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Assessment methods in human nutrition and metabolism for the monitoring of non-communicable chronic diseases

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Assessment methods in human nutrition and metabolism for the monitoring of non-communicable chronic diseases

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Associations of α-linolenic acid dietary intake with very short sleep duration in adults

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Objectives: This study aimed to investigate the association of α-linolenic acid (ALA; 18:3 ω-3) dietary intake with very short sleep duration (<5 h) in adults based on the CDC's National Health and Nutrition Examination Survey data.

Methods: Multinomial logistic regression was used to explore the association of ALA intake with very short sleep. To make the estimation more robust, bootstrap methods of 1,000 replications were performed. Rolling window method was used to investigate the trend of the odds ratios of very short sleep with age. A Kruskal–Wallis test was applied to estimate the differences in the ORs of very short sleep between genders and different age groups.

Results: Compared with the first tertile, the ORs of very short sleep and the corresponding 95% CIs for the second and the third tertile of dietary ALA intake in males were 0.618 (0.612, 0.624) and 0.544 (0.538, 0.551), respectively, and in females were 0.575 (0.612, 0.624) and 0.432 (0.427, 0.437). In most cases, the differences between different ages were more significant than those between different sexes. Men's very short sleep odds ratios for the second tertile of ALA intake increased linearly with age before 60.

Conclusions: The risk of a very short sleep duration was negatively related to the dietary intake of ALA. The effect of ALA on very short sleep is significantly different among groups of different genders and ages.

KEYWORDS

α-linolenic acid, sleep duration, odds ratio, bootstrap methods, trend analysis

1. Introduction

α-linolenic acid (ALA; 18:3 ω-3) is an essential fatty acid that cannot be synthesized by the human body and must be ingested through the diet. ALA is found in many seeds such as chia, flax, and hemp. Many plant foods (e.g., walnuts, soybeans, spinach, kale, and purslane) are also high in ALA. It additionally occurs in some seed oils, such as flaxseed and rapeseed (canola) oil, as well as some animal fats. Studies have shown that eating a diet rich in ALA and taking ALA supplements could reduce the risk of obesity (1), heart disease (2), cancer (3), and diabetes (4). ALA deficiency can lead to reduced vision, inability to walk, weakness, scaliness of skin, excessive cholesterol and inflammation, and pain in the legs (5, 6). To prevent deficiencies, between 0.2 and 0.3% of the total calories in a diet should contain ALA (5).

Short sleep problems are becoming more and more common in the United States. Over one-third of American adults reported sleeping <7 h in a 24 h period. Insufficient sleep can negatively affect energy, mood, concentration, and overall health. Short sleep duration is associated with higher mortality rates from ischemic heart disease, cancer, and stroke (7, 8), and increases the risks of hypertension, coronary heart disease, and diabetes mellitus (9–11). Dr. Thomas Roth said in Matthew Walker's book, Why We Sleep (12), "The number of people who can survive on 5 h of sleep or less without any impairment, expressed as a percent of the population, and rounded to a whole number, is zero." Most adults need 7–9 h of sleep every night to best function, and an average of 8 h and 10 min to avoid neurobehavioral impairment. Adjusting one's diet to improve sleep is a feasible, convenient, and low-cost strategy.

ALA, eicosapentaenoic acid (EPA; 20:5 ω-3) and docosahexaenoic acid (DHA; 22:6 ω-3) are the three most important types of ω-3 fatty acids. Some ALA can be converted into EPA and then to DHA, but only in very small amounts (13, 14). Numerous studies have documented the relationship between sleep and ω-3 fatty acids (15–18). Researchers have found that DHA increased sleep efficiency and reduced sleep latency in healthy young adults (15). These results supported Yehuda animal models (19). Further, low levels of ω-3 fatty acids intake have previously been associated with sleep problems in children and obstructive sleep apnea in adults (18, 20).

Studies have shown that EPA has beneficial effects on regulating a healthy sleep cycle and reducing the risk of very long and very short sleep durations (15, 17, 21). Researchers found that DHA was beneficial for sleep in people of all ages (15–18). Although consumption of ω-3 fatty acids is known to have positive effects on sleep, prior literature has overlooked the importance of ALA, the only member of the ω-3 family considered essential. This study aims to explore the association of the dietary intake of ALA with very short sleep (<5 h). The differences in the effects of ALA on very short sleep between different genders and age groups were analyzed, respectively. The trend of ALA's effect on very short sleep with age was illustrated by rolling window.

2. Materials and methods

2.1. Participants

We studied and implemented data from the six cycles of the US National Health and Nutrition Examination Survey (NHANES; 2007–2008, 2009–2010, 2011–2012, 2013–2014, 2015–2016, and 2017–2018). The NHANES is a continuous major program that has a continually updating focus on various health and nutrition measurements to meet emerging needs; the data are released on a 2-year cycle. For a complete description of the NHANES program, see: https://www.cdc.gov/nchs/nhanes/ or previous literature (22). We deleted data from participants who lacked information about sleep-related questions, individuals under 18 years old, those using sedative-hypnotic drugs, and those with 24 h dietary recall status that did not meet the reliable or minimum standards. Many activity-related measures, including vigorous and moderate work-related activities, walking, or bicycling for transportation, and vigorous and moderate leisure-time physical activities, were summarized into a new measure named "total activity," using the recommended MET scores. All other variables used raw data from NHANES. Figure 1 shows the detailed screening procedure. Finally, a total of 17,771 participants were involved in this study.

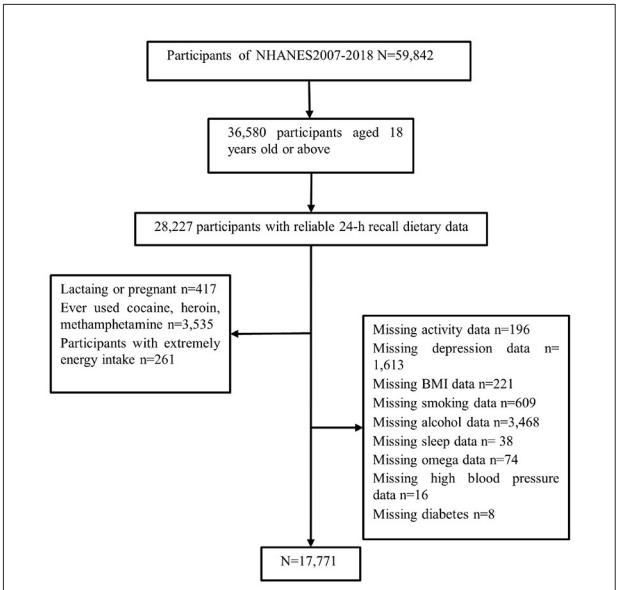

FIGURE 1
Flow chart of the screening process for the selection of eligible participants.

2.2. Sleep duration measurements

Data on sleep duration were based on the respondents' answers to the following question: "How much sleep do you usually get at night on weekdays or workdays?" They were further divided into very short (<5 h), short (5–7 h), normal (7–9 h), and long (≥9 h) sleep duration (23). "Normal" was set as the reference level.

TABLE 1 Classification and types of covariates.

Covariates	Classifications				
Age group	18–44 years		44–59 years		≥60 years
Gender	Males	Females			
Race/ethnicity	Mexican American	Other Hispanic	Non-Hispanic White	Non-Hispanic Black	Other races
Marital status	Married/Living with partner		Windowed/divorced/separated /Nevermarried		
Educational level	Below high school		High school		Above high school
Annual family income	<$20,000		≥$20,000		
Body mass index	< 25 kg/m^2		25–<30kg/m^2		≥30 kg/m^2
Smoking at least 100cigarettes in life	Yes		No		
Everhave 4/5 or more drinks every day	Yes		No		
Hypertension	Yes		No		
Diabetes	Yes		No		
Sedentary activity	Continuous variable				
Total activity score	Continuous variable				
Caffeine intake	Continuous variable				
Energy intake	Continuous variable				

2.3. Dietary intake of ALA

Dietary intake of ALA was obtained through two 24 h dietary recalls. In this study, the average daily intake of dietary ALA was adjusted for participants' body weights.

2.4. Covariates

To control for potential confounding effects, we included the following covariates: age, gender, marital status, body mass index, annual family income, race/ethnicity, educational level, smoking status, drinking status, caffeine intake, diabetes, and hypertension. As stated above, recreational and work-related physical activities were summarized into a new variable, "total activity." The classifications of the above covariates were consistent with previous studies (24, 25) and are illustrated in Table 1.

2.5. Statistical analysis

In descriptive statistical analysis, counts (percentage) and medians (interquartile range) were used to describe qualitative and quantitative data, respectively. The Wilcoxon rank-sum test was adopted to compare continuous variables for participants with different sleep duration. The chi-square test was applied to compare qualitative variables. Body weight-adjusted intake of ALA was divided into tertiles, and the lowest tertile (tertile 1) was set as the reference. To make the estimates representative of the non-institutionalized civilian population of the United States, we weighted the analysis using the NHANES weighting guide. Multinomial logistic regression analysis was used to examine the association of sleep duration with the intake of ALA with normal sleep duration (7–9 h) as the reference. Throughout the study, the models were adjusted for sex, age, educational level, annual family income, race/ethnicity, marital status, smoking status, drinking status, caffeine intake, hypertension, diabetes, body mass index, total activity scores, and sedentary activity. Among these adjustments, the total activity scores were calculated using recommended MET scores from the following five variables: vigorous work-related activity, moderate work-related activity, walking or bicycling for transportation, vigorous leisure-time physical activity, and moderate leisure-time physical activity. Given the differences in sleep conditions between gender and age groups, we conducted a stratified analysis of sleep duration by gender and age group. We used boxplots to visualize the odds ratios (ORs) of very short sleep for male, female, and different age groups. We applied a Kruskal-Wallis test (a nonparametric statistical method) to estimate the differences in the ORs of very short sleep between genders and different age groups. To make our estimation more robust, we used bootstrap methods of 1,000 replications. Analysis was implemented using R version 4.0.3 (R Core Team, 2020) and RStudio version 1.3.1093.

2.6. Rolling window method

To visualize the trend of the ORs of very short sleep duration for ALA intake with age, we conducted the following analysis: To establish a multinomial logistic regression model, we took each age from 18 to 70 years as the starting age and the subsequent 15 years of rolling window data. For example, when the starting age was 18 years, we selected individuals between 18 and 33 years as the analysis object and calculated the regression coefficient of ALA intake in the multinomial logistic regression model. When the starting age was 19 years, we selected participants aged 19–34 as the analysis object, and so on.

3. Results

Table 2 shows the characteristics of the study participants by sleep durations. Among the 17,771 participants, the ratio of men to women was approximately 1:1. The proportion of very short sleepers was about 4.63%. Moreover, compared with normal sleepers, they tended to be older, non-Hispanic black, have lower educational level, widowed/living-alone, lower family income, higher BMI index, hypertension, diabetes, more alcohol drinks, less sedentary activity, fewer activity scores, and depression. The median dietary intake of ALA and the corresponding 95% CI were 1.34 and (0.86, 2.00) mg/day, respectively, for participants with very short sleep, compared to 1.68 (1.13, 2.43) mg/day for those with normal sleep. It indicated that participants with very short sleep had significantly lower levels of ALA dietary intake.

Figure 2 illustrates the boxplots of the bootstrapped ORs of very short sleep for the second and highest tertiles of ALA obtained from the bootstrapped samples of men and women. We used the Kruskal–Wallis test to check whether the ORs of very short sleep for different genders had the same median. Figure 2 shows that the ORs of different genders were significantly different for both the second and highest tertiles of ALA dietary intake. In general, the ORs of very short sleep duration for the dietary intakes of ALA in women were lower than those in men, which was more pronounced for the third tertile of ALA intake.

Figure 3 shows the boxplots of the bootstrapped ORs of very short sleep for ALA intake in different age groups. For the second tertile of dietary intakes of ALA, the difference in the ORs between people aged 45–59 and those over 60 years was not apparent. In contrast, the differences in other pairwise comparisons were apparent. The ORs of very short sleep for both the second and the highest tertiles of dietary intakes of ALA in young people ages 18–44 were relatively low. People 60 years or older were more sensitive to high levels of dietary intake of ALA.

We built regression models to investigate the effect of ALA dietary intake on very short sleep in men and women. To make the estimations more robust, we used the bootstrap method. We resampled the male and female groups 1,000 times each. Further, we performed multinomial logistic regressions for each subsample. We recorded the regression coefficients (i.e., ORs of very short sleep for the second and third tertile of dietary ALA intake) with the first tertile of ALA intake being the reference. As shown in Table 3, dietary ALA intake was negatively related to very short sleep risk in both women and men, and the ORs in women were lower than those in men for both the second and the third tertiles of ALA intake. To estimate the differences in the effect of ALA dietary intake on very short sleep between men and women, we used the bootstrap method again. Specifically, we resampled each gender group 1,000 times and calculated the regression equations' ALA intake coefficients (i.e., OR). Further, we used the ORs' distribution of very short sleep for both the second and third tertiles of ALA dietary intake for each group to calculate the difference and its 95% CI between any two adjacent groups. The difference in the ORs of very short sleep for the third tertile of ALA intake was significantly higher than the second, which means that by increasing the dietary intake of ALA, women can improve very short sleep more effectively than men.

Similarly, we explored the impact of ALA dietary intake on very short sleep for people of different ages using three age groups. Table 4 lists the differences between the adjacent two age groups, denoted as Difference 1 and Difference 2. Difference 1 represents the difference between the 18–44 and 45–60 years groups; Difference 2 is the difference between the 44–60 and 60+ years groups. In most cases, the differences between different ages were more significant than those between different sexes. The smallest difference in the ORs of very short sleep for adjacent age groups was the difference between the 44–60 and 60+ years groups for the third tertile of ALA dietary intake. The difference, −0.220 (−0.230, −0.210), between the 18–44 and 44–60 years groups for the second tertile of ALA dietary intake was the largest.

We noted statistically significant differences in the effects of ALA dietary intake on very short sleep duration for different genders and ages. To further analyze the trend of the ORs of very short sleep for the second and the highest tertiles of dietary ALA intake with age in different sexes, we conducted the rolling window method to visualize the trend of the ORs of very short sleep duration for ALA intake with age. Figures 4, 5 show the regression coefficients, that is, the ORs of very short sleep for the second and the third tertiles of ALA intake in women and men with age. Figure 4 shows that the ORs of men's very short sleep for the second tertile of ALA intake increased linearly with age before the age of 60 and then decreased. Before age 45, there was no significant difference in the ORs of very short sleep duration in men for the second and the third tertiles of ALA intake. From age 45, the ORs in men for the third tertile of ALA intake were lower than those for the second tertile. For women under 55 years of age (Figure 5), the ORs of very short sleep for the second tertile of ALA intake were not significantly different from those for the third tertile. The ORs for the highest tertile were significantly lower than those for the second.

TABLE 2 Sample characteristics by sleep durations, NHANES 2007–2018 (N = 17,771).

Characteristic	7–<9 h, N = 9,644[a]	<5 h, N = 822[a]	5–<7 h, N = 5,176[a]	≥9 h, N = 2,129[a]	p-value[b]
Gender (%)					<0.001
Male	4,863 (50%)	385 (47%)	2,726 (53%)	989 (46%)	
Female	4,781 (50%)	437 (53%)	2,450 (47%)	1,140 (54%)	
Age (year) (%)					<0.001
18-44	3,920 (41%)	291 (35%)	2,136 (41%)	775 (36%)	
44-59	2,159 (22%)	237 (29%)	1,356 (26%)	332 (16%)	
60-	3,565 (37%)	294 (36%)	1,684 (33%)	1,022 (48%)	
Race/ethnicity (%)					<0.001
Mexican American	1,410 (15%)	81 (9.9%)	666 (13%)	293 (14%)	
Other hispanic	909 (9.4%)	91 (11%)	597 (12%)	198 (9.3%)	
Non-hispanic white	4,608 (48%)	284 (35%)	1,958 (38%)	1,014 (48%)	
Non-hispanic black	1,645 (17%)	302 (37%)	1,474 (28%)	424 (20%)	
Other race	1,072 (11%)	64 (7.8%)	481 (9.3%)	200 (9.4%)	
Educational level (%)					<0.001
Above	5,684 (60%)	360 (44%)	2,847 (56%)	1,050 (52%)	
Below	1,813 (19%)	230 (28%)	1,062 (21%)	467 (23%)	
High school	1,986 (21%)	225 (28%)	1,198 (23%)	514 (25%)	
Unknown	161	7	69	98	
Marital status (%)					<0.001
Married/Cohabitation	6,052 (64%)	428 (53%)	3,009 (59%)	1,168 (57%)	
Windowed/Living alone	3,433 (36%)	387 (47%)	2,099 (41%)	865 (43%)	
Unknown	159	7	68	96	
Annual family income (%)					<0.001
<20,000	1,753 (19%)	269 (34%)	1,083 (22%)	549 (27%)	
≥20,000	7,445 (81%)	520 (66%)	3,877 (78%)	1,485 (73%)	
Unknown	446	33	216	95	
Body mass index (%)					<0.001
<25	2,865 (30%)	185 (23%)	1,285 (25%)	630 (30%)	
Between	3,257 (34%)	221 (27%)	1,714 (33%)	674 (32%)	
Above30	3,522 (37%)	416 (51%)	2,177 (42%)	825 (39%)	
Caffeine intake (mg/d)	104 (34, 204)	86 (22, 190)	102 (32, 206)	88 (24, 182)	<0.001
Total energy (kcal/day)	1,917 (1,492, 2,427)	1,735 (1,320, 2,300)	1,910 (1,467, 2,477)	1,822 (1,402, 2,298)	<0.001
Hypertension (%)					<0.001
Yes	3,355 (35%)	387 (47%)	1,983 (38%)	880 (41%)	
No	6,289 (65%)	435 (53%)	3,193 (62%)	1,249 (59%)	
Diabetes (%)					<0.001
Yes	1,166 (12%)	145 (18%)	713 (14%)	353 (17%)	
No	8,258 (86%)	657 (80%)	4,324 (84%)	1,729 (81%)	
Borderline	220 (2.3%)	20 (2.4%)	139 (2.7%)	47 (2.2%)	
Smoked at least 100 cigarettes in life (%)					<0.001
Yes	4,035 (42%)	450 (55%)	2,311 (45%)	962 (45%)	
No	5,609 (58%)	372 (45%)	2,865 (55%)	1,167 (55%)	
Ever have 4/5 or more drinks every day (%)					<0.001
Yes	1,137 (12%)	165 (20%)	715 (14%)	299 (14%)	
No	8,507 (88%)	657 (80%)	4,461 (86%)	1,830 (86%)	

(Continued)

TABLE 2 Continued

Characteristic	7–<9 h, N = 9,644[a]	<5 h, N = 822[a]	5–<7 h, N = 5,176[a]	≥9 h, N = 2,129[a]	p-value[b]
Sedentary activity (minutes/day)	360 (180, 480)	300 (180, 480)	300 (180, 480)	360 (240, 480)	0.007
Total activity (per week)	880 (0, 2,760)	360 (0, 2,640)	840 (0, 2,880)	540 (0, 2,160)	<0.001
Unknown	1,225	146	794	230	
Depression					<0.001
Normal	9,112 (94%)	599 (73%)	4,710 (91%)	1,931 (91%)	
Depression	532 (5.5%)	223 (27%)	466 (9.0%)	198 (9.3%)	
α-Linolenic Acid (mg/pound/day)	1.68 (1.13, 2.43)	1.34 (0.86, 2.00)	1.58 (1.05, 2.30)	1.62 (1.09, 2.38)	<0.001

[a]n (%); Median (IQR).
[b]Pearson's Chi-squared test; Kruskal–Wallis rank sum test.

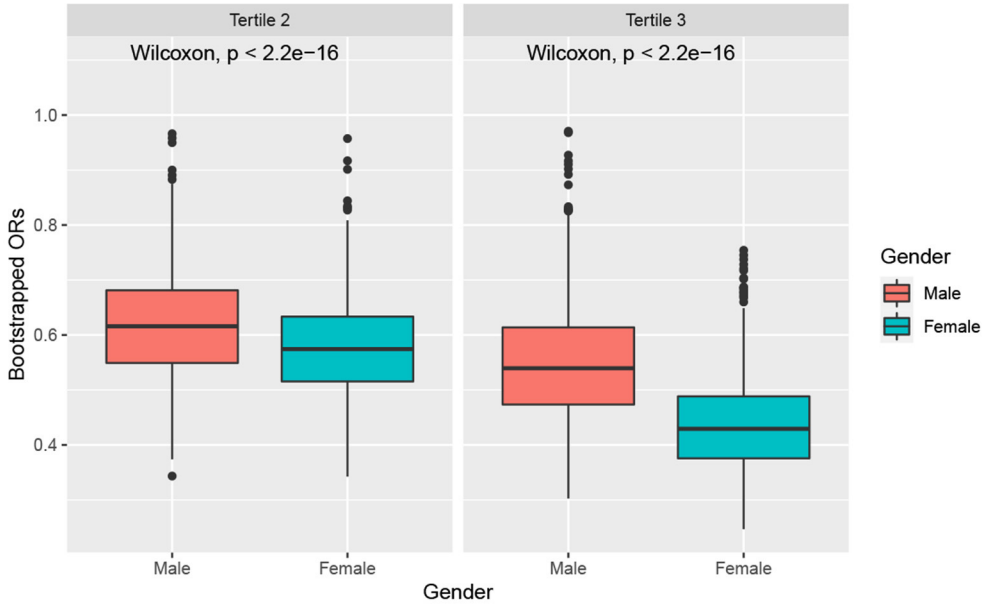

FIGURE 2
Boxplot of bootstraped ORs by gender. The model was adjusted for age, race, educational level, marital status, annual family income, BMI, caffeine intake, total energy intake, hypertension, diabetes, smoking status, drinking status, sedentary activity, and total activity per week.

In this paper, the rolling window data analysis used 15 years as the window width. We noticed that even though 15 was not the only choice, a short window width would lead to unstable results. On the other hand, a long window width would increase the calculation time. To illustrate this, we built a Shiny app at http://shiny.statlearning.com.cn/TrendAnalysisALA/. Figure 6 shows the interface of the app. It shows the ORs trend of tertile 2 (pink) and tertile 3 (sky blue) of ALA intake for any given window width and gender.

4. Discussion

To our knowledge, this is the first study that uses the bootstrap method to estimate the differences in the impact of ALA dietary intake on very short sleep duration between different genders and age groups. We proposed a new method to visualize the influence of ALA dietary intake on the risk of short sleep duration in men and women with age. Moreover, this is the first study based on a large, nationally representative sample of American adults that explores the association of dietary ALA intake with sleep disorders and very short sleep. Unlike previous studies (24, 26), we used body weight instead of energy intake to adjust dietary ALA intake. Our analysis results were statistically more significant; therefore, they were more reliable for application. In addition, since body weight is easier to measure than energy intake, it was more convenient for individuals to apply our analysis results to adjust their daily dietary ALA intake according to their body weights. Our

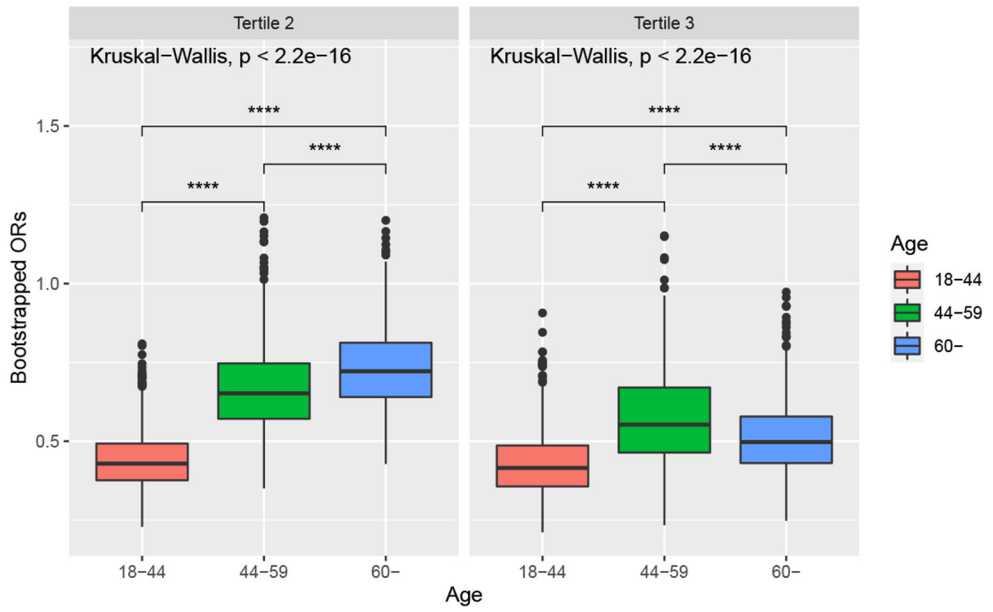

FIGURE 3
Boxplot of bootstrapped ORs by age. The model was adjusted for sex, race, educational level, marital status, annual family income, BMI, caffeine intake, total energy intake, hypertension, diabetes, smoking status, drinking status, sedentary activity, and total activity per week. Note: * $p <$ 0.05, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

TABLE 3 Bootstrapped odds ratios (95% confidence intervals) of very short sleep (reference, 7–9 h/night) across tertiles of body weight-adjusted dietary ALA intake and differences between odds (95% bootstrapped percentile confidence intervals), stratified by gender, NHANES 2007–2018.

	Male	Difference between genders	Female
Very short sleep duration (<5 h/night)			
≤ 1.27	1.00 (ref)		1.00 (ref)
(1.27, 2.08]	0.602***	0.018***	0.583***
	(0.596, 0.608)	(0.01, 0.027)	(0.578, 0.588)
> 2.08	0.533***	0.087***	0.445***
	(0.527, 0.539)	(0.079, 0.095)	(0.439, 0.45)

$^{+}$ $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
Calculated using multinomial logistic regression models, adjusted for age, race, educational level, marital status, annual family income, BMI, caffeine intake, total energy intake, hypertension, diabetes, smoking status, drinking status, sedentary activity, and total activity per week.

estimations were based on the bootstrap method, making them more robust than previous studies.

Our analysis indicated that the dietary ALA intake was generally negatively correlated with the risk of sleep disorders and very short sleep duration. Researchers reported that a mixture of linoleic and ALA with a ratio of 4:1 was the most effective in improving sleep (19). Prior study also showed the effects of a mixture of ALA and linoleic acid on behavioral variables related to anxiety (mood, poor sleep, appetite, fatigue, mental concentration, and academic organization) (27). Our findings are consistent with these previous conclusions. We also found that the ORs for ALA intake were generally lower and more significant in the sleep duration analysis than those in the sleep disorder analysis. This result might be because the self-reported binary response data on whether there is a sleep disorder is not as accurate as the self-reported sleep duration data. This is because the answers to questions about sleep disorders mainly emphasized whether a sleep disorder had ever been experienced. However, the responses to sleep duration were current objective sleep measurements.

We concluded that dietary ALA intake was negatively related to very short sleep risk. The following reasons may explain this. First, the high serum ALA and linoleic acid levels in free fatty acids were linked to decreased odds of depressive symptoms in Japanese adults, supporting a protective role of ALA and linoleic acid against depression (28). In a longitudinal study of women, supplementation of ALA in the diet reduced symptoms of depression (29). Some of the core features of depression include sleep problems. Second, oral consumption of ALA increases serum brain-derived neurotrophic factor (BDNF) levels in healthy adult humans (30). BDNF has an essential role in cognitive function and has been linked to clinical insomnia (31). Third, a previous study showed that dietary ALA and linoleic acid elevated melatonin activity in the hepatoma of rats (32). Melatonin is a "sleep hormone" that can help people fall asleep faster (33, 34). Many of these studies reported significant

TABLE 4 Bootstrapped odds ratios (95% confidence intervals) of very short sleep (reference, 7–9 h/night) across tertiles of body weight-adjusted dietary ALA intake and differences between adjacent age groups (95% bootstrapped percentile confidence intervals), stratified by age, NHANES 2007–2018.

	18–44 years	Difference 1	44–60 years	Difference 2	60+ years
Very short sleep duration (<5 h/night)					
≤ 1.27	1.00 (ref)		1.00 (ref)		1.00 (ref)
(1.27, 2.08]	0.441***	−0.203***	0.648***	−0.091***	0.737***
	(0.435, 0.447)	(−0.213, −0.193)	(0.639, 0.657)	(−0.102, −0.079)	(0.73, 0.745)
> 2.08	0.442***	−0.1***	0.546***	0.018**	0.525***
	(0.436, 0.449)	(−0.111, −0.089)	(0.537, 0.555)	(0.007, 0.03)	(0.517, 0.532)

$^{+}$ $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Calculated using multinomial logistic regression models, adjusted for sex, race, educational level, marital status, annual family income, BMI, caffeine intake, total energy intake, hypertension, diabetes, smoking status, drinking status, sedentary activity, and total activity per week.

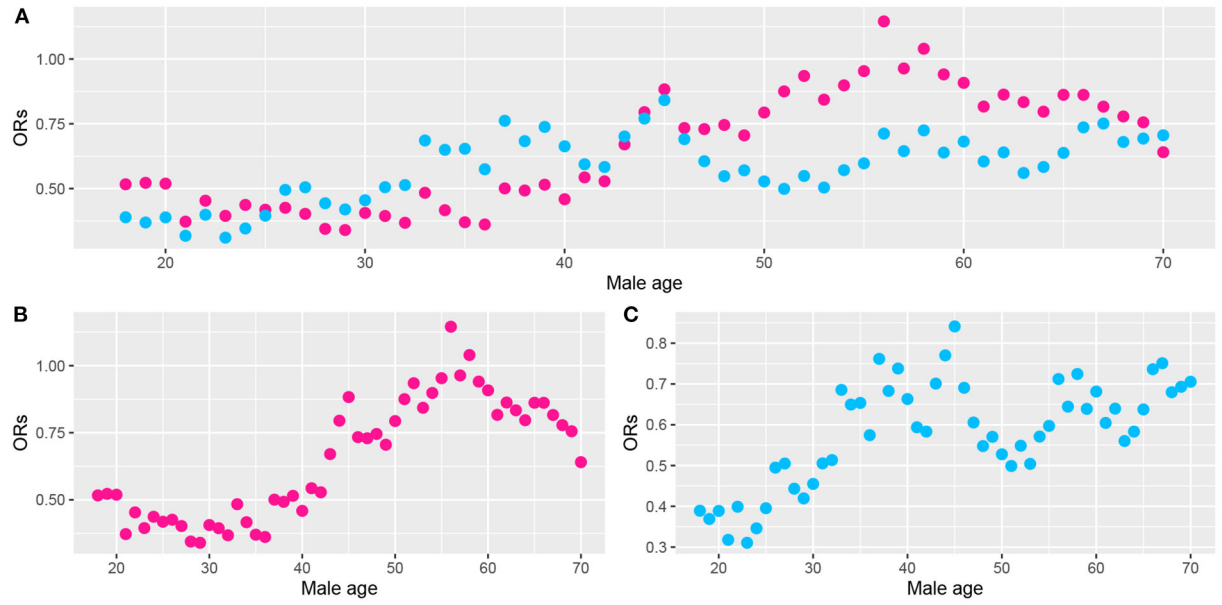

FIGURE 4
Scatter plot of the odds ratios (ORs) calculated from male data (aged from 18 to 70) in a rolling window with a 15-year time interval. **(B)** ORs of men's very short sleep for the second tertile of ALA intake. **(C)** ORs for the third tertile. **(A)** ORs for the second (pink) and the third tertile (skyblue) of ALA intake. Models were adjusted for race, educational level, marital status, annual family income, BMI, caffeine intake, total energy intake, hypertension, diabetes, smoking status, drinking status, sedentary activity, and total activity per week.

improvements in sleep quality (35). Fourth, poor sleep is related to inflammation (36, 37), and ALA can be used as an effective anti-inflammatory agent (38–40). Fifth, 5-hydroxytryptamine (5-HT) receptor 1A and 5-HT receptor 2A levels were enhanced by ALA-rich diets (41). 5-HT was considered as an important substance for sleep preparation, triggering and maintenance (42).

We found significant differences in the impact of ALA dietary intake on the risk of very short sleep duration for different genders and age groups. The trend analysis showed that the pattern for men by age differs from that of women, which may be related to sex hormones. Compared to men, women use a smaller proportion of α-linoleic acid as the substrate for β-oxidation, and a higher proportion is converted into long-chain fatty acids ω-3, possibly due to the regulation of estrogen (43). Previous studies have shown that ω-3 fatty acids improved sleep problems (24, 44). Furthermore, we found that higher dietary intake of ALA was associated with significantly lower ORs of very short sleep duration for older women and men and middle-aged men. The aging process, along with lifestyle changes and multiple comorbidities, can bring about many changes in sleep (45). Hormonal shifts during menstrual cycles and menopause may also play a role. During menopause, night sweats and hot flashes often disrupt sleep. ALA has significant effects on postmenopausal symptoms such as hot flashes, insomnia, and headaches, as well as balancing sex hormone levels in women with polycystic ovary syndrome (46).

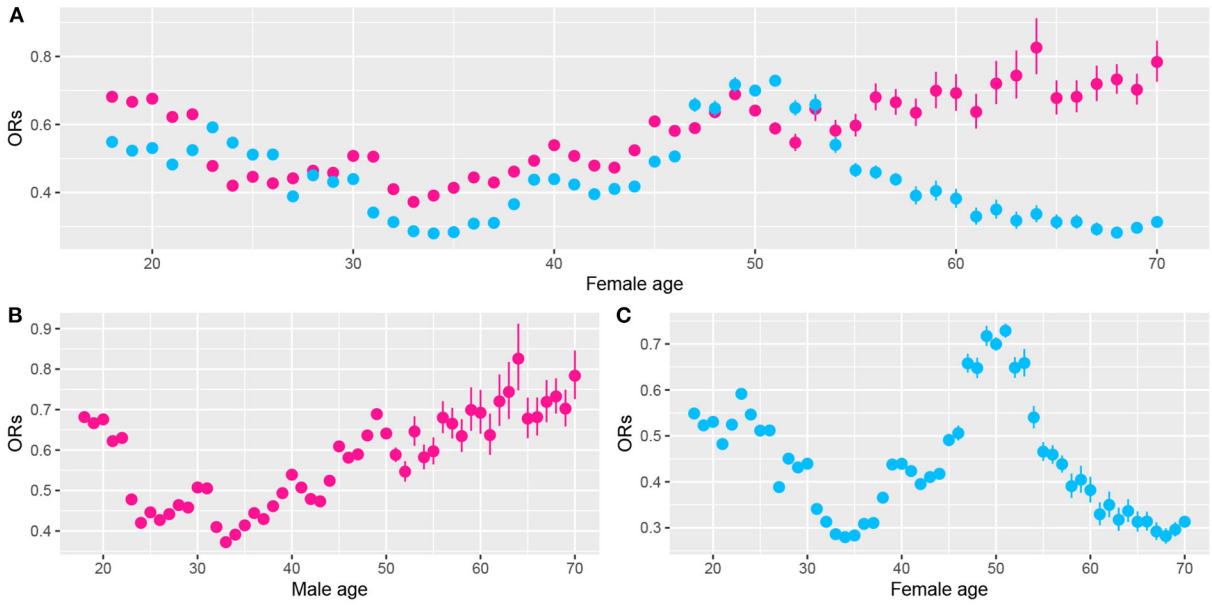

FIGURE 5
Scatter plot of the odds ratios (ORs) calculated from female data (aged from 18 to 70) in a rolling window with a 15-year time interval. **(B)** ORs of women's very short sleep for the second tertile of ALA intake. **(C)** ORs for the third tertile of ALA intake. **(A)** ORs for the second (pink) and the third tertile (sky-blue) of ALA intake. Models were adjusted for race, educational level, marital status, annual family income, BMI, caffeine intake, total energy intake, hypertension, diabetes, smoking status, drinking status, sedentary activity, and total activity per week.

Our results suggest that adequate dietary ALA intake may significantly improve very short sleep problem in middle-aged men, as well as older women and men. Our results also provide a theoretical reference for how to improve the issue of very short sleep duration through diet. Our findings can contribute theoretically and practically to the scientific fields of diet and sleep.

In 2002, the Food and Nutrition Board of the U.S. Institute of Medicine established adequate intake levels for 1.6 and 1.1 g/day ALA intake in male and female adults, respectively (47). In 2009, The European Food Safety Authority published its recommendations for 2 g/day ALA intake (48). Dietary intake of ALA intake should take these limitations into account.

Our research has several limitations and we need to interpret the conclusions with caution. First, the methods used in this paper, such as bootstrap and setting the rolling window to 15 years, can control the influence of confounding factors to a certain extent. However, due to the availability of data, some confounding factors, such as relative changes in sleep duration, use of sleep medication, etc., are not taken into account in the model. Second, the data on sleep disorders and durations were collected based on self-reported sleep wellness in personal interviews rather than physical examinations or medical chart reviews. Self-reported sleep duration is commonly used in population health monitoring studies because it offers several advantages (e.g., non-invasive, inexpensive, and logistically easy to manage for large individual samples). However, self-reported sleep duration usually overestimates actual sleep duration (49); it may also contain recall and reporting biases and may not reflect the actual sleep conditions. Third, participants' primary care providers may not have diagnosed some sleep disorders, so the reported prevalence of disorders may be lower than the objective prevalence. Finally, although the correlations between sleep disorders, very short sleep durations, and many covariates were explained as a factor in this study, our study was cross-sectional and could not confirm causation. Further longitudinal studies should validate the causes behind these associations.

5. Conclusions

ALA is considered a fundamental member of the ω-3 family, but its importance in regulating sleep is overlooked. Our study provides evidence supporting the role of ALA in improving very short sleep. Further, we found that the effects of ALA on sleep are age- and gender-dependent. Our findings can be used in dietary ALA intake guidelines to regulate very short sleep problems in different gender and age groups. Our use of the bootstrap method resulted in more robust and reliable findings. To our knowledge, no literature used the bootstrap method to investigate the relationship between sleep and specific dietary factors. Moreover, we built a Shiny app at http://shiny.statlearning.com.cn/TrendAnalysisALA/ for comparing the trends of ORs with different window widths.

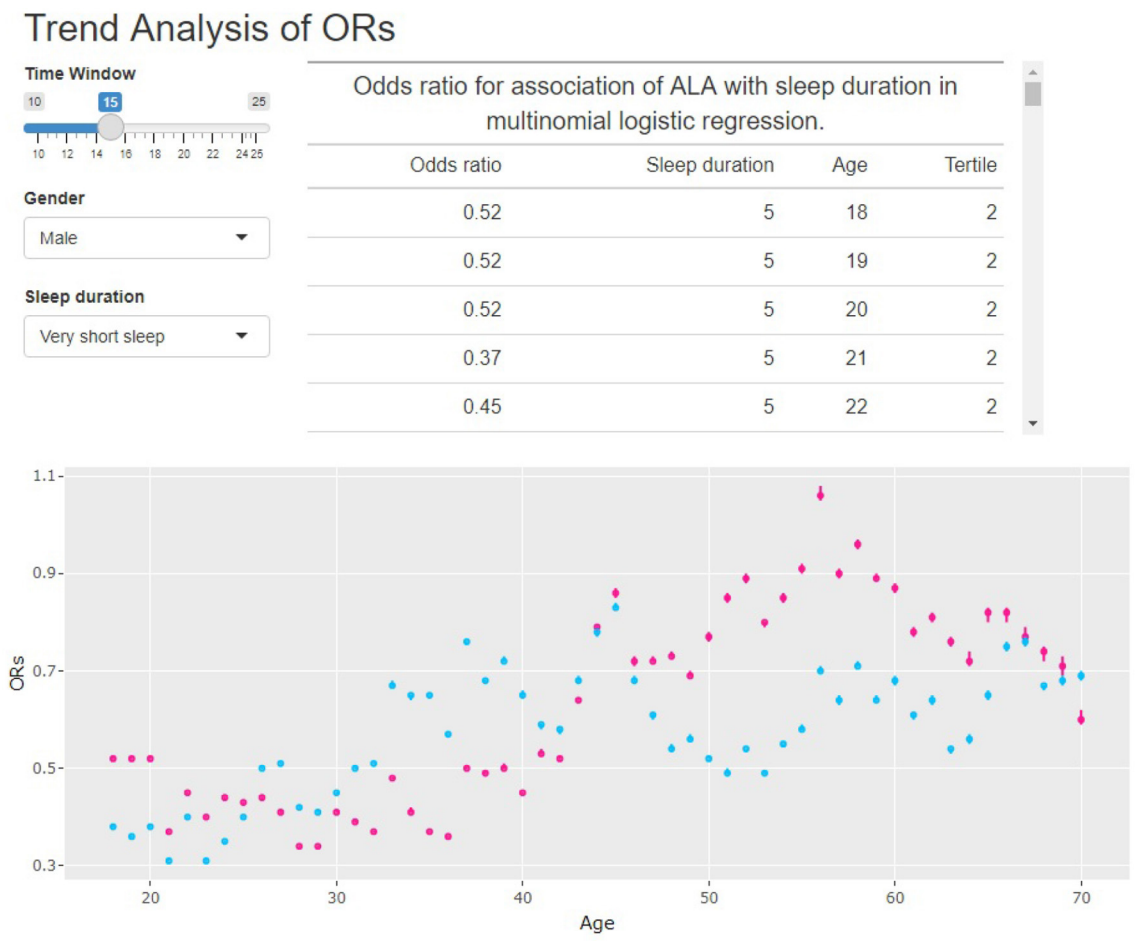

FIGURE 6
Interface of the Shiny APP.

Data availability statement

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

Author contributions

QL: study design, data analysis, and manuscript preparation. QS: methodology, software, and validation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Association between famine exposure in early life and risk of hospitalization for heart failure in adulthood

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Background: Few studies have reported the association of early life exposure to famine with the risk of heart failure. The current study aimed to investigate whether exposure to famine in early life is associated with a higher risk of hospitalization for heart failure in adulthood.

Methods: We used data from participants included in the sub-cohort of the China Patient-centered Evaluative Assessment of Cardiac Events Million Persons Project in Guangdong Province. Specific years of birth were used to define the famine-exposed group (born during the famine of 1959–1962), the pre-famine group (born before the famine [1954–1957], and the post-famine group (born after the famine [1964–1967]). Multivariable-adjusted generalized linear models were used to examine the associations of early life famine exposure with the risk of hospitalization for heart failure.

