419	1.00
W/h ratio	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.092	0.094
MetS	63.6%	75.0%	100.0%	0.097	0.027
WC	100.0%	100.0%	100.0%	–	–
Hypertension	72.7%	100.0%	100.0%	0.032	0.062
Hyperglycaemia	54.5%	58.3%	81.8%	0.346	0.170
HyperTG	27.3%	25.0%	45.5%	0.525	0.375
Low HDL-C	27.3%	41.7%	81.8%	0.029	0.010
FGF21 (ng/ml)	246.5 (140.6–315.3)	214.5 (180.64–367.7)	148.5 (70.7–196.2)	0.038	0.045
Glucose (mg/dL)	101.0 (88.0–108.0)	99 (94.75–109.5)	103 (94–117)	0.794	0.532
Insulin (μUI/ml)	17.4 (12.3–26.3)	17.8 (14.7–19.8)	15.1 (13.2–27.6)	0.784	0.870
HOMA-IR	5.4 (2.6–6.8)	4.4 (3.8–5.5)	3.8 (2.9–6.9)	0.963	0.974
Albumin (g/dL)	43.8 ± 2.6	43.9 ± 2.7	45.3 ± 2.4	0.324	0.586
AST (U/L)	22 (14–25)	19 (15.2–23.7)	20.0 (17–22)	0.460	0.154
ALT (U/L)	27 (21–42)	21.5 (17.5–36.5)	22 (18–31)	0.456	0.307
CRP (mg/dL)	4.4 (2.2–11)	3.3 (2.1–7.7)	2.5 (0.7–3.7)	0.154	0.071
Lean Mass (kg)	59.7 ± 8.5	60.3 ± 7.2	63.3 ± 12.3	0.637	1.000
Fat Mass (kg)	40.1 ± 8.0	40.1 ± 8.5	33.7 ± 10.1	0.165	0.306

BMI, body mass index; WC, waist circumference; W/h ratio, waist circumference/Hip circumference ratio; Mets, metabolic syndrome; HDL-C, HDL cholesterol; FGF21, fibroblast growth factor 21; HOMA-IR, homeostasis model assessment-insulin resistance; AST, aspartate transaminase; ALT, alanine transaminase; CRP, C-reactive protein.

upon dieting will obtain more profound weight loss. Moreover, the gut microbiome of the metabolically unhealthy individual has a specific signature, and it was previously reported that this represents a significant predictor of weight loss upon dieting, with specific gut bacteria likely synergizing with or counteracting macronutrients to influence weight loss (42). We previously reported that a VLCKD modulates the microbiome toward a healthier phenotype (43). We therefore suggest that under the role of W/H ratio as predictor of weight loss may also lie a fine interaction between dietary factors and the gut microbiome in determining the weight loss trajectory, but further studies are needed to confirm this.

We found that male gender predicted greater weight loss. As men have a larger amount of central fat, we cannot exclude that the finding may be pulled by this aspect, with the previously mentioned implications. In addition, men also have higher lean mass. Organs and tissues that constitute lean mass have high metabolic activity, accounting for ∼70% of the variance in resting energy expenditure (44), making it the most important determinant of energy expenditure in sedentary subjects with obesity. As the caloric intake prescribed was similar across patients, it accounted for a more profound energy deficit in those with higher energy expenditure (i.e., men), likely leading to larger weight loss. Confirming this, greater weight loss was observed in patients with higher lean mass at baseline. The finding was therefore expected and in line with previous studies (45). Noteworthy, studies investigating predictors of weight loss following bariatric surgery or pharmacologic treatment showed contrasting data, with some identifying male sex as a negative predictor of weight loss (46, 47), and others suggesting that males lose more weight (48). This is likely due to the heterogeneity in the cohorts and treatments evaluated, and no definitive conclusion may be drawn in this regard.

We report decreased circulating FGF21 after a VLCKD intervention, in line with previous studies, and that lower baseline FGF21 predicts greater weight loss even after adjustment for age, sex and baseline body weight. In humans, circulating FGF21 levels are regulated by different conditions, from the fasting to the refed state (49, 50), high carbohydrate and fructose consumption (51, 52), or dietary protein restriction (53). However, the mechanism underlying these effects is not entirely clear (54). Furthermore, higher FGF21 level was detected in different metabolic disorders, including type 2 diabetes (55), obesity and liver steatosis (56, 57), pancreatitis (58), and primary mitochondrial dysfunction (59). In a previous