Results: A total of 36,212 participants were enrolled in this analysis with a median age of 57.4 years and 37.5% of them were men. Compared with the post-famine group, famine births and pre-famine births were associated with increased risk of heart failure (OR: 1.96 [1.56–2.48] and OR: 1.62 [1.07–2.47], respectively). When compared with the age-balanced non-exposed group, the famine-exposed group was also significantly associated with increased risk of heart failure (OR: 1.32 [1.11–1.57]). The associations were stronger in participants with better economic status and in participants with hypertension, diabetes, and dyslipidemia (P for interaction < 0.05).

Conclusion: Early life exposure to the Chinese famine is associated with an elevated risk of hospitalization for heart failure in adulthood.

KEYWORDS

Chinese famine, heart failure, economic status, hypertension, diabetes, dyslipidemia

Introduction

Heart failure (HF) represents the advanced manifestation of various heart diseases and is one of the leading causes of mortality and disability around the world (1, 2). It is estimated that 64.3 and 8.9 million people are suffering from HF worldwide and in China, respectively (3, 4). Other than known risk factors such as high blood pressure, diabetes, and obesity, an increasing body of evidence, mostly from animal models, has shown the implications of exposure to poor nutrition early in life in the growth and developments of cardiometabolic outcomes (5–7). Although such studies in human beings is challenging to conduct, episodes of famine in recent history have provided some "natural" experimental settings to explore the role of undernutrition in early life in the development of cardiometabolic diseases in adulthood (8–10).

Previous famine studies have indicated significant associations of famine exposure with well-defined cardiometabolic risk factors such as obesity, hypertension, dyslipidemia, and diabetes, as well as cardiovascular diseases (CVD) including coronary heart disease, myocardial infarction, and stroke (11–18). However, the association between early life exposure to famine and risk of HF in adulthood has not been well-studied. Using sub-cohort of the China Patient-Centered Evaluative Assessment of Cardiac Events Million Persons Project (PEACE MPP) in Guangdong Province, we therefore investigated the associations of early life exposure to the Chinese famine of 1959–1962 with the risk of hospitalization for HF in adulthood.

Methods

Study population

The China PEACE MPP is a nationwide, government-funded, and population-based CVD screening study for identifying individuals with high CVD risk. The design and methods of China PEACE MPP have been described elsewhere (19–21). The current study was conducted in a sub-cohort of the China PEACE MPP, and 102358 participants were initially enrolled in 8 sites across Guangdong Province from 1 January 2016 to 31 December 2020. The inclusion criteria consisted of (1) registered in the local registration records, (2) community-dwelling residents who settled locally more than 6 months, and (3) aged 35 to 75 years. We excluded participants born in 1958 and 1963 from the analysis to minimize potential misclassification ($N = 6{,}638$), as previous studies have suggested (10). This is because the exact start or end date of Chinese famine is not clear according to nationally mortality rates around those years (22, 23). Among eligible participants, those who were born between 1954 and 1967 were included in the current study ($N = 36{,}212$) (Figure 1). We compared the characteristics of participants who were included in the present study or not and found no significant difference between them (all $P > 0.05$). This study was approved by both the Central Ethics Committee at the China National Center for Cardiovascular Disease and the Ethics Committee of Guangdong Provincial People's Hospital [No. GDREC2016438H (R2)]. Written informed consents were obtained from all participants.

Famine definition

As one of the greatest famines in human history, the Chinese famine of 1959 to 1962 affected mainland China and caused ~30 million deaths (24). Consistent with previous Chinese famine studies (10, 25), participants were categorized into 3 groups using birth year as the proxy variable of exposure to famine: pre-famine group (born in 1954–1957), famine-exposed group (born in 1959–1962), and post-famine group (born in 1964–1967). The birth date for each participant was obtained from their resident identification card.

Study outcomes

The main outcome of this study was hospitalization for HF. For both famine-exposed group and control group, participants' inpatient records from the Hospital Discharge Register System were reviewed and identified by trained staff who were blinded to the exposure status as well as other individual information. Events of hospitalization for HF were ascertained using code of I50 of the Tenth Revision of International Classification of Diseases (ICD-10) (26). All events were independently reviewed and verified by a panel of three experienced experts, including two cardiologists and one statistician.

Assessment of covariates

We assessed covariates that included age, sex, education, occupation, economic status (annual income), marriage, smoking and drinking status, self-reported history of hypertension, diabetes, and dyslipidemia, and self-reported current use of antihypertensive, antidiabetic, lipid-lowering, and antiplatelet medications, and statin therapy. Smoking and drinking status were collected by asking the question "Do you currently smoke cigarettes or drink alcohol?" For each participant, physical examinations were also performed to measure systolic blood pressure (SBP), diastolic blood pressure (DBP), height, weight, and waist circumference. Blood pressure was measured twice on the right upper arm after 5 min of rest in a seated position using an electronic blood pressure monitor (Omron HEM-7430; Omron Corporation, Kyoto, Japan) and a standard protocol. Fasting blood glucose (FBG) was measured

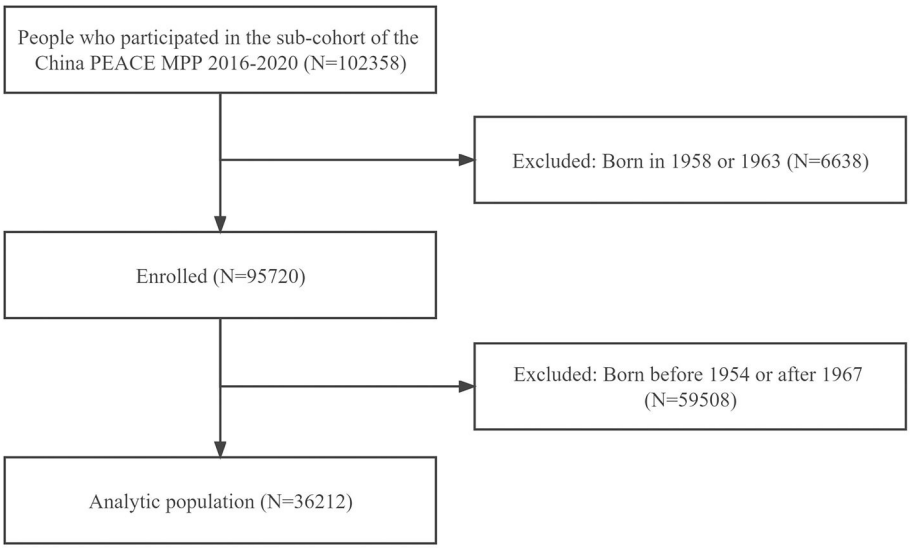

FIGURE 1
Flow chart of study participants. PEACE MPP, Patient-Centered Evaluative Assessment of Cardiac Events Million Persons Project.

using fingertip blood samples (BeneCheck BK6–20M Multi-Monitoring System, Suzhou Pu Chun Tang Biotechnology, China). Lipid profile including triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured by a rapid lipid analyzer (CardioChek PA Analyzer; Polymer Technology Systems, Indianapolis, Indiana, USA). Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of height in meters. Hypertension was determined based on self-reported using of antihypertensive drugs, or SBP $\geq$ 140 mmHg and/or DBP $\geq$ 90 mmHg (27). Dyslipidemia was determined based on self-reported using of lipid-lowering medications, or TC $\geq$ 6.2 mmol/L and/or LDL-C $\geq$ 4.1 mmol/L (28). Diabetes was determined based on self-reported using of antidiabetic drugs, or FBG $\geq$ 7.0 mmol/L (29).

Statistical analysis

Continuous variables were described as median (interquartile range) for non-normal distribution. Categorial variables were described as number and percentage. We compared the characteristics of study participants according to famine exposure status using chi-squared test, Wilcoxon rank sum test, one-way ANOVA or Kruskal-Wallis H test as appropriate. Multivariable-adjusted generalized linear models were used to estimate the odds ratio (OR) and 95% confidence interval (CI) of HF for famine group and pre-famine group compared with post-famine group. Model 1 was adjusted for age and sex; Model 2 with additional adjustment for marital status, educational status, occupation, economic status, smoking, drinking, and BMI; Model 3 with additional adjustment for hypertension, diabetes, dyslipidemia, and current use of antiplatelet medications and statin therapy. We conducted a series of stratification analyses by sex (men vs. women), economic status (annual income <50,000 vs. $\geq$ 50,000 yuan), current smoking status (no vs. yes), hypertension (no vs. yes), diabetes (no vs. yes), dyslipidemia (no vs. yes), and BMI (<24 vs. $\geq$ 24 kg/m^2). In the sensitivity analysis, to reduce the age gap between groups, we combined the pre-famine and post-famine groups together as an age-balanced non-exposed group to test the robustness of the main results, as suggested by previous studies (10, 30, 31). All analyses were conducted with R statistical software version 3.33 (R Project for Statistical Computing). $P < 0.05$ was considered significant.

Results

Characteristics of study participants

A total of 36,212 participants were included in the current study, of which 37.5% were men and the median age was 57.4 years. The prevalence of HF in the pre-famine group, famine group, and post-famine were 0.9, 2.0, and 2.8%, respectively. Compared with the pre-famine group, the famine group and post-famine group had higher prevalence of traditional CVD risk factors such as smoking, hypertension, diabetes, and dyslipidemia (Table 1). Of participants included, 669 (1.85%) had events of hospitalizations for HF, and those with HF were older and more likely to be men, had higher prevalence of

TABLE 1 Characteristics of study participants according to famine exposure among 36,212 participants.

Characteristics	Total	Pre-famine group	Famine group	Post-famine group	*P*-value
Number	36,212	12,542	10,493	13,177	
Age, y	57.4 (53.5–61.7)	52.6 (51.6–53.7)	57.2 (56.1–58.5)	62.5 (61.4–63.5)	<0.001
Men, *n* (%)	13,570 (37.5)	4,704 (37.5)	3,655 (34.8)	5,211 (39.5)	<0.001
Educational status (high school or above), *n* (%)	8,953 (24.7)	2,976 (23.7)	3,107 (29.6)	2,870 (21.8)	<0.001
Occupation (Farmer), *n* (%)	5,202 (14.4)	1,547 (12.3)	1,516 (14.4)	2,139 (16.2)	<0.001
Economic status (annual income ≥50,000 yuan), *n* (%)	15,899 (43.9)	5,695 (45.4)	4,574 (43.6)	5,630 (42.7)	<0.001
Marriage (married), *n* (%)	33,096 (91.4)	11,577 (92.3)	9,585 (91.3)	11,934 (90.6)	<0.001
Current smoker, *n* (%)	6,365 (17.6)	2,101 (16.8)	1,793 (17.1)	2,471 (18.8)	<0.001
Current drinker, *n* (%)	2,010 (5.6)	686 (5.5)	535 (5.1)	789 (6.0)	<0.001
BMI, kg/m^2	24.1 (22.0–26.3)	24.2 (22.2–26.4)	24.1 (22.1–26.3)	23.9 (21.8–26.2)	<0.001
Waist circumference, cm	84.0 (78.0–90.0)	84.0 (78.0–90.0)	84.0 (78.0–90.0)	85.0 (78.0–90.9)	<0.001
SBP, mm Hg	131.0 (119.5–143.5)	127.5 (117.0–140.0)	131.0 (119.5–144.0)	133.5 (122.0–147.0)	<0.001
DBP, mm Hg	80.0 (72.5–87.5)	79.5 (72.5–87.5)	80.0 (72.5–87.5)	80.0 (72.5–87.0)	0.596
FBG, mg/dL	102.6 (91.8–115.2)	100.8 (91.8–113.4)	102.6 (91.8–115.2)	102.6 (91.8–115.2)	<0.001
TG, mg/dL	122.1 (90.3–176.1)	120.5 (88.6–172.8)	124.9 (92.1–179.9)	123.2 (92.1–177.2)	<0.001
TC, mg/dL	191.8 (162.5–223.9)	189.6 (161.0–220.2)	194.7 (164.9–227.2)	192.7 (162.9–226.0)	<0.001
LDL–C, mg/dL	106.2 (82.2–133.2)	104.5 (81.3–130.0)	108.7 (84.0–137.0)	106.8 (82.4–135.1)	<0.001
HDL-C, mg/dL	54.8 (44.8–67.6)	55.0 (44.5–67.3)	55.0 (44.9–67.7)	55.7 (45.3–68.1)	<0.001
Hypertension, *n* (%)	16,385 (45.2)	4,791 (38.2)	4,796 (45.7)	6,798 (51.6)	<0.001
Diabetes, *n* (%)	6,699 (18.5)	2,025 (16.1)	2,083 (19.9)	2,591 (19.7)	<0.001
Dyslipidemia, *n* (%)	8,033 (22.2)	2,349 (18.7)	2,561 (24.4)	3,123 (23.7)	<0.001
Current use of antihypertensive drugs, *n* (%)	7,942 (21.9)	2,120 (16.9)	2,324 (22.1)	3,498 (26.5)	<0.001
Current use of antidiabetic drugs, *n* (%)	2,965 (8.2)	764 (6.1)	962 (9.2)	1,239 (9.4)	<0.001
Current use of lipid-lowering drugs, *n* (%)	1,614 (4.5)	396 (3.2)	504 (4.8)	714 (5.4)	<0.001
Current use of statin therapy, *n* (%)	275 (0.8)	59 (0.5)	90 (0.9)	126 (1.0)	<0.001
Current use of antiplatelet drugs, *n* (%)	197 (0.5)	41 (0.3)	47 (0.4)	109 (0.8)	<0.001

Data are presented as median (IQR) for non-normally distributed variables, and numbers (percentages) for categorical variables. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

cardiovascular risk factors such as smoking, BMI, hypertension, diabetes, and dyslipidemia, and had higher rate of current use of antihypertensive, antidiabetic, lipid-lowering, antiplatelet drugs, and statin therapy (Table 2).

Famine exposure and HF

Both the famine group and pre-famine group were associated with increased risk of hospitalization for HF compared with the post-famine group (OR = 1.96 for famine births, 95% CI: 1.56–2.48, and OR = 1.62 for pre-famine births, 95% CI: 1.07–2.47), after adjusting for age, sex, marriage, educational status, occupation, family annual income, smoking, drinking, BMI, hypertension, diabetes, dyslipidemia, statin therapy, and current use of antiplatelet drugs (Table 3). In the sensitivity analysis, famine group was also associated with elevated risk of HF compared with the age-balanced non-exposed group (multivariable-adjusted OR = 1.32, 95% CI: 1.11–1.57) (Supplementary Table 1).

Stratification analyses

Stratification analyses by sex, smoking status, and BMI did not show significant differences in the associations of early life exposure to the Chinese famine with hospitalization for HF in those subgroups. However, stratification analyses by economic status showed that, compared with the post-famine group, the associations between famine group and risk of HF were stronger in those with income ≥50,000 yuan per year than in those with income <50,000 yuan ($OR_{\geq 50,000yuan}$: 2.48 [1.72–3.64] vs. OR $_{<50,000yuan}$ 1.71 [1.27–2.30], P for interaction = 0.010). The associations of famine group with HF compared with the post-famine group were also stronger in those with hypertension

TABLE 2 Characteristics of study participants with or without hospitalization for heart failure among 36,121 participants.

Characteristics	Total	Without HF	With HF	*P*-value
Number	36,212	35,543	669	
Age, y	57.4 (53.5–61.7)	57.3 (53.5–61.7)	60.4 (56.5–62.7)	<0.001
Men, *n* (%)	13,570 (37.5)	13,182 (37.1)	388 (58.0)	<0.001
Educational status (high school or above), *n* (%)	8,953 (24.7)	8,783 (24.7)	170 (25.4)	0.71
Occupation (Farmer), *n* (%)	5,202 (14.4)	5,102 (14.4)	100 (14.9)	0.71
Economic status (annual income ≥ 50,000 yuan), *n* (%)	15,899 (43.9)	15,605 (43.9)	294 (43.9)	0.99
Marriage (married), *n* (%)	33,096 (91.4)	32,487 (91.4)	609 (91.0)	0.79
Current smoker, *n* (%)	6,365 (17.6)	6,170 (17.4)	195 (29.1)	<0.001
Current drinker, *n* (%)	2,010 (5.6)	1,962 (5.5)	48 (7.2)	0.08
BMI, kg/m2	24.1 (22.0–26.3)	24.1 (22.0–26.3)	24.9 (22.6–27.2)	<0.001
Waist circumference, cm	84.0 (78.0–90.0)	84.0 (78.0–90.0)	88.0 (80.0–94.0)	<0.001
SBP, mm Hg	131.0 (119.5–143.5)	131.0 (119.5–143.5)	135.0 (122.0–151.5)	<0.001
DBP, mm Hg	80.0 (72.5–87.5)	80.0 (72.5–87.5)	81.0 (73.0–89.0)	0.009
FBG, mg/dL	102.6 (91.8–115.2)	101.2 (91.8–115.2)	106.2 (91.8–127.8)	<0.001
TG, mg/dL	122.4 (90.5–176.5)	122.4 (90.5–176.5)	128.6 (94.0–190.7)	0.008
TC, mg/dL	192.3 (162.9–224.5)	192.3 (163.3–224.5)	174.5 (142.8–214.4)	<0.001
LDL-C, mg/dL	109.9 (84.4–131.2)	110.3 (84.4–131.6)	98.7 (72.4–122.3)	<0.001
HDL-C, mg/dL	55.0 (44.9–67.7)	55.3 (44.9–67.7)	49.5 (40.6–61.9)	<0.001
Hypertension, *n* (%)	16,385 (45.2)	15,950 (44.9)	435 (65.0)	<0.001
Diabetes, *n* (%)	6,699 (18.5)	6,469 (18.2)	230 (34.4)	<0.001
Dyslipidemia, *n* (%)	8,033 (22.2)	7,838 (22.1)	195 (29.1)	0.009
Current use of antihypertensive drugs, *n* (%)	7,942 (21.9)	7,639 (21.5)	303 (45.3)	<0.001
Current use of antidiabetic drugs, *n* (%)	2,965 (8.2)	2,829 (8.0)	136 (20.3)	<0.001
Current use of lipid-lowering drugs, *n* (%)	1,614 (4.5)	1,512 (4.3)	102 (15.2)	0.009
Current use of statin therapy, *n* (%)	275 (0.8)	255 (0.7)	20 (3.0)	<0.001
Current use of antiplatelet drugs, *n* (%)	197 (0.5)	178 (0.5)	19 (2.8)	<0.001

Data are presented as median (IQR) for non-normally distributed variables, and numbers (percentages) for categorical variables.
PEACE MPP, Patient-Centered Evaluative Assessment of Cardiac Events Million Persons Project; HF, heart failure; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

(OR: 2.80 [2.08–3.88] in hypertensive participants vs. 1.73 [1.29–2.35] in non-hypertensive participants, P for interaction = 0.024), in those with diabetes (OR: 2.55 [1.71–3.89] in diabetic participants vs. 1.73 [1.10–2.30] in non-diabetic participants, P for interaction = 0.010), and in those with dyslipidemia (OR: 2.18 [1.66–2.89] in participants with dyslipidemia vs. 1.55 [1.01–2.39] in participants without dyslipidemia, P for interaction = 0.012) (Figure 2). The results were similar for the pre-famine group when comparing to the post-famine group and remained robust when using the age-balanced control group as reference (Supplementary Figure 1).

Discussion

Our large population-based study revealed that early life exposure to the Chinese famine of 1959–1962 was significantly associated with increased risk of hospitalization for HF in adulthood.

Although the associations between famine exposure early in life and self-reported cardiovascular diseases have been recognized in previous studies, evidence about the associations with rarely reported cardiometabolic outcomes such as hospitalization for HF is still lacking (15, 17, 32). Among 5,772 participants in the China Health and Retirement Longitudinal Study (CHARLS), Shi and colleagues (33) observed that early life exposure to the Chinese famine increased the risk of self-reported composite CVD events (OR = 2.87, 95% CI: 1.16–7.07). In the REACTION (Risk Evaluation of Cancers in Chinese Diabetic Individuals) study of 259,657 community-dwelling adults, Du et al. (17) showed that early life famine exposure was associated with higher risk of self-reported total CVD, coronary heart disease, myocardial infarction, and stroke. Similarly, among 92 284 participants from the China Kadoorie Biobank, Meng et al. (15) found that early life exposure to the Chinese famine was associated with increased risks of ischemic heart disease, cerebrovascular disease, and ischemic stroke, which were defined using local disease national health insurance

TABLE 3 Odds ratio with 95% CI of hospitalization for heart failure according to famine exposure among 36,212 participants.

	Post-famine group	Famine group	Pre-famine group
Case (%)	118 (0.9)	211 (2.0)	340 (2.6)
Model 1	1.0	2.13 (1.70–2.69)	1.70 (1.12–2.59)
Model 2	1.0	2.14 (1.70–2.70)	1.68 (1.11–2.56)
Model 3	1.0	1.96 (1.56–2.48)	1.62 (1.07–2.47)

Model 1: adjusted for age and sex.
Model 2: adjusted for age, sex, smoking, drinking, marriage, educational status, occupation, economic status, and body mass index.
Model 3: adjusted for age, sex, smoking, drinking, marriage, educational status, occupation, economic status, body mass index, hypertension, diabetes, dyslipidemia, statin therapy, and current use of antiplatelet drugs.

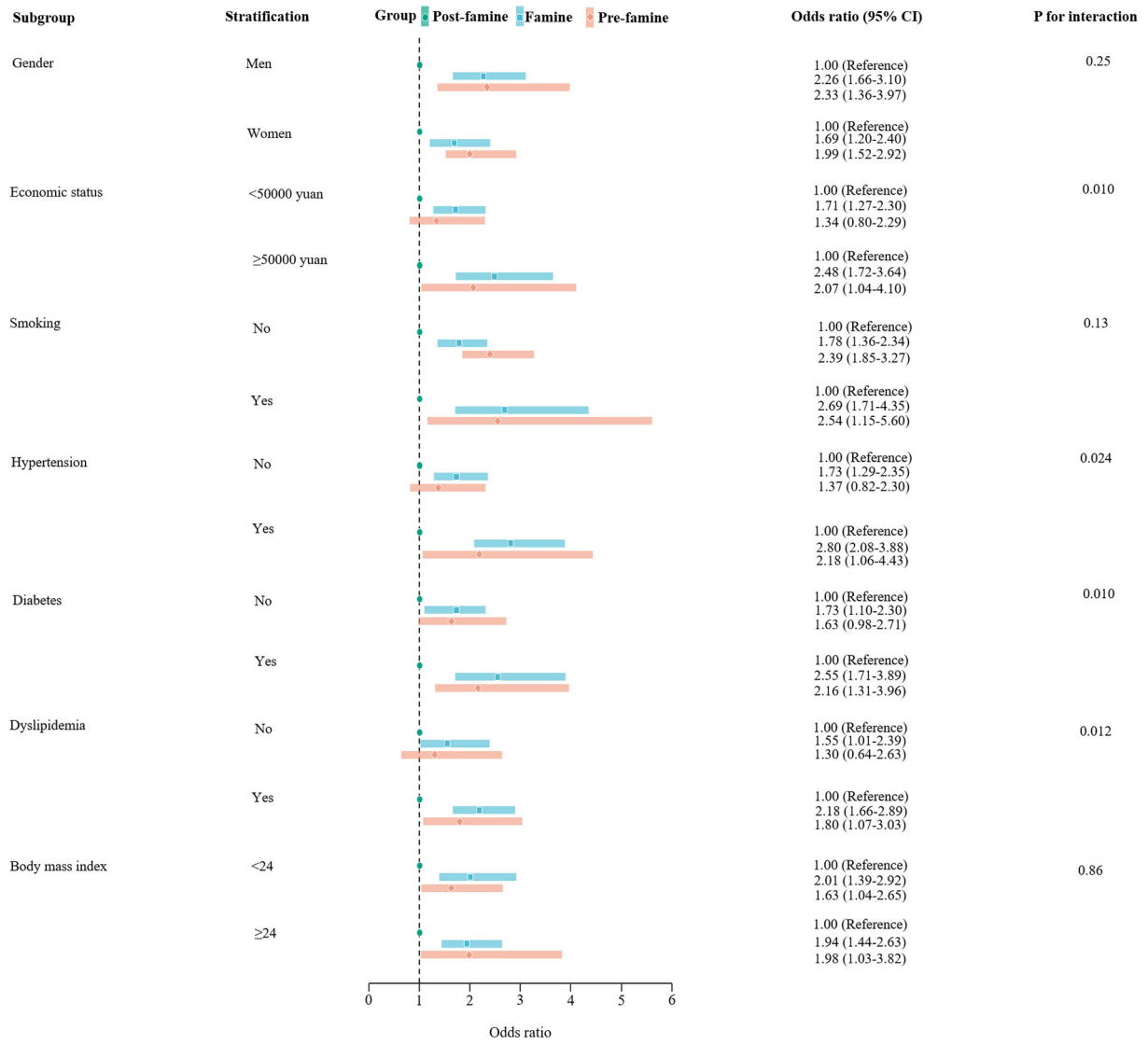

FIGURE 2
Stratification analysis of associations of pre-famine and famine births with risk of hospitalization for heart failure compared with post-famine births. Presented were multivariable-adjusted generalized linear models with adjustment for age, sex, marriage, educational status, occupation, economic, smoking, drinking, body mass index, hypertension, diabetes, dyslipidemia, current use of antiplatelet medications, and statin therapy. The square in the middle represents the odds ratio of the risk estimation, and the bar represents its 95% CI.

system and ICD-10 codes. Our study including over 35,000 participants added the evidence on the positive relation between famine exposure early in life and risk of hospitalization for HF, which is the final stage of various kinds of CVD (2).

Inconsistent with previous studies that revealed sex differences in the associations between famine exposure and cardiometabolic outcomes such high blood pressure (10, 34), diabetes (35, 36), and self-reported CVD events (17), we found similar association between famine exposure to the Chinese famine and HF risk in men and women. The study design, participant selection, and definitions of exposed and non-exposed groups may contribute to these reported inconsistent findings. However, we found in the current study that participants with better economic status had increased risk of HF when exposed to famine in early life. One explanation is that socioeconomic status plays an important role in developing CVD, and it even had bigger effect than healthy lifestyles in adulthood (37). Indeed, Wang et al. (38) found that early-life famine exposure was positively associated with hyperuricemia in subjects with high economic status rather than in those with low economic status. These findings conformed the Barker hypothesis (39), which demonstrated that if the utero development of a thrifty phenotype mismatched the later plentiful environment, infants suffering from undernutrition will be more prone to cardiometabolic disease in later life. Additionally, we found that hypertensive and diabetic participants who were exposed to famine in early life had higher risk of HF than non-hypertensive participants. These findings were consistent with results from two representative cohorts of Chinese adults that provided evidence that early exposure to the Chinese famine of 1959–1962 exacerbated the association of hypertension, diabetes, and risk of CVD in later life (33, 40). In addition, we found that in the present study, dyslipidemia modified the effect of early life exposure to famine on later risk of HF, which was inconsistent with the REACTION study that found non-significantly stronger association between early life famine exposure and CVD risk in participant without dyslipidemia (17). This difference could be explained to the complex mediation of metabolic syndrome on the relationship between famine and CVD and the clustering of multimorbidity (17).

Although not well-understood yet, several plausible mechanisms can be responsible for the adverse associations of famine exposure early in life with HF risk in adulthood. First, animal experiments have revealed that prenatal and postnatal malnutrition can elevate blood pressure by altering the renin-angiotensin system (41, 42), and increase blood glucose by destroying pancreatic β-cell function (43, 44), which can then increase cardiovascular risk. Second, nutritional deficiency early in life can result in limited development in multiple organs and tissues, such as pancreas, adipose, and kidney, and then increased risk of cardiac diseases later in life (45, 46). Third, it has been reported that fetal exposure to famine was associated with changes in DNA methylation of genes involved in inflammation, adipogenesis, and glycolysis (47–49), therefore, epigenetic modifications may be a plausible mechanism linking famine exposure and cardiovascular health.

Several limitations should be kept in mind when interpreting our findings. First. although the birth date was commonly used to define famine exposure in this research field, this method may lead to misclassification bias and underestimated associations. Second, residual confounding was still likely given the nature of observational study design. Third, data relevant to dietary patterns, physical activity, and birth weight was not collected and thus cannot be adjusted in our analysis. Finally, we were unable to distinguish either subgroups of HF (i.e., HF with reduced or preserved ejection fraction) or causes of HF, and then unable to further explore the relationships between early life famine exposure and specific HF outcomes.

Conclusion

Taken together, our study revealed that early life exposure to the Chinese famine of 1959–1962 was associated with increased risk of hospitalization for HF in adulthood. These associations were stronger in those with better economic status and those with hypertension, diabetes, or dyslipidemia.

Data availability statement

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

Ethics statement

This study was approved by both the Central Ethics Committee at the China National Center for Cardiovascular Disease and the Ethics Committee of Guangdong Provincial People's Hospital [No. GDREC2016438H (R2)]. The patients/participants provided their written informed consent to participate in this study.

Author contributions

C-lC and Y-qF: conceptualization and study design. C-lC, J-bW, and Y-qF: paper preparation. C-lC and J-bW: statistical analysis and data interpretation. All authors: investigation and reviewed and approved this manuscript.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.973753/full#supplementary-material

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Body fat and muscle were associated with metabolically unhealthy phenotypes in normal weight and overweight/obesity in Yi people: A cross-sectional study in Southwest China

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This study aimed to determine the association between the absolute mass, distribution, and relative ratio of body fat and muscle with the metabolically unhealthy (MU) phenotypes in normal weight and overweight/obesity in Yi people in China. The cross-sectional data from the Yi Migrants Study was used, which included 3,053 Yi people aged 20–80 years from the rural and urban sets. Participants were classified according to body mass index and metabolic status. Body composition including body fat percentage (BFP), fat mass index (FMI), visceral fat grade (VFG), muscle mass index (MMI), and muscle/fat ratio (M/F) were measured by bioelectrical impedance analysis. Restricted cubic spline and logistics regression models were used to test the associations between body composition parameters with MU phenotypes. Receiver-operating characteristic curves (ROC) were used to analyze the predictive value of MU phenotypes. Among the normal weight and overweight/obesity, 26.31% (497/1,889) and 52.15% (607/1,164) were metabolically unhealthy. Stratified by BMI, covariance analysis showed higher body fat (BFP, FMI, and VFG) and MMI in MU participants than in healthy participants. BFP, FMI, VFG, and MMI were positively associated with MU phenotypes both in normal weight and overweight/obesity after adjustment. M/F was significantly lower than MU participants and was negatively associated with MU phenotypes. BFP, FMI, VFG, and M/F could better predict MU phenotypes than BMI. We concluded that

BFP, FMI, and VFG were positively associated with MU phenotypes, while M/F was negatively associated with MU phenotypes across the BMI categories in Yi people. Body fat and muscle measurement could be a valuable approach for obesity management.

KEYWORDS

body composition, metabolically healthy overweight/obesity, Yi people, bioelectrical impedance analysis, muscle-to-fat ratio

Introduction

The prevalence of overweight and obesity has been sharply increasing in the last three decades worldwide including in China (1). The prevalence of obesity in Chinese adults was reported to increase from 3.6% in 1992 to 16.4% in 2015–19 (1). Obesity individuals are at higher risk of developing a wide range of diseases including cardiovascular disease (CVD), diabetes mellitus, chronic kidney disease, and some types of cancer (2). Nevertheless, the phenotypes of overweight and obesity are heterogeneous. Emerging evidence indicates that not all obese individuals develop metabolic disorders, and this subgroup of obese individuals is considered metabolically healthy obesity (MHO) (3). They have normal insulin sensitivity and inflammatory response and are usually free from cardiovascular metabolic risk factors (4). Similarly, the part of the normal-weight individuals with adverse metabolic status has also been reported, which is called metabolically unhealthy normal-weight (MUNW) (5).

Recent studies reveal the fact that the favorable metabolic profile might be a temporary status. A 0.5 million Chinese adults cohort showed that over one-third of the overweight or obese individuals converted from metabolic healthy to unhealthy phenotypes through 10 years of follow-up (6). Interestingly, participants with a stable MHO phenotype were found to have a comparable risk of CVD as metabolically healthy normal-weight (MHNW) individuals (7). Therefore, maintaining a healthy metabolic profile appears to be a valid approach to preventing cardiometabolic diseases (8).

The exact mechanisms underlying the metabolically healthy vs. unhealthy phenotypes remain to be explored. The dietary factors, physical activity, inflammation, and genetic factors were reported to contribute to MHO (9–12). Recent human studies suggest that adipose tissue function, body fat distribution, skeletal muscle et al. may be key factors in insulin sensitivity and metabolic phenotypes (13). Whether the effect of body composition indicators on metabolic health is consistent across all BMI categories, and whether the body fat and muscle predict metabolic phenotypes more precisely than BMI remain to be discussed.

In this present cross-sectional study, using population-based data, we aimed to determine the association between the absolute mass, distribution, and relative ratio of body fat and muscle with the metabolically unhealthy phenotypes in normal weight and overweight/obesity in Yi people in China. We also hypothesized that body fat and muscle indicators were more precise predictors than BMI in predicting metabolic phenotypes.

Materials and methods

Study population

The current study was based on a cross-sectional survey–The Yi Migrant Study, which was carried out in Liangshan Yi Autonomous Prefecture, Sichuan, China in 2015. The Yi Migrant Study was initiated in the 1980's and was designed to assess the cardiovascular risks as well as the determinants in Yi people using a migration epidemiology study design. Continuous field works have been conducted in different periods following the same procedure. A stratified cluster sampling method was used to recruit participants aged 20 to 80 years. Details of the sampling procedures have been described previously (14, 15).

In this migration epidemiology study, Yi people whose parents were both of Yi ethnicity can be included. Yi farmers were defined to be Yi people living in rural areas since birth. Yi migrants were defined to be Yi people who were born in rural areas and then migrated and had been living in an urban area for at least 1 year and were still living in the urban area. All participants provided written informed consent before the survey.

Data collection

Demographic characteristics (age, sex, education level, personal annual income, et al.), disease history (hypertension, diabetes, cardiovascular disease, et al.), and health-related lifestyle factors (smoking status, drinking status, physical activity, et al.) were collected by face-to-face interviews. A standard questionnaire was administered by well-trained staff to obtain the above information (15).

Anthropometric measurements including height, weight, and body composition were taken using calibrated instruments

with standard protocols. Weight and body composition were measured in light clothing by bioelectrical impedance analysis (BIA) using the body composition analyzer (BC-420, TANITA, Japan). The measurements were recorded with an accuracy of 0.1. Standing height was measured barefoot by a wall-mounted stadiometer with an accuracy of 0.1 cm.

Blood pressure was measured using a digital sphygmomanometer (Omron HEM907, Japan). For each participant, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured three times at one-minute intervals, after at least 5 min of rest in a seated position. The average of the three measurements was recorded.

A 9-ml venous blood sample with at least 8 h of fasting overnight was collected. The samples were centrifuged, aliquoted, and immediately frozen for future tests. Fasting blood glucose (FBG, mmol/L), triglyceride (TG, mmol/L), total cholesterol (TC, mmol/L), high-density lipoprotein cholesterol (HDL-C, mmol/L), and low-density lipoprotein cholesterol (LDL-C, mmol/L) levels were tested in Beijing Hepingli Hospital.

Definition

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). In this study, BMI was categorized into normal-weight (<24 kg/m^2) and overweight/obesity (≥ 24 kg/m^2) according to the criteria for Chinese (16).

Body fat percentage (BFP, %), fat mass index (FMI, kg/m^2), visceral fat grade (VFG), and muscle mass index (MMI, kg/m^2) were body composition parameters of interest. BFP and VFG were directly measured by the body composition analyzer. FMI and MMI were calculated as fat mass and muscle mass in kilograms divided by the square of height in meters. The muscle/fat ratio (M/F) was calculated as fat mass divided by muscle mass.

Four cardiometabolic risk factors were used to determine metabolically unhealthy phenotypes in this study: (1) elevated blood pressure (SBP $\geq$ 130 mmHg and/or DBP $\geq$ 85 mmHg) or use of antihypertensive drugs; (2) impaired fasting glucose (IFG): FBG $\geq$ 5.6 mmol/L or use of medications for diabetes; (3) TG $\geq$ 1.7 mmol/L; (4) HDL-C $<$ 1.03 mmol/L in men or $<$ 1.30 mmol/L in women. Metabolically unhealthy was defined as meeting two or more of the cardiometabolic risk factors (17).

Combining the BMI and metabolic phenotypes, participants were divided into four categories: metabolically healthy and normal-weight (MHNW), metabolically unhealthy and normal-weight (MUNW), and metabolically healthy over-weight/obesity (MHO), and metabolically unhealthy overweight/obesity (MUO).

The definition and classification of other covariates including education, income, smoking, drinking, and physical activity were described in our previous publications (18).

Statistical analysis

All analyses were performed using SAS statistical software (Version 9.4; SAS Institute Inc., Cary, NC, USA). A two-tailed *P*-value of <0.05 was considered statistically significant for all analyses.

Descriptive statistics were performed stratified by the four metabolic phenotypes. Summary results were presented as mean $\pm$ standard deviation (SD) for continuous variables and number (percentage, %) for categorical variables. Differences between phenotypic categories were tested using variance analysis or the Chi-square test. The standardized prevalence of MU phenotypes was calculated based on the sex and age distributions of the 2010 China census population.

Covariance analysis was used to compare the continuous metabolic components (BMI, BP, FBG, TG, and HDL-C) and body composition parameters (BFP, VFG, FMI, MMI, and M/F) to adjust for age, sex, and residence. Data were presented as least-square mean $\pm$ standard error (SE).

To examine the association between rural-to-urban migration, age, and BMI with metabolically unhealthy (MU) phenotypes, logistics regression models were used and the following modeling strategies were applied. Model 1 included rural-to-urban migration (Yi migrants vs. Yi farmers) as the variable of interest, model 2 and model 3 included BMI and age plus model 1 in sequence and model 4 adjusted for all covariates of concern (model 3 plus sex, education, income, smoking status, drinking status, occupational physical activity, and leisure-time exercise).

To examine the linear association between body composition parameters with MU phenotypes, restricted cubic spline (RCS) functions with three knots at the 5^{th}, 50^{th}, and 95^{th} quantiles were fitted, in which BFP, VFG, FMI, MMI, and M/F were included as independent variables, and covariates of concern were included for adjustment. Due to the significant difference in body fat and muscle between men and women, all the analysis was performed stratified by sex. We then divided the parameters into three categories according to data distribution and integers, and use logistics regression models to assess the odds ratios (OR) and 95% confidence intervals (CI) of each parameter, with adjustment for covariates.

Finally, to evaluate whether body composition parameters perform better in predicting MU phenotypes than BMI, the receiver-operating characteristic curves (ROC) were used and

TABLE 1 Demographic and metabolic characteristics in participants from the Yi Migrant Study.

	MHNW	MUNW	P	MHO	MUO	*P*
N (%)	1,392 (73.69)	497 (26.31)		557 (47.85)	607 (52.15)	
Sex, *n* (%)			0.2502			0.1700
Men	459 (32.97)	178 (35.81)		177 (31.78)	216 (35.58)	
Women	933 (67.03)	319 (64.19)		380 (68.22)	391 (64.42)	
Age (years)	44.16 ± 13.71	52.72 ± 13.78	<0.0001	44.05 ± 11.97	49.92 ± 11.7	<0.0001
Age (years), n (%)			<0.0001			<0.0001
20∼29	191 (13.72)	22 (4.43)		52 (9.34)	14 (2.31)	
30∼39	398 (28.59)	76 (15.29)		160 (28.73)	111 (18.29)	
40∼49	379 (27.23)	112 (22.54)		195 (35.01)	181 (29.82)	
50∼59	192 (13.79)	104 (20.93)		77 (13.82)	160 (26.36)	
60∼80	232 (16.67)	183 (36.82)		73 (13.11)	141 (23.23)	
Residence, *n* (%)			0.0005			0.0008
Farmers	1,019 (73.2)	323 (64.99)		288 (51.71)	254 (41.85)	
Rural-to-urban migrants	373 (26.8)	174 (35.01)		269 (48.29)	353 (58.15)	
Education, *n* (%)			0.8738			0.1806
Illiterate	838 (60.20)	300 (60.36)		290 (52.06)	287 (47.28)	
Primary or middle school	429 (30.82)	149 (29.98)		168 (30.16)	190 (31.30)	
High school or above	125 (8.98)	48 (9.66)		99 (17.77)	130 (21.42)	
Income (CNY/y), *n* (%)			0.9423			0.6041
<5,000	524 (37.64)	188 (37.83)		116 (20.83)	119 (19.60)	
≥5,000	868 (62.36)	309 (62.17)		441 (79.17)	488 (80.40)	
Smoking status, *n* (%)			0.0935			0.0510
Never	940 (67.53)	311 (62.58)		404 (72.53)	403 (66.39)	
Former	37 (2.66)	19 (3.82)		25 (4.49)	41 (6.76)	
Current	415 (29.81)	167 (33.60)		128 (22.98)	163 (26.85)	
Drinking status, *n* (%)			0.3919			0.0019
Never	940 (67.53)	325 (65.39)		372 (66.79)	364 (59.97)	
Former	93 (6.68)	42 (8.45)		28 (5.03)	62 (10.21)	
Current	359 (25.79)	130 (26.16)		157 (28.19)	181 (29.82)	
Occupational physical activity, *n* (%)			<0.0001			0.0028
Light	505 (36.28)	259 (52.11)		281 (50.45)	366 (60.3)	
Moderate	131 (9.41)	34 (6.84)		70 (12.57)	56 (9.23)	
Heavy	756 (54.31)	204 (41.05)		206 (36.98)	185 (30.48)	
Leisure-time exercise, *n* (%)			<0.0001			<0.0001
Light	1,163 (83.61)	369 (74.25)		397 (71.27)	338 (55.78)	
Moderate	112 (8.05)	48 (9.66)		69 (12.39)	110 (18.15)	
Heavy	116 (8.34)	80 (16.1)		91 (16.34)	158 (26.07)	
BMI (kg/m^2, mean ± SE)*	20.60 ± 0.06	21.54 ± 0.09	<0.0001	26.66 ± 0.11	27.43 ± 0.12	<0.0001
SBP (mmHg, mean ± SE)*	113.44 ± 0.44	123.79 ± 0.69	<0.0001	120.58 ± 0.73	129.60 ± 0.75	<0.0001
DBP (mmHg, mean ± SE)*	69.24 ± 0.30	75.45 ± 0.47	<0.0001	73.17 ± 0.46	79.44 ±0.47	<0.0001
FBG (mmol/L, mean ± SE)*	5.15 ± 0.04	6.09 ± 0.06	<0.0001	5.16 ± 0.10	6.16 ± 0.09	<0.0001
TG (mmol/L, mean ± SE)*	1.07 ± 0.02	1.87 ± 0.03	<0.0001	1.14 ± 0.05	2.19 ± 0.04	<0.0001
HDL (mmol/L mean ± SE)*	1.33 ± 0.01	1.07 ± 0.01	<0.0001	1.27 ± 0.01	1.03 ± 0.01	<0.0001

MHNW, metabolically healthy and normal-weight; MUNW, metabolically unhealthy and normal-weight; MHO, metabolically healthy overweight/obesity; MUO, metabolically unhealthy overweight/obesity; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TG triglyceride; HDL, high-density lipoprotein; SE, standard error. *Covariance analysis adjusted for age, sex, and residence.

the area under the curve (AUC) was calculated, and the Z-tests were used to compare the AUCs.