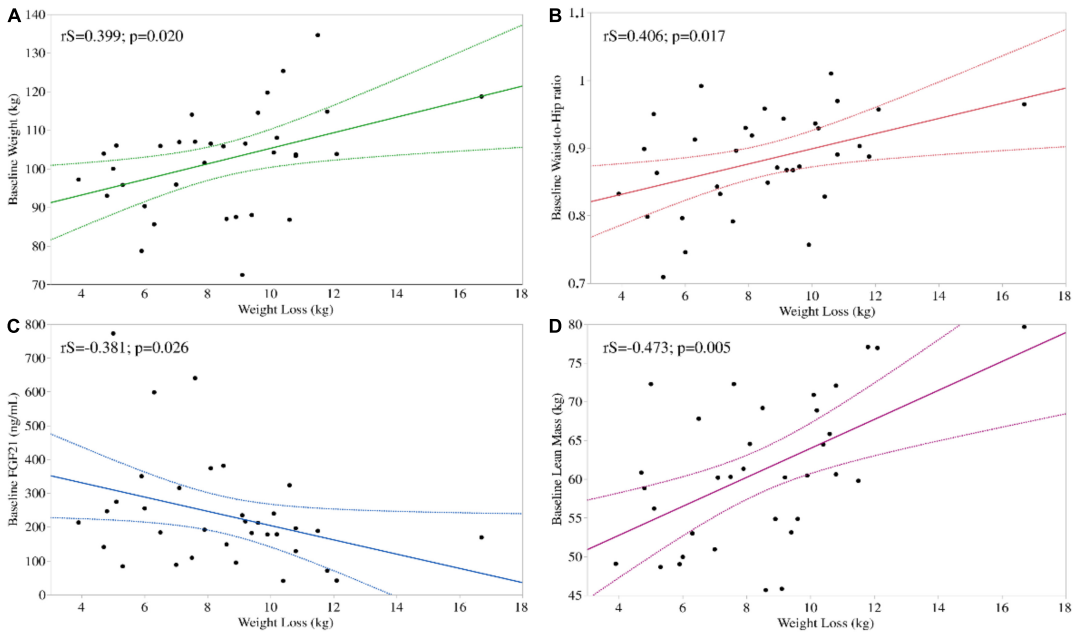

FIGURE 1
Significant univariate associations between baseline parameters and weight loss following a VLCKD. Weight loss correlated positively with baseline body weight **(A)** and W/H ratio **(B)** and negatively with baseline circulating FGF21 **(C)**. Regarding body composition, weight loss positively correlated with baseline lean mass **(D)**.

study, Crujeiras et al. proved a significant reduction in FGF21 in patients who lost weight using different diet types, including VLCKD. Conversely, they found increased levels in patients who underwent bariatric surgery, suggesting that FGF21 could represent a nutritional stress marker (60). The reason why patients losing more weight presented lower baseline FGF21 is unclear, but an explanation may be hypothesized. It has been reported by Fisher et al. for the first time, that obesity may be an FGF21-resistant state (61). Similar to the mechanism of insulin resistance (62), patients with lower FGF21 levels, possibly reflecting lower FGF21 resistance, could lose weight more easily as a consequence of a healthier and more flexible metabolism. However, in our study the extent of weight loss inversely correlates with FGF21 but not with HOMA-IR. HOMA-IR is a widely accepted marker of insulin resistance and it is influenced by the metabolic status of adipocytes (63), liver (64), and muscle cells (65). Differently, FGF21 mainly reflects liver metabolism (21, 22). Based on these differences, we could speculate that the residual liver function could play a key role in the extent of the weight loss following VLCKD, more than the levels of adipokines secretion or the metabolic status of muscle cells. However, further studies comparing the response to VLCKD of patients with or without hepatic disorders or with different stage of liver disease are needed to test our hypothesis.

TABLE 3 Multiple regression analysis to assess baseline predictors of body weight loss.

Panel A	B	S.E.	Beta	*p*
Age	−4.43E-5	0.003	−0.002	0.987
Male sex	0.066	0.064	0.230	0.316
Baseline W/H ratio	0.675	0.414	0.340	0.115
Baseline body Weight (kg)	0.002	0.002	0.171	0.336
Baseline FGF21[#]	−0.199	0.075	−0.410	0.012
Panel B	**B**	**S.E.**	**Beta**	***p***
Age	0.001	0.003	0.060	0.700
Male sex	0.135	0.050	0.472	0.011
Baseline body Weight (kg)	0.001	0.002	0.099	0.573
Baseline FGF21[#]	−0.198	0.077	−0.407	0.015
Panel C	**B**	**S.E.**	**Beta**	***p***
Age (years)	0.000	0.003	0.007	0.962
Baseline W/H ratio	0.954	0.311	0.481	0.005
Baseline body Weight (kg)	0.003	0.002	0.266	0.336
Baseline FGF21[#]	−0.183	0.073	−0.376	0.018

Independent variables evaluated: age, male sex, baseline body weight, baseline FGF21, baseline waist-to-hip circumference ratio (W/H ratio) (panel A). Panel (B) reports the model after the removal of W/H ratio. Panel (C) reports the model after the removal of the sex variable. SE, standard error. [#]Log transformed variables.

In addition, ketogenic diets exert immunomodulatory actions (2, 66, 67). Chronic low-grade inflammation is the culprit of metabolically unhealthy obesity (68), and FGF21 is secreted by the liver upon inflammatory *stimuli* (69, 70). The changes observed upon VLCKD treatment in terms of FGF21, as well as the possibly weight loss predictive role of baseline FGF21, may not currently have clinical implications, but they suggest that FGF21 could represent a link in the chain connecting the metabolic and immune system, although further mechanistic studies are needed to elucidate this.

Our study has some limitations. First, the absence of a control group following an isocaloric dietary intervention with different macronutrient composition did not allow to investigate the effect of the macronutrient ratio/nutritional ketosis as opposed to calorie restriction derived weight loss. The results observed are therefore the consequence of the dietary treatment as a whole, without speculations possibly being made on whether it was the very low-calorie content

or the nutritional ketosis achieved to impact the weight or other metabolic parameters. Although a control group would have allowed to assess whether different diets have the same predictors of weight loss, this was beyond the scope of the present study, and we aim at further pursuing this important aspect. Second, capillary BHB was not measured to confirm ketosis, although less reliable urinary acetoacetate confirmed ketonuria throughout the study in all subjects. Third, the sample size enrolled in the study was relatively small. However, *a priori* sample size was calculated allowing for sufficient power. Fourth, the duration was short, and no long term follow up was conducted. No direct evaluation of energy expenditure was adopted, neither was a validated questionnaire to monitor physical activity administered. It was therefore not possible to accurately calculate total daily energy expenditure, and the prescribed energy deficit was hence different across patients, possibly accounting for some bias. Finally, no basal metabolic rate or respiratory quotient were measured in our patients, not allowing the evaluation of the impact of energy delta between diet calorie consumption and expenditure upon weight loss.

5. Conclusion

In conclusion, men with central obesity and lower circulating FGF21 may benefit more than others in terms of weight loss obtained following this diet. Further controlled, larger and longer studies investigating whether this is specific to the VCLKD or to any caloric restriction need to be conducted before any definitive conclusion may be drawn.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethical Committee of Sapienza University of Rome (rif. 5475, date of approval 24-10-2019). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SB and FB: conceptualization. SB, GS, and LG: methodology. RR, MW, AP, IE, and DT: software. MW, SB, LG, AG, and CL: validation. IE, FB, and MW: formal analysis. SB, AP, SM, and EC: investigation. AP and RR: data curation. MW, IE, and DT: writing—original draft preparation. MW, LG, SB, SM, and CL: writing—review and editing. LG: supervision. SB: project administration. SB, GS, LG, and MW: funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

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

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Effects of the ketogenic diet on bone health: A systematic review

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Objective: To carry out a systematic review of published studies to evaluate the relationship between different type of ketogenic diet (KD) and bone health as supported by the scientific literature.

Methods: The study involved all articles that assessed the relationship between the use of KD for the treatment of overweight or obesity and bone health. The quality assessment was evaluated with using the Cambridge Quality Checklists. The search strategy included the following combination of Medical Subjects Headings terms and keywords: "osteoporosis", "bone health, "bone function", "bone mineral density", and "ketogenic diet".

Results: Seven trials were identified and reviewed. No significant changes in bone mass density (BMD) were observed after KD. The results showed no significant effect on bone resorption by measuring urinary N-telopeptide levels, on bone formation by measuring bone-specific alkaline phosphatase, or alterations in overall bone turnover in patients who followed KD. Only in female subject after a 10% weight loss, bone resorption increases while new bone synthesis decreases, but without increasing the risk of osteoporosis. Finally, patients on KD lost significantly more weight than controls, associated with an increase in serum vitamin D levels and a reduction in plasma parathyroid hormone (PTH) levels.

Conclusion: No human studies have currently been conducted with adequate and powerful experimental designs to definitively understand the impact of KD therapy on bone health.

KEYWORDS

ketogenic diet, low-calorie ketogenic diet, very-low-calorie ketogenic diet, bone health, osteoporosis, bone mineral density

1 Introduction

Low-calorie ketogenic diet (LCKD) and very-low-calorie ketogenic diet (VLCKD) are diets low in carbohydrates and high in lipids, which have been shown to be effective in losing weight quickly and safely, as well as being able to improve body composition (1), athletic performance (2), and markers of cardiovascular and metabolic health (3). Over the last few years, KD has been widely accepted as an efficient method for the treatment of obesity and body weight management. The ketogenic diet is usually characterized by providing less than 20% of daily caloric intake as

carbohydrates, more than 50% from lipids, and a moderate but variable amount of proteins (4). This type of distribution of macronutrients preserves glycogen and lean tissue protein utilization, increases fatty acid oxidation, and generates marked elevation of plasma ketone bodies (KB), such as acetate, acetone, and β-hydroxybutyrate (3βOH-B), known to be an effective alternative fuel source for tissues (5). Furthermore, β-hydroxybutyrate has been shown to have anti-inflammatory and anti-catabolic effects on skeletal muscle by inhibiting activation of the Nf-κB pathway (6). Several studies have shown that KD is effective in reducing body weight and fat mass, without inducing loss of muscle mass and fat-free mass (FFM), thus preventing the risk of sarcopenia (7, 8) and promoting the preservation of muscle strength (9). However, the preservation of muscle mass, known to be involved in glucose metabolism, in KD patients remains debatable.