Results

Characteristics of the participants

The flowchart Supplementary Figure S1 illustrates the study sample selection. Table 1 lists the demographic and metabolic characteristics of the study participants by metabolic phenotypes. A total of 3,053 Yi people aged 20–80 years were enrolled in this study, among whom 1,884 were Yi farmers and 1,169 were rural-to-urban migrants. Of all the participants, 497 (26.31%) out of the 1,889 normal weight and 607 (52.15%) out of the 1,164 overweight/obesity were metabolically unhealthy. The age, occupational physical activity, and leisure-time exercise were significantly different between metabolically healthy and unhealthy participants both in normal weight and overweight/obesity. With the covariance analysis adjusted for age, sex, and residence, all the metabolic factors were found to be significantly different in metabolically healthy and unhealthy participants.

Prevalence of metabolically unhealthy phenotypes in Yi people

Stratified by BMI, the crude prevalence of MUNW and MUO in Yi farmers was significantly lower than in Yi migrants. After the adjustment for age and sex, the standardized prevalence of MUNW in Yi farmers and Yi migrants was still significantly different, while the standardized prevalence of MUO in the two groups was no different (see Supplementary Figures S2, S3).

The results of logistics regression models in Figure 1 show the association between rural-to-urban migration, BMI, and age with metabolically unhealthy phenotypes in Yi people. Without adjustment, Yi migrants were at nearly 1.5-fold higher odds of MU than Yi farmers. With the sequential adjustment for BMI and age, the ORs of rural-to-urban migration gradually decreased and were no more significant. With the full adjustment for demographic characteristics and health-related lifestyle factors, the OR of MU was 1.26 per 1 kg/m^2 of BMI and 1.67 per 10 years of age in normal weight. In overweight/obesity, the ORs were 1.12 and 1.55 for BMI and age, respectively.

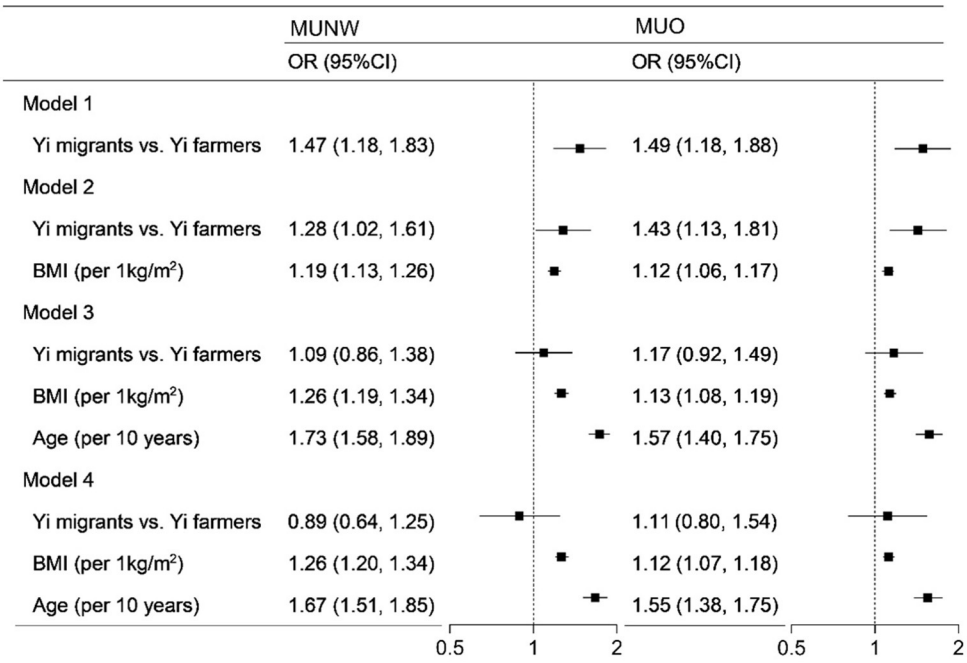

FIGURE 1
Logistics regression analysis of the association between rural-to-urban migration, age, and BMI with metabolically unhealthy phenotype. MUNW, metabolically unhealthy and normal-weight; MUO, metabolically unhealthy overweight/obesity; OR, odds ratio; BMI, body mass index. In the MUNW models, the MHNW was the control, and in the MUO models, the MHO was the control. Model 1 included rural-to-urban migration. Model 2 included model 1 plus BMI. Model 3 included model 2 plus BMI and age. Model 4 included model 3 plus sex, education, income, smoking status, drinking status, occupational physical activity, and leisure-time exercise.

TABLE 2 Covariance analysis of body composition between metabolically healthy and unhealthy participants by sex and BMI.

	BMI < 24 kg/m^2			BMI ≥ 24 kg/m^2		
	MHNW	MUNW	P	MHO	MUO	*P*
Men						
BFP (%)	17.08 ± 0.22	19.24 ± 0.32	<0.0001	25.69 ± 0.22	26.67 ± 0.21	0.0007
FMI (kg/m^2)	3.59 ± 0.06	4.21 ± 0.09	<0.0001	6.92 ± 0.11	7.33 ± 0.11	0.0043
VFG	6.81 ± 0.17	8.48 ± 0.25	<0.0001	13.19 ± 0.14	13.73 ± 0.14	0.0035
MMI (kg/m^2)	16.12 ± 0.05	16.47 ± 0.08	<0.0001	18.74 ± 0.08	18.87 ± 0.07	0.1644
M/F	4.80 ± 0.07	4.15 ± 0.11	<0.0001	2.80 ± 0.03	2.65 ± 0.03	0.0003
Women						
BFP (%)	28.28 ± 0.16	30.50 ± 0.25	<0.0001	37.94 ± 0.18	39.03 ± 0.20	<0.0001
FMI (kg/m^2)	5.91 ± 0.05	6.63 ± 0.08	<0.0001	10.19 ± 0.11	10.85 ± 0.12	<0.0001
VFG	4.09 ± 0.05	4.81 ± 0.09	<0.0001	7.70 ± 0.08	8.16 ± 0.08	<0.0001
MMI (kg/m^2)	13.88 ± 0.03	14.09 ± 0.04	<0.0001	15.52 ± 0.03	15.71 ± 0.04	<0.0001
M/F	2.48 ± 0.02	2.18 ± 0.04	<0.0001	1.56 ± 0.01	1.49 ± 0.01	<0.0001

MHNW, metabolically healthy and normal-weight; MUNW, metabolically unhealthy and normal-weight; MHO, metabolically healthy overweight/obesity; MUO, metabolically unhealthy overweight/obesity; BMI, body mass index; BFP, body fat percentage; FMI, fat mass index; VFG, visceral fat grade; MMI, muscle mass index; M/F, muscle-to-fat ratio. Covariance analysis adjusted for age and residence. Data were shown as mean ± SE (standard error).

Association between body composition with metabolically unhealthy phenotypes

Covariance analysis of body composition between metabolically healthy and unhealthy phenotypes was shown in Table 2. After adjustment for age and residence, the BFP, FMI, VFG, and MMI in metabolically healthy participants were significantly lower than in those metabolically unhealthy both in men and women. The MMI in overweight/obese men was an exception, the difference between the phenotypic groups was not significant. The M/F was found to be significantly higher in MHNW and MHO than in MUNW and MUO.

The RCS analysis shows the linear relationship between body composition parameters and MU phenotypes by sex and BMI (see Figure 2 and Supplementary Figures S4–S7). With the adjustment for demographic characteristics and health-related lifestyle factors, BFP, FMI, VFG, and MMI show positive relationships with MHNW and MUO both in men and women. Figure 2 shows that with the increase in muscle/fat ratio, the ORs of MUNW and MUO descend.

The continuous body fat and muscle parameters were divided into three categories according to tertiles and their association with MU phenotypes was assessed using logistic regression models by sex (see Figure 3). Each of the parameters was evaluated in a multivariable model separately, with the adjustment for demographic characteristics and health-related lifestyle factors. In men participants, the higher BFP and FMI categories were positively associated with MUNW and MUO. VFG and MMI were associated with higher odds of MUNW but not MUO. The higher M/F categories were associated with decreased odds of both MUNW and MUO. In women, all these parameters were significantly associated with MU phenotypes. The association between BFP, FMI, VFG, and MMI was positive while the association of M/F was negative. As the results show the consistent association between body fat and muscle with MU across the BMI categories, we then evaluated the association in the whole population. Supplementary Table S1 shows the strong association between BFP, FMI, VFG, MMI, and M/F with MU phenotype both in men and women.

The ROCs of the body fat and muscle for predicting MU phenotypes by sex and BMI are shown in Figure 4. These parameters did not show a favorable predicting value of MUNW and MUO in men and women (~0.6–0.7). But they have better performance in prediction than BMI except for MMI ($P < 0.05$ for all comparisons). A better performance was found in normal weight than that in overweight/obesity.

Discussion

In this cross-section study, we demonstrated the prevalence of metabolically unhealthy phenotypes and evaluated the association of absolute mass (BFP, FMI, MMI), distribution (VFG), and relative ratio (M/F) of body fat and muscle with the MU phenotypes in normal weight and overweight/obesity in Yi people. Our results verified the consistent effects of body fat and muscle mass on metabolic health across the normal-weight and overweight/obesity categories, and also illustrated the better performance of body composition indicators than BMI in predicting MU phenotypes.

The idea and the clinical implication of MHO have drawn much discussion in the past few years (19). The research on MHO is important because the existence of a metabolic healthy phenotype among obesity might provide an

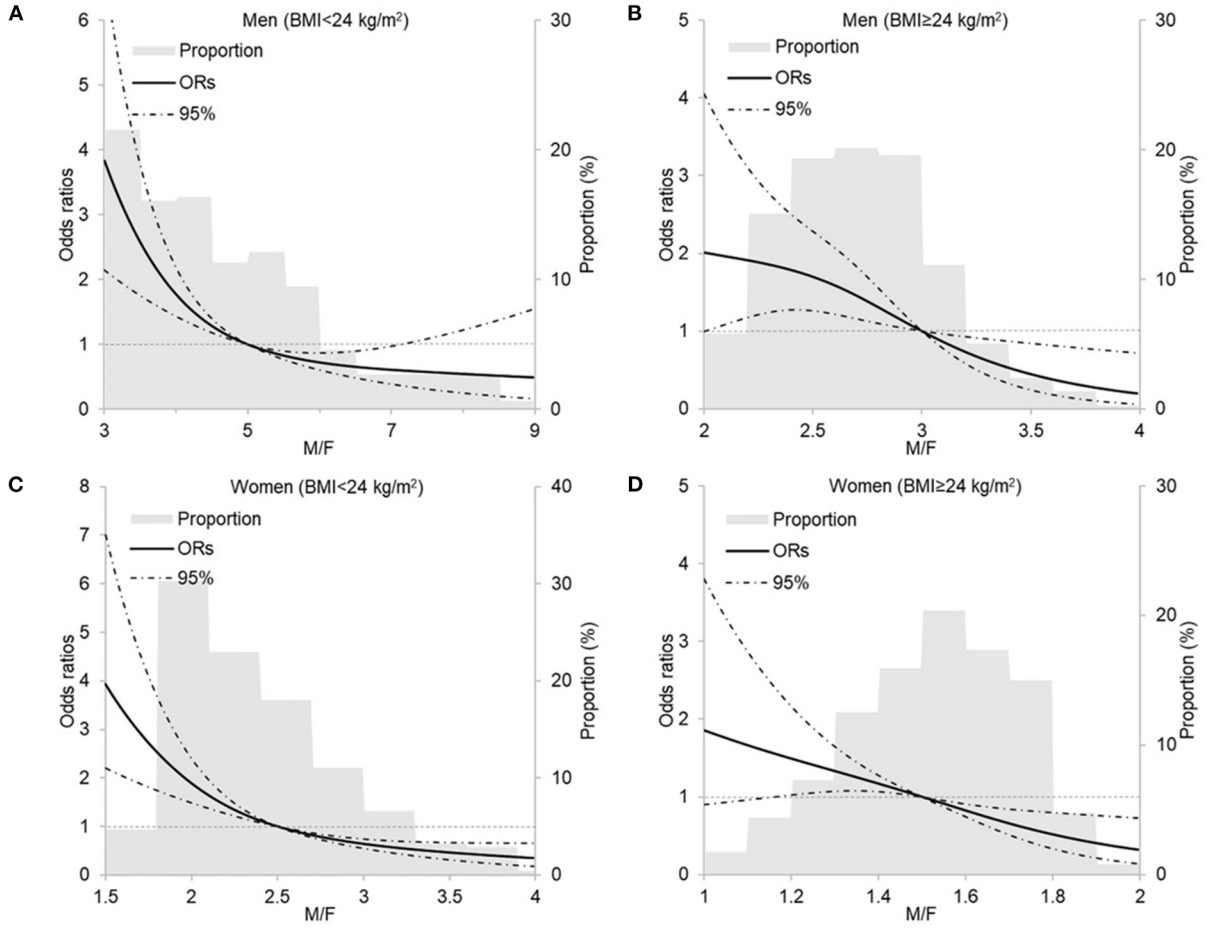

FIGURE 2
Restricted cubic spline analysis of the relationship between M/F with metabolically unhealthy phenotype by sex and BMI. **(A)** men, BMI < 24 kg/m^2, P $_{linear}$ < 0.0001; **(B)** men, BMI ≥ 24 kg/m^2, P $_{linear}$ = 0.7122; **(C)** women, BMI<24 kg/m^2, P $_{linear}$ < 0.0001; **(D)** women, BMI ≥ 24 kg/m^2, P $_{linear}$ = 0.2234. M/F, muscle-to-fat ratio; BMI, body mass index. Models included age, residence, education, income, smoking status, drinking status, occupational physical activity, and leisure-time exercise.

effective way for obesity management and treatment. Due to the lack of standard criteria to define metabolically healthy obesity, the prevalence of MHO can be greatly varied (20, 21). In this study, we assessed that approximately half of the overweight/obese adults in Yi people were metabolically healthy. We also found that although normal-weight, 1/4 to 1/3 of participants had unhealthy metabolic phenotypes. The proportions differed by residence and increased by age and BMI. Even though this study and our previous studies showed that rural-to-urban migrants were at higher risk of metabolic disorders (22–24), the variance could be partly attributed to demographic and increased body weight. The results showed that the disparities between Yi farmers and rural-to-urban migrants were no more significant after adjusting for age and BMI, which indicated the essential role of age and body weight increase on metabolic unhealthy phenotypes.

Along with genetic and lifestyle factors (25, 26), BMI and total adiposity are positively correlated with cardiometabolic disease risk at the population level (27). BMI is a widely used parameter for evaluating obesity, while BMI is unable to distinguish the relative mass of body fat and lean mass, nor estimate the body fat distribution. In this study, using the bioelectrical impedance analysis method, we assessed the mass (BFP and FMI) and distribution (VFG) of body fat. The results indicated that no matter the BMI categories and sex, both mass and distribution of body fat showed a positive relationship with metabolically unhealthy phenotypes. A recent study in China showed coincident results of the relationships (28). Lv et al. (28) measured the body composition indices by quantitative computed tomography and found that total adipose tissue (TAT), visceral adipose tissue (VAT), and VAT/TAT were positively associated with a higher risk of MU phenotypes across BMI categories. In our study, we did not assess the subcutaneous

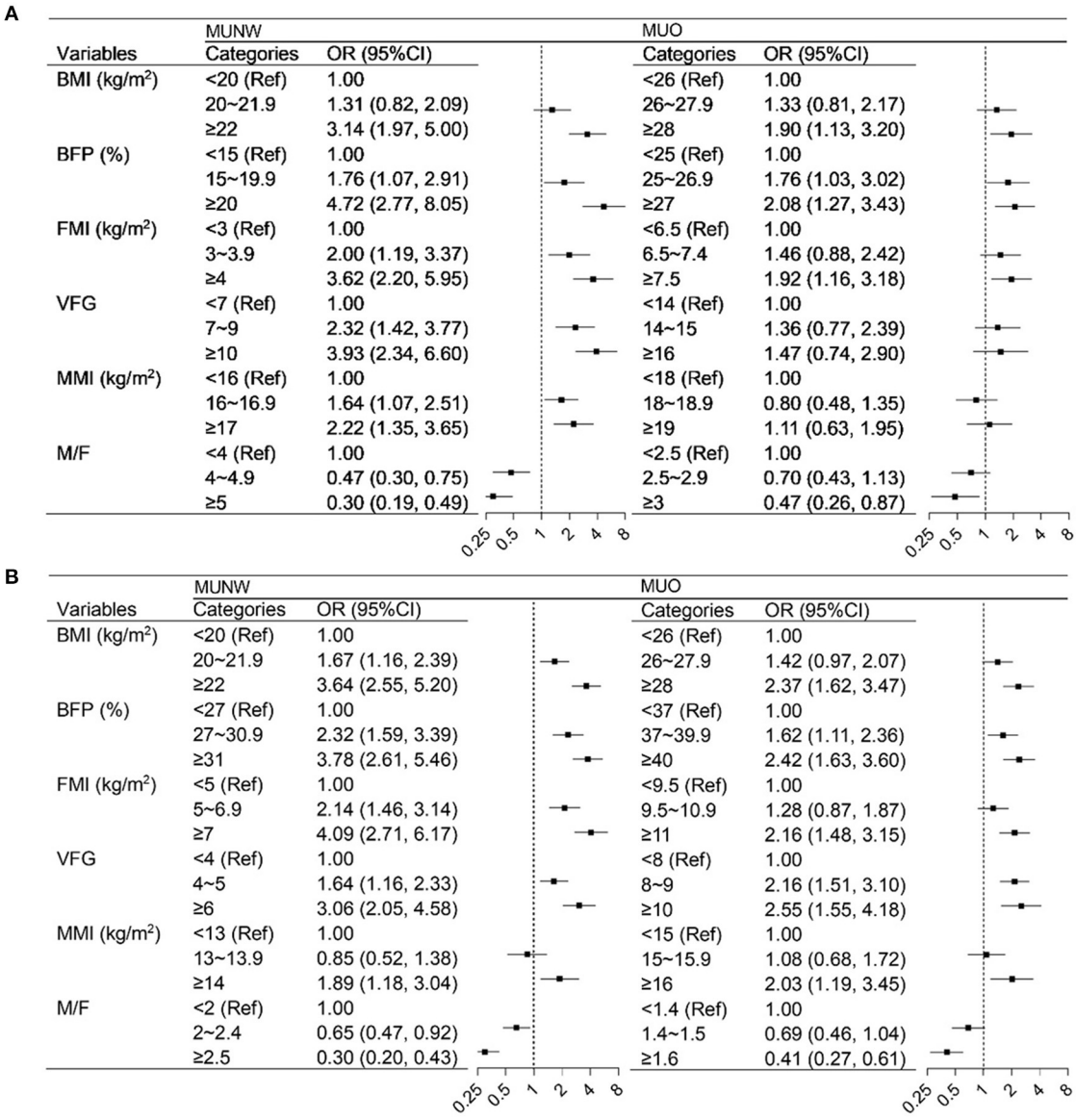

FIGURE 3
Logistics regression analysis of the association between body composition with metabolically unhealthy phenotype by sex and BMI. **(A)** men, **(B)** women. BMI, body mass index; BFP, body fat percentage; FMI, fat mass index; VFG, visceral fat grade; MMI, muscle mass index; M/F, muscle-to-fat ratio. In the MUNW models, the MHNW was the control, and in the MUO models, the MHO was the control. Models were adjusted for age, residence, education, income, smoking status, drinking status, occupational physical activity, and leisure-time exercise.

fat, which showed a significant difference between metabolically healthy and unhealthy participants (28, 29). Another parameter of fat distribution in this study, VFG, showed a strong association with metabolic health and showed better predicting performance than the other indicators. The effect of visceral fat has also been proved in previous studies (30, 31).

Muscle mass was regarded as a protective factor against MU phenotypes in previous studies (32, 33). However, in this study, the highest category of muscle mass index was at increased risk of being metabolically unhealthy. It might be due to the concomitance of muscle and fat mass at the individual level. Kim et al. (32) compared the muscle mass and quality between metabolically healthy and unhealthy phenotypes and concluded that not only muscle mass but also muscle quality are associated with metabolic health. In the present study, we calculated the muscle-to-fat ratio to assess the effect of the relative ratio of muscle and fat on metabolic health. The results showed that increased M/F values were beneficial to a healthier metabolic phenotype. The fat-to-muscle ratio has been introduced as a new anthropometric indicator by several

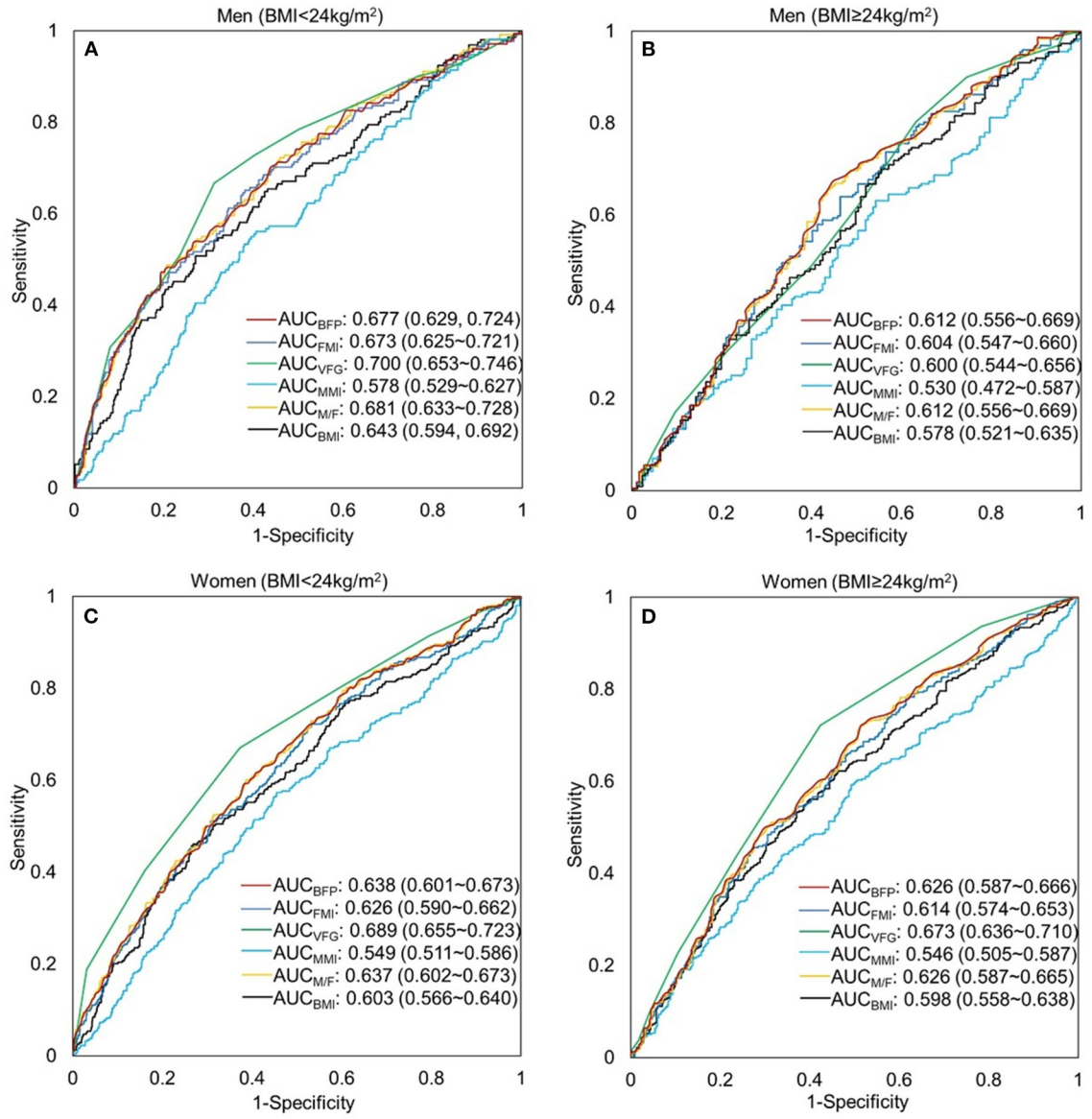

FIGURE 4
Receiver-operating characteristic (ROC) curve of the body composition indicators to predict metabolically unhealthy phenotype by sex and BMI. **(A)** men, BMI < 24 kg/m^2; **(B)** men, BMI ≥ 24 kg/m^2; **(C)** women, BMI<24 kg/m^2; **(D)** women, BMI≥24 kg/m^2. BFP, body fat percentage (%); FMI, fat mass index (kg/m^2); VFG, visceral fat grade; MMI, muscle mass index (kg/m^2); M/F, muscle-to-fat ratio; BMI, body mass index (kg/m^2).

researchers (34–36). However, we regard the muscle-to-fat ratio as a more significant indicator because it provided the approach to reducing metabolic disorder risks by elevating the muscle mass and simultaneously reducing the fat mass. A previous study also suggested that the muscle-to-fat ratio was a better indicator than the fat-to-muscle ratio in quantifying insulin resistance (37).

Our study has a few importance for clinical and public health implications. The study verified the heterogeneity of obesity, and also reminded the implicit cardiometabolic disease risk in the normal-weight. We emphasized the importance of body weight management by monitoring BMI at the population level. Despite the weakness of distinguishing body composition, BMI is an intuitive and economical approach for indicating the exceeded body adipose tissues. The study confirmed the linear positive association between body fat (BFP, FMI, and VFG) with MU phenotypes, and also proved a better performance in predicting MU phenotypes than BMI. The results called for the utilization of body composition measurement for a more precise obesity evaluation and prevention when the health resources

were accessible. Additionally, since 1/4 to 1/3 of the normal-weight participants were metabolically unhealthy, it is essential to maintain a healthy lifestyle and monitor blood pressure, glucose, and lipid metabolism at an appropriate interval in this cardiometabolic low-risk population.

One of the limitations of our study lies in the cross-sectional nature of the study design. The statistical association can be found, but, no causal inference can be reliably established. Secondly, the measurements of the body fat and muscle were dependent on the bioelectrical impedance analysis method, rather than the more accurate dual-energy X-rays absorptiometry (DXA). While the BIA has the advantage of safety, cost, and portability over DXA in the large-scale population-based field survey. And fortunately, BIA shows satisfying agreement with DXA in the real-world setting (38). We also acknowledge that AUCs around 0.6 in our study were not good enough to distinguish MUNW and MHO. More precise indicators were pending to be discovered in the future. Finally, we did not collect data on diet, which is a major contributor to metabolic phenotypes. The lack of dietary data limits the detection of risk factors and the essential adjustment in the multivariable models. Further prospective studies are needed to collect comprehensive data and verify the relationship between body fat and muscle with metabolic trajectories.

Conclusions

In this cross-sectional study, using the population-based data, we observed that the metabolically unhealthy phenotypes were prevalent both in normal-weight and overweight/obesity in Yi people. The results showed the positive association of BFP, FMI, VFG, and the negative association of M/F with MU phenotypes across the BMI categories in Yi people. Measurement of body fat and muscle could provide a more precise approach for the management and prevention of obesity-related cardiometabolic risks.

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 bio Ethical Committee of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. The patients/participants provided their written informed consent to participate in this study.

Author contributions

GS designed the study and supervised data collection. YW analyzed the data, interpreted results, and drafted the manuscript. LP, SW, WY, FY, ZL, and ZY participated in data collection. All authors have approved the submitted versions.

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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/fpubh.2022.1020457/full#supplementary-material

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Trend dynamics of gout prevalence among the Chinese population, 1990-2019: A joinpoint and age-period-cohort analysis

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Background: The burden of gout is increasing worldwide, which places a heavy burden on society and healthcare systems. This study investigates the independent effects of age, period, and cohort on the gout prevalence from 1990 to 2019 in China, compares these effects by gender and then predicts the future burden of gout over the next decade.

Methods: The data were obtained from the Global Burden of Disease (GBD) study in 2019. Joinpoint regression model was employed to calculate the annual percentage change (APC) in gout prevalence, and the age-period-cohort analysis was utilized to estimate the independent effects of age, period, and cohort. ARIMA model was extended to predict the gout epidemic in 2020–2029.

Results: In 2019, there were 16.2 million cases of gout in China, with an age-standardized prevalence rate (ASPR) of 12.3‰ and 3.9‰ in men and women, respectively. During 1990–2019, the ASPR of gout was increasing significantly, with an average APC of 0.9%. The periods of 2014–2017 and 2001–2005 were "joinpoint" for men and women (APC: 6.3 and 5.6%). The age-period-cohort analyses revealed that the relative risk (RR) of developing gout increased with age, peaking at 70–74 years in men ($RR_{age(70-74)} = 162.9$) and 75–79 years in women ($RR_{age(75-79)} = 142.3$). The period effect trended upward, with a more rapid increase in women ($RR_{period(2019)} = 2.31$) than men ($RR_{period(2019)} = 2.23$). The cohort effect generally peaked in the earlier cohort born in 1905–1909 for both sexes. Gout prevalence showed a strong positive correlation with the consumption of meat and aquatic products ($r_{meat} = 0.966$, $r_{aquatic\ products} = 0.953$). Within 2029, the ASPR of gout was projected to be 11.7‰ and 4.0‰ in men and women, respectively.

Conclusion: The prevalence of gout is increasing at an alarming rate in China; thus, it is necessary to provide targeted health education, regular

screening, and accessible urate-lowering therapy healthcare to prevent and protect against gout in China, particularly in older women.

KEYWORDS

gout, hyperuricemia, epidemiology, joinpoint regression, age-period-cohort analysis, ARIMA model

Introduction

Gout is a common metabolic disease, that results from purine metabolism disorders and/or decreased uric acid excretion (1, 2). It is manifested as hyperuricemia, acute gouty arthritis, chronic gouty arthropathy, renal functional impairment, urolithiasis and obstructive uropathy (3). There have been significant changes in people's lifestyles and eating habits recently with the rapid development of the worldwide economy. It resulted in the increased prevalence of gout and hyperuricemia, which places a heavy burden on society and healthcare systems. According to the 2017 global burden of disease (GBD) study, the prevalence of gout worldwide was 7.9‰ and 2.5‰ in men and women, respectively (4). The US National Health and Nutrition Examination Survey reported that the prevalence of gout increased from 2.9% in 1988–1994 to 3.9% in 2007–2008 (5). In the UK, the gout prevalence was 24.9‰ in 2012, with a 64% increase from 1997 to 2012 (6). In Korea, the gout prevalence increased from 3.5‰ in 2007 to 7.6‰ in 2015 (7). However, there is no national epidemiological survey on the prevalence of gout in China, but meta-analyses estimated that the pooled prevalence of gout is 1.1% (8). The risk of gout increases with age; thus, it is more common in aging populations (9). Previous studies revealed that gout and hyperuricemia were associated with genetic background, a high-purine diet (particularly meat and seafood) and consumption of alcohol and sugar-sweetened beverages (10–13). Moreover, early-life exposure to famine was more likely to develop hyperuricemia in adulthood (14, 15). These age, period, and cohort effects together contributed to the high burden of gout. However, to date, no comprehensive study has explored the longitudinal trends of gout from age, period, and cohort dimensions. Age-period-cohort analysis can estimate these effects on disease, especially in the context of complex historical events and environmental factors (16). Therefore, this study aimed to investigate the independent effects of age, period, and cohort on gout from 1990 to 2019 in China, compare these effects by gender using the GBD 2019 data and then predict the future prevalence of gout over the next decade. The study findings will provide a reliable epidemiology basis for further gout prevention, facilitate adequate healthcare resource planning, and avoid disability in the elderly.

Abbreviations: AAPC, average annual percent change; ACF, autocorrelation function; AIC, Akaike information criterion; APC, annual percentage change; ARIMA, autoregressive integrated moving average; ASPR, age-standardized prevalence rate; BMI, body-mass index; CPR, crude prevalence rate; CI, confidence interval; DALYs, disability-adjusted life-years; GBD, global burden of disease; IE, intrinsic estimator; PACF, partial autocorrelation function; RR, relative risk; SDI, sociodemographic index; UI, uncertainty interval; ULT, urate-lowering therapy; WHO, world health organization; YLDs, years lived with disability; YLLs, years of life lost.

Methods

Data sources

Data on gout prevalence during 1990–2019 were retrieved from the world health organization (WHO) GBD estimates (https://ghdx.healthdata.org/). The latest GBD study in 2019 covered 204 countries and territories, providing a standardized and comprehensive estimation of 369 diseases and injuries and 87 risk factors (17, 18). It estimated incidence, prevalence, mortality, years lived with disability (YLDs), years of life lost (YLLs), and disability-adjusted life-years (DALYs) for different age groups, genders, geographical units, time periods, and cause levels. A total of 86,249 sources were used in the GBD estimation process, including censuses, household surveys, civil registration, vital statistics, disease registries, health service use, air pollution monitors, satellite imaging, disease notifications, and other sources (17). In China, GBD data were from the national population consensus, disease surveillance points, maternal and child health surveillance system, chronic disease and risk factor surveillance, as well as surveys (19). The data reliability and population representativeness have been officially recognized, and multiple studies using China GBD data have been published in the top research journals (20, 21). Gout is defined as the presence of characteristic urate crystals in the joint fluid, and/or a tophus proved to contain urate crystals by chemical or polarized light microscopic means, and the presence of six of the twelve gout clinical, laboratory, and X-ray phenomena (22). In this study, we filtered the disease as "gout (B.11.5)," location as "China," metrics as "prevalence" and "DALY," and set other options to select all. The crude prevalence rate (CPR) refers to the actual

prevalence of all-age populations, and the age-standardized prevalence rate (ASPR) was based on GBD 2019 global age-standard population. Gout prevalence was expressed as a per thousand (‰). Since gout is a non-fatal disease, the estimates of mortality and YLLs were inaccessible in GBD 2019 database. We used the DALYs to measure the healthy life lost due to gout. Based on 10,000 iterations, the uncertainty interval (UI) is defined by the 2.5th and 97.5th draw values, representing the 2.5th and 97.5th percentiles. The sociodemographic index (SDI) of China was also extracted from GBD website (https://vizhub.healthdata.org/gbd-results/). SDI summarizes the level of national development, which is closely related to the resident's health status. The value range of SDI is (0, 1), with higher scores representing higher per capita income and education levels, but lower fertility rates. Moreover, we extracted the national per capita consumption of major foods, including grains, fresh vegetables, vegetable oil, meat (pork, beef, mutton, poultry) and aquatic products, from China Statistical Yearbook 1990–2019 (http://www.stats.gov.cn/tjsj/ndsj/).

Statistical analysis

Joinpoint analysis was applied to estimate the trends of gout prevalence from 1990 to 2019. As proposed by Kim in 2000 (23), it can divide the longitudinal variations into different segments by piecewise regression and identify the segment trends with statistical significance. Regression fitting was performed on the natural logarithm of the prevalence and mortality rate in different segments, and then the annual percentage change (APC) and its 95% confidence interval (CI) were calculated for each period. The global trend was described by average annual percent change (AAPC). APC and AAPC were considered statistically significant by non-overlapping 95% CI and $p < 0.05$ compared to the null hypothesis of having no variation.

An age-period-cohort model was applied to assess the impact of age, period, and cohort effects on health outcomes (24). The age effect refers to the differences in gout prevalence across age groups caused by aging-related factors. The period effect refers to the influence of human factors on gout prevalence, such as diagnosis development. The cohort effect refers to the change in gout prevalence due to different exposures to risk factors among people of different birth years. Age and period were first divided into 5-year continuous intervals from 15–19 to 85–89, and from 1994–1999 to 2014–2019, respectively. Twenty birth cohorts were summarized from 1905–1909 to 2000–2004. The intrinsic estimator (IE) method was integrated into the age–period–cohort model to estimate the net effects for three dimensions (25). The relative risk (RR) and 95% CI were then calculated based on the estimated coefficients to quantify the effects of age, period, and cohort on gout prevalence. The first groups of 15–19 years, 1994–1999 period, and 1905–1909 birth cohort were defined as the reference groups. Moreover, we applied the Pearson correlation coefficient (r) to evaluate the linear association of gout prevalence and SDI and the national per capita consumption of major foods.

The autoregressive integrated moving average (ARIMA) model was applied to predict future trends of gout prevalence over the next decade. The model expression is ARMIA (p, d, q), where p is the autoregressive order, d is the number of differences, and q is the moving average order (26). The difference method was employed to transform the non–stationary data into stationary data. The autocorrelation function (ACF) and the partial autocorrelation function (PACF) were then plotted to check the stationary of the sequence after differencing, and *auto.arima()* was used to establish the optimal model according to the Akaike information criterion (AIC) value. The *auto.arima()* function is suitable for different ARIMA models of univariate time series data, searches the models according to the provided constraint order, and determines the optimal model (27, 28). The normality of model residuals was tested through QQ plots, ACF and PACF plots. The Ljung–Box test for white noise was used to test whether the residuals have serial correlations. The predictive capacity of ARIMA models was estimated by using mean error (ME), root mean squared error (RMSE), mean absolute error (MAE), mean percentage error (MPE), mean absolute percentage error (MAPE), and mean absolute scaled error (MASE).

The joinpoint analysis was run in the joinpoint regression 4.9 software (Statistical Research and Applications Branch, National Cancer Institute, USA). The age–period–cohort model was established in the Stata 14.0 software (StataCorp LP, TX, UA). The ARIMA analysis and plot drawing were mainly conducted in the R 4.1 software (R core team) using the packages of "forecast," "tseries" and "ggplot2." A p–value of < 0.05 was considered statistically significant.

Results

Description analysis of gout prevalence in China

In 2019, there were 16.2 million (95% UI: 12.8–20.4) cases of gout in China, with men and women patients accounting for 12.1 million (95% UI: 9.6–15.2) and 4.1 million (95% UI: 3.2–5.2), respectively. The CPR and ASPR trends from 1990 to 2017 for gout among Chinese adults were presented in Figure 1 and Supplementary Table 1. In general, the CPR and ASPR of gout were both higher in men than in women. CPR in men increased from 7.38‰ in 1990 to 17.76‰ in 2017 and then decreased to 16.70‰ in 2019. CPR in women gradually increased from 2.38‰ to 5.81‰ during the same period in China. The trends of ASPR by gender were similar to that of CPR but with mild fluctuation. Over the past thirty years, the

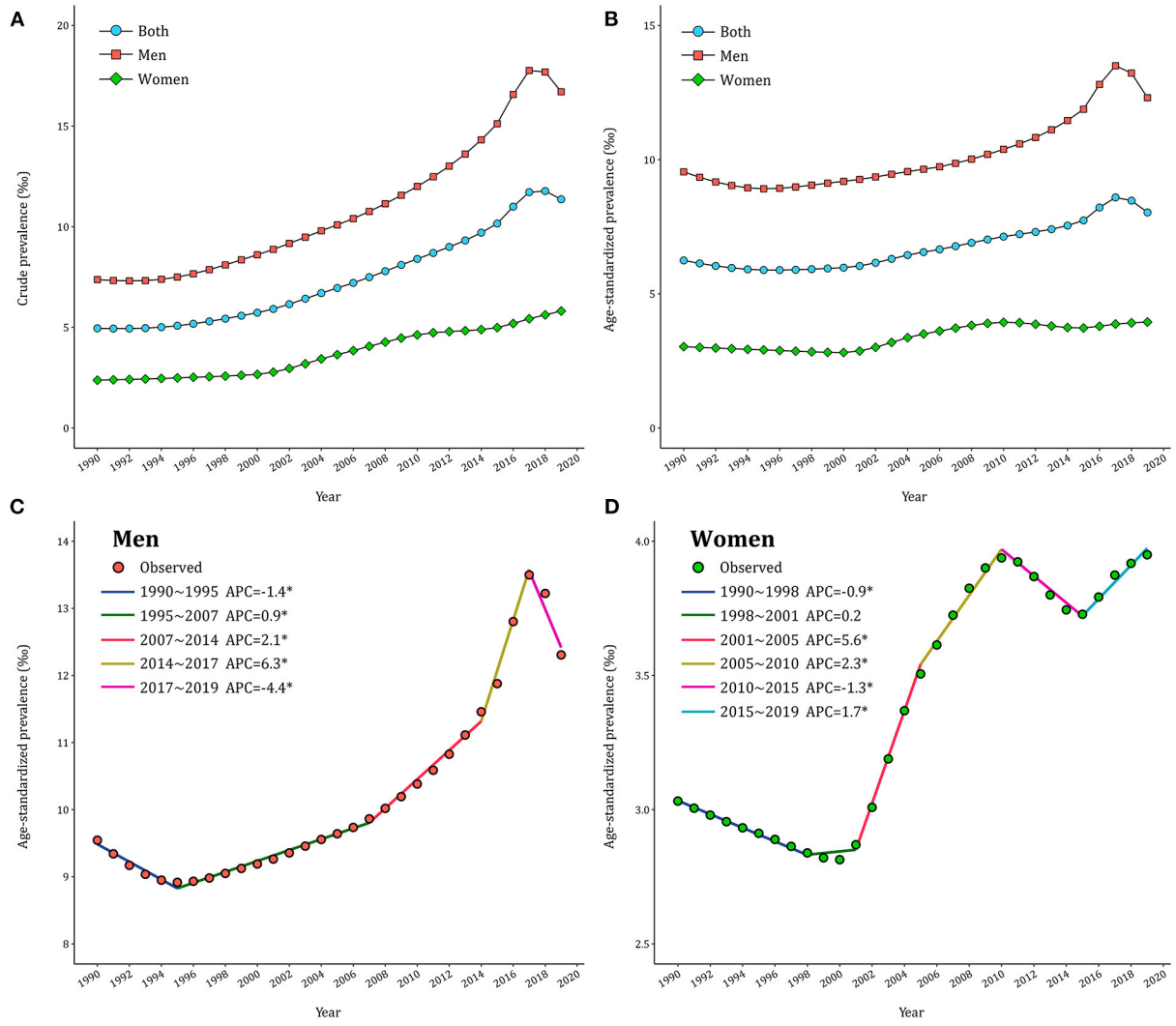

FIGURE 1
Trends of gout prevalence in China during 1990–2019. **(A)** Crude prevalence of gout. **(B)** Age–standardized prevalence of gout. **(C,D)** APCs of the age–standardized prevalence of gout for men and women.

crude DALYs of gout increased by 124% in men and 141% in women, but the age–adjusted DALYs showed a less marked increase (Supplementary Figure 1).