Although the higher reduction in body fat and the cardiometabolic benefits, the use of low-carbohydrate diets has been associated with various adverse outcomes. KD causes alters vitamin D levels, lowers growth factors and a high "acid load" *via* the ketone bodies, these contribute to an increased risk for bone mineral density (BMD) loss (10). KD has previously been studied for its impact on bone mineral content (BMC), osteopenia, and osteoporosis, as well as common consequences related to this dietary treatment, such as hypercalciuria, urine acidification, and hypocitraturia (11). Evidence shows that this occurs due to the renal response, which consists of increased excretion of acid to compensate for the dietary acid overload. In turn, the skeleton acts as a buffer system and through its active resorption causes hypercalciuria and a negative effect on bone quality (12). Given the role of crosstalk between adipose tissue and bone, it is important to evaluate the effects of KD on bone metabolism and the possible mechanisms underlying the onset of osteopenia and osteoporosis.

Based on these premises, this study aimed to carry out a systematic review of published studies to evaluate the relationship between low-calorie and very-low-calorie ketogenic diet and bone health as supported by the scientific literature.

2 Methods

2.1 Search strategy

A systematic search was performed from January 2022 to November 2022, through Pubmed and Scopus databases from the earliest available date to November 2022, using Medical Subjects Headings (MeSH) indexes and keyword searches. The string used included the entry term "ketogenic diet*", which was searched in combination (AND) with the terms "osteoporosis" OR "bone health" OR "bone function" OR "bone mineral density" OR "BMD". Alternative entry term was "VLCKD", "LCKD", and "KD". The query "LIMIT-TO (DOCTYPE, "ar"))" was used to limit retrieve only original studies. The same combination of terms was used for all the databases. The search strategy was performed in compliance with the Meta-Analysis and Systematic Reviews of Observational Studies (MOOSE) guidelines (13) (Supplementary Table 1) and the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) (14) (Supplementary Table 2). Abstracts of the retrieved articles were independently screened by two researchers in duplicate (V.G. and F.B). Disagreements were resolved by a third person (R.A.C.).

2.2 Selection criteria

This systematic review included all published articles that evaluated the relationship between the use of KD for the treatment of overweight or obesity and bone health. All eligible studies were selected following the PICOS (Population, Intervention, Comparison/Comparator, Outcomes, Study design) (15) model (Table 1). Ketogenic diet consists of an extreme reduction in carbohydrate intake (<50 g/day) with a consequent increase in protein and fat intake. The aim of the ketogenic diet is to decrease appetite and increase lipolysis, resulting in increased use of fats as an energy source. There are different types of carbohydrate-restricted diets with varying protein and fat intake (16). Studies conducted on children treated with anti-epileptic drugs and athletes were excluded, given the impact on bone metabolism of epilepsy therapies and physical activity. Only articles in English reporting complete data of clinical relevance for the present review were included in the analysis. Duplicates have carefully been checked and removed.

2.3 Quality assessment

The quality of evidence (QoE) of the studies was evaluated by 3 investigators (VG, FB and RC), by using the Cambridge Quality Checklists (17).

TABLE 1 Inclusion and exclusion criteria of the current systematic review, according to the PICOS model (15).

	Inclusion	Exclusion
Population	Patients with obesity or overweight	Children, patients with hypogonadism, Cushing syndrome, hyperparathyroidism, renal failure, and other comorbilities capable of impacting on the bone density
Intervention	Ketogenic diet	Non ketogenic diets
Comparison	Non ketogenic diets (e.g. Mediterranean diet, fat diet) or no treatment	–
Outcome	BMD, UNTx, BSAP, PINP, β-Crosslaps, BMC	–
Study Type	Randomized controlled studies, case-control studies, cohort studies	*In vitro*, animal studies, case reports, editorials, communications, reviews, meta-analysis

BMC, bone mineral content; BMD, bone mineral density; BSAP, bone-specifc alkaline phosphatase; PICOS, Population, Intervention, Comparison/Comparator, Outcomes, Study design; PINP, procollagen type I N-propeptide; UNTx, urinary N-telopeptide.

3 Results

The aforementioned search strategy identified a total of 95 records. After the exclusion of 36 duplicates, the remaining 59 articles were considered potentially relevant for this review. After reading the abstracts, twenty-two articles were excluded because they were concerned about the correlation between epilepsy and ketogenic diet in children and adults, and 5 studies were conducted in animals. the remaining 23 articles were excluded as not pertinent. The full-text of the remaining 9 articles were downloaded and read carefully: two of these were excluded because one study evaluated the effects of a low-carbohydrate ketogenic diet in endurance-trained women and another focused on administering 3βOH-B. In conclusion, 7 studies were considered for this systematic review (Figure 1).