Temporal trends of gout prevalence in China

The joinpoint models were applied to divide the temporal trends of gout prevalence into several segments and estimate the APCs by gender. As displayed in Figure 1C, ASPR of gout in men declined first ($APC_{1990-1995} = -1.4\%$), then significantly increased ($APC_{1995-2007} = 0.9\%$ and $APC_{2007-2014} = 2.1\%$), peaking in 2017 ($APC_{2014-2017} = 6.3\%$) and then decreased thereafter ($APC_{2017-2019} = -4.4\%$). By contrast, ASPR underwent three significant increases in women ($APC_{2001-2005} = 5.6\%$, $APC_{2005-2010} = 2.3\%$ and $APC_{2015-2019} = 1.7\%$) and two significant declines ($APC_{1990-1998} = -0.9\%$ and $APC_{2010-2015} = -1.3\%$ in 2010–2015) in Figure 1D. Over the entire study period, AAPC was 0.9% (95% CI: 0.8%−1.1%) in men and 0.9% (95% CI: 0.8%−1.0%) in women. Further analyses in CPR of gout across gender exhibited similar patterns (Supplementary Table 2).

Age, period, and cohort trends of gout prevalence

The age–specific gout prevalence was approximated by a linear distribution in different periods (Figures 2A,B). It

accelerated with age, reaching a peak in the group aged 85–89 years, and this age pattern was consistent across both men and women. The period variations of gout prevalence in men were relatively stable in the younger groups and trended upward over the period in the 85–89 age group (Figure 2C). In women, by contrast, a period–specific trend with three inflection points became apparent after the groups aged 60–64 years (Figure 2D). The birth cohort of each age group revealed that the gout prevalence in the early period was lower than that in the later period (Figures 2E,F). For men in the 85–89 age group, the gout prevalence increased with the birth cohorts. For women in the 85–89 age group, the gout prevalence increased first and then decreased with the birth cohort. Starting in the 35–39 age group, such trends were leveling–off.

The age, period, and cohort effects on gout prevalence

RRs of age, period, and cohort effects of gout prevalence for both sexes were presented in Figure 3 and Supplementary Tables 3–5. After controlling for period and cohort factors, age was significantly associated with gout prevalence, with the risk increasing with advancing age and then remaining stable thereafter. Regarding the reference group of 15–19 years, RR values peaked at 70–74 years in men ($RR_{age(70-74)} = 162.9$, 95% CI: 124.6–213.4) and 75–79 years in women ($RR_{age(75-79)} = 142.3$, 95% CI: 96.1–210.6). The period effect of gout prevalence for both men and women trended upward between 1994 and 2019, with a faster increase in women ($RR_{period(2019)} = 2.31$, 95% CI: 2.30–2.31) than men ($RR_{period(2019)} = 2.23$, 95% CI: 2.23–2.24). The cohort effect of gout prevalence showed a significant downward trend and was slightly lower in men than women. The early birth cohort had a greater impact on the risk of gout ($RR_{cohort(1910-1914)} = 0.91$, 95% CI: 0.90–0.92), which continued to decline in the recent birth cohorts ($RR_{cohort(2000-2004)} = 0.12$, 95% CI: 0.05–0.27).

Gout burden and its associated factors

High body–mass index (BMI) and kidney dysfunction were identified as major risk factors for gout, contributing to 22.5% and 9.1% of gout DALYs, in 2019. As shown in Figure 4, the gout prevalence was positively correlated with SDI, meat, aquatic products, and oil consumption, with a correlation coefficient ranging from 0.903 to 0.966. In contrast, the gout prevalence showed a negative correlation with the consumption of grain and vegetables ($r_{grain} = -0.887$, $r_{vegatables} = -0.812$).

Predicted trends of gout prevalence in 2020–2029

The gout prevalence data from 1990 to 2019 was then applied to quantitatively predict future trends over the next decade in ARIMA models. As presented in Supplementary Figures 2A–C, 3, the longitudinal ASPRs of gout were non–stationary; therefore, first–order differencing was performed to stabilize the variance of the series (Supplementary Figures 2D,F, 4). The differential time series were further verified as non–random series through the white noise test (Supplementary Table 6). Filtered by the *auto.arima()* function, the optimized parameters for ARIMA model were chosen to be (2,1,1) for both men and women, with AICs of 250.78 and 130.02, respectively. Q–Q plots, ACF and PACF plots revealed that the residual error was normally distributed (Supplementary Figure 5). The Ljung–Box test confirmed that ARIMA models were robust and the residuals were white noise ($\chi^2 = 0.040/0.004$, $p = 0.842/0.949$). The calibration plots suggested that the true value agreed well with the predicted value (Supplementary Figure 6). ARIMA (2,1,1) models were then used to predict ASPR of gout from 2020 to 2029 by gender, as displayed in Figure 5 and Supplementary Figure 7. ASPR in men is expected to increase from 11.47‰ in 2020 to 11.95‰ in 2025, and then decrease to 11.67‰ in 2029, whereas ASPR in women will remain stable in the next decade, ranging from 3.97 to 4.02‰. The predictive capacity of ARIMA models was listed in Supplementary Table 7.

Discussion

The present study analyzed the temporal trends in gout prevalence in China from 1990 to 2019 and found that it increased significantly over the past three decades. In 2019, there were 16.2 million (95% UI: 12.8–20.4) cases of gout in China, with an ASPR of 12.31‰ (men) and 3.95‰ (women). It was higher than the global estimates (10.31/3.03‰) and other Asian countries (Japan: 11.91/2.72‰, South Korea: 11.60/2.62‰) (29). The increased gout burden is correlated with lifestyle changes, increased life expectancy, and a high prevalence of obesity and other comorbidities (30, 31). Asymptomatic hyperuricemia is a "subclinical or hidden" stage of gout, and its early prevention and treatment are usually neglected (32). Our previous meta–analysis estimated the prevalence of hyperuricemia in China to be 16.4% (33). When uric acid crystals involved musculoskeletal structures, gout can affect patients' ability to perform normal self–care activities, recreational and social activities and work (34, 35). Furthermore, gout–related disability is an underestimated and understudied problem, and there were almost 1.3 million YLDs due to gout in 2017 (36), incurring substantially greater direct and indirect costs ($172 to $6179 per capita) (37). Gout is more prevalent

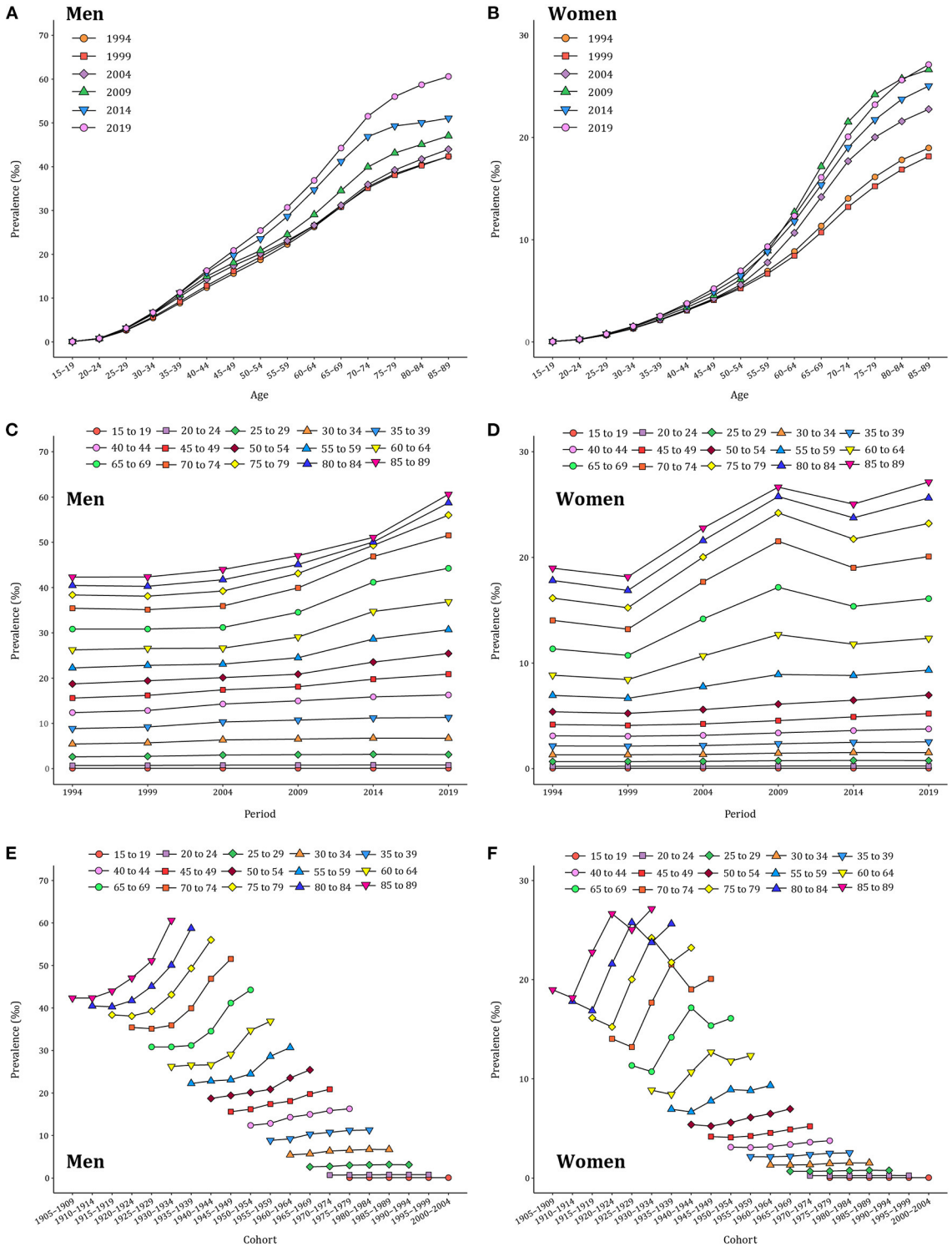

FIGURE 2
Long–term trends of age–specific, period–based, and cohort–based variation of gout prevalence in China during 1990–2019. **(A,B)** Age–specific prevalence of gout for men and women. **(C,D)** Period–based prevalence of gout for men and women. **(E,F)** Cohort–based prevalence of gout for men and women.

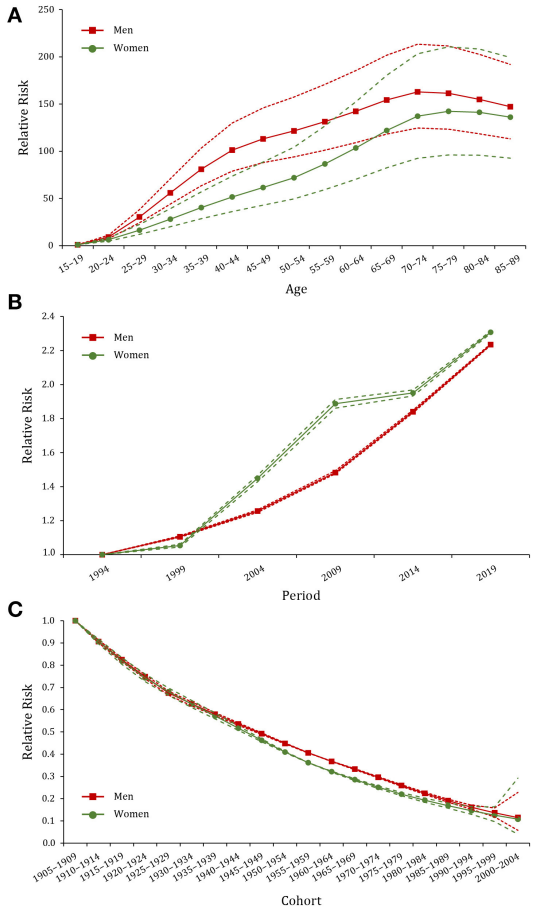

FIGURE 3
Age, period, and cohort effects on the prevalence of gout in China during 1990–2019. **(A–C)** Age, period, and cohort effects, respectively; the red dot line represents the 95% confidence interval (CI) for men, and the green dot line represents the 95% CI for women.

in men than women. This sex disparity was due to estrogen and progesterone promoting uric acid excretion (38), as well as men's greater exposure to risk factors such as smoking, alcohol consumption and obesity (12, 39). Joinpoint analysis revealed that the gout ASPR increased in men and women initially from 2000 to 2010. Subsequently, the prevalence in men began to decline but continued to rise in women and peaked in 2019. The National Health and Nutrition Examination Survey (NHANES) in the United States also showed that the annual increase of gout was only observed in women (from 2.0% in 2007 to 2.7% in 2015) rather than men (40). This may be because women's exposure to unhealthy diets and lifestyles is starting to approach that of men. Although effective and low–cost urate–lowering therapy (ULT) has been available for decades, gender inequities exist in gout management (41). Federica et al. found that women were less likely to participate in clinical trials of serum uric acid lowering drugs (42). Language barriers, disparities in socioeconomic position and cultural practices that limited their participation in ULT need to be acknowledged. In addition, physician attitudes, communication styles and perceptions of patient distrust have been also shown to have a negative impact. Therefore, we should pay more attention to the prevention and control of gout in women, the ever–growing group of gout patients.

Due to the intricate interaction among age, period, and cohort factors, we applied the age–time–cohort model and IE algorithm to quantify their net effects on gout prevalence. It was observed that the age effect increased from the youngest age group to the 70–74 age group and subsequently remained stable. Aging can cause renal morphologic and pathophysiologic dysfunction, resulting in impaired uric acid excretion and elevated serum levels. Moreover, elderly people usually suffer from multiple diseases, such as hypertension, diabetes and cardiovascular diseases, which are involved in gout development (43). Stratified by gender, RRs of the age effect increased faster in women than men in the 55 ∼ age group, suggesting that the age effect may be intensified in older women. The decline in estrogen function is the main cause of gout for postmenopausal women. The hyperuricemia and inflammation of gout put women, but not men, at higher risk for osteoporotic fractures (44). Previous studies proved that postmenopausal hormone therapy modestly reduces the risk of gout (45).

In addition, the period effect on gout prevalence remarkably increased in China, which may be explained by the changes in dietary patterns and the increasing obese population. Over the past decades, consumption of energy/fat–dense foods like meat, seafood, alcohol and sugar beverages has increased significantly, with dietary patterns switching from a predominantly plant–based diet to a Western–style diet high in fat and animal–based foods (46). These dietary factors can increase urate concentrations, hence the risk of gout occurrence and progression (47). We also found that gout prevalence showed a strong positive correlation with the consumption of meat and aquatic products ($r_{meat} = 0.966$, $r_{aquatic\ products} = 0.953$). A prospective cohort study demonstrated that a DASH–style diet (high intake of fruits, vegetables, nuts and legumes, low–fat dairy and whole grains, and low intake of sodium, sweetened beverages, red and processed meats) could reduce uric acid levels in individuals with hyperuricemia, thereby reducing the risk of gout (48, 49). Obesity, especially abdominal obesity, is also closely linked to gout. According to the China Chronic Disease and Risk Factors Surveillance, the BMI levels rose from 22.7 kg/m^2 in 2004 to 24.4 kg/m^2 in 2018 and obesity prevalence from 3.1% to 8.1% (50). Available evidence supports weight loss in overweight/obese gout patients to reduce serum uric acid and gout (51). This study determined high BMI as the major risk factor for gout, contributing to 22.5% of the gout DALYs in 2019. The increasing prevalence of gout makes it

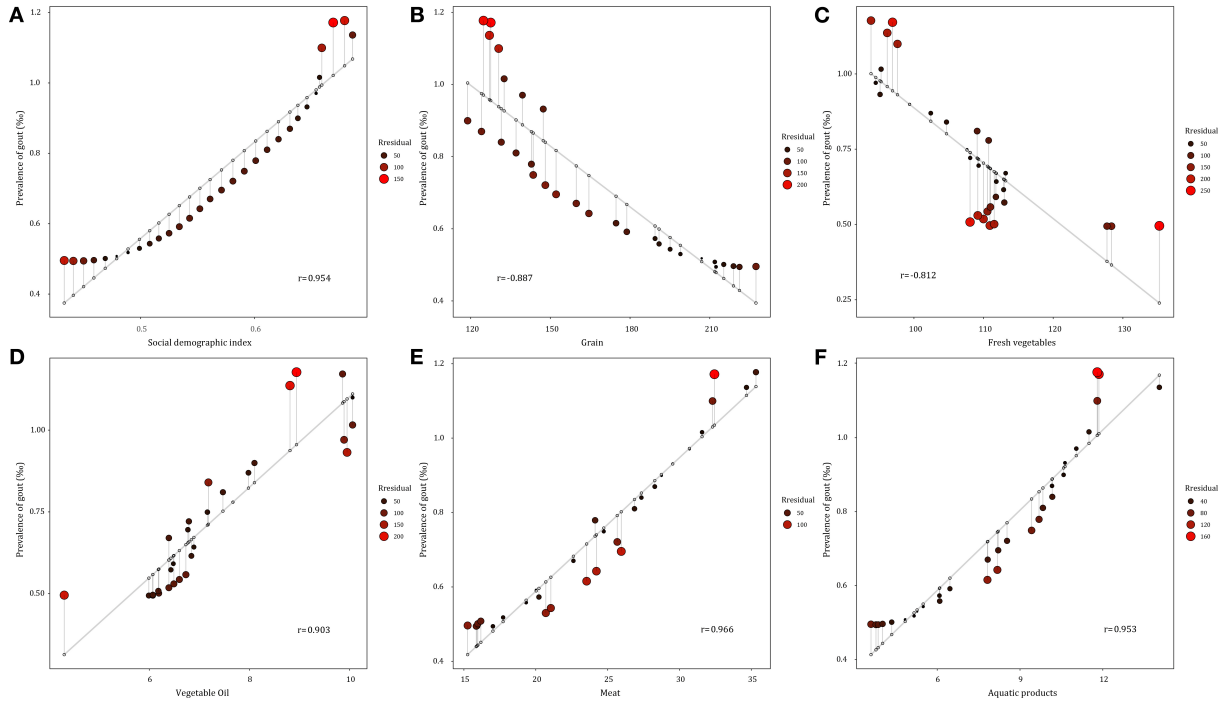

FIGURE 4
Correlation between gout prevalence and social demographic index and major food consumption from 1990 to 2019 in China. **(A)** Social demographic index. **(B)** Grain consumption. **(C)** Fresh vegetable consumption. **(D)** Vegetable oil consumption. **(E)** Meat consumption. **(F)** Aquatic products consumption.

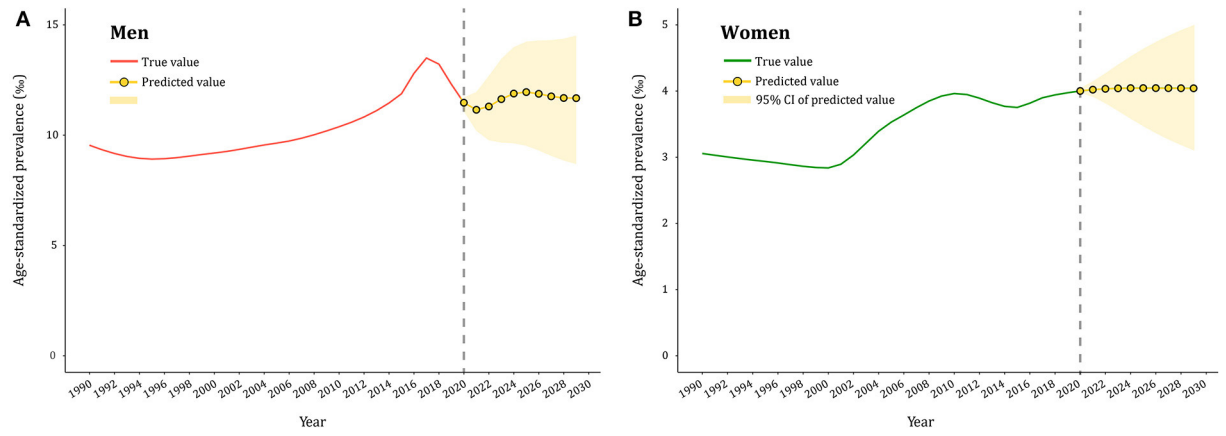

FIGURE 5
Predicted trends of gout prevalence in China over 10 years (2020–2029). **(A,B)** the red and green lines represent the true trend of age–standardized prevalence of gout during 1990–2019 for men and women; the yellow lines represent the predicted trend and the light–yellow shaded regions represent the 95% confidence interval of predicted values; the gray dot vertical line split data into true value (1990–2019) and predicted value [2020–2029)].

necessary to strengthen preventive actions, such as promoting a rational diet, appropriate exercise, and controlling alcohol and tobacco consumption.

The cohort effect represents early socioeconomic, behavioral, and environmental factors on the risk of gout. RRs of the cohort effect initially peaked in the earliest birth cohort (1905–1909) and exhibited a downward trend until the most recent birth cohort (2000–2004). This decline was the result of China's socioeconomic development and medical advancement. However, the gout prevalence still increased

over time in the cohort from 1905–1090 to 1949–1950, mainly because of social upheaval, poor nutrition intake, and healthcare conditions before establishing the People's Republic of China in 1949. Since the reform and opening–up policy in China, the prevalence has become stable after the 35–39 age group, whose earliest birth cohort was recorded in 1955–1959. In addition, health awareness has improved among the younger generations, and they devote more attention to chronic disease prevention.

Predicting the gout epidemic is useful for informing the disease burden and assisting in decision–making for health resource allocation. In 2029, ASPR of gout is expected to rise to 11.7‰ and 4.0‰ in men and women, respectively. However, gout is often misdiagnosed as a sprain or infection at the first presentation, or the diagnosis is delayed in many cases (52). Furthermore, the proportion of gout patients receiving ULT was also low, with an overall adherence rate of 47% (53). Studies in China revealed that the rate of ≥80% ULT adherence ranged from 9.6% to 21.9% (54, 55). Absent or delayed use of ULT increases the risk of gout attacks and joint inflammation and destruction, as well as the long–term deleterious effects on the cardiovascular and renal systems. Accordingly, a comprehensive strategy, including risk factor prevention, regular monitoring of uric acid levels, and the popularity of ULT, is necessary to slow the gout epidemic and achieve better health outcomes for gout patients.

This study has several limitations. First, the gout data were extracted from GBD 2019, which had varied data sources including surveillance system data and individual–level survey data, implying that the selection bias could affect the certainty of gout burden estimates. Second, the ASPR of gout was calculated using the GBD standard population rather than the Chinese population. While it is favorable for horizontal comparison with other countries, it might underestimate the actual prevalence due to the larger proportion of the aging population in China. Third, due to the unavailability of provincial data, this study did not have a geographic description of the burden of gout. It prevents us from validating the ARIMA predictions at the provincial level. Our previous meta–analysis found that hyperuricemia prevalence was higher in southern and southwestern China, possibly attributed to regional eating habits (seafood and hot pot) (33). Lastly, the age–period–cohort model was analyzed at the population level, so that it may be subject to ecological fallacy.

Conclusion

The prevalence of gout in Chinese men and women increased at an alarming rate from 1990 to 2019, with age being the critical factor affecting the gout epidemic. China's population growth and aging also amplified this effect, and there was a persistent impact of an unhealthy diet and obesity on gout prevalence in China. Therefore, it is necessary to provide targeted health education, regular screening and accessible ULT healthcare to reduce the gout disease burden. Moreover, preventing and protecting against gout should be reinforced for older women.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here the Global Burden of Disease study 2019 is an open–access resource, data are available at https://vizhub.healthdata.org/gbd-results/.

Author contributions

YL and XD contributed to the conception and design of the work. BZ, YW, and WZ contributed to the acquisition, analysis, and interpretation of data for the work. SJ, ZS, and HZ participated in data management. BZ, YW, and YL drafted the manuscript. XZ, XD, and YL critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1008598/full#supplementary-material

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Comparison of three nutritional screening tools for detecting sarcopenia in patients with maintenance hemodialysis

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Background: Malnutrition, dynapenia, and sarcopenia are prevalent conditions among patients with maintenance hemodialysis (MHD). They are related to numerous adverse health outcomes. The aim of this study was to compare the effect of three nutritional screening tools on predicting the risk of dynapenia and sarcopenia in patients with MHD.

Methods: From July 2020 to April 2021, a total of 849 patients with MHD were enrolled at seven different healthcare facilities in Shanghai, China in this multi-center cross-sectional study. Geriatric nutritional risk index (GNRI), malnutrition inflammation score (MIS), and creatinine (Cr) index were used for nutritional assessment. The cutoff values of muscle mass and strength to define dynapenia, pre-sarcopenia, and sarcopenia were based on the consensus by the Asia Working Group of Sarcopenia in 2019.

Results: Among 849, almost 60% were malnourished with the majority suffering from dynapenia (27.7%), followed by sarcopenia (22.7%), and pre-sarcopenia (6.2%).The area under the receiver–operating characteristic curve for GNRI was 0.722 [95% confidence interval (CI) = 0.684–0.760] and 0.723 (95% CI = 0.663–0.783) in predicting sarcopenia and pre-sarcopenia. The GNRI [odds ratio (OR) =6.28, 95% CI: 4.05–9.73], MIS (OR =1.91, 95% CI: 1.31–2.78), and the Cr index (OR =2.73, 95% CI: 1.71–4.34) were all significantly associated with the risk of sarcopenia. More importantly, the sarcopenia predictability of the GNRI appears greater than the MIS and Cr index, while MIS was similar to the Cr index. Similarly, the superiority of GNRI prediction was also found in pre-sarcopenia, but not in dynapenia.

Conclusion: All the three nutritional screening tools were significantly associated with an increased risk of sarcopenia. The sarcopenia predictability of the GNRI was greater than the MIS and Cr index.

KEYWORDS

dynapenia, geriatric nutritional risk index, hemodialysis, nutritional screening tool, pre-sarcopenia, sarcopenia

Introduction

Sarcopenia is a clinical condition characterized by an aged-related decrease in skeletal muscle mass and low muscle strength and/or physical performance (1). Muscle mass and muscle strength have been discussed together since 2010 when the European Working Group on Sarcopenia in Older People (EWGSOP) defined pre-sarcopenia and sarcopenia (2). Evidence is accumulating that both low muscle quantity and quality might relate to adverse clinical outcomes, including increased hospitalization, poorer quality of life, and increased mortality in patients with maintenance hemodialysis (MHD) (3–5). Interestingly, however, several recent studies suggest that the loss of muscle strength without low muscle mass as dynapenia, is an important risk for mortality in patients with MHD (6). Hence, there is a great interest in correctly differentiating the loss of muscle mass from strength. In fact, this deviation between the association of muscle mass with muscle strength and clinical adverse outcomes is a matter of interest and debate in the international scientific community (7, 8).

The patients with MHD are related to a range of causes of muscle mass and function, such as decreased physical activity and nutrition intake, hormone dysfunction, and chronic inflammation (9). Among these, malnutrition is a significant risk factor for the development of sarcopenia (10). A wide variety of nutritional screening markers and tools are available to assess the nutritional status of patients with MHD (11), such as body mass index (BMI), serum creatinine (Cr), and serum albumin. These markers are insufficient when used alone, so many clinicians usually use them in combination in clinical practice. The malnutrition–inflammation score (MIS) (12) is a valid diagnostic tool for evaluating nutritional status in patients with MHD. However, the MIS is time-consuming and cumbersome, because it requires a subjective evaluation. Indeed, several simple and completely objective nutritional screening tools can also be used to evaluate the nutritional risk of patients with MHD. The Cr index and geriatric nutritional risk index (GNRI) are recommended as the simple risk indexes for nutritional status assessment among patients with MHD (13). These two markers are objective and do not need special skills or experience but instead can easily calculated from the results of routine blood tests obtained at the bedside. Previous reports have reported the association between the nutritional marks and sarcopenia among the hospitalized older adults (14) and kidney transplant recipients (15). The conclusions are inconsistent as different nutritional indicators were used. To date, there are no research on the association between dynapenia and nutritional status in the dialysis population, so further study is needed.

In addition, few studies have compared the usefulness and predictive ability of these three nutritional indexes (MIS, GNRI, and Cr index) regarding dynapenia, pre-sarcopenia and sarcopenia in this population. It is clinically important to directly compare these three indexes in the hemodialysis population comprising a relatively large number of patients with MHD. Thus, the aim of this study was to evaluate the ability of the three nutritional screening tools (MIS, GNRI, and Cr index) to identify dynapenia, pre-sarcopenia, and sarcopenia in patients with MHD.

Methods

Subjects

A multi-center cross-sectional study was conducted in Shanghai between July 2020 and April 2021and included seven hemodialysis centers. The inclusion were (1) age $\geq$ 18 years, (2) receiving maintenance hemodialysis for at least 3 months, (3) able to provide informed consent, while the exclusion criteria included (1) missing data on diagnostic criteria for sarcopenia, (2) did not complete date on the nutrition assessments, (3) clinical instability (presented with an infection, pulmonary edema, amputated limb, or malignancy), and (4) unable to communicate with the researchers or refusal to participate in this study. We excluded patients who: (1) refused to undergo body composition examinations ($n = 11$); (2) unable to complete the handgrip strength test because of hand disability ($n = 2$); (3) unable to walk due to disability or complete wheelchair dependence and unable to perform gait speed tests ($n = 6$); and 4) absence of results of relevant nutritional blood tests ($n = 12$). The remaining 849 patients were included in the final analytic sample (Figure 1). The Ethics Committee of Shanghai University of Medicine and Health Sciences approved this study (number 2019-A4-2621-19-201001-03-12010419771113601X), and all of the patients provided written informed consent to take part in this study. These methods were implemented in accordance with the principles of the Declaration of Helsinki.

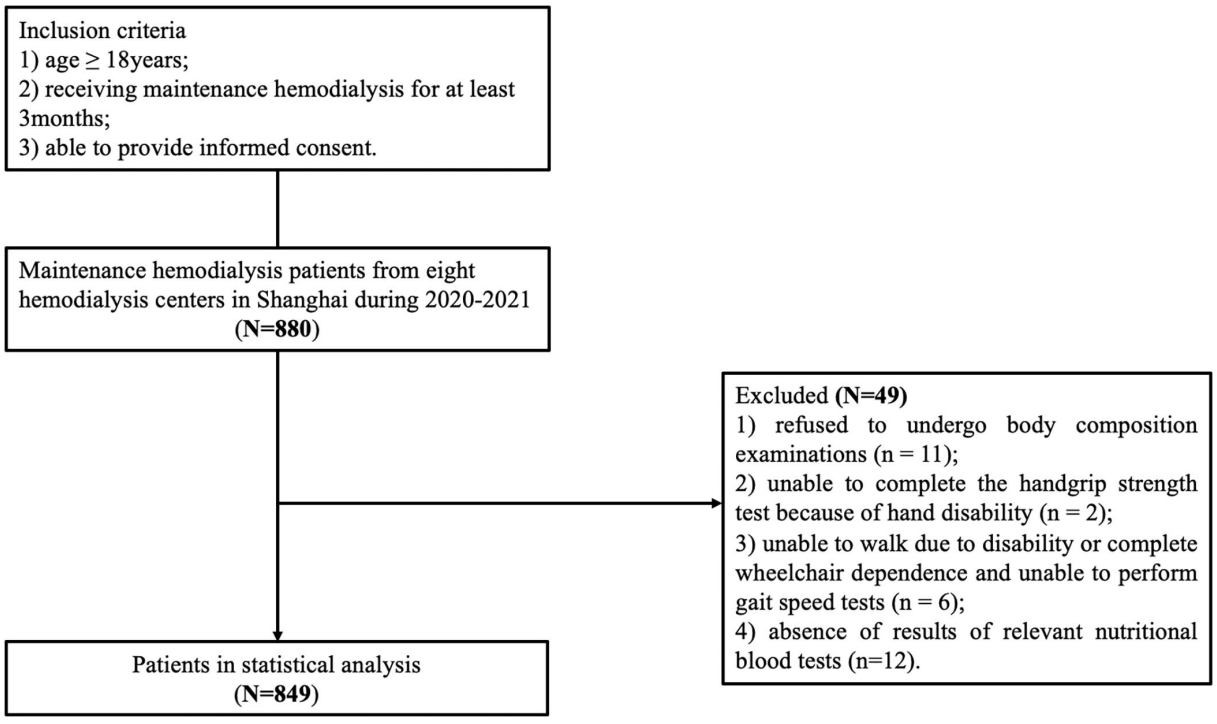

FIGURE 1
Flow chart of the study.

Covariates

All the patients were invited to a face-to-face interview to answer a standardized questionnaire. Covariates included socio-demographic characteristics and chronic disease status. Demographic characteristics comprised of age, sex, post-dialysis weight, dialysis vintage, and education level. The International Physical Activity Questionnaire (IPAQ) (16) was used to evaluate the physical activity. Comorbidity was assessed using the Charlson Comorbidity Index (CCI), which accounted for multiple comorbidity by creating a summation score based on 19 comorbidity conditions (17). Moreover, all the blood samples were taken prior to dialysis. The dialysis quality was assessed by fractional clearance index for urea (Kt/V), which was monitored in every dialysis treatment using the OCM® (On-line Clearance Monitor; Fresenius Medical Care, Bad Homburg, Germany).

Definition of dynapenia, pre-sarcopenia, and sarcopenia

A direct segmental multi-frequency bioelectrical impedance analysis (BIA; InBody S10; Biospace, Seoul, Korea) was used to measure muscle mass (18). Low muscle mass was defined as skeletal muscle index (SMI) lower than 7.0 and 5.7 kg/m^2 in man and women, respectively. The dynamometer (GRIP-D; Takei Ltd, Niigata, Japan) on the non-fistula hand before a dialysis session to evaluate the muscle strength.

For patients with an indwelling dialysis catheter, we used the dominant hand to test muscle strength. Low muscle strength was defined as handgrip strength < 28 or < 18 kg in males and females. Usual gait speed was measured to assess poor-physical performance, which was less than 1.0 m/s in both males and females. All the patients were measured for these tests using the same measurement by a physical therapist at each facility on dialysis day before dialysis treatment.

Dynapenia was defined as patients with decreased muscle strength and normal muscle mass (8). A low SMI with normal handgrip strength and normal gait speed were to defined pre-sarcopenia (2). Sarcopenia was diagnosed based on 2019 new version of the Asian Working Group for Sarcopenia (AWGS) criteria. These criteria included both low muscle mass and low muscle strength and/or poor-physical performance (1).

Assessment of nutritional status

Malnutrition–inflammation score

The Malnutrition–Inflammation Score (MIS) is a comprehensive scoring system that is associated with expected

hospitalization rates, mortality rates, and nutritional measures of patients with MHD (12). The 10 items about the patient's with MHD nutritional and functional state were included in the MIS. Each component of the MIS has four severity levels, ranging from 0 (normal) to 3 (severely abnormal). The total score ranges from 0 to 30. The higher score suggests a more severe degree of malnutrition status.

Geriatric nutritional risk index

In the several previous studies, Geriatric Nutritional Risk Index (GNRI) has been shown to be an important predictor of morbidity and mortality and associated with a variety of nutrition-related markers (19, 20). The GNRI was calculated using the following equation (21):

$$\begin{aligned}\text{GNRI} = &[14.89\times\text{albumin(g/dl)}]\\ &+[41.7\times(\text{bodyweight/idealbody weight})]\end{aligned}$$

Ideal body weight was defined as having a body mass index (BMI) value of 22 kg/m^2.

Creatinine index

Creatinine (Cr) index was calculated based on known sex differences as follows (13, 22):

$$\begin{aligned}&\text{Crindex for men} = 16.21 + 1.12 - 0.06\times[\text{age (year)}]\\ &-0.08\times(\text{single pool Kt/V}) + 0.009\\ &\times[\text{serum creatinine}\ (\mu\text{mol/L})],\\ &\text{Cr index for women} = 16.21 - 0.06\times[\text{age (year)}]\\ &-0.08\times(\text{single pool Kt/V}) + 0.009\\ &\times[\text{serum creatinine}\ (\mu\text{mol/L})].\end{aligned}$$

Statistics

Independent *t*-test (numeric variables) or by chi-square test (categorical variables) were used to compared patients with and without sarcopenia. Numeric variables with a normal distribution are expressed as the mean ± SD, while with a non-normal distribution are expressed as the median, with the 25–75% interquartile ranges given in parentheses. Categorical variables used an absolute number and proportions. The receiver operating characteristic curve (ROC) was a graph of sensitivity plotted against (1–specificity) overall possible diagnostic cut points. The cutoff points of dynapenia, pre-sarcopenia and sarcopenia were determined by the maximal Youden's index, which was calculated as (sensitivity + specificity −1) and the greatest combination of sensitivity and specificity. Logistic regression analyses were used to assess the relationships between three nutritional screening tools (MIS, GNRI, and Cr index) and dynapenia, pre-sarcopenia, or sarcopenia. Adjusted model included age, sex, Kt/V, IPAQ, CCI, Cr, PTH, and phosphorus. We further used Harrell's C-statistics to confirm which nutritional markers were best for identifying dynapenia, pre-sarcopenia, and sarcopenia. $P < 0.05$ was considered statistically significant. IBM SPSS Statistics v26.0 (SPSS Inc., Chicago, IL, United States) was used to perform all the statistical analyses.

Results

Characteristics of the patients for sarcopenia

Among 849 participants (520 men, 329 women; mean age 61.4 ±12.6 years), and there were 235 (27.7%) patients with dynapenia, followed by 193 (22.7%) patients with sarcopenia, and 53 (6.2%) with pre-sarcopenia, respectively. Table 1 shows the socioeconomic and health-related characteristics of patients with MHD stratified by sarcopenic status. Age, post-dialysis weight, BMI, Kt/v, handgrip strength, SMI, gait speed, IPAQ, nutritional factors (MIS, GNRI, and Cr index), CCI, and laboratory parameters (albumin, Cr, PTH, and phosphorus) significantly differed between groups ($P < 0.05$, Table 1).

Evaluations of three nutritional screening tools on ROC curve

The ROC curves were created to quantify sensitivity, specificity, areas under the ROC curves (AUC), and optimal cutoff points of three nutritional screening tools. Table 2 shows that the AUC of GNRI toward sarcopenia and pre-sarcopenia were lager than Cr index and MIS. The AUC of MIS, GNRI, and Cr index toward sarcopenia were 0.640, 0.722, and 0.700, respectively (Figure 2), while pre-sarcopenia was 0.517, 0.723, and 0.558, respectively. Meanwhile, the cutoff points of GNRI toward pre-sarcopenia and sarcopenia were 103 and 102.4, respectively.

Relationships of the three nutritional screening tools and dynapenia, pre-sarcopenia, and sarcopenia

The relationships between the three nutritional screening tools and dynapenia, pre-sarcopenia, and sarcopenia are shown in Table 3. After adjustment for age, sex, Kt/V, IPAQ, CCI, Cr, PTH, and phosphorus, a significantly higher risk of sarcopenia was found in patients in poor-nutrition categories identified by GNRI [odds ratio (OR) = 6.28, 95%CI: 4.05, 9.73) than Cr index (OR = 2.73, 95%CI: 1.71, 4.34) and MIS (OR = 1.91, 95%CI: 1.31, 2.78). The OR for pre-sarcopenia was higher in Cr index in

TABLE 1 Baseline characteristics of study participants according to the presence of sarcopenia.

Characteristics	Non-sarcopenia ($n = 656$)	Sarcopenia ($n = 193$)	*P*-value
Age (y)	59.4 ± 12.3	68.3 ± 11.3	<0.001
Male (%)	404(61.6)	116(60.1)	0.710
Post-dialysis weight (kg)	65.2 ± 12.0	53.5 ± 7.9	<0.001
BMI (kg/m^2)	24.0 ± 3.8	21.1 ± 2.9	<0.001
Vintage (months)	46.2(22.8,91.4)	48.2(30.1,105.3)	0.062
Kt/v	1.33 ± 0.33	1.49 ± 0.30	<0.001
Handgrip strength	26.6 ± 8.7	18.8 ± 6.2	<0.001
SMI (kg/m^2)	7.3 ± 1.1	5.8 ± 0.8	<0.001
Gait speed (m/s)	1.04 ± 0.28	0.78 ± 0.31	<0.001
IPAQ (Met-min/wk)	1,508(693.3492)	783(0,2079)	0.001
Nutritional factors			
GNRI	104.0 ± 9.8	97.8 ± 7.2	<0.001
MIS	3.9 ± 2.8	5.4 ± 3.3	<0.001
Cr index	22.4 ± 3.0	20.4 ± 2.5	<0.001
Number of medications (n)	4.4 ± 2.4	4.5 ± 2.5	0.709
CCI	3.8 ± 1.6	4.2 ± 1.8	0.001
Laboratory parameters			
Hemoglobin (g/dL)	110.9 ± 15.7	111.3 ± 16.3	0.720
Albumin (g/L)	39.8 ± 3.5	38.8 ± 3.4	0.001
Cr (μmol/L)	1013.5 ± 276.6	864.1 ± 223.5	<0.001
PTH (pg/dL)	375.2 ± 332.7	303.4.3 ± 288.0	0.007
Calcium (mg/dL)	2.27 ± 0.25	2.25 ± 0.27	0.180
Phosphorus (mg/dL)	1.99 ± 0.63	1.84 ± 0.65	0.004

BMI, body mass index; Kt/V, fractional clearance index for urea; ESRD, end stage renal disease; SMI, skeletal muscle index; GNRI, geriatric nutritional risk index; MIS, malnutrition inflammation score; Cr, creatinine; IPAQ, international physical activity questionnaire; Met-min/wk, metabolic equivalent task minutes per week; CCI, Charlson comorbidity index; PTH, parathyroid hormone.

pre-sarcopenia (OR = 5.49, 95% CI: 1.88, 16.02) than GNRI (OR = 5.29, 95% CI: 2.69, 10.39). However, the OR for dynapenia was slightly higher in MIS (OR = 2.12, 95% CI: 1.42, 3.17) than GNRI (OR = 1.71, 95% CI: 1.02, 2.86), and Cr index (OR = 1.73, 95% CI: 1.09, 2.73).

Discrimination tests

Harrell's C statistic were used to evaluate the discrimination of each nutritional screening tool. The Harrell's C statistics of the GNRI and Cr index were significantly higher than the MIS (GNRI vs. MIS: $P = 0.001$; GNRI vs. Cr index: $P = 0.001$), and there was no significant difference between the MIS and the Cr index in the values of the Harrell's C statistic for sarcopenia ($P = 0.436$, Table 4). Pre-sarcopenia also showed the similar results; however, there were no significant difference among the three nutritional screening tools in the values of the Harrell's C statistic for dynapenia (Table 4).

TABLE 2 Receiver operating characteristic curve for the nutritional factors to estimate the probability of dynapenia, pre-sarcopenia, and sarcopenia.

Variables	Bootstrap ROC curve				
	AUC (95%CI)	*P*-value	Cutoff	Sensitivity (%)	Specificity (%)
Dynapenia					
MIS	0.582(0.535,0.628)	<0.001	5.5	33.6	82.6
GNRI	0.471(0.425,0.517)	0.208	94.4	14.9	90.2
Cr index	0.606(0.562,0.651)	<0.001	22.5	67.2	50.8
Pre-sarcopenia					
MIS	0.517(0.443,0.592)	0.674	3.5	56.6	52.9
GNRI	0.723(0.663,0.783)	<0.001	102.4	77.4	63.2
Cr index	0.558(0.490,0.626)	0.161	24.1	92.5	28.4
Sarcopenia					
MIS	0.640(0.596,0.684)	<0.001	4.5	54.4	66.2
GNRI	0.722(0.684,0.760)	<0.001	103.0	79.3	56.6
Cr index	0.700(0.660,0.740)	<0.001	21.1	67.4	66.5

ROC, receiver operating characteristic curve; AUC, area under the curve; CI, confidence interval; GNRI, geriatric nutritional risk index; MIS, malnutrition inflammation score; Cr, creatinine.

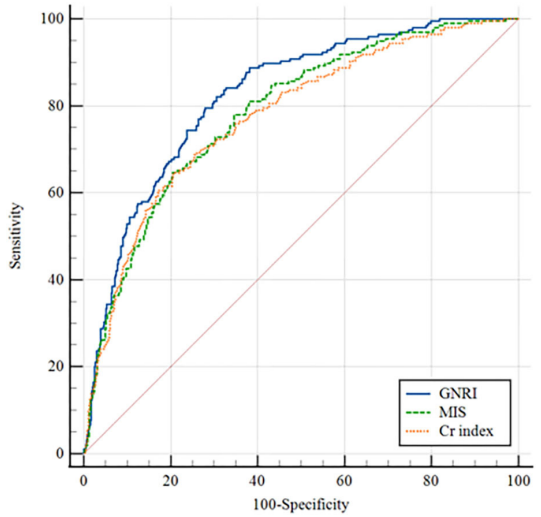

FIGURE 2
Receiver operating characteristic (ROC) curve for geriatric nutritional risk index (GNRI), malnutrition inflammation score (MIS), and Creatinine (Cr) index according to sarcopenia.

Discussion

Our study suggested that each of the three nutritional screening tools was significantly associated with an increased risk of dynapenia, pre-sarcopenia, and sarcopenia in Chinese patients with MHD. Moreover, the sarcopenia predictability of

TABLE 3 Multivariable logistic regression analysis evaluating nutritional factors associated with dynapenia, pre-sarcopenia, and sarcopenia.

Variables	OR (95%CI)			
	Crude	*P*	Adjusted model	*P*
Dynapenia				
MIS	2.41(1.67,3.47)	<0.001	2.12(1.42,3.17)	<0.001
GNRI	1.53(0.96,2.46)	0.075	1.71(1.02,2.86)	0.040
Cr index	2.06(1.48,2.85)	<0.001	1.73(1.09,2.73)	0.019
Pre-sarcopenia				
MIS	1.47 (0.83,2.58)	0.186	1.57(0.87,2.83)	0.132
GNRI	5.82(3.00,11.31)	<0.001	5.29(2.69,10.39)	<0.001
Cr index	3.86(1.51,9.87)	0.005	5.49(1.88,16.02)	0.002
Sarcopenia				
MIS	2.33(1.68,3.23)	<0.001	1.91(1.31,2.78)	0.001
GNRI	4.86(3.33,7.09)	<0.001	6.28(4.05,9.73)	<0.001
Cr index	3.98(2.84,5.60)	<0.001	2.73(1.71,4.34)	<0.001

Adjusted model: Adjusted for age, sex, Kt/V, IPAQ, CCI, Cr, PTH and phosphorus. GNRI, geriatric nutritional risk index; MIS, malnutrition inflammation score; Cr, creatinine; Kt/V, fractional clearance index for urea; IPAQ, international physical activity questionnaire; CCI, Charlson comorbidity index; PTH, parathyroid hormone.

TABLE 4 Results of the Harrell's C statistic.

Variables	Harrell's C statistic (95%CI)	SE		*P*
Dynapenia				
MIS	0.713(0.677,0.747)	0.021	Ref.	0.267
GNRI	0.705(0.664,0.746)	0.021	0.267	Ref.
Cr index	0.703(0.667,0.737)	0.021	0.322	0.982
Pre-sarcopenia				
MIS	0.662(0.624,0.698)	0.040	Ref.	0.004
GNRI	0.757(0.722,0.790)	0.030	0.004	Ref.
Cr index	0.701(0.664,0.736)	0.034	0.184	0.120
Sarcopenia				
MIS	0.785(0.756,0.813)	0.018	Ref.	0.001
GNRI	0.828(0.801,0.853)	0.016	0.001	Ref.
Cr index	0.779(0.749,0.806)	0.019	0.436	0.001

GNRI, geriatric nutritional risk index; MIS, malnutrition inflammation score; Cr, creatinine.

the GNRI appears greater than Cr index and MIS, while Cr index similar to the MIS. Thus, GNRI is considered a useful tool for predicting sarcopenia in this group.

In our study, the prevalence of sarcopenia was 22.7%, which is consistent with the previously reported sarcopenia prevalence in dialysis patients (20.0%) (6), as well as the results of a recent systematic analysis of 30 studies in dialysis patients (sarcopenia prevalence = 28.5%) (23). Based on the cut-off values of muscle mass and function recommended by existing guidelines for the diagnosis of sarcopenia in the general population, the prevalence of sarcopenia in hemodialysis patients ranges from 4 to 68% (6, 23–25). This wide range is partly representative of the lack of recognized definition for diagnosing low muscle mass and low muscle strength (24). On the other hand, sarcopenia, as defined in the healthy population, may not apply to dialysis population. Therefore, more research is needed in the future to determine the appropriate diagnostic cut-point values for sarcopenia in the dialysis population.

Consistently, previous studies found that malnutrition was significantly associated with sarcopenia in patients with MHD (26). With regard to the indicators used for nutritional assessment, studies have shown that the GNRI (27) and MIS (10) were associated with sarcopenia in patients with MHD. However, the relationship between the Cr index and sarcopenia remains scarce. Furthermore, the GNRI has the best discrimination to predict sarcopenia, while the Cr index similar to the MIS. However, Beberashvili et al. (28) reported that MIS is more comprehensive than GNRI in monitoring of nutritional status in patients with MHD. In addition, another study found that lower GNRI and Cr index values were both independently and equally associated with an increased risk of all-cause mortality in a multivariable-adjusted model (13). Differences of sample size, dialysis vintage, or population characteristics could explain this inconsistent finding. Our findings and hypotheses warrant further confirmation.

In our study, the cut-off value for estimating GNRI for sarcopenia was 103. In a previous study of Japanese dialysis patients, the cut-off value for GNRI was 90 when the outcome was mortality (19). The cut-off value in our study for sarcopenia was slightly higher, possibly due to the lower prevalence of malnutrition among patients in this population. The reason may be that universal health insurance system in Shanghai of China allows all the dialysis patients to treat complications early, and it should be recognized that many patients may be not malnourished due to their relative financial wealth. In addition, several research on the effectiveness of the GNRI in patients with MHD have shown that the GNRI is more sensitive and specific than other assessment tools in predicting mortality (29). Nutritional screening tool should be simple, fast, and reproducible (intra-rater and interrater reliability) and sufficiently discriminating. MIS is time-consuming and cumbersome because it requires a subjective evaluation. Moreover, GNRI requires only two parameters while the Cr index needs four parameters, making GNRI easier. The simpler of the two would be more practical and useful in the clinical setting, which is consistent with our results. Hence, GNRI may be used as a screening indicator based on medical record information in place of complex diagnostic criteria described in the consensus for estimating sarcopenia in patients with MHD.

This study has important implications for clinical practice and future research. First, it highlights that screening poor nutrition status is usually highly predictive of sarcopenia in patients with MHD. Second, it is strongly recommended to

maintain sufficient calorie and protein intake especially physical therapy to avoid or reverse sarcopenia (26). However, efforts to increase food intake in patients with MHD with sarcopenia often are unsuccessful because of the persistent anorexia arising from sarcopenia-related inflammatory status (30). Finally, physical therapy may increase appetite in the short term by improving the patient's mood and increase metabolic rate in the long term by activating muscle metabolism.

Some limitations should be considered in our study. First, it is not possible to elucidate clear causal associations between three nutritional screening tools and sarcopenia in the patients with MHD because this study is a cross-sectional design. Second, the prevalence of malnutrition in this population is relatively low and few patients had severe malnutrition. Future studies with longitudinal designs are required to confirm the causal association between different nutritional screening tools and sarcopenia.

In conclusion, MIS, GNRI, and Cr indexes were almost equally and significantly associated with an increased risk of dynapenia, pre-sarcopenia, and sarcopenia. The sarcopenia predictability of the GNRI was greater than the Cr index and MIS, while the Cr index similar to MIS. Our findings provide further evidence for the selection of the nutritional screening tool to predict sarcopenia in patients with MHD. If further confirmed, GNRI as a simple tool could be used to identify patients with MHD at high risk of sarcopenia. Multiple interventions targeting these high-risk patients and implementing early intensification can help reduce their risk of sarcopenia and other clinical complications in the future.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Shanghai University of Medicine and Health Sciences. The patients/participants provided their written informed consent to participate in this study.

Author contributions

XC, PH, and QG: study concept and design. PS, YZ, HZ, CY, JN, WD, and JZ: acquisition, analysis, and interpretation of data. XC and PH: drafting of the work. XZ, LZ, HQ, SZ, and QG: critical revision of the manuscript. All the authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

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Evaluation of psychometric properties of dietary habits, lifestyle, food frequency consumption, and nutritional beliefs (KomPAN) questionnaire in Iranian adults

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Background: Adherence to unhealthy dietary patterns is a major cause of overweight and obesity in adults. Therefore, it is recommended that assessment and modification of unhealthy lifestyle should be included in prevention programs. To achieve this goal, it is necessary to evaluate the status of dietary patterns in adults with valid and reliable tools. Thus, the aims of the present study were to translate the KomPAN questionnaire, evaluate its psychometric properties in Iranian adults and measure 4 dietary indices including high-saturated-fats-Diet-Index-8 (hSFDI-8), high-Sugar-Diet-Index-4 (hSDI-4), low-Glycaemic-Diet-Index-4 (LGIDI-4) and high-Glycaemic-Diet-Index-7 (hGIDI-7) based on 3 groups of body mass index (BMI) (BMI = 18.5–24.9, BMI = 25–29.9 and BMI ≥ 30), gender, educational level, income status, and age.

Methods: The KomPAN questionnaire included 4 scales nutrition beliefs (NB), lifestyle, food frequency consumption (FFC), dietary habits (DH) and after its translation from English into Persian, the psychometric properties of all 4 scales (face and content validity) were evaluated. For both FFC and NB scales, the construct validity was assessed through exploratory factor analysis (EFA), confirmatory factor analysis (CFA) and convergent and discriminant validity, the internal consistency was evaluated using the Cronbach's alpha coefficient, McDonald's omega (Ω) and Theta coefficient (θ), as well as the stability was assessed *via* intraclass correlation coefficient (ICC). Cross-classification and Kappa statistics were evaluated for both DH and lifestyle scales. Then, 4 dietary indices were measured in terms of demographic variables.

Results: The cross-classification of DH (93.96%) and lifestyle (95.87%) scales indicated the percentage of correct classification in the test-retest scales.

The Kappa statistic was >0.4 and its value was acceptable. The mean Kappa statistics were 0.734 and 0.865 for the DH and lifestyle scales, respectively. The fit indices showed that the two-factor construct of the FFC scale and the one-factor construct of the NB scale had a good and acceptable fit among the Iranian adults. The FFC and NB scales had acceptable internal consistency and stability.

Conclusion: It is recommended that other researchers use the KomPAN questionnaire to identify DH, FFC, NB and lifestyle as well as measure diet quality scores in the adult community.

KEYWORDS

diet quality index, dietary habits, lifestyle, nutrition knowledge, psychometric evaluation

Background

A varied, balanced and healthy dietary pattern is one of the most important aspects of a lifestyle that is useful for maintaining health and preventing chronic diseases (1). A rational diet is a key determinant of adult health. However, the prevalence of unhealthy food choices is high among adults (2). The results of studies show that unhealthy dietary patterns are associated with obesity, insulin resistance, metabolic syndrome and other risk factors for cardiovascular disease (3–5).

In recent years, the use of dietary pattern analysis in various groups including adults has received much attention. Dietary patterns can summarize the combined effects of common foods used by the community (6) and reflect that the food interests and preferences of individuals are influenced by cultural, economic, social and lifestyle factors (7, 8).

The American Dietetic Association in healthful eating messages to the public suggests that it should be emphasized dietary patterns rather than foods or meals because assessing dietary patterns can help assess nutrients or the amount of food received (9). On the other hand, since nutrients and foods are not consumed separately, nutritionists have also suggested that in order to achieve a broader picture of the diet, it is necessary to evaluate a person's dietary patterns (10).

Studies have demonstrated that adherence to unhealthy dietary patterns such as high-calorie foods, increased consumption of fast foods and sedentary lifestyle is a major cause of overweight and obesity in adults (5, 7).Therefore, it is recommended that assessment and modification of unhealthy lifestyle should be included in prevention programs including increasing physical activity, increasing the quality of the diet, modifying the diet such as consumption of foods with the low glycemic index, saturated fat, low sugar and weight loss (11). To do so, it is necessary to evaluate the status of dietary patterns in different communities with valid and reliable tools. One of the available tools is the Dietary Habits, Lifestyle, food frequency consumption and Nutritional Beliefs Questionnaire (KomPAN questionnaire), which was developed in 2014 by the Committee of Human Nutrition, Polish Academy of Science, in two interviewer-administered (IA-Q) and self-administered (SA-Q) versions (12). The KomPAN questionnaire is a reliable and valid tool and has been used in some sections of several published studies (11–14). One of the advantages of using this tool is that the diet quality scores (DQS) can be calculated by evaluating the food frequency consumption (FFC). DQS is a very important predictor of non-communicable diseases (15), and its analysis reflects people's dietary patterns in real life and their dietary habits (11). Calculating DQS may be particularly useful in nutrition education interventions or clinical settings (16, 17). Other benefits of using this tool are simplicity, applicability, nutritional habits and beliefs, comprehensiveness in assessing the various dimensions of dietary patterns, as well as lifestyle in adults. The KomPAN questionnaire is the first questionnaire to evaluate dietary habits (DH), FFC, nutrition beliefs (NB) and lifestyle that has been confirmed to be reproducible in people of a wide age range (in healthy individuals and individuals with chronic diseases) (12). Although the findings of the research lead to

Abbreviations: hSFDI-8, high-saturated-fats-Diet-Index-8; hSDI-4, high-Sugar-Diet-Index-4; LGIDI-4, low-Glycaemic-Diet-Index-4; hGIDI-7, high-Glycaemic-Diet-Index-7; BMI, Body mass index; NB, Nutrition beliefs; FFC, Food frequency consumption; DH, Dietary habits; EFA, Exploratory factor analysis; CFA, Confirmatory factor analysis; ICC, Intraclass correlation coefficient; IA-Q, Interviewer-administered; SA-Q, Self-administered; DQS, Diet quality scores; pHDI, pro-Healthy-Diet-Index; nHDI, non-Healthy-Diet-Index; CVR, Content validity ratio; CVI, Content validity index; KMO, Kaiser-Meyer-Olkin; PAF, Principal axis factoring; GFI, Goodness of fit indices; CFI, Comparative fit index; TLI, Tucker–Lewis index; SRMR, Standardized root mean square residual; RMSEA, Root mean square error of approximation; CI, Confidence interval.

valid and reliable evidence if the variables are measured using tools consistent with the culture of communities (18). The KomPAN questionnaire is not localized in Iranian adults. The psychometric properties of this tool have not been evaluated in other studies.

Objectives

Thus, the aims of the present study were to translate the Dietary Habits, Lifestyle, food frequency consumption and Nutritional Beliefs Questionnaire (KomPAN questionnaire), evaluate its psychometric properties in Iranian adults and measure 4 dietary indices including high-saturated-fats-Diet-Index-8 (hSFDI-8), high-Sugar-Diet-Index-4 (hSDI-4), low-Glycaemic-Diet-Index-4 (LGIDI-4) and high- Glycaemic-Diet-Index-7 (hGIDI-7) based on 3 groups of body mass index (BMI) (BMI = 18.5–24.9, BMI = 25–29.9 and BMI $\geq$ 30), gender, educational level, income status, and age.

Materials and methods

Design and setting

In this validation study, the setting of the study was comprehensive health service centers in the cities of Mazandaran province, Iran. The choice of this setting was due to the availability of samples with relatively common cultural structures and lifestyles. This study with the ethics code of IR.MUBABOL.HRI.REC.1400.046 was conducted in 2022.

Measures

In the current study, two questionnaires were used to collect data:

Demographic characteristics questionnaire: This questionnaire included age, gender, educational level, marital status, place of residence, occupational status, economic status, number of people in the household, weight, height and BMI.

KomPAN questionnaire: In the present study, the self-administered questionnaire (SA-Q) version of this questionnaire was used. In the ongoing study, according to the nutritional culture, lifestyle of the target community and opinions of the panel of experts (research team), items were added to different parts of the KomPAN questionnaire or items were combined with each other, which was explained in each section. KomPAN questionnaire consisted of 4 scales.

Dietary habits (DH)

DH contained 10 multiple-choice items (one or more than one correct answer) and investigated regular consumption of meals, snacks, soft drinks or ready meals, sweets and so on.After translating the tool and checking its items by the research team, 4 items were added to the DH scale according to the food culture of Iranian adult society.

In the current study, four food groups including "Do you use smoked foods such as smoked rice and smoked fish?," "Do you use food seasonings such as sour, salt, pepper, cinnamon and ginger?," "Do you add rice bran to your food? " and "What kind of snack do you usually eat between meals during the day?" were added to this scale. The data of this scale had qualitative characteristics and its analysis was performed by cross-classification analysis and Kappa statistics.

Food frequency consumption (FFC)

FFC consisted of 33 items including cereals (4 items), fruits/vegetables/legumes/potatoes (5 items), dairy products (4 items), meat/fish/eggs (6 items), fats (3 items), drinks (7 items), sweets and other products (4 items). The FFC was determined by respondents based on a 6-point Likert scale (never, 1–3 times a month, once a week, several times a week, once a day or several times a day). Numerical values were assigned to FFC (once a day = 1, few times a day = 2, few times a week = 0.5, once a week = 0.14, 1–3, times a month = 0.06, and never = 0).

In the present study, the item "How often do you eat fish?" was combined with the item "How often do you eat white meat example chicken, turkey and rabbit?" as well as the item "How often do you consume nuts, sunflower seeds, pistachios, hazelnuts and walnuts?" was added to this scale. Kowalkowska et al. (12), according to FFC items, determined two nutritional indices including pro-Healthy-Diet-Index (pHDI) (whole-wheat bread; whole-wheat cereals, oatmeal or whole-wheat pasta; milk; fermented milk drinks; cottage cheese; white meat; fish; dishes with legumes; fruits and vegetables) and non-Healthy-Diet-Index (nHDI) (white bread; white rice, pasta or fine-ground groats; fast food; fried dishes; butter; lard; cheese; cold meats, smoked sausages or hot-dogs; red meat dishes; sweets; tinned meats; sweetened carbonated and non-carbonated drinks; energy drinks and alcoholic beverages). In the current study, *via* reviewing the texts and using the opinions of nutritionists, the items of instant soups or ready-made soups (example tinned, jar and concentrates), tinned (jar) vegetables (example pickles), still beverages and sweetened hot beverages were added to nHDI as well as the items of vegetable oils, eggs, vegetable juices, fruit and vegetable juices, potato and water were added to pHDI. Therefore, the number of items and score levels were changed as follows:

pHDI-15: The pHDI-15 included whole-wheat bread; whole-wheat cereals, oatmeal or whole wheat pasta; vegetable

oils; milk; fermented milk drinks; cottage cheese; white meat; nuts; vegetable juices, fruit and vegetable juices; eggs; dishes with legumes; potato; fruits; vegetables; vegetable juices or fruit juices and water. The total score range was 0–30 points divided into three categories: low (0–10.0), moderate (10.1–20.0) and high (20.1–30.0). PHDI is interpreted in such a way that the higher the value represents the greater the intensity of the FFC desirable characteristics.

nHDI-18: It consisted of white bread; white rice, pasta or fine-ground groats; fast food; fried dishes; butter; lard; cheese; cold meats, smoked sausages or hot dogs; red meat dishes; sweets; instant soups or ready-made soups (example tinned, jar and concentrates); tinned meats; tinned (jar) vegetables (example pickles), still beverages; sweetened hot beverages sweetened carbonated and non-carbonated drinks; energy drinks and alcoholic beverages. The total score range was 0–36 points categorized into three categories: low (0–12.0), moderate (12.1–24.0) and high (24.1–36.0).

The interpretation of nHDI is such that the higher the value represents the greater the intensity of the FFC undesirable characteristics. In the present study, according to the classification of Bykowska-Derda et al. (19) four indices including hGIDI-7, LGIDI-4, hSDI-4 and hSFDI-8 were evaluated (11). Moreover, the values of these indices were compared based on 3 groups of normal (BMI = 18.5–24.9), overweight (BMI = 25–29.9) and obese (BMI $\geq$ 30) BMIs, gender, educational level, income status, and age:

hGIDI-7: The hGIDI-7 was composed of items including white bread; white rice, pasta or fine-ground groats; fruits, sweets, juices, sweetened hot drinks as well as sweetened carbonated and non-carbonated drinks.

LGIDI-4: The LGIDI-4 comprised items of whole-wheat bread; whole-wheat cereals, oatmeal or whole-wheat pasta; dishes with legumes and vegetables.

hSDI-4: The hSDI-4 involved items such as sweets, juices, sweetened hot drinks as well as sweetened carbonated and non-carbonated drinks.

hSFDI-8: The hSFDI-8 included items of fast food; fried dishes; butter; lard; cheese; cold meats, smoked sausages or hot dogs; red meat dishes and tinned meat. Based on the following formula and using the data of this scale, the DQS was calculated as dietary indexes (20).

$$\text{Diet Quality Index} = \frac{100^* \sum A}{\sum B} [\%]$$

Where A is the sum of the reported daily intake of all items listed in specific food groups (e.g., low GI), for example, $\Sigma = 0 + 0{\cdot}14 + 0{\cdot}06 + 0{\cdot}5$. B is the sum of the maximum possible to report daily intake of the same (low GI) foods, determined for one product as 2 (e.g., $\Sigma = 2 + 2 + 2 + 2$). The total score of diet quality was from 0 to 100 (20). The total score of nutritional trait intensity was classified into low (0–33.32% points), moderate (33.33–66.65% points) and high (66.66–100% points) (11).

Nutrition beliefs (NB)

NB consisted of 25 items, each item is scored with 3 response categories (true = 1, false = 0 and not sure = 0). The scores of all items were summed (the total NB score range: 0–25 points). The response to the items was divided into three categories: insufficient (0–8), sufficient (9–16) and good (17–25) (21).

Lifestyle

This scale comprised 16 items that included different aspects of lifestyle such as diet, drinking alcohol, smoking, sleep, screen time, recreational physical activity and type of water consumed. Lifestyle items were scored differently.

For example, the physical activity was scored based on a 3-point Likert scale including low = 1, moderate = 2 and high = 3, or self-declared by the respondent of nutritional knowledge was scored based on a 4-point Likert scale including insufficient, sufficient, good and very good (12). In the current study, two items including "Are you currently using drugs such as poppy, opium, opium juice (Shireh), methadone, bupropion and heroin?" and "Are you currently using industrial addictive substances such as hashish, marijuana, drug flowers, crack and methamphetamine" were added to the lifestyle. NB scale data had qualitative characteristics and were analyzed by cross-classification analysis and Kappa statistics.

Cross-cultural adaptation of KomPAN questionnaire and content validity

In the development of the Persian version of the KomPAN questionnaire based on the WHO protocol (2015), the forward-backward translation technique was used (12). Firstly, written permission was obtained from Professor Marzena Jezewska-Zychowicz who designed this tool *via* email for translating and validating the tool. The cross-cultural adaptation process of the KomPAN questionnaire and the evaluation of content validity were done in three steps:

Step 1: Forward translation (Translation of the original questionnaire):

The English KomPAN questionnaire was translated into Persian by two translators, independently.

Step 2: Reconciliation of forward translations (Synthesis of the translations):

The two Persian translations were reviewed by the research team, and finally, a single Persian version of the questionnaire was prepared,

Step 3 Backward translation (Back translation of the consolidated version):
The backward translation (from Persian into English) was performed independently by two other translators. The two English (backward) translations were reviewed and compared with the original (English) questionnaire by the research team. After the necessary corrections, the final version of the questionnaire was sent to Marzena Jezewska-Zychowicz *via* email for approval.

Pre-test

The forward translation (final version) of the KomPAN questionnaire was completed by 30 adults selected based on inclusion criteria. This part of this study was done by the corresponding author. The reactions of these individuals during responding to items such as long pauses in responding to each item, changes in response to items and symptoms such as confusion when responding to items were examined. All adults completed the questionnaire in the pre-test stage. Some of the items were not easy for them to understand; therefore, some modifications were made to items based on their suggestions.

Assessment of the validity (face and content construct), convergent and divergent validity, reliability, cross-classification, and Kappa of the KomPAN questionnaire

Face validity

Face validity was qualitatively and quantitatively evaluated for all 4 scales of the KomPAN questionnaire. For qualitative face validity, 10 persons of the target group were asked to comment on the levels of difficulty, appropriateness and ambiguity of each item through individual and face-to-face interviews. Proposed corrections were made to the items. In the current study, the impact score was calculated using the following formula:

$$\text{Impact item} = \text{frequency}\ (\%) \times \text{importance}$$

Items with $\geq$ 1.5 were appropriate, and other items were removed (22).

Content validity

Content validity was assessed both qualitatively and quantitatively for all 4 scales of the KomPAN questionnaire. To do so, the questionnaire was sent to 10 experts (8 persons who had experience in performing qualitative studies and making tools as well as 3 nutritionists) *via* email. These individuals were asked to evaluate grammar, wording, item allocation and scaling of tools. All changes suggested by experts were made to the items. Content validity ratio (CVR) and content validity index (CVI) were evaluated in the quantitative part (23). To calculate the CVR, experts (the same persons invited to review the quality of the content validity) were asked to comment on each item based on a three-point scale (from "not essential" to "essential"). Then, the CVR was calculated using the following formula:

$$\text{CVR} = \frac{\text{ne} - (\frac{\text{N}}{2})}{(\frac{\text{N}}{2})}$$

ne = the number of experts selected an item "essential," N = the total number of experts evaluated all items.

The minimum acceptable CVR was determined based on the Lawshe (1975) table. The number of experts was 10, so the acceptable value of CVR was $\geq$ 0.62 (24).

To evaluate CVI, Waltz & Bausell method was used (25). Thus, 10 experts (the same ones invited to assess the quality of the content validity) were asked to determine the relevance of each item based on a four-point Likert scale ranging from 1 = irrelevant, 2 = somewhat relevant, 3 = quite relevant to 4 = highly relevant. The CVI was then calculated using the following formula:

$$\text{CVI} = \frac{\text{The number of the experts who checked option 3 and 4}}{\text{The total number of experts}}$$

The acceptable value of CVI was >0.79, and if the CVI value was between 0.70 and 0.79, the item was revised (25).

Construct validity

For both NB and FFC scales, the construct validity was assessed using Exploratory Factor Analysis (EFA) and Confirmatory Factor Analysis (CFA).

A cross-sectional study was conducted to evaluate the construct validity. The study population included all adults referred to comprehensive health service centers. Samples were selected using the convenient sampling method. Inclusion criteria were 18–60-year-old persons having literacy, no chronic diseases (having no specific diet), no allergy to one type of food, no specific diet program (People working in a system that uses a special diet.), no anorexia nervosa and no bulimia nervosa. Exclusion criteria included not completing the questionnaires completely and the person who refused to continue working with the research team. To evaluate the validation of two scales of FFC and NB, all samples (N = 1,400) were randomly divided into two subgroups of 700 persons. The first subgroup included 386 females and 314 males (M_{age} = 37.92, SD = 11.59; M_{BMI} = 26.60, SD = 6.01) and the second subgroup was 335 women and 365 men (M_{age} = 37.57, SD = 10.49; M_{BMI} = 27.53, SD = 4.75). Only for both NB and FFC scales, the construct validity was evaluated using EFA and CFA:

EFA

EFA was performed on the samples of the first subgroup ($N = 700$) for FFC and NB instruments. The Kaiser-Meyer-Olkin (KMO) and Bartlett's sphericity tests were utilized to assess sample adequacy and sphericity, respectively. Then, the latent factors of both sections were extracted using the principal axis factoring (PAF) method, Varimax rotation and scree plot. The presence of a single item in the factor was ~0.3 based on the following formula:

$$CV = 5.152 \div \sqrt{(n - 2)}$$

The CV is the number of extractable factors and n is the sample size of the study.

Therefore, items with factor loadings lower than 0.3 are eliminated in the EFA (26).

CFA

CFA was conducted on the samples of the second subgroup (N = 700). Model fitting was carried out using the goodness of fit indices (GFI), Satorra–Bentler scaled chi-square test (S–B χ^2), comparative fit index (CFI), Tucker–Lewis index (TLI), standardized root mean square residual (SRMR), root mean square error of approximation (RMSEA) and confidence interval (CI) of 90%. The cut-off points of CFI and TLI>0.9, SRMR≤0.08 and RMSEA≤0.08 were considered as the acceptable limit. The cut-off points of CFI and TLI>0.9 as well as SRMR and RMSEA≤0.08 were considered acceptable.

Convergent and discriminant validity

At this stage, the correlation between FFC and NB with age, gender and BMI status was investigated. Positive and significant values (>0.3) indicated appropriate convergent validity (27). In addition, two indices of average variance extracted (AVE) and construct reliability (CR) were used to evaluate the convergent validity. Values of AVE > 0.5 and CR > AVE represented acceptable convergent validity (28).

Reliability assessment

In the present study, several methods were utilized to evaluate the reliability:

Cross-classification analysis and Kappa statistics were applied to assess the reliability of DH and lifestyle scales. Kappa values >0.4 were considered acceptable agreement (29). To measure cross-classification and kappa statistics, 150 participants (73 females and 77 males ($M_{age} = 37.75$, SD = 10.01; $M_{BMI} = 26.98$, SD = 3.31) were selected based on inclusion criteria, and on two occasions (with an interval of 4 weeks), they responded to items on the dietary habits and lifestyle scales.

The reliability of the FFC and NB scales was evaluated using the internal consistency [Cronbach's alpha coefficient (α), McDonald's omega (Ω) and Theta coefficient (θ)] and stability reliability [intraclass correlation coefficient (ICC)]. ICC >0.8 (30) and α > 0.7 (31) and were considered acceptable levels.

Data analysis

In the present study, to evaluate EFA, the $R_{4.5}$ software (Psych and Polycor packages) was used for validation of two FCC and NB scales, and to assess CFA, the MPlus6.1 software was applied. Convergent and discriminant validity was performed through the Pearson correlation test and the Fornell-Larcker criterion (1981) (28). The cross-classification analysis and Kappa statistics in SPSS26 were used to validate DH and lifestyle scales.

Results

Characteristics of the participants

The results suggested that the mean age, weight, height and BMI of participants were 37.75 ± 11.05 years, 76.37 ± 13.37 kg, 168.30 ± 9.75 cm and 26.60 ± 6.01, respectively. Among these adults, 51.5% and 48.5% were females and males. Moreover, 56.4, 28.3, 7.8 and 7.5% were married, single, widowed and divorced, respectively. Among them, 48.4 and 51.6% were employed and unemployed as well as 42.5 and 57.5% had diploma and academic degrees, respectively. The economic status of 73.6 and 26.4% was sufficient and insufficient, respectively. Totally, 80 and 20% of them lived in urban and rural areas. In addition, 37.8% of individuals had normal BMI, as well as 37.6 and 24.6% were overweight and obese, respectively. None of them had BMI <18.5 (underweight).

Dietary habits

The DH scale had 14 items, of which 6 items were analyzed and the other items were not analyzed because each participant could choose more than one option. According to test-retest results, the percentage of participants classified at the item level was on average 93.96%. So that among the studied adults, the highest cross-classification agreement was 98 and 95.3%, for the items "What type of milk (pasteurized high- or low-fat milk or whole milk) and dairy products do you usually consume?" and "Do you add sweeteners to hot drinks like tea, hot chocolate and coffee?," respectively. Cross-classification analysis showed that on the DH scale, the percentage of correct classification was high, and the percentage of misclassification was very low among the studied persons. Kappa value ranged from 0.968 (for item

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TABLE 1 Agreement and misclassification in test-retest for dietary habits scale.

No.	Questionnaire items	Cat.	Total agreement	Misclassification			Kappa
				1 ± cat.	2 ± cat.	3 ± cat.	
1	What type of milk (pasteurized high- or low-fat milk or whole milk) and dairy products do you usually consume?	3	98	2			0.968
5	Do you add sweeteners to hot drinks like tea, hot chocolate and coffee?	4	95.3	0.67	2	2	0.927
6	Do you use smoked foods such as smoked rice and smoked fish?	3	93.3	4	2	0.7	0.894
7	Do you use food seasonings such as sour, salt, pepper, cinnamon and ginger?	2	93.3	6.7			0.851
8	Do you add salt to meals after cooking?	3	92.6		4	3.4	0.879
9	Do you add rice bran to your food?	3	91.3	7.3	0.7	0.7	0.856

No., item number in the questionnaire. Cat., number of response categories in the question. Evaluated in 3 categories: low (0–33 points), moderate (34–66 points), high (67–100 points).

"What type of milk (pasteurized high- or low-fat milk or whole milk) and dairy products do you usually consume?") to 0.851 (for item "Do you use food seasonings such as sour, salt, pepper, cinnamon and ginger?"), respectively. The Kappa statistic was >0.4 for all analyzed items and its value was acceptable (Table 1).

Food frequency consumption

Construct validity

EFA

The results of evaluating sampling adequacy indices (KMO) (0.892) and Bartlett's sphericity test ($\chi^2 = 13{,}480.37$, $P < 0.001$) indicated that the data were suitable for EFA. Two factors (nHDI and pHDI) with eigenvalue (λ) values >1 were identified for the FFC scale and confirmed based on the scree plot diagram. The data were rotated by Varimax rotation, and in total, two factors of FFC were 44.85% of the total variance. Totally, the first 15-item factor "pHDI" and the second 16-item factor "nHDI" allocated 25.72% ($\lambda = 7.973$) and 19.133% ($\lambda = 5.931$) of the total variance, respectively. Additionally, there was a weak correlation (<0.3) between the two factors. The findings revealed that factor loadings of all items (except for two items "How often do you use lard to flavor your bread or for cooking? (Q8)" and "How often do you eat tinned meats? (Q25)") were >0.3; therefore, these two items were deleted. The correlation between all items and the total score was >0.3 (Table 2).

CFA

The fit indices demonstrated that the two-factor construct of FFC had a good and acceptable fit in the Iranian adult community (S-B $\chi 2 = 1{,}099.864$, DF = 428, P <0.001, CFI = 0.963, TLI = 0.948, RMSEA = 0.047 (90% CI: 0.042-0.051), SRMR = 0.031). All factor loadings of items on the FFC scale were significant on their factors (all ps < 0.001) (Table 2, Figure 1).

Reliability (internal consistency and stability) and convergent validity

The internal consistency (Cronbach's alpha, McDonald's Ω, and θ), CR and ICC values of the two FFC subscales (pHDI and nHDI) are listed in Table 3. The AVE values of the two FFC subscales were >0.5 and <CR>0.7 (Table 2). There was a moderate and negative correlation between the two FFC subscales as well as there was a moderate to a high correlation between the pHDI and nHDI subscales with the NB scale (Table 3).

The results demonstrated that there was a significant difference between the 4 dietary indices (hSFDI-8, hSDI-4 LGIDI-4 and hGIDI-7) in terms of BMI and education.

TABLE 2 Exploratory and confirmatory factor analyses of the food frequency consumption scale.

No	Items	Internal consistency (α; ITC)	EFA Sample 1 $n = 700$			CFA Sample 2 $n = 700$	
			Factor loading	h^2	Eigenvalue (%variance explained)	λ_X	(AVE; CR)
	pHDI						
2	How often do you consume whole-wheat breads such as homemade bread, barbari, sangak, taftoon and toast?	(0.936; 0.666)	0.692	0.497	7.973 (25.721%)	0.68	0.525;0.942
4	How often do you consume whole cereals such as oats, barley and wheat (whole or oatmeal), corn, popcorn and brown rice?	(0.935; 0.703)	0.724	0.528		0.72	
9	How often do you consume vegetable oils (such as sesame oil, olive oil, sunflower oil, canola oil), animal butter and margarine butter for cooking?	(0.936; 0.669)	0.693	0.482		0.70	
10	How often do you drink regular milk (plain milk without additives) or flavored milks such as cocoa milk, coffee milk, date milk, banana milk, honey milk and strawberry milk?	(0.933; 0.801)	0.828	0.686		0.84	
11	How often do you consume dairy products such as yogurt, buttermilk, curd and ice cream?	(0.934; 0.757)	0.779	0.607		0.77	
12	How often do you use high-fat cheese?	(0.936; 0.669)	0.697	0.508		0.70	
16	How often do you eat white meat such as chicken, ostrich, turkey, quail and partridge and seafood such as fish and shrimp?	(0.932; 0.820)	0.846	0.718		0.84	
17	How often do you consume nuts, sunflower seeds, pistachios, hazelnuts and walnuts?	(0.934; 0.726)	0.742	0.552		0.74	
18	How often do you eat bird eggs such as chicken, quail, partridge, duck and goose eggs?	(0.934; 0.753)	0.775	0.604		0.77	
19	How often do you eat legumes such as split pea, bean, chickpea, mung bean, lentil, red lentil, broad bean and soybean?	(0.941; 0.523)	0.537	0.291		0.54	
20	How often do you eat potatoes but not crisps?	(0.933; 0.798)	0.825	0.681		0.81	
21	How often do you eat fruit (raw and dried fruit)?	(0.936; 0.674)	0.706	0.498		0.69	
22	How often do you eat raw vegetables (lettuce, carrots, cabbage, pumpkin, onions, mushrooms, cauliflower, broccoli, celery, spinach, vegetables, tomatoes, cucumbers) and cooked vegetables?	(0.934; 0.734)	0.765	0.585		0.75	
28	How often do you drink vegetable juices or fruit and vegetable juices?	(0.937; 0.622)	0.642	0.417		0.65	
32	How often do you drink water?	(0.939; 0.577)	0.601	0.361		0.61	

(Continued)

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TABLE 2 (Continued)

No	Items	Internal consistency (α; ITC)	EFA Sample 1 $n = 700$			CFA Sample 2 $n = 700$	
			Factor loading	h^2	Eigenvalue (%variance explained)	λ_X	(AVE; CR)
	pHDI						
	nHDI				5.931 (19.133%)		0.535;0.948
1	How often do you use white bread (lavash) or bakery products such as baguettes and toast?	(0.889; 0.556)	0.601	0.363		0.65	
3	How often do you use white rice or white pasta?	(0.891; 0.479)	0.497	0.247		0.70	
5	How often do you eat fast food such as French fries, burgers and pizza?	(0.881; 0.768)	0.820	0.672		0.81	
6	How often do you consume fried foods such as fried meat or fried sweets such as dumplings?	(0.887; 0.580)	0.606	0.368		0.77	
7	How often do you use butter (animal or vegetable) or oils such as homemade oils to flavor your bread?	(0.893; 0.422)	0.444	0.198		0.62	
13	How often do you use processed cheeses?	(0.885; 0.652)	0.702	0.492		0.75	
14	How often do you consume smoked sausages, cold meats and hot dogs?	(0.890; 0.517)	0.542	0.298		0.70	
15	How often do you consume red meat and offal of livestock and poultry such as heart, liver, gizzard, Kalle pache (Khash), kidney, tripe and abomasum?	(0.883; 0.725)	0.783	0.615		0.81	
23	How often do you consume sweets such as pastries, biscuits, Sohan, cakes, chocolates, jams and fruit compotes?	(0.883; 0.690)	0.745	0.556		0.78	
24	How often do you use ready-made or instant soups such as noodle, mushroom, chicken or vegetable soups?	(0.890; 0.514)	0.522	0.276		0.74	
26	How often do you eat tinned (jar) vegetables such as pickles and peas?	(0.889; 0.522)	0.531	0.282		0.65	
27	How often do you drink still beverages	(0.883; 0.692)	0.741	0.551		0.77	
29	How often do you drink sweetened hot beverages such as black tea, coffee and herbal or fruit teas?	(0.889; 0.538)	0.577	0.335		0.62	
30	How often do you drink sweetened carbonated and non-carbonated drinks like Coca, Pepsi, Lemonade or Fanta?	(0.891; 0.484)	0.500	0.250		0.75	
31	How often do you drink energy drinks like Red Bull, Life, Monster or Rockstar?	(0.896; 0.288)	0.536	0.089		0.84	
33	How often do you drink alcoholic beverages?	(0.890; 0.500)	0.545	0.298		0.70	

EFA, Exploratory Factor Analysis; CFA, Confirmatory Factor Analysis; Internal Consistency (Cronbach's Alpha if item deleted and Item-Total Correlation [ITC]); λx, standardized coefficients; h2, Communalities.