3.1 Quality of evidence of included studies

The 7 included studies were evaluated using the Cambridge quality checklist. Although this scale does not establish a precise threshold to differentiate between high- and low-quality studies, out of a total score of 15, five studies scored > 10, while only two studies scored 6 to 10 (Table 2).

3.2 Specific results

The main characteristics of the included studies are reported in Table 3. All of them evaluated the effects of KD on obese or overweight subjects. Among the included studies, all studies evaluated different parameters regarding body composition. Five studies (19–22, 24) used a dual-energy X-ray absorptiometry (DEXA) to assess bone mineral density (BMD) and bone mineral content (BMC). Two studies (18, 21) used β-Crosslaps, a collagen-degradation product that represents a biochemical marker of bone turnover. Only one study used different bone markers such as urinary N-telopeptide (UNTx) and bone-specific alkaline phosphatase (BSAP) to evaluate bone turnover in patients doing VLCKD, but only mean changes are reported (23). Procollagen type I N-propeptide (PINP) used to assess bone synthesis (18).

All the seven studies showed a significant reduction in body mass index (BMI) after KD. No significant change in BMD and BMC was observed after KD (Table 3). Only one study observed a minimal decrease in total body BMD, but this decrease was not significantly different from the control (21), while patients who did not receive a calcium supplement during the diet had a BMC reduction (24). Two studies reported an increase in serum vitamin D levels (20, 24) and a decrease in HOMA index after KD. Regarding bone markers, no effect was reported neither on bone resorption [the mean UNTx decreased by 2.2 n/m (95% CI ± 27.2)], nor on bone formation [the mean BSAP decreased by 0.53 ug/l (95% CI ±2.96)]. Also, no alteration in overall bone turnover [the bone turnover ratio increased by 0.08 (95% CI ±0.81)] in patients who followed KD was described (23). In female subjects after a 10% weight loss PINP, the marker of bone synthesis, decreased remarkably (Δchange% 17.6%, p=0.000) and β-Crosslaps, the marker of bone absorption, increased remarkably (Δchange% −9.8%, p=0.035), but there was no increased risk of osteoporosis (18). Increase of β-Crosslaps has also been reported by Brinkworth et al. In two studies (18, 24) has been observed a significant decrease in serum parathyroid hormone (PTH).

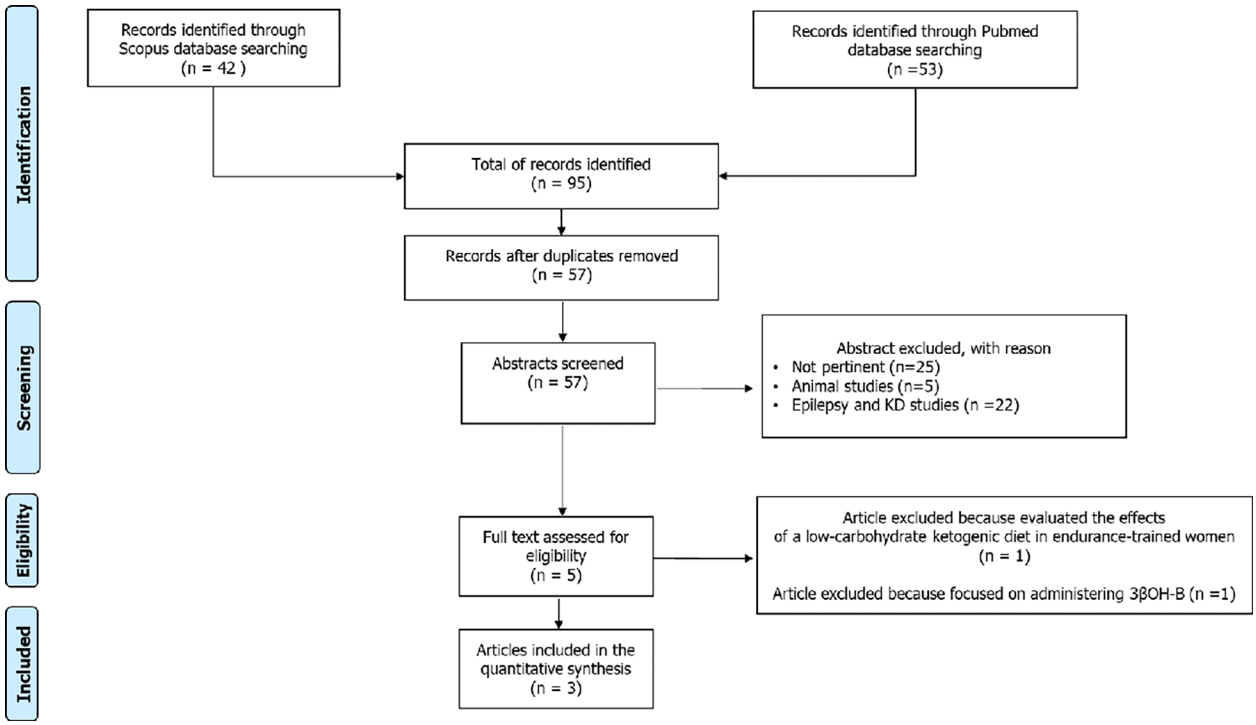

FIGURE 1
Study flowchart.

TABLE 2 Quality of evidence assessment of the included studies [results of the Cambridge Quality Checklist (17)].

Study name	Type of study	Cambridge Quality Checklists		
		Checklist for correlates	Checklist for risk factors	Checklist for causal risk factors
Yu et al., 2022 (18)	CCT	2	3	3
Perissiou et al., 2020 (19)	RCT	3	3	7
Colica et al., 2017 (20)	RCT	4	3	7
Brinkworth et al., 2016 (21)	RCT	3	3	7
Foster et al., 2010 (22)	RCT	2	3	7
Carter et al., 2006 (23)	CCT	2	3	4
Jensen et al., 2001 (24)	RCT	3	3	7

CCT, Controlled Clinical Trial; RCT, randomized controlled trial.

4 Discussion

In the last two decades, the use of KD therapy has spread widely. The prevalence of some diseases, such as type 2 diabetes mellitus (T2DM), hypertension, dyslipidemia, sleep apnea, fatty liver disease, osteoarthritis, stress incontinence, gastroesophageal reflux, and polycystic ovary syndrome, could be reduced by weight loss (25). Specific metabolic disorders, in which KD is indicated in the clinical management of overweight/obese patients, have been listed by several guidelines, including that of the Italian Society of Endocrinology (26). Despite various hypotheses about the correlation between ketogenic diet (KD) and bone health in children, to date it remains unclear whether KD has any effect on bone health in adults. The considerations available are derived from studies conducted primarily in adults undergoing KD, similar to what was reported in children, although many of these studies do not provide relevant data on bone involvement. Clinical studies on the possible effects of KD on human bone health are poor and there are not many data on the long-term risk of osteoporosis in patients undergoing KD (26).

Although few studies on KD and skeletal metabolism are available, chronic metabolic acidosis is known to increase calcium excretion in the urine without increasing intestinal calcium absorption, leading to bone calcium loss by acute physicochemical dissolution and chronic increased bone resorption (27, 28). In an *in vitro* model of osteoblasts (OBL) cultures, the presence of certain types of ketone bodies affects different activities of alkaline phosphatase and mineralization. In particular, the mineralization activity of OBL appears to be upregulated by acetoacetate and downregulated by 3βOH-B (29). Therefore, all types of KDs that lead to metabolic acidosis could damage BMC. However, no studies

TABLE 3 Main characteristics of the studies included in the analysis.

	Type of study	Age (y)	Population (n)		BMI (Kg/mq)		Type of intervention	BMD (g/cm^2)			
			Diet	Controls	Diet	Controls		Baseline		KD	
								Cases	Controls	Cases	Controls
Yu et al. 2022 (18)	CCT	35	75	NR	30.5	NR	LCKD	NR	NR	NR	NR
Perissiou et al. 2020 (19)	RCT	35 ± 9	31	33	31.2 ± 3	30.8 ± 4	VLCKD and prescribed exercise for 8 weeks	1.10 ± 0.05	1.12 ± 0.07	NR	NR
Colica et al 2017 (20)	RCT	45.4 ± 14.2	VLCKD1 = 20 VLCKD2 = 20	NR	30.45 ± 2.64	NR	Two types of VLCKD (with synthetic amino acids and with placebo) for 3 weeks	1.17 ± 0.19	1.19 ± 0.19	1.19 ± 0.18	1.19 ± 0.22
Brinkworth et al. 2016 (21)	RCT	51.6 ±6.5	32	33	33.7 ±4	33.2±4	LC diet for 52 weeks	1.26 ±0.10	1.26±0.09	1.22 ±0.09	1.23±0.08
Foster et al. 2010 (22)	RCT	46.2	153	154	36.1	36.1	LC diet for 104 weeks	1.1	1.1	0.00	0.00
Carter et al. 2006 (23)	CCT	40.8	13	13	33.51 ± 11.76	29.03 ± 3.88	LC diet for 12 weeks	NR	NR	NR	NR
Jensen et al. 2001 (24)	RCT	NR	62	NR	34	NR	LCKD with or without calcium supplement	NR	NR	NR	NR

BMD, bone mineral density; BMI, body mass index; CCT, controlled clinical trial; LC, very low carbohydrate diet; NR, not reported; RCT, randomised controlled trial; VLCKD, very low carbohydrate ketogenic diet.

have explored the effects of KD on bone health, and its does not lead to metabolic acidosis. Additionally, studies investigating the metabolic consequences of KD on calcium loss and bone health have not been conducted for longer than 3-4 months. Therefore, in patients using KD for prolonged periods or repeatedly in a cyclic manner for short periods, data on the impact of increased calcium loss on bone health are scarce.