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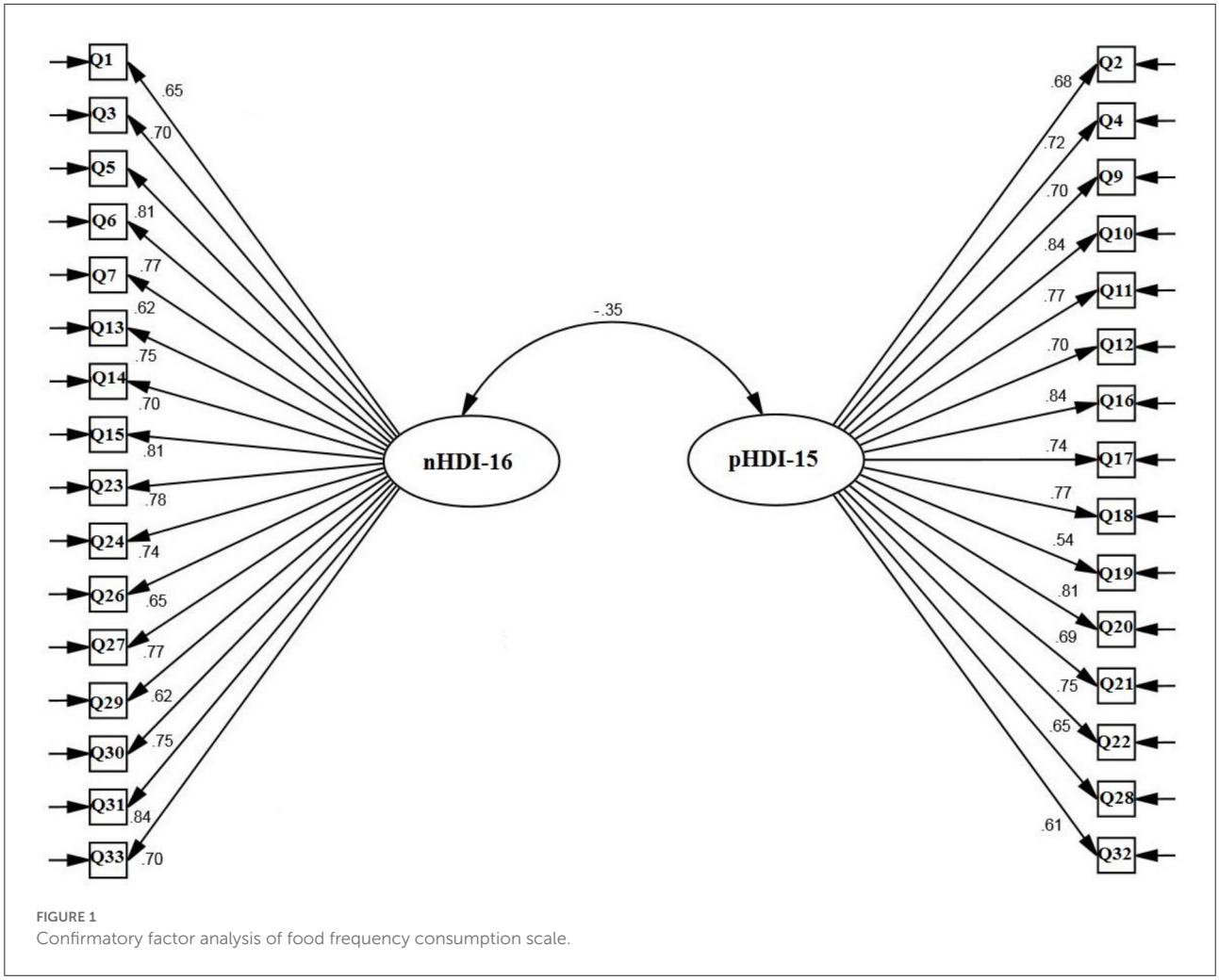

FIGURE 1
Confirmatory factor analysis of food frequency consumption scale.

Scores on these indices were higher in those with bachelor's and higher degrees than in those with less than a bachelor's degree, and scores on the hSFDI-8, hSDI-4, and hGIDI-7 indices were higher in obese adults than in others. The hGIDI-7 index was higher for men than for women, higher for divorced and widowed persons than for married and single persons, and higher for adults with insufficient income than for persons with other income levels. The LGIDI-4 index was higher for women than for men and higher for persons with sufficient or higher income than for persons with other income levels. The hSDI-4 index was higher for men than for women, higher for divorced and widowed adults than for married and single adults, higher for persons with insufficient income than for persons with other income levels, and higher for adults aged 30–40 than for other age groups. The hSFDI-8 index was higher for divorced and widowed individuals than for married and single persons, and for adults with sufficient income than for individuals with other income levels. Based on the results, the dietary indices differed significantly in the levels of the demographic variables. Therefore, these indices together with two pHDI and nHDI indices could be considered as dietary indices (Table 4).

Nutrition beliefs (NB)

Construct validity

EFA

The results of evaluating sampling adequacy indices (KMO) and Bartlett's sphericity test were 0.955 and 9,974.22 ($P < 0.001$), respectively. One factor with a eigenvalue values >1 was identified for the NB scale and confirmed based on the screen plot diagram. The data were rotated by Varimax rotation, and in total, one factor of NB was 46.61% of the total variance.

The findings suggested that factor loadings of all items (except items 55 and 56) were >0.3; hence, two items "once-daily consumption of cereals is sufficient (Q1)" and "Only children and adolescents

TABLE 3 Correlation and reliability (internal consistency and test-retest) of two scales of food frequency consumption and nutrition beliefs.

Variable	α	Θ	Ω	ICC(95% CI)	*P*-value	1	2
pHDI-15	0.939	0.936	0.932	0.928 (0.918–0.935)	<0.001	1	
nHDI-16	0.896	0.894	0.890	0.846 (0.831–0.863)	<0.001	−0.302	1
Nutrition beliefs	0.935	0.933	0.930	0.917 (0.901–0.947)	<0.001	0.556	−0.447

α, Cronbach's alpha coefficients; θ, theta coefficient; Ω, McDonald omega coefficient; ICC, intra-class correlation.

TABLE 4 Changes in dietary indexes (hSFDI-8, hSDI-4, LGIDI-4, hGIDI-7), at the levels of demographic variables.

Variable		Dietary indexes			
		hgidi-7 (%)	**LGIDI-4 (%)**	**hSDI-4 (%)**	**hSFDI-8 (%)**
BMI[a]	Normal weight	18.72 (0.71)	19.22 (0.82)	16.54 (0.69)	9.05 (0.4)
	Overweight	17.49 (0.6)	17.92 (0.63)	16.39 (0.65)	12.10 (0.45)
	Obese	20.10 (0.78)	15.14 (0.56)	20.96 (0.92)	15.73 (0.69)
P value		0.043	<0.001	<0.001	<0.001
Gender[b]	Male	20.37 (0.61)	16.19 (0.47)	18.93 (0.62)	11.39 (0.39)
	Female	16.92 (0.52)	18.13 (0.58)	16.30 (0.58)	12.26 (0.43)
P value		<0.001	0.011	0.002	0.143
Marital status[a]	Married	17.82 (0.53)	16.86 (0.48)	16.95 (0.57)	11.12 (0.38)
	Single	17.89 (78)	16.70 (0.74)	16.09 (0.79)	11.13 (0.53)
	Widowed	21.28 (1.16)	18.31 (1.25)	21.52 (1.35)	15.86 (1.12)
	Divorced	24.30 (1.56)	20.37 (1.56)	23.80 (1.53)	15.75 (1.07)
P value		<0.001	0.079	<0.001	<0.001
Education level[a]	Diploma and under diploma	16.73 (0.56)	14.55 (0.47)	15.54 (0.6)	11.03 (0.44)
	Associate Degree	19.25 (1.02)	16.21 (0.91)	18.39 (1.07)	13.04 (0.75)
	Bachelor	19.16 (0.75)	19.48 (0.74)	18.12 (0.78)	11.77 (0.52)
	Master of arts	22.72 (1.34)	22.07 (1.38)	22.16 (1.43)	13.22 (0.89)
P value		<0.001	<0.001	<0.001	0.033
Income status[a]	Insufficient	22.54 (0.93)	15.33 (0.63)	20.90 (0.91)	10.23 (0.48)
	Sufficient	17.35 (0.45)	17.89 (0.48)	16.39 (0.5)	12.52 (0.38)
	>sufficient	15.62 (1.20)	17.51 (1.52)	16.33 (1.41)	11.42 (0.9)
P value		<0.001	0.013	<0.001	0.003
Year (age)[a]	<30	18.77 (0.75)	17.56 (0.65)	17.53 (0.79)	12.03 (0.49)
	30–40	19.11 (0.55)	16.95 (0.53)	18.73 (0.58)	12.01 (0.41)
	>50	16.53 (0.91)	17.20 (0.94)	13.84 (0.95)	10.92 (0.77)
P value		0.082	0.773	<0.001	0.404

High-Glycemic-Diet-Index-7 (hGIDI-7); Low-Glycemic-Diet-Index-4 (LGIDI-4); High-Sugar Diet-Index-4 (hSDI-4); High-Saturated-Fats-Diet-Index-8 (hSFDI-8). [a]Analysis of Variance (ANOVA). [b]Independent Samples T Test.

should drink milk (Q2)" were deleted. The correlation between all items and the total score was >0.3 (Table 5).

CFA

The fit indices displayed that the one-factor construct of NB had a good and acceptable fit in the Iranian adult community (S-B χ^2 = 446.304, DF = 217, P < 0.001, CFI = 0.923, TLI = 0.920, RMSEA = 0.039 (90% C.I: 0.03-0.05), SRMR = 0.031). All factor loadings of items on the NB scale were significant (all ps <0.001) (Table 5; Figure 2).

Reliability (internal consistency and stability) and convergent validity

The internal consistency (α, Ω, and θ) and CR values of the NB scale were >0.7. For the NB scale, the ICC was 0.917

TABLE 5 Results of the exploratory and confirmatory factor analysis of the nutrition beliefs scale.

No	Items	Internal consistency (α: ITC)	EFA sample 1 $n = 700$			CFA sample 2 $n = 700$	
			Factor loading	h^2	Eigenvalue (%variance explained)	λ_X	(AVE; CR)
3	once-daily consumption of cereals is sufficient	(0.930; 0.754)	0.795	0.632	10.722 (46.615%)	0.77	0.512 (0.959)
4	Eating moldy bread can lead to food poisoning caused by Salmonella.	(0.931; 0.719)	0.763	0.583		0.75	
5	High salt intake prevents highpertention.	(0.932; 0.666)	0.721	0.519		0.71	
6	Limiting the intake of high-fat foods prevents cardiovascular disease.	(0.931; 0.734)	0.778	0.605		0.76	
7	Frequent consumption of fatty fish (such as salmon) can lead to clogged arteries.	(0.931; 0.731)	0.781	0.611		0.78	
8	Frequent consumption of grilled meats can lead to cancer.	(0.930; 0.754)	0.793	0.629		0.78	
9	A vegetarian diet increases the risk of anemia.	(0.931; 0.693)	0.751	0.564		0.75	
10	Natural yogurts contain beneficial intestinal bacteria.	(0.930; 0.736)	0.780	0.608		0.78	
11	Vegetable and olive oils are high in cholesterol.	(0.931; 0.669)	0.723	0.523		0.74	
12	Whole-wheat bread has more fiber than white bread.	(0.935; 0.437)	0.457	0.209		0.51	
13	Fruits and vegetables are calorie free.	(0.931; 0.704)	0.756	0.571		0.76	
14	Enriched animal and vegetable butters contain high amounts of vitamins A and D.	(0.935; 0.398)	0.404	0.163		0.56	
15	Low-fat cheeses have less calcium than regular cheeses.	(0.931; 0.735)	0.782	0.612		0.78	
16	Kalle pache (Khash) has high levels of cholesterol (harmful fats).	(0.935; 0.447)	0.462	0.213		0.51	
17	In a healthy diet, complex carbohydrates such as whole cereals (rice and pasta) should be replaced with simple sugars (sugar, cakes and pastries).	(0.931; 0.707)	0.759	0.576		0.75	
18	In a balanced diet, the main source of energy should be provided through protein intake.	(0.930; 0.781)	0.815	0.663		0.79	
19	Inadequate intake of niacin leads to skin inflammation and diarrhea.	(0.930; 0.768)	0.808	0.653		0.80	
20	sunlight exposure increases the synthesis of vitamin D in the body.	(0.935; 0.426)	0.440	0.194		0.59	
21	Phosphorus is one of the main components of neural tissue.	(0.930; 0.756)	0.797	0.635		0.78	
22	In a healthy diet, the ratio of calcium to phosphorus should be equal.	(0.936; 0.368)	0.382	0.146		0.54	
23	Consumption of fruits containing high amounts of vitamin C leads to increased absorption of iron in the body.	(0.935; 0.401)	0.419	0.176		0.57	
24	Cooking vegetables in cold water helps preserve the nutrients.	(0.936; 0.387)	0.390	0.152		0.74	
25	Sweets and animal fats are rich in nutrients.	(0.932; 0.651)	0.696	0.484		0.68	

EFA, Exploratory Factor Analysis; CFA, Confirmatory Factor Analysis; Internal Consistency (Cronbach's Alpha if item Deleted and Item-Total Correlation [ITC]); λx, standardized coefficients; h2, Communalities.

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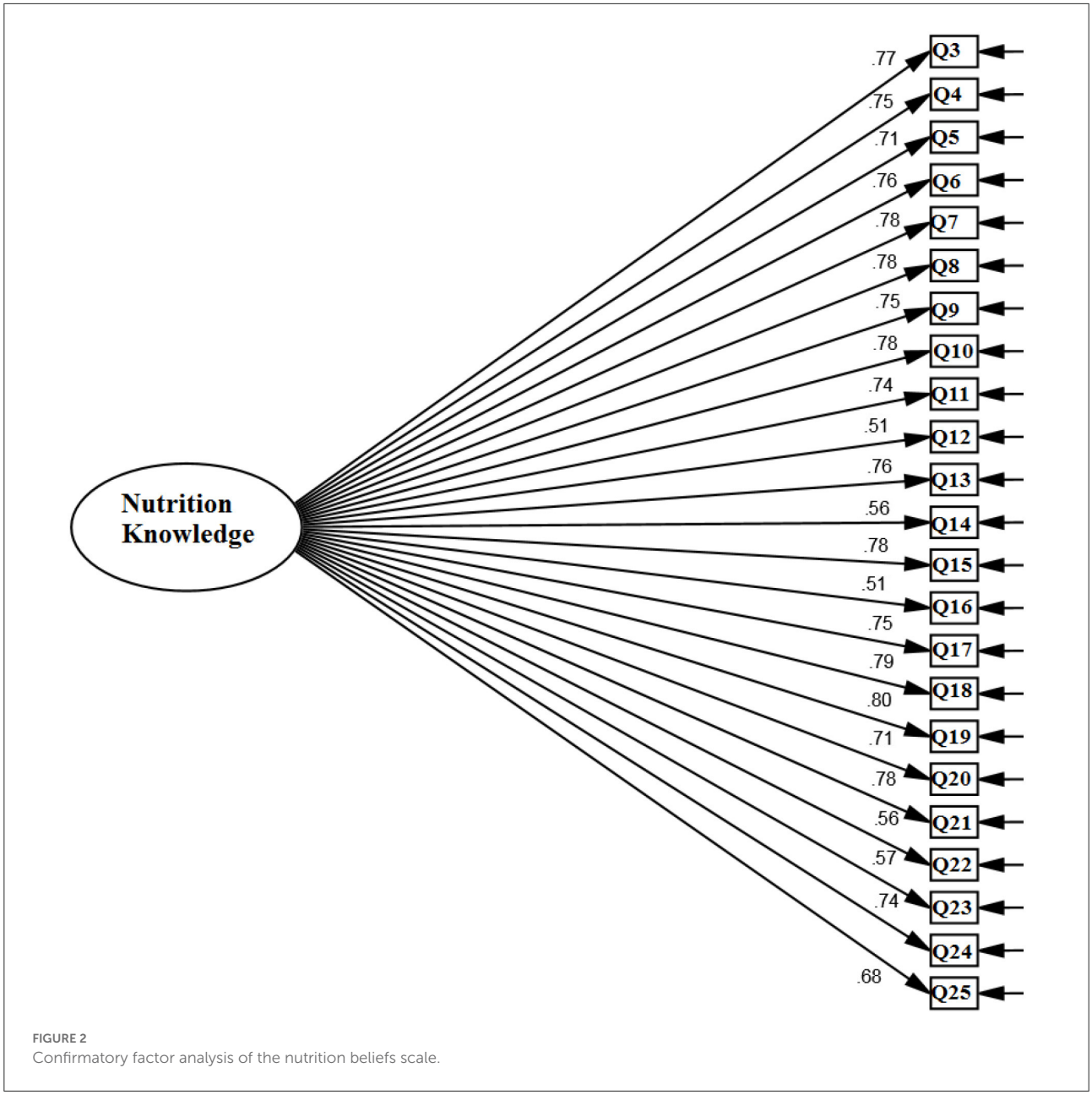

FIGURE 2
Confirmatory factor analysis of the nutrition beliefs scale.

(Table 3). The AVE values for the NB scale were >0.5 and CR>0.7 (Table 5).

Life style

The lifestyle scale had 18 items, of which 16 items were analyzed, and two items "Please provide the type of diet and" How long have you been following this diet?" were not analyzed because each participant could choose more than one option. According to test-retest results, the percentage of participants classified at the item level was on average 93.96%. Among the studied adults, the highest cross-classification agreement was 99.3 and 89.3% for the items "How would you describe your knowledge of nutrition?" and "Are you currently on a special diet (to lose or gain weight)?," respectively. Cross-classification analysis demonstrated that on the DH scale, the percentage of correct classification was high, and the percentage of misclassification was very low among the studied persons. Kappa value ranged from 0.772 (for item "Are you currently using industrial addictive substances such as hashish, marijuana, drug flowers, crack and methamphetamine?" to 0.989 (for item "How would you describe your knowledge of nutrition?"), respectively. The

TABLE 6 Agreement and misclassification in test-retest for lifestyle scale.

No.	Questionnaire items	Cat.	Total agreement	Misclassification			Kappa
				1 ± cat.	2 ± cat.	3 ± cat.	
1	Are you currently on a special diet (to lose or gain weight)?	3	89.3	10	0.7		0.481
4	How often do you eat out, for example in a restaurant, cafe or canteen?	7	95.3	1.4	2	1.3	0.929
5	Do you currently drink alcohol?	2	96.6	2.7	0.7		0.765
6	Do you currently smoke cigarettes, pipe or hookah?	2	94.6	5.4			0.706
7	Did you use to smoke cigarettes, pipe or hookah	2	98.6	1.4			0.882
8	Are you currently using drugs such as poppy, opium, opium juice (Shireh), methadone, bupropion and heroin?	2	95.3	4.7			0.813
9	Are you currently using industrial addictive substances such as hashish, marijuana, drug flowers, crack and methamphetamine?	2	94	6			0.772
10	How many hours do you sleep on weekdays?	3	97.3	2.7			0.953
11	How many hours do you sleep on the weekends?	3	96.6	3.4			0.947
12	How many hours a day do you spend watching TV/using a computer or mobile phone for entertainment or work?	6	98	2			0.969
13	How would you describe your physical activity at work/school or university?	3	97.3		0.7	2	0.955
14	How would you describe your physical activity in your spare time?	3	97.3	2.7			0.955
15	How would you describe your health compared to your peers?	3	94.6	2	3.4		0.904
16	How would you describe your knowledge of nutrition?	4	99.3			0.7	0.989
17	How would you describe your diet?	4	96.6	1.3	1.3	0.8	0.938
18	How would you describe your diet on weekdays compared to weekends?	3	93.3	0.7	4	2	0.895

No., item number in the questionnaire. Cat., number of response categories in the question. evaluated in 3 categories: low (0–33 points), moderate (34–66 points), high (67–100 points).

Kappa statistic was >0.4 for all analyzed items and its value was acceptable (Table 6).

Discussion

The aims of the current study were to translate the KomPAN questionnaire and evaluate its psychometric properties in Iranian adults and measure DQS and 4 indicators including hGIDI-7, LGIDI-4, hSDI-4 and hSFDI-8 based on 3 groups of body mass index (BMI) (BMI = 18.5–24.9, BMI = 25–29.9 and BMI ≥ 30), gender, educational level, income status, and age.

Because of cultural and social differences between our samples and the ones form Kowalkowska's study (12) some changes were made to the items. Some of the food categories in the original set was accessible to Iranian adults, hence the categories that were more accessible and commonly used among Iranians were substituted. This made the tool to become a more general and practical too especially among Iranian and Spanish communities. In this study, some of the items were eliminated during the psychometric process and some items were added to the tool. Before the psychometric process and after the translation. This has been added to the discussion section.

DH

The results of the cross-classification analysis showed that most of the items on the DH scale were correctly classified in two repetitions and only 6.04% of the items were misclassified, indicating that the items were capable of measuring the DH construct. Among the studied adults, the highest cross-classification agreement was related to the items "What type of milk (pasteurized high- or low-fat milk or whole milk) and dairy products do you usually consume?" and "Do you add sweeteners to hot drinks like tea, hot chocolate and coffee?," respectively, representing that these two items were more important than other items in measuring DH. Kappa values for all items analyzed were >0.4, illustrating the acceptable reliability of this scale (29), which is consistent with the results of the study of Kowalkowska et al. (12).

FFC and NB

The results of KMO and Bartlett's sphericity test suggested that the data for FFC and NB scales were appropriate for EFA. Klein (32) found that EFA and CFA require 10 samples per item

and a minimum of 200 samples, respectively. The fit indices showed that the two-factor construct of the FFC scale and one-factor construct of the NB scale had a good and acceptable fit in the Iranian adult community. On the FFC scale except for two items "How often do you use lard to flavor your bread or for cooking? " and "How often do you eat tinned meats?" as well as on the NB scale, except for two items "once-daily consumption of cereals is sufficient." and "Only children and adolescents should drink milk.," the correlation between all items and the total score was higher than the minimum acceptable value of >0.3 (27). The internal consistency and CR values of the two FFC subscales (pHDI and nHDI) and NB scale were higher than the recommended value of 0.7 (33), indicating good reliability of these two scales. The ICC values for the FFC subscales and NB scale were higher than the recommended value of 0.8 (31), representing the repeatability of these two scales. AVE and CR values of two FFC subscales and NB scale were >0.5 and >0.7, respectively. In addition, for both scales, CR values were >AVE, and according to the Fornell-Larcker criterion (1981) criteria, these two scales had good convergent validity. The results of the ongoing study revealed that there was a moderate and negative correlation between the two subscales of pHDI and nHDI, displaying that the FFC scale consisted of two independent constructs. Moreover, a significant positive and negative correlation was found between pHDI and nHDI with the NB scale, respectively, indicating that both FFC and NB scales had good discriminant validity (27).

Lifestyle

The results of the cross-classification analysis showed that most items of the lifestyle scale were correctly classified in the primary class in two repetitions and only 4.13% of items were incorrectly classified in another class in the second time. This shows that the items of lifestyle structure have an acceptable reproducibility. Among the studied adults, the highest cross-classification agreement was related to the items "How would you describe your knowledge of nutrition?" and "Are you currently on a special diet (to lose or gain weight)?," indicating that these two items are more important than other items in measuring lifestyle. Kappa values for all analyzed items were >0.4, representing the acceptable reliability of this scale (29). which is consistent with the results of the study of Kowalkowska et al. (12).

Conclusion

It is recommended that other researchers use the KomPAN questionnaire due to its simplicity, comprehensibility, multidimensionality and acceptable validity and reliability to identify DH, FFC, NB and lifestyle as well as measure DQS in the adult community. Moreover, it is proposed to use hGIDI-7, LGIDI-4, hSDI-4 and hSFDI-8 indices as dietary indices in addition to pHDI and nHDI indices.

Strengths of the study

The translation and evaluation of the psychometric properties of the KomPAN questionnaire in the current study enable other researchers to utilize this tool to identify dietary patterns in adults. DQS can be measured in the target population using the FFC scale of this questionnaire. A large sample size for EFA and CFA is another strength of the present study. The new classification of the items of the FFC scale based on the food culture and lifestyle of the Iranian community in this study will help researchers to measure FFC more accurately and comprehensively. In the present study, other dietary indices including hGIDI-7, LGIDI-4, hSDI-4 and hSFDI-8 were evaluated and had a good validity based on the analysis of known groups (BMI, gender, educational level, income status, and age). Using a weight estimator for CFA is another strength of the current study.

Limitations

The self-report version of the KomPAN questionnaire was applied to assess dietary habits, FFC, NB and lifestyle in healthy individuals. Therefore, it is proposed to use two IA-Q and self- SA-Q versions of the KomPAN questionnaire to increase the validity of the data. It is also recommended that the psychometric properties of the KomPAN questionnaire should be evaluated in other people with physical problems. The use of a convenient sampling method may limit the generalizability of the results to adults living in other regions of Iran. It is recommended to evaluate the psychometric properties of the KomPAN questionnaire in a more heterogeneous population, in different regions of Iran with various cultures and different social and demographic characteristics. In the present study, the measurement invariance, concurrent validity and common factor bias of the KomPAN questionnaire were not investigated. Hence, it is recommended that these indices be evaluated to increase the validity of the tool in future studies in different populations. Another limitation of the ongoing study is that the number of items in the KomPAN questionnaire is very large, which may cause respondents to become tired while completing the questionnaire, especially in the last parts of the instrument. Therefore, it is recommended to change the

order of the sections of the KomPAN questionnaire during data collection.

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 current study was approved by the Ethics Committee of Babol University of Medical Sciences (IR.MUBABOL.HRI.REC.1400.046). The patients/participants provided their written informed consent to participate in this study.

Author contributions

KS and FG conceptualized the research project. FG, KJ, FC, MK, and KS performed data curation, analysis, interpreted analysis results, and wrote first draft of the manuscript. FC, KJ, AS, RG, MK, and ZP reviewed and edited the manuscript. All authors read, revised, and approved the final manuscript.

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Conflict of interest

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

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Comparison of longitudinal changes in four surrogate insulin resistance indexes for incident T2DM in middle-aged and elderly Chinese

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Aims: Previous studies suggested a significant relationship between four surrogate indexes of insulin resistance and subsequent type 2 diabetes mellitus (T2DM). But the association of longitudinal changes (denoted as *-D*) in CVAI (Chinese visceral adiposity index), LAP (lipid accumulation product), TyG (triglyceride-glucose), and TG/HDL-C (triglyceride/ high-density lipoprotein cholesterol) indexes with the risk of T2DM remained uncertain. We aimed to compare the changes in those four surrogate indexes for predicting T2DM in middle-aged and elderly Chinese.

Methods: We extracted data from the China Health and Retirement Longitudinal Study (CHARLS). Multivariate logistic regression models were used to estimate odds ratio (OR) with 95% confidence interval (CI) of incident T2DM with four surrogate indexes. The restricted cubic spline analysis was used to examine potential non-linear correlation and visualize the dose-response relationship between four indexes and T2DM. The receiver operator characteristic curve was used to compare the performance of the four indexes to predict T2DM.

Results: We enrolled 4,596 participants in total, including 504 (10.97%) with T2DM. Analysis results showed that four surrogate indexes were associated with T2DM, and the multivariate-adjusted ORs (95% CIs) of T2DM were 1.08 (1.00–1.16), 1.47 (1.32-1.63), 1.12 (1.00–1.25), and 2.45 (2.12–2.83) for each IQR (interquartile range) increment in CVAI-D, LAP-D, TG/HDLC-D, and TyG-D, respectively. Restricted cubic spline regression showed a non-linear correlation between four surrogate indexes and the risk of T2DM (p for non-linear < 0.001). From the ROC (receiver operating characteristic) curve, TyG-D had the highest AUC (area under curve), and its AUC values were significantly different from other three indexes both in male and female (all $P < 0.001$).

Conclusion: Compared with other indexes, TyG-D was a better predictor in the clinical setting for identifying middle-aged and elderly Chinese with T2DM. Monitoring long-term changes in TyG might help in the early identification of individuals at high risk of T2DM.

KEYWORDS

type 2 diabetes mellitus, longitudinal changes, triglyceride glucose index, Chinese visceral adiposity index, lipid accumulation product, triglyceride/high-density lipoprotein cholesterol, area under curve, surrogate index

Introduction

The prevalence of type 2 diabetes mellitus (T2DM) is a growing global health issue (1, 2). Presently, about 1 in 11 adults have diabetes mellitus (DM), 90% of whom develop T2DM (3). Asia is a significant region of the fast spreading T2DM global epidemic. During the past few decades, the prevalence of T2DM has increased significantly in China (4).

T2DM is known as a complex and heterogeneous disease, for which obesity is one of the strongest risk factors (5). Increased (mostly visceral) adipose tissue mass-related insulin resistance (IR) has been recognized as a critical component that may contribute to concurrent increases in the prevalence of T2DM (6). IR is present in many metabolic disorders, such as T2DM and metabolic syndrome (7, 8). However, due to its complicated process, lengthy laboratory test, and high cost, the method for the IR measurement is not widely used in clinical trials or sizable population-based studies (9). Excessive abdominal fat accumulation has been linked to glucose and lipid metabolic problems (10). In particular, increased visceral adipose tissue (VAT) has been linked to IR (11). In diabetic studies, the Chinese visceral adiposity index (CVAI) and lipid accumulation product (LAP) have been employed as trustworthy indicators of visceral adiposity (12–14). Triglyceride-glucose (TyG) index and the triglyceride-to-HDL-cholesterol (TG/HDL-C) ratio have also emerged as straightforward, affordable, repeatable, and reliable surrogates for IR measurement (15).

Previous studies show that high TyG index is a risk factor for incident DM and cardiovascular disease (16, 17). However, a drawback of those studies is that they only examine data from one moment in time and do not examine how the index changes over time. Given that each individual has unique underlying conditions, assessing dynamic changes may be more beneficial than measuring data at a single time point (18). We calculate the difference in index value at the end of follow-up (2015) minus that at baseline (2011) for analysis, denoted as *-D*. This research aims to assess the applicability of longitudinal changes in CVAI, LAP, TyG, and TG/HDL-C index in predicting T2DM in middle-aged and elderly Chinese population in a large community-based prospective cohort study.

Abbreviations: DM, Diabetes mellitus; T2DM, Type 2 diabetes mellitus; CVAI, Chinese visceral adiposity index; LAP, Lipid accumulation product; TyG, Triglyceride-glucose; TG, Triglyceride; HDL-C, High-density lipoprotein cholesterol; BMI, Body mass index; WC, Waist circumference; DBP, Diastolic blood pressure; SBP, Systolic blood pressure.

Methods

Study population

Our study obtained data from 2011 wave and 2015 wave of the CHARLS survey completed by the National School of Development at Peking University, which was open to the public (http://charls.pku.edu.cn/). CHARLS was a large-scale, multidisciplinary survey conducted for nation-wide residents aged 45 and older in 450 villages and communities across 28 provinces (autonomous regions and municipalities). It collected data on a wide range of topics, including basic demographics, family, health status and function, cognition and depression, health care and insurance, work and retirement, pensions, income expenditure and assets, and housing. It is a trustworthy resource for researching middle-aged and elder people's health statuses and contributing elements. By the year 2015, 12,241 households with a total of 21,097 residents were represented in the survey.

Professional staff took venous blood samples from participants in 2011 and 2015 after overnight fasting for at least 12 h. Complete blood count was performed right away at the survey sites. The full blood samples were kept at 4°C while the other samples were delivered to the central laboratory in Beijing (Youanmen Centre for Clinical Laboratory of Capital Medical University) for further testing. An enzymatic-colourimetric test was used to determine the levels of glucose, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). The levels of glycated hemoglobin (HbA1c) were measured using high-performance liquid chromatography with boronate affinity.

The initial approval of CHARLS was granted by Peking University's Ethical Review Committee in 2008 (IRB00001052–11,015). The research methods followed all applicable CHARLS requirements and guidelines. Before participating willingly in CHARLS, each individual completed informed consent forms.

We finalized the inclusion criteria for the participants in this study: 45 years and older; complete demographic data including gender, level of education, marital status, and residential location; complete fasting blood glucose levels. Based on those inclusion criteria, a total of 4,596 participants from 17,708 individuals were included.

Measurement

Assessment of four surrogate indexes of insulin resistance

CVAI

Male: CVAI = −267.93 + 0.68 × age + 0.03 × BMI + 4.00 × WC (cm) + 22.00 × log10(TG) (mmol/L)– 16.32 × HDL-C (mmol/L)

Female: CVAI = −187.32 + 1.71 × age + 4.23 × BMI + 1.12 (cm) × WC + 39.76 × log10(TG) (mmol/L) – 11.66 × HDL-C (mmol/L) (19).

LAP

Male: LAP = [(WC (cm) – 65)×TG (mmol/L)]

Female: LAP = [(WC (cm) – 58)×TG (mmol/L)] (20).

TyG

Ln [fasting triglycerides (mg/dL) × fasting glucose (mg/dL)/2] (21).

TG/HDL-C

Ratio of serum triglycerides to high-density lipoprotein cholesterol (22).

CVAI-D, LAP-D, TyG-D, TG/HDLC-D

Changes in CVAI, LAP, TyG, TG/HDL-C indexes were calculated with their levels measured in 2015 minus that in 2011 (baseline).

Assessment of T2DM

We defined T2DM in Accordance with the 2005 American Diabetes Association criteria: a HbA1c level of 6.5 percent or higher; a fasting blood glucose level of 126 mg/dL (7 mmol/L) or higher; a random blood glucose level of 200 mg/dL (11.1 mmol/L) or higher, and/or self-reported diagnosis ("Have you ever been diagnosed with diabetes or hyperglycemia?").

The analyses were adjusted for anthropometric data, health-related activities, and socio-demographic factors. Age, gender, education (primary school or below, high school, college or above), location (city/town, village), and marital status (married, non-married), were all analyzed as demographic variables. As for factors of health-related behavior, we analyzed smoking status ("non-smoker," "ex-smoker," "current smoker"), drinking status ("never", "less than once a month", "more than once a month"), and sleep time. Those data came from self-reported questionnaires and were gathered with the aid of skilled interviewers. Athropometric data such as systolic blood pressure (SBP) and diastolic blood pressure (DBP) were the means calculated from three measurements using Omron HEM-7200 sphygmomanometer.

Statistical analysis

Classified variables were represented by percentages, while continuous variables were represented by means and standard deviation (SD) for normally distributed data and median (interquartile range, IQR) for non-normally distributed data. Based on the quartiles of four surrogate indexes (Q1, Q2, Q3, Q4), baseline characteristics and diabetes incident rate were compared using the One-Way ANOVA, Kruskal-Wallis H test or chi-square test, if necessary. In order to calculate the odds ratio (OR) of diabetes with a 95% confidence interval (CI) for four surrogate indexes as continuous (per IQR increment) or categorical (quartiles) variables, we employed logistic models. Three models were used to explore the relationship between four surrogate indexes and T2DM, including unadjusted crude model (Model 1); model adjusted for age, gender, education level, location and marital status (Model 2); model further adjusted for smoking status, drinking status and sleep time (Model 3). The results were presented with ORs and 95% CIs. The restricted cubic spline analysis was also performed to examine possible non-linear correlation and visualize the dose–response relationship between four surrogate indexes and T2DM. Additionally, to assess the predictive performance of LAP-D, TyG-D, CVAI-D, and TG/HDLC-D for T2DM, The area under the receiver operating characteristic (ROC) curve (AUC) value were determined. DeLong's non-parametric method was used to compare the AUC between TyG-D and other indexes (23). The optimal cutoff values of LAP-D, TyG-D, CVAI-D, and TG/HDLC-D for predicting T2DM were identified based on the maximum value of the youden idnex.

All statistical analyses were performed using R 4.1.3. The package "rms" was used for analyzing with restricted cubic splines. MedCalc version 13.0 for Windows (MedCalc Software, Mariakerke, Belgium) was used to perform significance tests for the comparison of AUCs. $P < 0.05$ for a two-tailed test denoted statistical significance.

Results

Baseline characteristics

Table 1 shows the characteristics of the study population. A total of 4,596 participants were included [median age = 58,

TABLE 1 The characteristics of the study participants grouped by T2DM at baseline ($N = 4,596$).

	Total ($n = 4,596$)	Non-T2DM ($n = 4,092$)	T2DM ($n = 504$)	p
Age	58.00 (52.00, 65.00)	58.00 (52.00, 65.00)	60.00 (53.00, 67.00)	<0.01
Gender				0.67
Female	2,444 (53.18)	2,181 (53.30)	263 (52.18)	
Male	2,152 (46.82)	1,911 (46.70)	241 (47.82)	
Marita				0.38
Non-married	516 (11.23)	453 (11.07)	63 (12.50)	
Married	4,080 (88.77)	3,639 (88.93)	441 (87.50)	
Education				0.39
Primary school or below	3,267 (71.08)	2,897 (70.8)	370 (73.41)	
High school	929 (20.21)	832 (20.33)	97 (19.25)	
College or above	400 (8.70)	363 (8.87)	37 (7.34)	
Location				0.67
City/Town	4,335 (94.32)	3,857 (94.26)	478 (94.84)	
Village	261 (5.68)	235 (5.74)	26 (5.16)	
Smoking				0.51
Non-smoker	2,803 (60.99)	2,507 (61.27)	296 (58.73)	
Current smoker	1,432 (31.16)	1,264 (30.89)	168 (33.33)	
Ex-smoker	361 (7.85)	321 (7.84)	40 (7.94)	
Drinking				0.13
Never	3,056 (66.49)	2,706 (66.13)	350 (69.44)	
Less than once a month	371 (8.07)	341 (8.33)	30 (5.95)	
More than once a month	1,169 (25.44)	1,045 (25.54)	124 (24.60)	
Sleep time	6.25 (5.00, 8.00)	6.50 (5.00, 8.00)	6.00 (5.00, 8.00)	0.91
BMI (kg/m^2)	22.83 (20.71, 25.28)	22.7 0 (20.64, 25.09)	23.80 (21.18, 26.61)	<0.01
WC (cm)	83.50 (77.00, 90.03)	83.00 (77.00, 90.00)	87.00 (79.00, 94.00)	<0.01
Glucose (mg/dl)	99.90 (93.24, 106.92)	99.36 (92.88, 106.20)	104.76 (96.66, 111.78)	<0.01
TG (mg/dl)	99.12 (71.68, 138.06)	97.35 (70.8, 136.29)	107.97 (80.54, 151.78)	<0.01
HDL-C (mg/dl)	51.03 (42.91, 61.47)	51.42 (43.3, 61.47)	48.33 (39.82, 59.63)	<0.01
SBP (mmHg)	125.33 (113.33, 139.67)	124.67 (112.67, 139.08)	130.00 (119.33, 143.67)	<0.01
DBP (mmHg)	74.00 (66.67, 82.33)	73.67 (66.33, 82.00)	76.33 (69.67, 85.00)	<0.01
CVAI-D	7.46 (−7.08, 21.39)	6.98 (−7.34, 20.95)	10.83 (−4.15, 25.87)	<0.01
LAP-D	17.37 (−23.42, 71.65)	15.19 (−24.76, 66.66)	41.16 (−11.95, 121.52)	<0.01
TG/HDLC-D	0.18 (−0.51, 0.86)	0.18 (−0.5, 0.83)	0.26 (−0.56, 1.26)	0.05
TyG-D	0.07 (−0.24, 0.38)	0.04 (−0.26, 0.35)	0.33 (0.02, 0.69)	<0.01

p-values were calculated from chi-square tests (categorical variables) or rank-sum tests (non-normally distributed continuous variables) or t-tests (normally distributed continuous variables).
T2DM, type 2 diabetes mellitus; BMI, body mass index; WC, waist circumference; DBP, diastolic blood pressure; SBP, systolic blood pressure; TG, triglyceride; HDL-C, high-density lipoprotein; CVAI-D, difference in Chinese visceral adiposity index; LAP-D, difference in lipid accumulation product; TG/HDLC-D, difference in triglyceride/high-density lipoprotein cholesterol ratio; TyG-D, difference in triglycerides/ glucose index.

including 2,152 (46.82%) male and 2,444 (53.18%) female]. Of all those included participants, 504 (10.97%) had T2DM while the rest had not. The baseline median of CVAI-D, LAP-D, TG/HDLC-D, and TyG-D in all participants was 7.46 (−7.08, 21.39), 17.37 (−23.42, 71.65), 0.18 (−0.51, 0.86), and 0.07 (−0.24, 0.38), respectively. Characteristics of participants with T2DM were obviously different from those without, specifically, the formers were more likely to be older, and with higher SBP, DBP, BMI, WC, Glucose, TG, CVAI-D, LAP-D, TG/HDLC-D, TyG-D and lower HDL-C.

Association and dose-response relationship between four surrogate indexes and T2DM

Table 2 shows the association between four surrogate indexes and the risk of T2DM, as well as their quartiles. Multivariable logistic regression analysis was performed after adjusting for age, gender, education level, location, marital status, smoking status, drinking status, sleep time, SBP and DBP. Results showed that four surrogate indexes were associated

TABLE 2 Association between four surrogate indexes and T2DM risk.