Carter and colleagues published a study that evaluated whether a low-carbohydrate diet would lead to increased bone turnover in humans by measuring bone turnover markers. Thirty patients (15 undergoing a low-carbohydrate diet and 15 controls with no dietary restriction) were recruited for 3 months. The results showed no significant effect on bone resorption by measuring urinary N-telopeptide (UNTx) levels, on bone formation by measuring bone-specific alkaline phosphatase (BSAP), or alterations in overall bone turnover (BSAP/UNTx ratio) in patients who followed the low-carbohydrate diet for 1 and 3 months. No increase in bone turnover markers compared with controls was found in patients on the low-carbohydrate diet even though these patients lost significantly more weight than controls (23).

Other two markers of bone turnover, PINP and β-Crosslaps, show respectively a reduction and an increase in female subjects after loss of 10% body weight. The decrease in bone formation and increase in resorption during the initial phase of weight loss could be due to the rapid weight loss and energy restriction induced by KD, causing a mechanical unloading on the bone and consequently an increase in bone turnover, but without increasing the risk of developing osteoporosis (18).

A placebo randomized-controlled trial study in forty-two patients of both sexes analyzed the possible effects of two arms with different dietary treatments for three weeks each with a three-week washout interval. The two VLCKD treatments (<800 kcal/day) differed in terms of protein content and quality, specifically, in the first VLCKD1 arm, 50% of protein intake was replaced by synthetic amino acids, while in the VLCKD2 arm, a placebo was used. Before and after each dietary treatment, all patients were evaluated for various health parameters (health and nutritional status, anthropometric analysis, DXA-assessed body composition, bioimpedance metering, biochemical evaluation, and PPARγ expression by transcriptomic analysis), also including DXA-BMD and -BMC. After 21-days VLCKD, no negative changes were observed in global measurements of nutritional state including sarcopenia, BMC, BMD, liver, kidney, and lipid profile. In contrast, the left femur BMC was significantly increased after VLCKD1 (20). However, the DXA bone scans, at enrolment and the end of each dietary treatment, were not performed appropriately regarding the time interval (three weeks) between the two scans to best interpret the observed changes. In addition, considering the heterogeneity by age (ranging from 18 and 65 years) of the population analyzed, the BMD of the lumbar spine (LS) should have been reported as a Z-score (i.e., BMD normalized for BMD of persons of the same sex and age in the same population) and not as a T-score (i.e., BMD normalized for BMD of healthy young adults in the same population), given the presence in the study of men younger than 50 years and premenopausal women (30).

Combination of VLCKD and physical training with aerobic and resistance exercises result in significant improvement in the cardiometabolic profile of obese subjects (19). Combined exercise training may have also attenuated muscle mass loss, commonly observed with VLCKD. In addition, no changes were observed regarding BMD.

Obese patients have higher concentrations of the parathyroid hormone and lower blood concentrations of 25(OH)-Vit D than non-obese people, despite a higher habitual intake of vitamin D. Body mass index (BMI), fat mass, and waist circumference seems to be inversely correlated with levels of serum 25(OH)-Vit D, probably due to the large amount of adipose tissue, which can sequester this micronutrient, reducing its bioavailability. Buscemi and colleagues found that serum levels of 25(OH)-Vit D were inversely correlated with measures of general adiposity as BMI and fat mass size, suggesting that adipose tissue is an important influencing factor. Indeed, following VLCKD and subsequent weight/fat loss, a significant increase in 25(OH)-Vit D concentrations has been observed, this is in agreement with the hypothesis that in people with obesity, low 25(OH)-Vit D concentrations are due to its uptake and storage by adipose tissue, with subsequent release following fat mass reduction. In particular, the change in fat mass was correlated solely with the change in 25(OH)-Vit D blood concentrations, indicating the prominent role of this parameter as a possible depot (31).

Calcium supplementation during KD reduces BMC and urinary calcium loss, resulting in reduced PTH levels and thus reduced bone loss (24).

Foster et al. and Brinkworth et al. compared the effects of a low-carbohydrate diet and a low-fat diet on bone health (21, 22). A small reduction of BMD was observed in both diets, without a corresponding change in BMC. One possible explanation could be an artefact due to the instrument's lack of sensitivity when weight and body composition vary and not a physiological change in BMD (32). An alternative explanation could be the longer duration of these studies compared to the others, which could lead to a greater loss of bone mass.