	Model 1	*P*	Model 2	*P*	Model 3	*P*
CVAI-D per IQR	1.06 (0.99, 1.14)	0.095	1.07 (1.00, 1.15)	0.048	1.08 (1.00, 1.16)	0.04
Quartiles of CVAI-D						
Q1	Reference	——	Reference	——	Reference	——
Q2	1.00 (0.76, 1.32)	1	1.03 (0.77, 1.36)	0.853	1.02 (0.77, 1.36)	0.869
Q3	1.21 (0.92, 1.58)	0.171	1.26 (0.96, 1.65)	0.102	1.27 (0.96, 1.67)	0.09
Q4	1.21 (0.92, 1.58)	0.002	1.58 (1.22, 2.06)	0.001	1.63 (1.25, 2.12)	<0.001
P for trend		<0.001		<0.001		<0.001
LAP-D per IQR	1.44 (1.30, 1.60)	<0.001	1.49 (1.34, 1.65)	<0.001	1.47 (1.32, 1.63)	<0.001
Quartiles of LAP-D						
Q1	Reference	——	Reference	——	Reference	——
Q2	0.99 (0.74, 1.32)	0.941	0.96 (0.72, 1.29)	0.804	0.98 (0.73, 1.31)	0.905
Q3	1.00 (0.75, 1.33)	1	1.02 (0.76, 1.36)	0.901	1.03 (0.77, 1.38)	0.844
Q4	2.15 (1.67, 2.78)	<0.001	2.28 (1.77, 2.95)	<0.001	2.24 (1.73, 2.90)	<0.001
P for trend		<0.001		<0.001		<0.001
TG/HDLC-D per IQR	1.11 (0.99, 1.24)	0.056	1.12 (1.00, 1.25)	0.047	1.12 (1.00, 1.25)	0.047
Quartiles of TG/HDLC-D						
Q1	Reference	——	Reference	——	Reference	——
Q2	0.87 (0.67, 1.14)	0.311	0.85 (0.65, 1.10)	0.219	0.86 (0.65, 1.12)	0.255
Q3	0.70 (0.53, 0.92)	0.012	0.69 (0.52, 0.91)	0.009	0.70 (0.53, 0.92)	0.012
Q4	1.31 (1.03, 1.68)	0.029	1.33 (1.04, 1.71)	0.023	1.33 (1.04, 1.71)	0.024
P for trend		0.076		0.057		0.06
TyG-D per IQR	2.38 (2.06, 2.74)	<0.001	2.45 (2.12, 2.83)	<0.001	2.45 (2.12, 2.83)	<0.001
Quartiles of TyG-D						
Q1	Reference	——	Reference	——	Reference	——
Q2	0.91 (0.65, 1.28)	0.606	0.91 (0.65, 1.28)	0.594	0.90 (0.64, 1.27)	0.56
Q3	1.83 (1.36, 2.47)	<0.001	1.85 (1.37, 2.50)	<0.001	1.79 (1.33, 2.42)	<0.001
Q4	3.58 (2.74, 4.75)	<0.001	3.71 (2.83, 4.92)	<0.001	3.72 (2.83, 4.93)	<0.001
P for trend		<0.001		<0.001		<0.001

Model 1 was crude model; Model 2 adjusted for age, gender, education level, location and marital status; Model 3 was further adjusted for smoking status, drinking status and sleep time. IQR, interquartile range; CVAI-D, difference in Chinese visceral adiposity index; LAP-D, difference in lipid accumulation product; TG/HDLC-D, difference in triglyceride/high-density lipoprotein cholesterol ratio; TyG-D, difference in triglycerides/glucose index.

with T2DM as continuous variables. Specifically, each IQR increment in CVAI-D was associated with 8% higher odds for T2DM (OR, 1.08; 95% CI, 1.00–1.16); each IQR increment in LAP-D was associated with 47% higher odds for T2DM (OR, 1.47; 95% CI, 1.32–1.63); each IQR increment in TG/HDLC-D was associated with 12% higher odds for T2DM (OR, 1.12; 95% CI, 1.00–1.25); each IQR increment in TyG-D was associated with 145% higher odds for T2DM (OR, 2.45; 95% CI, 2.12–2.83). The risk of T2DM increased gradually with the quartiles of the CVAI-D, LAP-D, AND TyG-D (*P* for trend < 0.001). Compared to the first quartile group (Q1), the last quartile group (Q4) in CVAI-D,LAP-D, TG/HDLC-D, and TyG-D presented the highest risk of T2DM (OR = 1.63, 95% CI = 1.25–2.12; OR = 2.24, 95% CI = 1.73–2.90; OR = 1.33, 95% CI = 1.04–1.71; OR = 3.72, 95% CI = 2.83–4.93, respectively).

Figure 1 shows the dose-response relationship between four surrogate indexes and the risk of T2DM. Restricted cubic spline regression showed a non-linear dose-response relationship between four surrogate indexes and the risk of T2DM ($P_{overall} < 0.001$, $P_{non-liner} < 0.001$). And we found that there was U-shaped association between four surrogate indexes and the risk of T2DM.

Predictive performance of the four surrogate indexes for T2DM by gender

Table 3 shows the predictive performance of four surrogate indexes for T2DM risk. TyG-D had the highest AUC [0.66 (0.63–0.69)], followed by LAP-D [0.59 (0.56–0.62)], CVAI-D [0.55 (0.52–0.57)], and TG/HDLC-D [0.53 (0.50–0.56)]. TyG-D had the highest sensitivity (0.669) and TG/HDLC-D had the highest specificity (0.890) of all. Gender-stratified analyses showed that TyG-D had the highest diagnostic value of all (Figure 2). Among the female participants, TyG-D had the highest AUC [0.65 (0.61–0.69)], followed by LAP-D [0.59 (0.55–0.63)], TG/HDLC-D [0.53 (0.49–0.57)], and

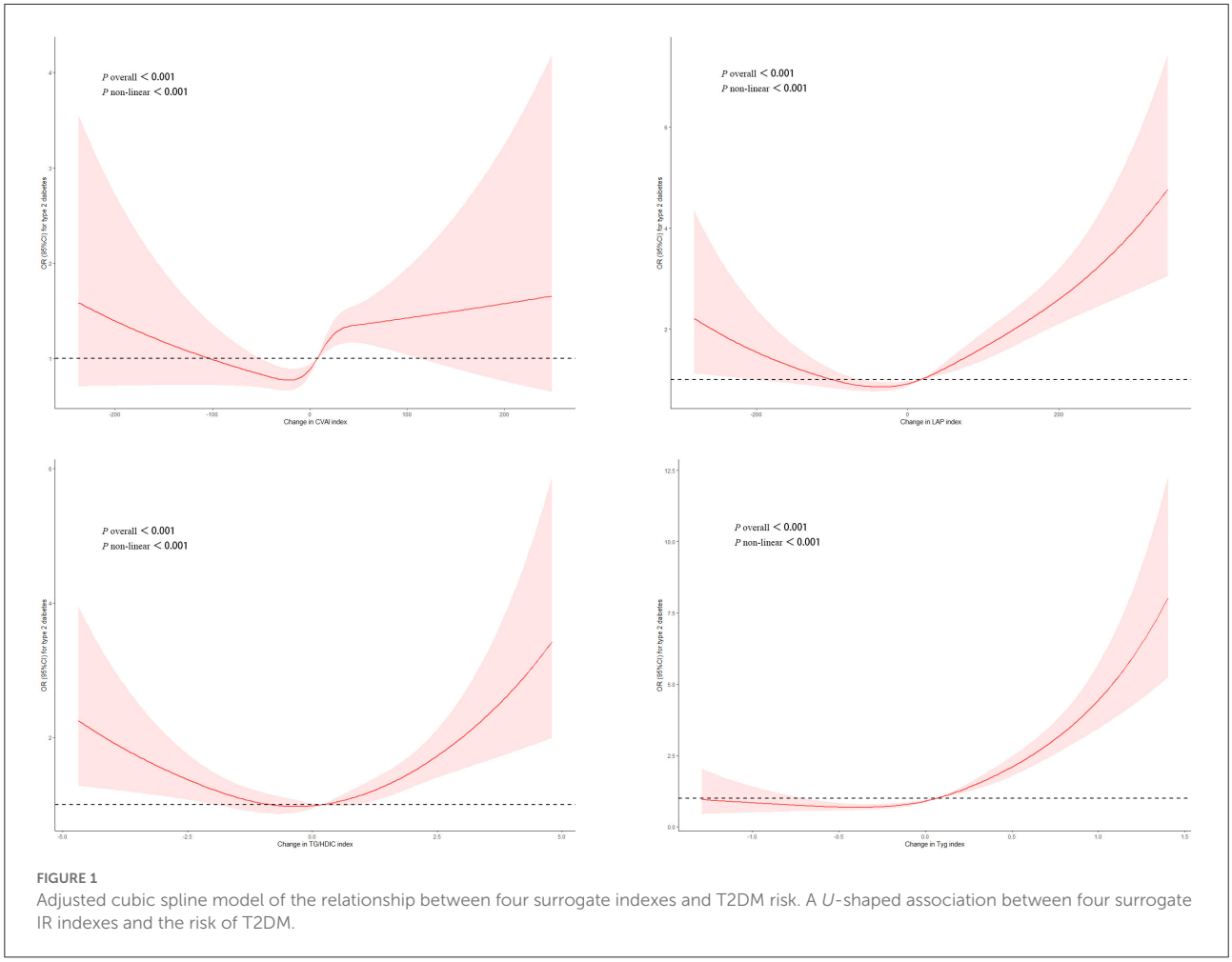

FIGURE 1
Adjusted cubic spline model of the relationship between four surrogate indexes and T2DM risk. A *U*-shaped association between four surrogate IR indexes and the risk of T2DM.

TABLE 3 Comparison of predictive accuracy and cut-off values of four surrogate indexes for T2DM by gender.

	Test	AUC	95 CI low	95 CI up	Cutoff value	Specificity	Sensitivity	PPV	NPV	*P*
Female	CVAI-D	0.51	0.48	0.55	13.05	60.89	43.73	0.12	0.90	0.484
	LAP-D	0.59	0.55	0.63	70.34	72.31	47.53	0.17	0.92	<0.001
	TG/HDLC-D	0.53	0.49	0.57	1.10	80.61	31.18	0.17	0.90	0.223
	TYG-D	0.65	0.61	0.69	0.12	55.66	69.58	0.16	0.94	<0.001
Male	CVAI-D	0.58	0.54	0.62	8.74	55.47	58.09	0.14	0.91	<0.001
	LAP-D	0.60	0.56	0.64	52.30	74.52	42.74	0.17	0.91	<0.001
	TG/HDLC-D	0.53	0.49	0.57	1.63	89.95	18.67	0.19	0.90	0.146
	TYG-D	0.68	0.64	0.71	0.14	61.02	64.73	0.17	0.93	<0.001
Overall	CVAI-D	0.55	0.52	0.57	13.06	61.61	47.02	0.13	0.90	<0.001
	LAP-D	0.59	0.56	0.62	67.07	75.22	42.06	0.17	0.91	<0.001
	TG/HDLC-D	0.53	0.50	0.56	1.62	89.03	20.83	0.19	0.90	0.066
	TYG-D	0.66	0.63	0.69	0.13	58.14	66.87	0.16	0.93	<0.001

IQR, interquartile range; CVAI-D, difference in Chinese visceral adiposity index; LAP-D, difference in lipid accumulation product; TG/HDLC-D, difference in triglyceride/high-density lipoprotein cholesterol ratio; TyG-D, difference in triglycerides/ glucose index.

CVAI-D [0.51 (0.48–0.55)]. Among the male participants, TyG-D also had the highest AUC [0.68 (0.64–0.71)], followed by LAP-D [0.60 (0.56–0.64)], CVAI-D [0.58 (0.54–0.62)], and TG/HDLC-D [0.53 (0.49–0.57)].

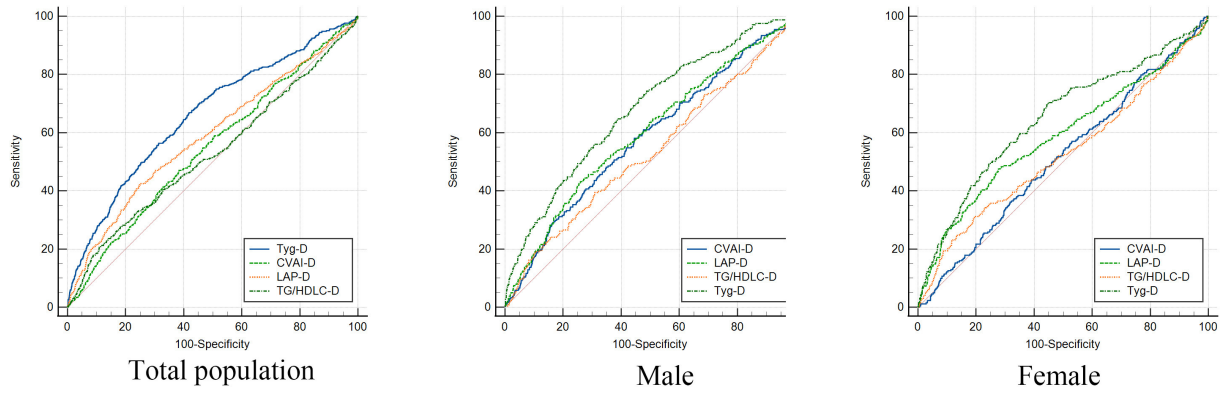

FIGURE 2
Receiver operating characteristic curves of four surrogate indexes in total population, male and female for incident T2DM. TyG-D presented the highest diagnostic value of all.

Comparison of AUC values between TyG-D and other indexes for T2DM by gender

Table 4 shows the differences in AUC values between TyG-D and other indexes for T2DM. We found that differences in AUC values between TyG-D and other three indexes were significant both in male and female (all $P < 0.001$). Results showed that TyG-D had the strongest predictive performance for T2DM compared with other three surrogate indexes.

TABLE 4 Comparison of AUC values between TyG-D and other indexes by genders.

	Difference between area (95%CI)	*P*-value
Female		
TyG-D vs. CVAI-D	0.134 (0.097, 0.171)	<0.001
TyG-D vs. LAP-D	0.061 (0.033, 0.088)	<0.001
TyG-D vs. TG/HDLC-D	0.122 (0.095, 0.148)	<0.001
Male		
TyG-D vs. CVAI-D	0.097 (0.053, 0.142)	<0.001
TyG-D vs. LAP-D	0.080 (0.046, 0.114)	<0.001
TyG-D vs. TG/HDLC-D	0.145 (0.117, 0.174)	<0.001
Overall		
TyG-D vs. CVAI-D	0.114 (0.084, 0.143)	<0.001
TyG-D vs. LAP-D	0.073 (0.051, 0.094)	<0.001
TyG-D vs. TG/HDLC-D	0.133 (0.114, 0.153)	<0.001

Discussion

In this prospective cohort study, we analyzed the baseline and follow-up data of 4,596 Chinese participants and found that longitudinal changes in the four surrogate IR indexes were significantly associated with the risk of T2DM. TyG-D showed the highest predictive accuracy of all the indexes.

Although relevant studies have been conducted previously, they have some limitations. Li et al. found the association between the TyG and new-onset diabetes was positive and linear, with an AUC of 0.597 (0.559–0.636) (16). Compared to Li's study, our research found that the AUC of the TyG-D was 0.66 (0.63–0.69), which was higher than the TyG index alone in the risk of incident diabetes. It was possible that TyG-D had higher predictive performance than TyG index alone for the risk of T2DM. Zhang et al. reported that the risk of T2DM increased with elevated TyG index in normal-weighted Chinese and suggested that TyG might be an important indicator in identifying population at high risk of T2DM. However, the applicability and utility of TyG index for predicting T2DM in normal-weighted people should be further confirmed in the entire population (24).

In Chinese adults, IR showed a stronger correlation with T2DM risk compared to β-cell dysfunction (25). In clinical practice, in addition to BMI, measurement of WC might be useful in identifying and managing overweight/obese population at high cardiometabolic risk (26). Anthropometric parameters (WC, waist-to-hip ratio, BMI), surrogate measurements of IR (fasting plasma glucose, insulin, fasting insulin-glucose product), fasting lipids, SBP and DBP were also important in predicting T2DM risk (27). Although BMI was a major risk factor of T2DM-related mortality, many people with normal BMI were referred to as "metabolically obese but normal weight (MONW)," which was characterized by a variety of metabolic risk factors and would significantly increased T2DM risk in many ethnic groups.

TyG, a combination of fasting blood glucose and TG, was considered a surrogate marker of IR. Previous studies on

middle-aged and elderly adults in China suggested that TyG could identify metabolic syndrome (Mets) (28, 29). There was a significant correlation between the TyG index and incidence of T2DM in individuals with obesity (including visceral fat obesity), fatty liver and normal BMI, which was not a measure of body fat distribution (30). In Japanese with normal glycemic levels, the longitudinal cohort research showed a positive correlation between baseline TyG-BMI and the risk of T2DM, which was noticeably higher in young people, women, non-hypertensive people, and non-drinkers (31). Previous studies suggested that TyG-waist-to-height ratio and TyG-BMI were both clinically effective markers for predicting diabetes in individuals with normal and impaired fasting glucose levels, respectively (16). Numerous studies on the TyG index in individuals of normal weight demonstrated the significance of the TyG index (23, 32).

For female, TyG, VAI and LAP indexes were considered better predictors for T2DM than conventional anthropometric and laboratory measures (27). Based on BMI, WC, TG, and HDL-C, VAI was a gender-specific indicator of visceral adiposity. The risk of new-onset T2DM was significantly correlated with baseline VAI and its transitions. Early prevention was required to reduce the risk of T2DM in Chinese with high VAI levels (33). LAP index, a phenotype of obesity produced by WC and fasting TG, was independently linked to T2DM in hypertensive female. Elevated LAP levels and patterns of low-to-high and maintained-high LAP transitions were significant risk factors for T2DM in women (34). Prevention was needed to combat T2DM at an early dyslipidemic stage. Previous studies suggested that TG/HDL-C was a better marker for assessing IR and DM in elderly Chinese when compared with other common lipid measurements (35, 36). For African American women, the TG/HDL-C ratio was a predictor of β-cell function (37), but it was not a predictor of IR (38). Although the TyG presented significantly better predictive performance than CVAI, LAP and TG/HDL-C in this study, it still required further research in other populations.

The data of this study were obtained from a cohort, so there was a strong causal inference. Compared to the traditional HOMA-IR detection method, surrogate IR indexes were cheaper, faster, and helpful to earlier prevention of T2DM. Researchers could fully utilize both the investigated data and laboratory data when considering the longitudinal changes of IR to reflect the dynamic changes of the human body. Calculating the difference of indexes could reduce the absolute difference between the data and avoid the influence of individual extreme values. This study provided a potential direction for predicting the risk of T2DM development. There were some limitations. First, the follow-up period of this study was not long enough. Second, as the majority of the study's participants were middle-aged and elderly individuals, further researches on other age groups would be necessary. Previous studies suggested that there was a slight difference in DM incidence between women and men. More researches should be encouraged to explore specific T2DM predictors in both women and men. Finally, we were unable to include some other potential confounders such as physical activity, diet and genes in our analysis, which were not contained in the database.

Conclusion

Our study found that TyG-D index was a stronger predictor in the clinical setting for identifying individuals with T2DM in middle-aged and elderly Chinese compared with other indexes. Among the four analyzed indexes, TyG-D presented the largest AUC value [0.66 (0.63–0.69)] and highest sensitivity.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: China Health and Retirement Longitudinal Survey (2020). Available online at: http://charls.pku.edu.cn/pages/data/111/zh-cn.html.

Author contributions

YH and LP designed the study. LL, HP, JL, HW, and GX contributed to data acquisition. MW and LP performed the statistical analysis. JH and YH contributed to the discussion. LP and YG drafted the manuscript. JH edited the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

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

Publisher's note

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Trend of metabolic risk factors among the population aged 25–64 years for non-communicable diseases over time in Vietnam: A time series analysis using national STEPs survey data

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Introduction: The study aims to examine the trends of 4 metabolic NCDs risk factors including raised blood pressure, increased blood glucose, elevated blood lipids and overweight/obesity over the last 10 years in Vietnam as well as examine these trends among different sub-population by geographical area, gender, and age groups.

Methods: The study combined the national representative data from three rounds of STEPs survey in Vietnam conducted in 2010, 2015, and 2020 on people aged 25–64 years. The overall prevalence of each metabolic factor together with 95% CI for each time point as well as the stratified prevalence by rural/urban, male/female, and 4 separated age groups were calculated and considered the sampling weight. Cochran–Armitage test for trend was used to test for the differences in the prevalence over time.

Results: The prevalence of hypertension, overweight/obesity, hyperglycemia, and hyperlipidemia among the population aged 25–64 years old was 28.3, 20.57, 6.96, and 15.63%, respectively in the year 2020. All NCD metabolic risk factors examined in this analysis show significantly increasing trends over time. For most age groups, the increasing burden of NCD metabolic risk factors was more significant during the period 2015–2020 compared to the period 2010–2015. Male population and population aged 55–64 experienced the most dramatic changes in the burden of all NCD metabolic risk factors.

Conclusion: To reverse the increasing trend of NCD metabolic factors in Vietnam, intervention, and policy need to apply a comprehensive life course approach.

KEYWORDS

NCD, metabolic risk factor, time series analyses, trend, STEPs

Introduction

Non-communicable diseases (NCDs) are one of the leading causes of death worldwide in both developing and developed countries. In addition, its incidence and mortality have an increasing trend in most countries around the world; it is responsible for 73% of the total deaths in 2018 (1). During 2010–2020, the mortality burden due to NCDs increased by 15% (equivalent to 44 million deaths). More significantly, deaths from NCDs in low-income countries will be eight times higher than those in developed countries in 2030 (2).

Vietnam achieved significant changes in the economic situation and became a middle-income country in 2008. Along with economic growth, Vietnam is also experiencing fast and wide urbanization and a population aging process. The rapid epidemiological and demographic transition in the last 20 years has resulted in a significant increase in the burden of NCDs in Vietnam. It is estimated that in 2016, the whole country had 549,000 deaths of all kinds, of which 77% were deaths due to NCDs, mainly cardiovascular diseases (31%), cancer (19%), diabetes (4%), and chronic obstructive pulmonary disease (6%) (1, 3).

With the high level of risk factors driven by the increasingly global economy and fast population aging, NCDs are expected to worsen in the future. For that reason, NCDs prevention and control has been one of Vietnam's health priorities (4). As many other countries, Vietnam has applied the STEPs survey introduced by WHO (5) as an effective information system to monitor trends of NCDs and their risk factors to provide evidence for developing policies and related interventions.

The STEPs survey is a comprehensive approach to measuring established core risk factors responsible for NCDs at the population level. The survey consisted of three steps: (1) STEP 1 was for collecting demographic information/behavioral risk factors in an interviewer-administered survey; (2) STEP 2 was for collecting physical measurements such as height/weight/blood pressure, and (3) STEP 3 was for obtaining blood samples to test for glucose/cholesterol and urine samples.

Vietnam completed three rounds of the STEP survey in the years 2010, 2015, and 2020. All previous studies using STEPs 2010 and 2015 data was cross-sectional study, thus, was not able to present and test for the significant changes of the patterns of NCD metabolic factors over time (6–8). This study attempts to combine the data from the three-round surveys to explore the trend of 4 metabolic NCDs risk factors, including raised blood pressure, overweight/obesity; increased blood glucose; and elevated blood lipids over the last 10 years in Vietnam as well as examine these trends among different sub-population by geographical area, gender, and age groups.

Methods

Data sources

The 2010 survey applied a three-stage sampling method. First, eight provinces were randomly selected, representing eight ecological regions of Vietnam. Within each province, 20 clusters were randomly selected (commune as sampling unit). Within each commune, study subjects were randomly selected. Stratified sampling by gender, age groups, and rural/urban was applied. Of the 22,940 eligible subjects selected for participation, 14,706 (64.1%) participated in this survey. The sampling method and the characteristics of the 2010 survey was presented in a previous study (6).

The 2015 step survey used a two-stage random systematic sampling method. A household's primary sampling unit (PSU) was identified in the first stage. The sample frame was 15% of the general population of Vietnam and represented all 63 provinces and cities. The original samples were stratified by gender and age group (18–29, 30–49, and 50–69 years). Of the 3,856 eligible subjects selected for participation, 2,816 participants had completed all stages of STEPs survey (response rate 73.0%). Details of the 2015 survey have been reported elsewhere (7).

The 2020 survey used the same sampling approach as the 2015 survey. The sample was based on the National Sampling Master Frame developed by the General Statistics Office (GSO). The PSU was Enumeration Areas (EAs). Of the 5,000 eligible subjects selected for participation, 3,712 subjects had completed all three stages of STEPs survey (response rate of 74.2%).

Study population

The study population for the STEPs survey in 2015 and 2020 was Vietnamese persons aged 18–69 years. The survey population in the year 2010, however, included only persons aged 25–64 years. To keep the survey comparable over three-time points, we selected data for the population aged 25–64 years in all three surveys. Thus, the selection criteria for this analysis were Vietnamese persons aged 25–64 years who were residing in Vietnam at the time of the survey. Exclusion criteria included (1) those who were not residing permanently in Vietnam, (2) in-patients who were treated at health facilities, and (3) people with impaired mental health. The survey was approved by all participating institutions. All survey participants were provided verbal and/or written informed consent.

Key measurement

The NCD's metabolic risk factors examined in this analysis included both physical and biochemical measurements. Physical

measurements were (1) body mass index (BMI) calculated as weight (kg)/height^2 (m); (2) Blood pressure (BP) measured by cuff at the midpoint of the right upper arm by trained staff using a validated digital BP monitor with participants seated and rested for at least 15 min (two BP readings were taken 3 min apart, and third reading was taken if there was a difference between the two readings of more than 25 mmHg for systolic BP or more than 15 mmHg for diastolic BP. If a third measurement was taken, the mean of the two closest measures was used; otherwise, the mean of the two measures was used). Biochemical measurements were: (1) total cholesterol (TC) and (2) blood glucose (BG) from whole capillary blood after overnight fasting.

Overweight/obesity was defined as study subjects with BMI $\geq$ 25.

Raised blood pressure: defined study subjects with SBP $\geq$140 mmHg, and/or DBP $\geq$90 mmHg, and/or currently on medicine for raised blood pressure.

Increased blood glucose: BG from whole capillary blood after overnight fasting $\geq$7 mmol/l and/or currently on medicine for diabetes.

Elevated blood lipids: TC from whole capillary blood after overnight fasting $\geq$6.2 mmol/l and/or currently on medicine for elevated blood lipids.

Statistical approach

Weights were calculated for STEPS 1, STEPS 2, and 3 separately. Base weight was first calculated based on the inverse of the probability of selection, and then a non-response adjustment was made for non-response at household and individual levels. Census data in relevant years was used to estimate the population aged 25–64 years. The population adjustment was made for 16 subgroups obtained from males-females; urban-rural, and four age groups: 25–34; 35–44; 45–54; 55–64 years.

The survey data (SVY) procedure in STATA 17 was used to estimate the overall prevalence of raised blood pressure, overweight/obesity, increased blood glucose, and elevated blood lipids. Suitable weights were selected for different outcomes. For raised blood pressure and overweight/obesity, STEP 2 weights were used for all the estimation. For increased blood glucose and elevated blood lipids. STEP 3 weights were used for all the calculation.

For all metabolic risk factors, the study estimated the weighted prevalence, 95% CI for year as well as the stratified prevalence's by rural/urban, male/female, and 4 separated age groups. The differences in burden of metabolic factors between male/female, urban/rural and across 4 age groups were tested by Chi-square ($p < 0.05$ indicated significant differences). Cochran–Armitage test for trend was used to test for the differences in the prevalence over time. The Cochran- Armitage was performed by using nptrend command in STATA. This procedure has an option to compute exact p-values based on Monte Carlo permutations ($p < 0.05$ indicated significant trend over time).

The logistic regression for survey data (i.e., svy: logit in STATA) was used to examine whether the trends of some groups varied over time. For instance, a logistics model for dependent variable raised blood pressure (1: yes, 0: no) with three independent variables: year, gender and product term of year and gender was used to test whether the trend of raised blood pressure among female population were different from that among male population. If the p-value of the product term (i.e., year* gender) was <0.05, these two trends were statistically different.

Results

Changes in the prevalence of raised blood pressure among the population aged 25–64 years over time

In 2020, the prevalence of raised blood pressure was 28.33% (95% CI: 26.34%; 30.32%).

Table 1 presents the prevalence of raised blood pressure over time and the differences between 2015/2010 and 2020/2015. The prevalence of raised blood pressure for all populations and for all sub-groups increased significantly over time (Cochran–Armitage test for trend, $p < 0.001$). The increasing trend was more significant between the years 2020 and 2015 than between the years 2015 and year 2010. Specifically, the absolute difference in the prevalence of raised blood pressure between 2010 and 2015 was only 4.94%, while this was 7.55% between 2015 and 2020.

For the rural population, the prevalence of raised blood pressure increased from 16.24% in 2010 to 21.01% in 2015 and 27.0% in 2020. For the urban population, the prevalence was 14.87%, 20.33%, and 30.6% in 2010, 2015, and 2020, respectively. Both urban and rural populations showed a significant increase in the prevalence of raised blood pressure over time. However, figures for urban demonstrates significantly higher increasing trend compared to that of rural (p-value = 0.027).

Data among the male population indicates much more dramatic changes over time compared to the female population (p-value for difference in trend between male and female=0.014). Among 4 age groups, the oldest group (aged 55–64 years) experiences the sharpest increase in the prevalence of raised blood pressure over time. For the age group 25–34 years and the age group 35–44 years, the differences between the years 2015 and 2020 were significantly higher than that between the year 2010–2015 ($p = 0.018$). However, for the age group 45–54 years, the difference between the years 2010-2015 was

TABLE 1 Absolute changes in the prevalence of raised blood pressure among the population aged 25–64 years over time.

Characteristics	Year 2010, Proportion (%) [95%CI]	Year 2015, Proportion (%) [95%CI]	Year 2020, Proportion (%) [95%CI]	Absolute differences (%, p2–p1)		*p*-value from Cochran–Armitage test for trend
				Between 2010 – 2015	Between 2015 – 2020	
All	15.85 [15.04; 16.65]	20.78 [18.95; 22.60]	28.33 [26.34; 30.32]	4.93	7.55	<0.001
Urban/rural						
Urban	14.87 [13.36; 6.38]	20.33 [17.59; 23.06]	30.6 [27.12; 34.09]	5.46	10.27	<0.001
Rural	16.24 [15.25; 17.22]	21.01 [18.62; 23.41]	27.04 [24.73; 29.35]	4.77	6.03	<0.001
Gender						
Male	19.81 [18.68; 20.94]	25.09 [22.01; 28.17]	36.75 [33.65; 39.84]	5.28	11.66	<0.001
Female	12.05 [11.22; 12.89]	16.67 [14.48; 18.86]	20.06 [17.76; 22.36]	4.62	3.39	<0.001
Age groups						
Age 25–34	5.86 [4.93; 6.80]	5.73 [3.49; 7.97]	10.69 [7.67; 13.70]	−0.13	4.96	<0.001
Age 35–44	13.25 [11.99; 14.50]	15.15 [12.22; 18.08]	23.89 [18.90; 28.89]	1.90	8.74	<0.001
Age 45–54	23.24 [21.73; 24.74]	31.76 [27.76; 35.76]	33.08 [28.97; 37.19]	8.52	1.32	<0.001
Age 55–64	35.86 [33.94; 37.79]	42.36 [37.61; 47.11]	55.26 [51.62; 58.90]	6.50	12.90	<0.001

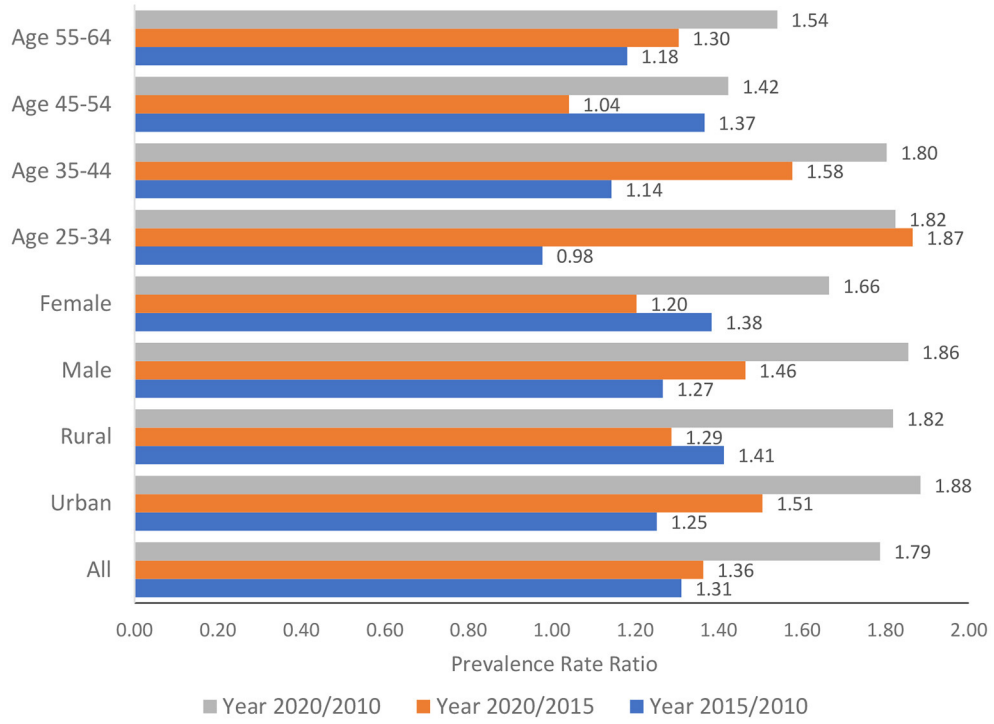

FIGURE 1
Relative changes in the prevalence of raised blood pressure among the population aged 25–64 years over time.

more noticeable compared to that between the years 2015–2020 (8.53 vs. 1.32%).

Four age groups showed significant differences in trend of raised blood pressure over time (p-value < 0.001).

Figure 1 presents the relative changes in the prevalence of raised BP among all populations aged 25–64 years over time and among subgroups by age group, gender, and urban/rural The relative changes were measured by the prevalence rate

TABLE 2 Changes in the prevalence of overweight/obesity over time.

Characteristics	Year 2010, Proportion (%) [95%CI]	Year 2015, Proportion (%) [95%CI]	Year 2020, Proportion (%) [95%CI]	Absolute differences (%, p2–p1)		*p*-value from Cochran–Armitage test for trend
				Between 2010 – 2015	Between 2015 – 2020	
All	10.48 [9.68; 11.29]	17.63 [15.67; 19.59]	20.57 [18.45; 22.68]	7.15	2.94	<0.001
Urban/rural						
Urban	15.9 [13.94; 17.86]	23.08 [19.76; 26.40]	25.86 [22.68; 29.04]	7.18	2.78	<0.001
Rural	8.32 [7.41; 9.22]	14.75 [12.37; 17.14]	17.58 [14.72; 20.43]	6.43	2.83	<0.001
Gender						
Male	10.84 [9.78; 11.91]	17.01 [14.16; 19.86]	20.19 [17.51; 22.86]	6.17	3.18	<0.001
Female	10.14 [9.18; 11.09]	18.24 [15.81; 20.66]	20.94 [17.59; 24.29]	8.10	2.70	<0.001
Age groups (in years)						
25–34	7.85 [6.64; 9.05]	13.56 [9.91; 17.22]	20.06 [15.18; 24.95]	5.71	6.50	<0.001
35–44	10.01 [8.78; 11.24]	17.43 [14.23; 20.63]	18.89 [15.85; 21.93]	7.42	1.46	<0.001
45–54	13.15 [11.88; 14.41]	21.02 [17.63; 24.42]	21.56 [18.08; 25.04]	7.87	0.54	<0.001
55–64	13.81 [12.17; 15.46]	20.62 [16.60; 24.65]	22.51 [19.28; 25.74]	6.81	1.89	<0.001

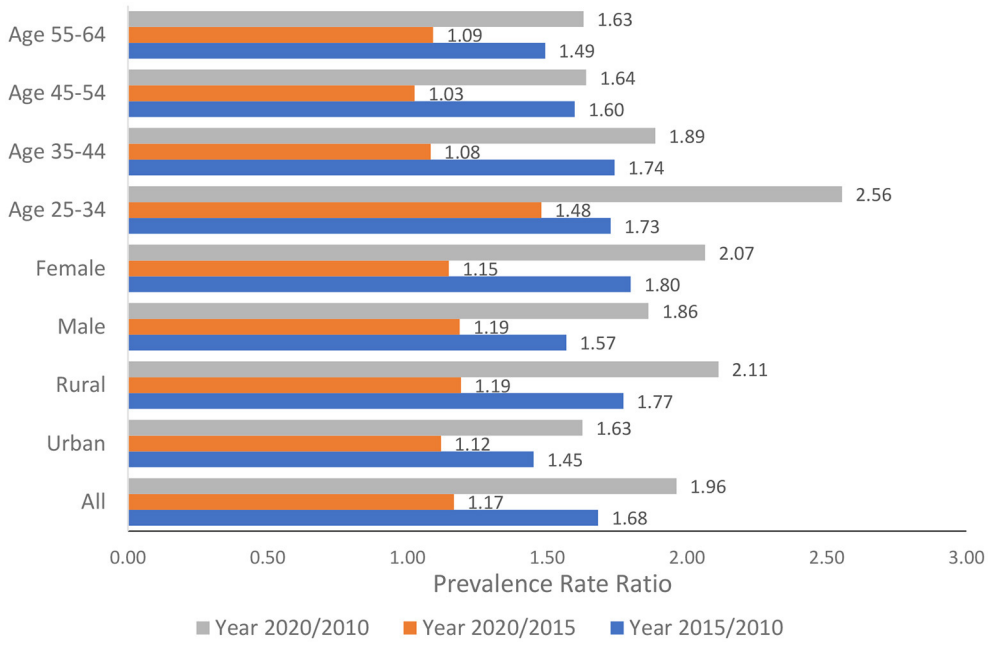

FIGURE 2
Relative changes in the prevalence of overweight/obesity among the population aged 25–64 years over time.

ratios (PRR) between 2015 and 2010, 2020 and 2015, and 2020 and 2010. If the ratio was >1, the burden of raised BP would increase over time. It can be observed that during the last 10 years (i.e., from 2010 to 2020), the burden of raised BP doubled among the population aged 25–64 years in the urban population.

Changes in the prevalence of overweight/obesity among the population aged 25–64 years over time

Table 2 examines the changes in the prevalence of overweight/obesity over the last 10 years. The overall prevalence

of overweight/obesity for all populations and for all sub-groups increased significantly over time (Cochran–Armitage test for trend, $p < 0.001$). In the year 2010, the prevalence of the population aged 25–64 years having BMI ≥25 (i.e., overweight/obesity) was 10.48%; this figure went almost doubled in the year 2020 (20.57%). The trends were not statistically significant between rural and urban (p-value = 0.17) and between males and females (p-value = 0.57). Among 4 age groups, the youngest group (aged 25–34 years) experienced the most dramatic changes in the burden of overweight/obesity. Trends of overweight/obesity among 4 age groups were statistically different (p-value = 0.015).

Overall, the period from 2010 to 2015 had a higher increase rate of overweight/obesity compared to the period from 2015 to 2020 (7.15 vs. 2.94%). This pattern was observed for almost all sub-population groups, except for the population aged 25–34 years, as the difference between the years 2010–2015 was 5.71%, but the difference between the years 2015–2020 was 6.5%.

Figure 2 presents the relative changes in the burden of overweight/obesity among all populations aged 25–64 years over time and among subgroups by age groups, gender, and geographical areas. All the PRR for the years 2020/2010 was >1.5. Three groups, females aged 25–64 years, rural population, and population aged 25–34 years, had the PRR ≥ 2, showing an outbreak in the burden of overweight/obesity.

Changes in the prevalence of increased blood glucose among the population aged 25–64 years over time

The changes over time in the prevalence of increased blood glucose (BG) among the population aged 25–64 years are presented in Table 3. This prevalence increased from 1.19% (95% CI: 0.89%; 1.48%) to 3.87% (95% CI: 3.05%; 4.70%) in 2015 and to 6.96% (95% CI: 5.89%; 8.03%) in 2020. The population aged 55–64 years experienced the sharpest increase in the burden of increased BG during the period 2015–2020 (i.e., from 7.59 to 15.07%). The Cochran–Armitage test shows that the increasing trends of increased BG for all populations and all sub-groups were statistically significant ($p < 0.001$).

The burden of increased BG among the urban population was steadily higher than that among the rural population over time, and the gap between urban/rural disease burdens also became bigger over time. The male and female populations all experienced a higher burden of increased BG over time, but the differences between males/females did not change.

The relative changes in the burden of increased BG over time among the population aged 25–64 years and for all the sub-groups analyses measured by the prevalence rate ratio were presented in Figure 3. Overall, the Vietnam population aged 25–64 years experienced a sharp increase in the burden of increased BG as the prevalence of increased BG in the year 2020 was 5.85 times higher than that in the year 2015. Sub-group analysis showed that among the male population and population aged 35–44 years, the prevalence of increased BG in the year 2020 was 7.55 times and 8.15 times higher than that in the year 2010.

In term of difference in trends over time, there was no statistically significant difference in trends between rural and ruban ($p = 0.43$), male and female ($p = 0.17$) and among four age groups ($p = 0.44$).

TABLE 3 Changes in the prevalence of increased blood glucose over time.