Studies on the effects of KD on the skeleton in adults are limited and are mainly conducted on narrow, specific, and particular populations, such as children with drug-resistant epilepsy. Several studies have explored the effect of KDs on skeletal development in children with epilepsy being treated with VLCKD. Bergqvist and colleagues demonstrated a reduction in BMC in children with epilepsy treated with KD, with follow-up after 15 months. The study protocol included questionnaires on daily calcium intake and assessment of BMC by DXA performed at a time interval appropriate enough for proper interpretation of the results (10). Combined treatment with anticonvulsant drugs and KD produces a greater degree of alteration in bone mineral metabolism than treatment with anticonvulsant drugs alone (33). AEDs appear to have a specific effect on the developing skeleton evidenced by the fact that epileptic adults treated with antiepileptic drugs (AEDs) since childhood have lower bone mass than epileptic adults who started AED therapy in adulthood (34).

Different studies in mice under KD treatment described low BMD and abnormal cancellous and cortical bone mass. Wu and colleagues showed that in mice the microarchitecture of the trabecular bone of the femur is impaired by KD to a level similar to that of ovariectomy

(OVX). Measuring and comparing levels of tartrate-resistant acid phosphatase, to measure activities of osteoclasts, collagen type I, an early-stage marker of osteoblasts activity, and osteocalcin, a late-stage marker of osteoblasts activity, in the four groups, they found that the results found indicate that KD has a negative effect on trabecular and cortical bone quality in mice in a manner similar to OVX, in that both conditions result in a promotion of bone uptake through activation of osteoclasts rather than an inhibition of osteoblast-mediated bone formation (35). Another study demonstrated a significant decrease in the total BMD of rats fed KD for 12 weeks, with no difference in the serum calcium and phosphate concentration between the KD and control groups. Specifically, using micro-CT, it was observed that KD led to bone loss in cancellous and cortical bones (humerus and tibia), with insignificant changes in L4 vertebral bone. In addition, the stiffness and compressive strength of appendicular and axial bones decreased with KD and were highly correlated with the microstructural parameters of cancellous and cortical bones, as demonstrated by simulated compression analysis using micro-FE analysis (36).

An interesting study in mouse models evaluated the effects of administration of metformin, an oral antidiabetic drug, on KD + OVX-induced bone loss. The authors suggested that KD-induced cancellous bone loss is effectively attenuated using metformin while maintaining the biomechanical properties of long bones. However, further studies are needed to confirm the use of metformin as a potential treatment to prevent KD-induced osteoporosis in younger skeletons (37).

In conclusion, there are currently no human clinical studies with powerful and adequate experimental designs to definitively understand the impact of KD therapy on bone health. The few articles included in this systematic review showed no significant changes in bone metabolism in patients treated with KD. In children with intractable epilepsy, the combination of KD and AED could explain the reduction in BMD and bone mass. Animal studies show low BMD and abnormal cortical and cancellous bone mass, but these results have not been reported in human studies. Due to the lack of clinical studies on the impact of KD on bone health conducted in adult men and its long-term effects, it is not possible to determine whether KD can result in osteopenia and osteoporosis.

Data availability statement

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

Author contributions

Abstracts of the retrieved articles were independently screened by two researchers in duplicate: VG and FB. Disagreements were resolved by a third person: RC. The manuscript was written by VG, AC and SL. Materials and methods and results were developed by VG, FB and RC. Supervision of the manuscript and research work was done by RC. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

Publisher's note

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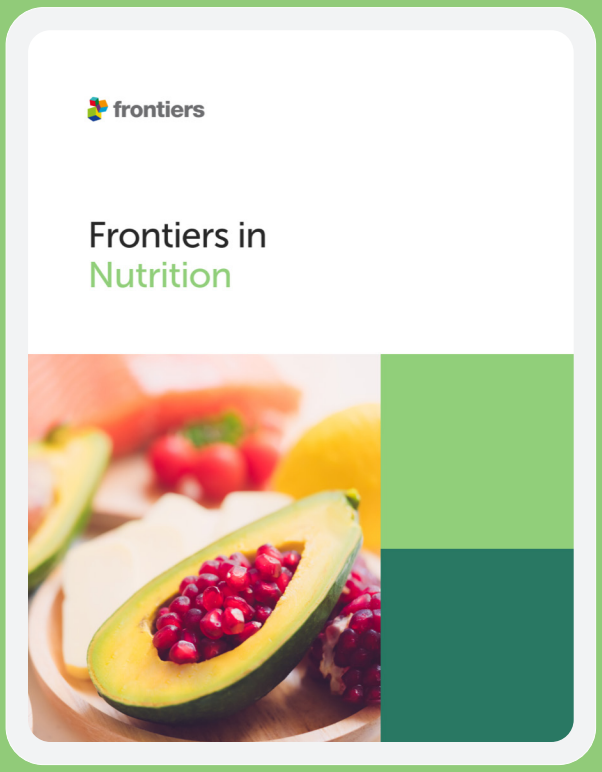

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