Characteristics	Year 2010, Proportion (%) [95%CI]	Year 2015, Proportion (%) [95%CI]	Year 2020, Proportion (%) [95%CI]	Absolute differences (%, p2–p1)		*p*-value from Cochran–Armitage test for trend
				Between 2010 – 2015	Between 2015 – 2020	
All	1.19 [0.89; 1.48]	3.87 [3.05; 4.70]	6.96 [5.89; 8.03]	2.68	3.09	<0.001
Urban/rural						
Urban	1.75 [0.86; 2.64]	5.56 [3.90; 7.21]	9.03 [7.21; 10.86]	3.81	3.47	<0.001
Rural	0.96 [0.76; 1.17]	2.99 [2.09; 3.89]	5.85 [4.56; 7.14]	2.03	2.86	<0.001
Gender						
Male	1.04 [0.80; 1.29]	4.16 [2.94; 5.39]	7.85 [6.22; 9.48]	3.12	3.69	<0.001
Female	1.32 [0.88; 1.77]	3.6 [2.60; 4.60]	6.11 [4.76; 7.46]	2.28	2.51	<0.001
Age groups (in years)						
25–34	0.71 [0.02; 1.40]	1.05 [0.29; 1.81]	2.29 [0.55; 4.03]	0.34	1.24	<0.001
35–44	0.78 [0.47; 1.09]	3.67 [2.15; 5.19]	6.36 [3.86; 8.85]	2.89	2.69	<0.001
45–54	1.59 [1.20; 1.98]	5.1 [3.38; 6.83]	6.77 [4.73; 8.80]	3.51	1.67	<0.001
55–64	2.74 [2.13; 3.35]	7.59 [5.03; 10.16]	15.07 [12.43; 17.70]	4.85	7.48	<0.001

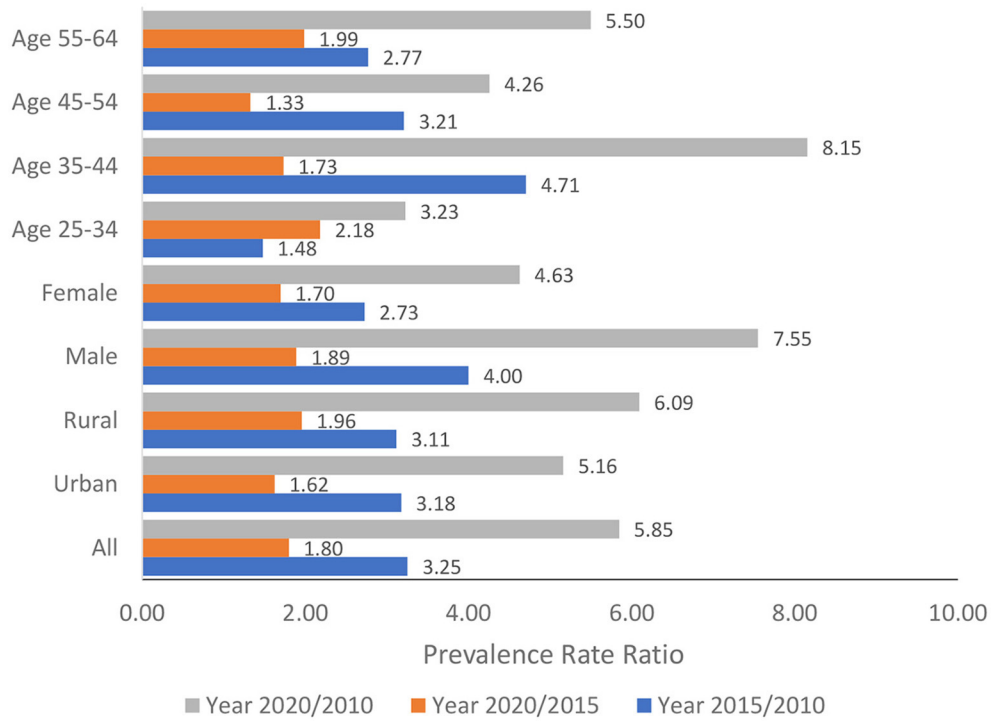

FIGURE 3
Relative changes in the prevalence of increased blood glucose over time.

Changes in the prevalence of elevated blood lipids among the population aged 25–64 years over time

Table 4 presents the prevalence of elevated blood lipids over time and the absolute differences between the years 2015/2010 and the year 2020/2015. The prevalence of elevated blood lipids among the population aged 25–64 years was 4.46% (95% CI: 4.01%; 4.90%). This figure went up to 10.31% (95%CI: 8.69%; 11.94%) in year 2015 and to 15.63% (95%CI: 13.65%; 17.61%) in year 2020. The Cochran–Armitage test shows that the increasing trends of elevated blood lipids for all populations and all sub-groups were statistically significant.

There was no statistically significant difference in trends between rural and ruban ($p = 0.72$) and male and female ($p = 0.78$). However, the p-value for testing differences in trends among 4 age groups was statistical significant ($p < 0.001$). Among 4 age groups, the two oldest groups (aged 45–54 and 55–64 years) show the highest burden of elevated blood lipids in the year 2020 and the most significant changes over time. For the age group 45–54 years, the prevalence of elevated blood lipids went from 6.6% in 2010 to 19.58% in 2020 (a change of 12.98%). Among the group aged 55–64 years, this figure increased from 9.22% in 2010 to 26.64% (a change of 17.42%). and among four age groups ($p = 0.44$).

The PRRs of elevated blood lipids over time for all populations aged 25–64 years and all sub-group analyses were presented in Figure 4. The PRR for all populations between 2020 and 2010 for elevated blood lipids was 3.50, indicating that in 2020 the prevalence of elevated blood lipids increased 3.5 times compared to the year 2010. The relative changes over time were similar for the male and female populations. The rural population showed bigger relative changes in the burden of diseases over time compared to the urban population during the last 10 years (PRR 3.91 vs. 2.77).

When examining the absolute changes over time, two older groups (aged 45–54 years and 55–64 years) showed the sharpest increase. However, when examining the relative changes, two younger groups (aged 25–34 years and 35–44 years) demonstrated stronger changes in the prevalence ratio than the older groups. Most significant, the prevalence of elevated blood lipids in the year 2020 was 4.91 times higher than that in the year 2010 among the population aged 25–34 years.

As visualized in Figure 5, the burden of all four risk factors has been increasing significantly over time among the studied population. For most groups (except the population aged 35–44 years and aged 45–54 years), the increasing burden of NCD metabolic risk factors was more significant during the period 2015–2020 compared to the period 2010–2015. The male population and population aged 55–64 years experienced the

TABLE 4 Absolute changes in the prevalence of elevated blood lipids over time.

Characteristics	Year 2010, Proportion (%) [95%CI]	Year 2015, Proportion (%) [95%CI]	Year 2020, Proportion (%) [95%CI]	Absolute differences (%, p2–p1)		*p*-value from Cochran–Armitage test for trend
				Between 2010 – 2015	Between 2015 – 2020	
All	4.46 [4.01; 4.90]	10.31 [8.69; 11.94]	15.63 [13.65; 17.61]	5.85	5.32	<0.001
Urban/rural						
Urban	6.84 [5.75; 7.93]	10.91 [8.55; 13.27]	18.92 [15.99; 21.85]	4.07	8.01	<0.001
Rural	3.52 [3.03; 4.01]	10 [7.85; 12.14]	13.77 [11.19; 16.34]	6.48	3.77	<0.001
Gender						
Male	3.87 [3.29; 4.44]	8.62 [6.49; 10.74]	13.32 [11.04; 15.61]	4.75	4.70	<0.001
Female	5.01 [4.39; 5.62]	11.94 [9.85; 14.02]	17.88 [15.05; 20.71]	6.93	5.94	<0.001
Age groups (in years)						
25–34	1.84 [1.28; 2.40]	4.96 [2.55; 7.36]	9.04 [5.19; 12.88]	3.12	4.08	<0.001
35–44	3.65 [2.89; 4.40]	8.8 [6.30; 11.30]	11.42 [8.53; 14.31]	5.15	2.62	<0.001
45–54	6.6 [5.69; 7.51]	13.84 [10.77; 16.91]	19.58 [15.79; 23.37]	7.24	5.74	<0.001
55–64	9.22 [8.06; 10.38]	17.72 [13.78; 21.66]	26.64 [23.15; 30.14]	8.50	8.92	<0.001

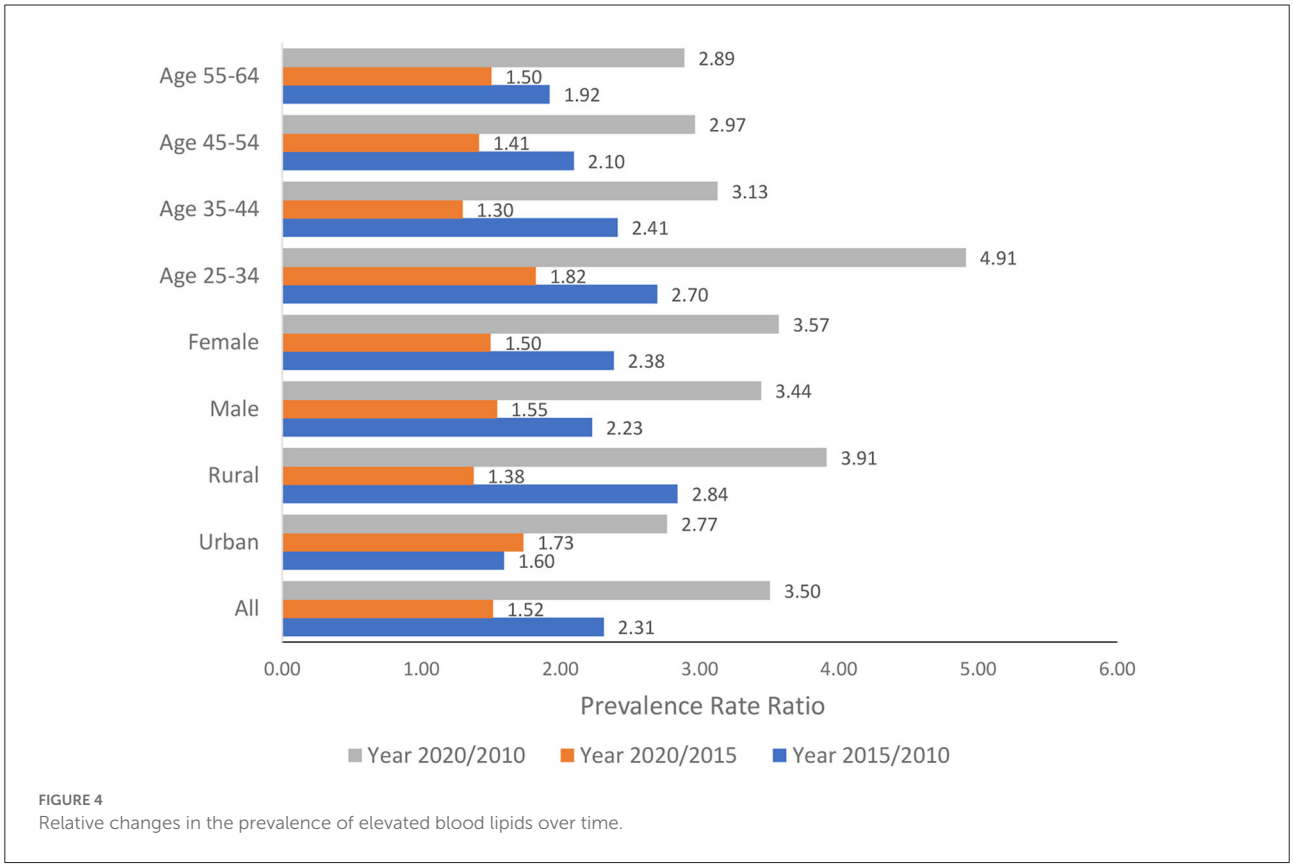

FIGURE 4
Relative changes in the prevalence of elevated blood lipids over time.

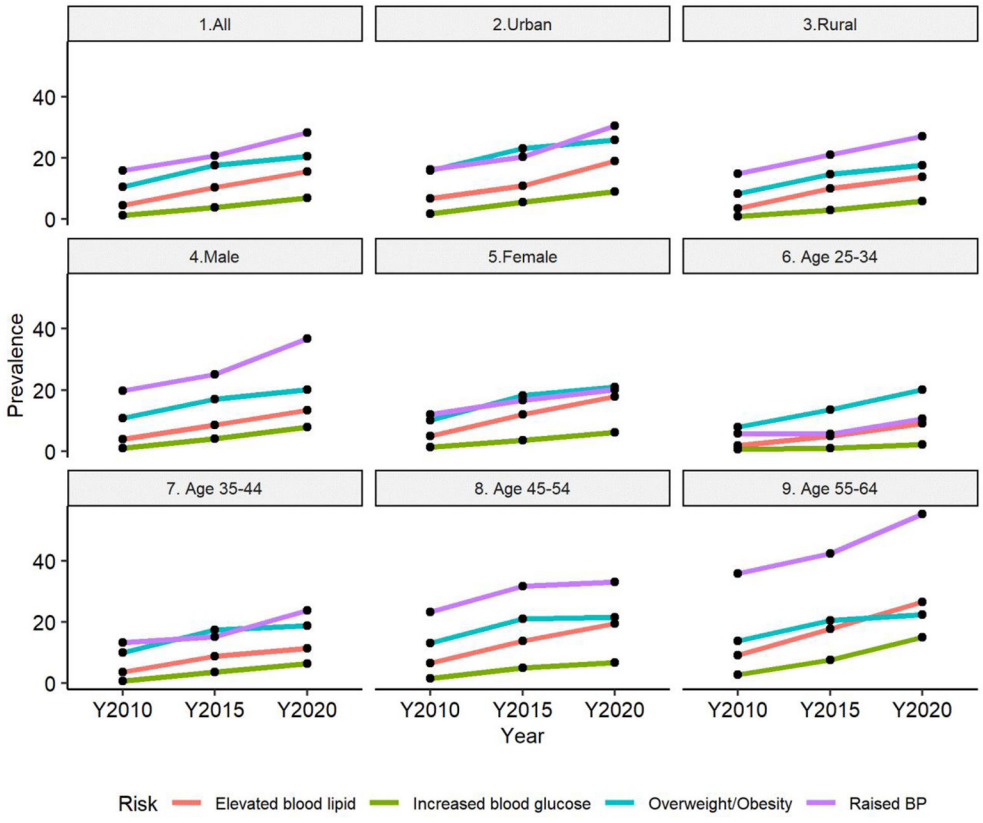

FIGURE 5
Trends of NCD metabolic risk factors over time across different population groups.

most dramatic changes in the burden of all NCD metabolic risk factors.

Discussion

This study combined the data from the three national STEPs survey in Vietnam conducted during the last 10 years to examine the trend of 4 major NCDs metabolic risk factors for sampled population aged 25–64 years and explore these trends across different sub-population groups. Data provided point estimates and 95% CI for each NCDs risk factor at three-time points, a statistical test for the trend, and the relative changes in the burden of risk factors over time.

Globally, the age-standardized point prevalence of type 2 diabetes was highest in Oceania, Central Latin America, and the Caribbean (ranging from 7.5 to 11.9%) in 2019 (9). For raised BP, the prevalence was highest throughout central and Eastern Europe, central Asia, Oceania, southern Africa, and some countries in Latin America and the Caribbean, with the global average prevalence of 32% in women and 34% in men aged 30–79 years (10). In Vietnam, the prevalence of raised BP, overweight/obesity, increased BG, and elevated blood lipids among the population aged 25–69 years was 28.3, 20.57, 6.96, and 15.63%, respectively, in the year 2020. Compared to the global situation, Vietnam did not have the highest burden of raised BP and diabetes. However, it is important to note that the burden of NCDs among the older population (aged 55–64 years) was very high, as 55.26% lived with raised BP, 26.6% with elevated blood lipids, and 15.07% with diabetes.

Most recent estimations showed a stable or decreased trend in the global age-standardized prevalence of raised BP. For instance, a pooled analysis of 1,201 population-representative studies with 104 million participants for the period 1990–2019 estimated that the global age-standardized prevalence of raised BP in adults aged 30–79 years was stable during the last 20 years (around 32–34%) due to the net effect of a decrease in high-income countries and an increase in some low-income and middle-income countries. The highest increase was from 10 to 15 percentage points (absolute %) (10). Another study reported that the global age-standardized mean SBP in 2015 was stable among men and slightly declined among women ≥18 years of age since 1975. For both sexes, the age-standardized prevalence of raised BP declined globally, from 29.5 to 24.1% among men

and from 26.1 to 20.1% among women during the period 1975–2015 (11). In Vietnam, the prevalence of raised BP among the population aged 25–64 years went from 15.85% in 2010 to 28.33% in 2020 (a change of nearly 13 percentage points). Thus, Vietnam currently does not have the highest burden of raised BP, but Vietnam is one of the countries with the most significant increasing trend of raised BP over time.

The prevalence of increased BG among the population aged 25–64 years in Vietnam increased from 1.19% in the year 2010 to 6.96% in the year 2020 (a net change of 5.85 percent point and a relative change of 4.9 times). This increasing trend was similar to other Asian countries. During the last 30 years, the incidence rate of increased BG in India increased by 110.9% and in China by 63.9% (12).

Previous studies attributed 80% of diabetes risk to an unhealthy diet, obesity, lack of exercise, and high triglyceride (13, 14). Multiple behaviors under modern lifestyles in Asian countries, such as exposure to industrial chemicals, smoking, and depression, were also indicated to have an impact on the high incidence of type 2 diabetes (15, 16).

The prevalence of elevated blood lipids increased sharply from 4.7% in 2010 to 15.6% in 2020. A recent global estimation pooled 1,127 population-based studies among 102.6 million people aged ≥18 and reported that the mean cholesterol levels did not change much over the four decades of follow-up. However, the trends in high-income and low-income countries are different, and high-income countries experience a significant decrease while low-income countries in Southeast Asia experience a most sharp increase in the mean of blood cholesterol (17).

The prevalence of overweight/obesity increased from 10.5% in 2010 to 20.6% in 2020. This trend was similar to other countries. For instance, the WHO European Region estimated that obesity prevalence rose by 21% in the 10 years before 2016 and by 138% since 1975; and for overweight (including obesity), by 8% in the 10 years before 2016 and by 51% since 1975 (18).

Globally, the increases in both plasma cholesterol and overweight/obesity were largely attributed to dietary habits and the adoption of unhealthy lifestyles (17). In Asia, these trends can be attributed to the increasing consumption of animal-source foods, refined carbohydrates, and palm oil (19, 20) and the low use of statin (21). Since 2010 Vietnam has seen a fast-food boom, with major chains like Pizza Hut, Domino's, Popeye's, Burger King, and KFC is entering the Vietnamese fast-food market (22). Many local brand names were also developed at the same time. This explanation was confirmed by the fact that the young population (aged 25–34 years), the main consumer of fast food in Vietnam, was the group that experienced the most significant relative changes in the prevalence of overweight/obesity (2.56 times higher) and elevated blood lipids (4.91 times higher).

Non-communicable diseases are affected by various factors, from nutritional and environmental to behavioral factors throughout the life course (11, 15, 23–25). To reverse the increasing trend of NCD metabolic factors in Vietnam, intervention and policy need to be applied to a comprehensive life course approach. For instance, intervention in early childhood, such as improved BMI and better nutrition, can shift the entire population's distribution of blood pressure and thereby change both the mean value and the prevalence of raised blood pressure (11).

This study was able to present the trends of 4 metabolic NCDs risk factors among population aged 25–64 years in Vietnam over time by combining data from three STEPs survey in 2010, 2015, and 2020. These trends provide important clues for the healthcare professionals to understand the unmet needs for care and the magnitudes of health problems (26). However, it should be noted that the three national surveys might have differences in sampling process, response rate and equipment for blood testing. Thus, all the interpretation of the results should consider these limitations.

Conclusion

The study used three rounds of national STEPs surveys in Vietnam conducted in 2010, 2015, and 2020 to examine the trend of NCD metabolic risk factors for all populations aged 25–64 years and explore these trends across different sub-population groups. All NCD metabolic risk factors examined in this analysis showed significantly increasing trends over time. For most age groups, the increasing burden of NCD metabolic risk factors was more significant during the period 2015–2020 compared to the period 2010–2015. To reverse the increasing trend of NCD metabolic factors in Vietnam, intervention and policy need to apply a comprehensive life course approach.

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 Ha Noi University of Public Health. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LV and QB designed and conceptualized the paper. LV and LK analyzed the data. All authors interpreted the results and prepared and reviewed the manuscript. All authors contributed

to the critical revision of the manuscript for important intellectual content read and approved the final manuscript.

Conflict of interest

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

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Dietary factors and the risk of lung cancer by epidermal growth factor receptor mutation status and histological subtypes

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Background: Previous studies have reported differential associations of certain dietary factors such as soy consumption by epidermal growth factor receptor mutant (*EGFR* +) subtype of non-small cell lung cancer (NSCLC). However, whether the other dietary factors including meat, fruits, and vegetables have differential risks on different histological and molecular subtypes of lung cancer remains unclear. Therefore, we conducted a case-control study to evaluate these associations.

Methods: A total of 3,170 cases and 4,238 controls from three different studies (Genes and Environment in Lung Cancer Study, Lung Cancer Consortium Singapore Study, and Multi-ethnic Cohort Study) were included. Information on demographics, lifestyle, and dietary consumption was obtained using questionnaires. Diet was assessed by using the number of standard servings of each item consumed per week. Multivariable logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between meat, vegetables, and fruits consumption with lung cancer risk after adjusting for potential confounders.

Results: We identified a significant inverse association between higher consumption of fruits and the risk of lung cancer (2nd tertile: OR = 0.54, 95%CI = 0.46–0.65; 3rd tertile: OR = 0.77, 95%CI = 0.65–0.91), compared with the lower (1st tertile) consumption of fruits. Higher vegetable consumption was significantly associated with a lower risk of *EGFR* + lung cancer (OR = 0.69, 95% CI = 0.54–0.88), however, this association was not significant among *EGFR* wild-type (–) lung cancer. Conversely, higher consumption of total meat (OR = 2.10, 95%CI = 1.58–2.79) was significantly associated with higher lung cancer risk, as compared with the lower consumption group.

Conclusions: Differential associations between vegetable consumption with *EGFR* mutation status in NSCLC were found. Further prospective studies are warranted to assess this association and elucidate the biological mechanisms.

KEYWORDS

diet, lung cancer, *EGFR*, case-control, non-small cell lung cancer

Introduction

Lung cancer is the leading cause of cancer death and disability-adjusted life-years (DALYs) worldwide (1–3). In 2019, there were an estimated 2.26 million incident cases of lung cancer and 2.04 million deaths that occurred globally, accounting for 45.9 million DALYs (3). Non–small cell lung cancer (NSCLC), including adenocarcinoma, squamous cell carcinoma, and large cell carcinoma histologic subtypes, accounted for approximately more than 80% of lung cancers (4). Different histological types of lung cancer have different age and sex distribution, smoking status, clinical performance status, biological pathways, and overall survival rate (5, 6). Epidermal growth factor receptor (*EGFR*), a transmembrane protein with tyrosine kinase activity, is one of the most well-documented and investigated pathways in NSCLC (7), and has been identified as an oncogenic driver, playing an important role in regulating the proliferation, survival, and differentiation of tumor cells (7, 8). *EGFR* mutations are found in approximately 60% of never-smoking Asian patients with adenocarcinomas compared to 5–10% in Caucasians, and thus represent a significant proportion of NSCLC in our local context (9, 10).

The associations between dietary factors and lung cancer risk have been explored by previous studies. A healthy dietary pattern was associated with a lower risk of lung cancer (11). For example, fruits and vegetables are a rich source of vitamin C, vitamin E, carotenoids, and other micronutrients, which are previously reported to have a protective association with the risk of lung cancer and other cancers (12). A meta-analysis showed that the highest consumption group of fruits and vegetables was inversely associated with the risk of lung cancer, as compared with the lowest consumption group (13). In contrast, the literature on the association between meats and lung cancer was conflicting. Some studies suggested that red meat and processed meat were both positively associated with the risk of lung cancer (14, 15), especially among never-smokers (16, 17). However, other studies revealed either a null association or a statistically significant inverse association between meat and the risk of lung cancer (18, 19). When stratified by the types of meat, a meta-analysis demonstrated an inverse association between poultry consumption and lung cancer, based on 11 studies, but not for total white meat or fish (16). A similar trend was identified among never-smokers; higher consumption of red meat was found to be associated with an increased risk of lung cancer, and no significant associations were observed between other types of meat and lung cancer risk (20).

Associations between dietary factors and lung cancer risk have been shown to vary by histological and molecular subtypes. Some studies demonstrated that when stratified by histological subgroups of lung cancer, including adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, the above-mentioned positive or inverse associations became statistically insignificant (13, 14). A previous study in Japan has reported differential associations of soy consumption by *EGFR* lung cancer subtypes (21); the protective effect of soybean products was found only among *EGFR* mutated lung cancer. Another study demonstrated that an alkaline diet prolonged overall survival among NSCLC patients with *EGFR* mutations (22). Furthermore, anthocyanidin extracted from fruits and vegetables was identified as an effective inhibitor of *EGFR* mutated cancers (23), and a low-protein diet combined with an *EGFR* inhibitor was reported to be a promising cancer therapy method (24). These studies demonstrated the potential differential associations between some dietary factors and *EGFR* lung cancer subtypes. *EGFR* can be abnormally activated by various mechanisms, and constitutive *EGFR* tyrosine kinase activation caused by mutations in the tyrosine kinase binding pocket is one of the key targets of specific small molecule inhibitors (25). *EGFR* tyrosine kinase inhibitors have been found to significantly improve outcomes in patients with advanced NSCLC that contain an activating *EGFR* mutation compared with platinum-based chemotherapy (26–29). However, whether other dietary factors are differentially associated with different histological and molecular subtypes of lung cancer remains unclear (13), particularly among the Asian population (21).

In this study, we evaluated the association between the consumption of meats, vegetables, and fruits with the risk of lung cancer by histological and molecular subtypes among Asians.

Materials and methods

Study population

A total of three studies were included: the Genes and Environment in Lung Cancer (GEL) Study (case-control), Lung

Cancer Consortium Singapore (LCCS) Study (case-only), and the Multi-ethnic Cohort Study (MEC) study (cohort). The LCCS is a case-only study of lung cancer with clinical data from three hospitals, including Singapore General Hospital (SGH), Changi General Hospital (CGH), and the National Cancer Center Singapore (NCCS). A total of 3,245 lung cancer patients, including 1,252 females and 1,993 males, with a diagnosis mean age of 63.4 years were included in the LCCS study between 2007 and 2017 (30).

The GEL study is a hospital-based case-control study of 815 controls and 399 cases recruited from 2005 to 2008, from Singapore public hospitals, including SGH, CGH, National University Hospital (NUH), and Tan Tock Seng Hospital (TTSH) (31, 32). Controls and cases were recruited from the same hospitals and frequency-matched by 10-year age groups. Controls were selected within one month after the date of diagnosis of the corresponding cases.

The MEC is a cohort study that was formed by combining two existing population-based studies, the Singapore Prospective Study Program (SP2) and the Singapore Cardiovascular Cohort Study (SCCS2), with additional recruitment of participants from 2007 to 2010 (33). The baseline of the MEC study recruited 13,777 participants. After excluding those who have been diagnosed with cancer at the baseline, a total of 13,149 cancer-free controls were included.

Lung cancer subtypes were extracted from medical records. Lung tumor tissues from the LCCS study were tested for their *EGFR* mutation status (mutation/+, or wildtype/-) using direct Sanger sequencing, or the real-time polymerase chain reaction (PCR) test. All *EGFR* tests were done at the Singapore General Hospital. Lung cancer cases in this current study were obtained from the LCCS and GEL studies. Healthy controls were obtained from the GEL and MEC baseline studies. Therefore, a total of 3,644 lung cancer cases and 13,964 controls were included in our study.

This current study of using three datasets was approved by the National University of Singapore Institutional Review Board (NUS-IRB Ref: N-20-053E). GEL study and MEC study (NUS-IRB Ref: 04-044; NUS-IRB Ref: 12–140 and CIRB Ref: 2001/001/C) were approved by the Institutional Review Board of the National University of Singapore and SingHealth Centralized Institutional Review Board (CIRB), and all participants gave informed consent prior to their participation. For the LCCS study, written informed consent was obtained from all patients and the study was approved by the SingHealth CIRB (CIRB Ref: 2018/2963).

Measurement of diet

Similar semi-quantitative Food Frequency Questionnaires (FFQ) were used in all three studies. For each study, consumption frequency and standard portion size were collected, and pictures of each item portion were used during the interview. Consumption frequency was converted into average frequency per week, and the portion size was converted into the number of standard servings. The average frequency per week and number of standard servings were multiplied to obtain the number of standard servings consumed per week (Supplementary Table S1). Fresh fruit consumption was the summed weekly consumption of fresh fruits. Vegetable consumption was defined as the sum of green, leafy, and other vegetables. Fish, chicken/poultry, pork/other meat, and preserved meat intake were summed as total meat consumption. Preserved meat was summed weekly consumption of bacon, ham, luncheon meat (canned), and sausages (Supplementary Table S2). The tertile cut-off values were chosen based on the consumption among the controls. Total energy intake per week was calculated based on the energy and nutrient composition of food by the Health Promotion Board (HPB) Singapore (34) (Supplementary Table S3). To reduce information bias, we calculated the total energy intake of participants and excluded outliers to improve the robustness of our study. Outliers were defined as those with a total energy intake $<$ 2.5th or higher than 97.5th centiles (35).

Covariate definition

All covariates were collected in the questionnaire and adjusted in all logistic models, including sex (male vs. female), age (years, continuous), ethnicity, educational level, family history of lung cancer, smoking status, body mass index (BMI), and total energy intake (kcal, continuous). Smoking status was divided into never and ever smokers. To avoid residual confounding by smoking, ever smokers were further categorized as smoking duration $<$20 years, 20–40 years, and $\geq$40 years. Ethnicity was categorized as Chinese, Malay, Indian and others. Body mass index (BMI, kg/m^2) was categorized as underweight ($<$ 18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obese ($\geq$ 30 kg/m^2). Educational level was categorized as 0 years, $\leq$6 years, and $>$6 years of education. Family history of lung cancer was categorized as no family history of any cancers, family history of lung cancer, and family history of other cancers.

Statistical analyses

Differences in baseline characteristics between the cases and controls were assessed using the *t*-test or Wilcoxon rank test based on the normality distribution for continuous variables, and Fisher's exact test for categorical variables. The multivariable logistic regression model was used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) of the association

between meat, vegetables, and fruits consumption with lung cancer risk.

In order to reduce the potential selection bias from the age difference between cases and controls, a sensitivity analysis was conducted to match cases and controls using the propensity score nearest neighbor matching (1:1 matching by age, no replacement) (36). The matching caliper width was set as 0.2 as suggested in the previous studies (37, 38). The conditional logistic regression was performed in the sensitivity analysis for matched cases and controls to estimate the ORs and 95% CIs.

Stratification analyses by smoking status, different subtypes of lung cancer (non-small cell lung cancer, adenocarcinoma, squamous cell carcinoma), and *EGFR* status were also conducted. We also did a further subgroup analysis among non-smoking Chinese females as they are at a higher risk of *EGFR*-positive lung cancer (10, 39). All statistical tests were conducted as two-sided, and a *P*-value < 0.05 was considered as being statistically significant. All analyses were performed in Stata 16.1 (Stata Corporation, College Station, Texas, USA).

Results

A total of 3,644 lung cancer cases and 13,964 controls were included. After excluding participants with missing information, 3,170 cases and 4,238 controls were included in the final analysis. As shown in Table 1, among cases with known *EGFR* status, *EGFR* mutation (*EGFR*+) was detected in 1,084 (57.29%) lung cancer cases, and *EGFR* wildtype (*EGFR*-) was detected in 808 cases (42.71%). Non-small cell lung cancer accounted for about 87.98% (2,789 cases) of the total lung cancer cases, of which the majority (2,242 cases, 80.39%) were adenocarcinoma. Compared with controls, lung cancer cases were significantly older and more likely to be males, have a family history of lung cancer, have lower educational levels, and lower BMI.

We found a significant inverse association between high fruit consumption (3rd tertile) and the risk of lung cancer (OR = 0.77, 95% CI = 0.65–0.91), as compared to low fruit consumption (1st tertile) (Table 2). Significant inverse associations were also observed among *EGFR*+ (OR = 077, 95% CI = 0.61–0.96) and *EGFR*- lung cancer (OR = 0.72, 95% CI = 0.55–0.94). For total vegetable consumption, as compared to low vegetable consumption (1st tertile), although the third tertile did not reach statistical significance (ORs = 0.85, 95% CI = 0.71–1.02), a significantly lower risk of lung cancer was observed among those with median consumption of vegetables, with an OR of 0.77 (95% CI = 0.65–0.91). A similar trend was observed in both *EGFR* + lung cancer and *EGFR*–lung cancer, however, the high consumption of total vegetables was statistically significant only among *EGFR*+ lung cancer (OR = 0.69, 95% CI = 0.54–0.88).

Overall, positive associations between total meat intake and lung cancer were reported in our study population. Compared with low meat consumption (1st tertile), a statistically significant positive association between higher consumption (3rd tertile) of total meat and the elevated risk of lung cancer was observed (OR = 2.10, 95% CI = 1.58–2.79). When the analysis was stratified by *EGFR* status, statistically significant positive associations were also found for *EGFR*+ lung cancer (OR = 2.20, 95% CI = 1.50–3.24) and *EGFR*- lung cancer (OR = 2.86, 95% CI = 1.84–4.47). In addition, we observed positive associations between higher consumption of fish (OR = 1.49, 95% CI = 1.20–1.85), pork and other meats (OR = 1.32, 95% CI = 1.08–1.62), preserved meat (OR = 3.02, 95% CI = 2.46–3.70), with the risk of lung cancer.

When stratified by different subtypes of lung cancer, we observed similar associations among non-small cell lung cancer, adenocarcinoma, and squamous cell carcinoma (Table 3). Higher fruit consumption was significantly and inversely associated with the risk of all subtypes of lung cancer. A statistically significant positive association between higher consumption of total meat and the elevated risk of non-small cell lung cancer (OR = 1.99, 95% CI = 1.49–2.67), and adenocarcinoma (OR = 2.06, 95% CI = 1.52–2.79) were observed, except for squamous cell carcinoma (OR = 1.21, 95% CI = 0.59–2.51).

Among never smokers, as compared to the lowest tertile, the highest total meat consumption group was associated with a higher risk of lung cancer across all strata of never smokers (never smokers, never-smoking females, and never-smoking Chinese females) (Table 4). No statistically significant associations between total vegetable consumption and risk of lung cancer were observed. For the age-matched sensitivity analyses 2,340 cases were age-matched with 2,340 controls. A total of 1,084 EGFR+ and 909 EGFR- cases were also age-matched with the same number of controls, respectively. Overall, the results were similar with the main analyses (Supplementary Tables S4–S6). Compared with low fruit consumption, a significant inverse association between high fruit consumption and the risk of lung cancer remained (OR = 0.79, 95% CI = 0.64–0.98). The statistically significant positive association between higher consumption (3rd tertile) of total meat and the elevated risk of lung cancer was also observed (OR = 1.92, 95% CI = 1.34–2.75), as compared with low meat consumption (1st tertile).

Discussion

In this study, we assessed the association between dietary factors and the risk of different histological and molecular subtypes of lung cancer. After adjusting for covariates, we identified higher consumption of total fresh fruits associated with a lower risk of lung cancer. In contrast, the higher total meat consumption, fish, pork, and preserved meat were statistically associated with elevated lung cancer risk. A significant inverse association between higher vegetable consumption and risk of

TABLE 1 Baseline characteristics of lung cancer cases and controls.

Variable	Controls ($N = 4,238$)		Cases ($N = 3,170$)		P-value [a]
	n	%	*n*	%	
Age at enrolment / Age at diagnosis, years					
Mean (SD)	44.18 (15.21)		63.67 (10.99)		**<0.001**
<50	2,778	65.55	330	10.41	
50–59.9	782	18.45	740	23.34	
60–69.9	382	9.01	1,077	33.97	
≥ 70	296	6.98	1,023	32.27	
Gender					**<0.001**
Male	1,504	35.49	1,712	54.01	
Female	2,734	64.51	1,458	45.99	
Ethnicity					**<0.001**
Chinese	1,747	41.22	2,699	85.14	
Malay	1,180	27.84	207	6.53	
Indian and others [b]	1,311	30.93	264	8.33	
Education					**<0.001**
0 years	328	7.74	425	13.41	
≤6 years	855	20.17	1,025	32.33	
>6 years	3,055	72.09	1,720	54.26	
Family history of cancer (first-degree)					
No	3,501	82.61	2,027	63.94	**<0.001**
Yes- Lung cancer	77	1.82	335	10.57	
Yes- Other cancers	660	15.57	808	25.49	
Smoking status					
Never smoker	2,753	64.96	1,632	51.48	**<0.001**
Ever smoker	1,485	35.04	1,538	48.52	
Smoking duration <20 years	646	15.24	160	5.05	
Smoking duration 20–40 years	381	8.99	443	13.97	
Smoking duration ≥40 years	84	1.98	831	26.21	
Unknown/missing	374	8.82	104	3.28	
Usual body mass index, kg/m^2					
Mean (SD)	25.54 (5.38)		22.98 (4.03)		**<0.001**
< 18.5	236	5.57	359	11.32	
18.5–24.9	1,965	46.37	1,999	63.06	
25.0–29.9	1,317	31.08	640	20.19	
≥ 30.0	720	16.99	172	5.43	
Total energy intake of fruits, vegetables, and meat, kcal/week					**<0.001**
Mean (SD)	2809.28 (1427.82)		2885.32 (1319.98)		
Total fruit consumption (standard servings/week)					**<0.001**
Mean (SD)	5.69 (5.54)		4.60 (4.18)		
Total vegetable consumption (standard servings/week)					**<0.001**
Mean (SD)	14.10 (12.66)		15.51 (12.58)		
Total meat consumption (standard servings/week)					**<0.001**
Mean (SD)	8.20 (5.22)		8.54 (4.66)		
Lung cancer types–*EGFR* status					
EGFR Mutant (+)	-	-	1084	34.19	
EGFR Wild type (-)			808	25.49	
Unknown/not tested			1278	40.32	

(Continued)

TABLE 1 (Continued)

Variable	Controls ($N = 4,238$)		Cases ($N = 3,170$)		*P*-value [a]
	n	%	*n*	%	
Lung cancer types–histologic types					
Non-small cell carcinoma	-	-	2789	87.98	
Adenocarcinoma			2242	80.39	
Squamous cell carcinoma			399	14.30	
Large cell carcinoma			21	0.75	
Unspecified NSCLC			127	4.55	
Small cell lung cancer			165	5.21	
Neuroendocrine carcinoma			37	1.17	
Others [c]			179	5.65	

SD, standard deviation; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer.
[a] P-values were obtained using the t-test or Wilcoxon rank test for continuous variables, and Fisher's exact test for categorical variables.
[b]Other ethnicity included Bangladeshi, Brunei, Burmese, Cambodian, Caucasian, Eurasian, Filipino, Indonesian, Korean, Pakistani, Sri Lanka, Thai, United Arab Emirates (UAE), and Vietnamese.
[c] Other lung cancer types included adenocarcinoma mixed with neuroendocrine carcinoma, adenosquamous carcinoma, clinical diagnosis only, lymphoepithelioma-like carcinoma, salivary gland-type tumors, sarcomatoid carcinoma, and other unspecified lung cancer.
Bold values refer to statistically significant results with $P < 0.05$.

EGFR+ lung cancer was identified, however, this association was not statistically significant among *EGFR*- lung cancer.

Consistent with previous studies, our findings showed that higher fruit consumption was correlated with a lower risk of lung cancer (40, 41). However, we did not find the monotonic decreasing ORs when comparing medium and higher fruit consumption groups. This may be attributed to the non-linear association reported in the previous study: lung cancer risk decreased for fruit consumption up to 200–300 grams per day, and no further decrease for higher consumption (42, 43). Compared with vegetables, we observed a pronounced association between fruits consumptions and lung cancer across all subtypes of lung cancer and among all subgroup populations. According to several previous studies, this pronounced protective evidence of fruits was repeatedly reported, however, the potential mechanisms still need to be investigated (41, 44, 45).

For vegetable consumption, our findings concur with previous work that an inverse association among higher consumption groups was reported (46), although we did not find a clear dose-response relationship. Similarly, a recent literature review by the World Cancer Research Fund supported the non-linear relationship between vegetable consumption and the risk of lung cancer, with decreasing risks for 300–400 grams per day and no further decrease for higher intake levels (42, 43). When stratified by smoking status, we did not find any significant associations among never-smokers, never-smoking females, or never-smoking Chinese females. Vieira et al. (13) and Smith-Warner et al. (47) also demonstrated that this protective effect was only significant among current smokers but was not statistically significant among former and never smokers. Interestingly, in the stratified analysis by *EGFR* status, a significantly decreased lung cancer risk was found only among *EGFR*+ lung cancer. Hamaguchi et al. reported that an alkaline diet (more vegetables and fruits, and less meat and dairy products) enhanced the effect of *EGFR*-TK inhibitor treatment in lung cancer patients with *EGFR* mutations (22). Our results may provide some insights into the potential mechanisms. Furthermore, the curcumin from turmeric (48, 49), Lupeol (a kind of phytosterol derived from fruits and vegetables) (50), and procyanidins-rich diets (51) have been shown to inhibit *EGFR* activation and have anti-cancer effects in lung cancer in multiple steps. However, we noted that the 95% CIs of the estimates in association for vegetable consumption and lung cancer by *EGFR* status were largely overlapping, i.e., 0.69 (0.54–0.88) and 0.76 (0.58–1.01). In the sensitivity analysis, no significant associations between vegetable consumption and *EGFR* +/- lung cancer were found. Therefore, this difference may be due to chance.

We found a significant positive association between total meat consumption and the risk of lung cancer after adjusting for covariates and total energy intake. Similar to our findings, a dose-response association was also found by Xue et al. with every increase of 120 g per day of red meat consumption, the risk of lung cancer increased by 35% (RR = 1.35, 95%CI = 1.25–1.46) (14). Lam et al. reported a significant positive association between higher meat intake and the risk of lung adenocarcinoma and squamous cell carcinoma (17). Similarly, our results also support the statistically significant relationship between higher meat intake and the risk of lung adenocarcinoma. As for the lack of statistically significant results for squamous cell carcinoma, this may be due to the limited number of cases in this group.

In this study, we were able to assess the effect of the consumption of total fruits, vegetables, and meat on the risk of lung cancer by specific subtypes. Based on our results, we

TABLE 2 Association between consumption of fruits, vegetables, and meat with risk of lung cancer subtypes.

Amount of food intake (Standard servings per week)	Controls ($N = 4,238$)		Cases ($N = 3,170$)		Adjusted OR (95% CI)	*EGFR* + Cases ($N = 1,084$)		Adjusted OR (95% CI)	*EGFR* − Cases ($N = 808$)		Adjusted OR (95% CI)
	n	%	*n*	%		*n*	%		*n*	%	
Fresh fruits [a]											
Low (≤2.5)	1,529	36.08	1,535	48.42	1	452	41.70	1	419	51.86	1
Medium (>2.5–≤6.9)	1,236	29.16	598	18.86	**0.54 (0.46–0.65)**	191	17.62	**0.47 (0.37–0.60)**	151	18.69	**0.57 (0.43–0.74)**
High (>6.9)	1,473	34.76	1,037	32.71	**0.77 (0.65–0.91)**	441	40.68	**0.77 (0.61–0.96)**	238	29.46	**0.72 (0.55–0.94)**
Vegetables [a]											
Low (≤7.5)	1,318	31.10	1,099	34.67	1	350	32.29	1	310	38.37	1
Medium (>7.5–≤15)	1,563	36.88	969	30.57	**0.77 (0.65–0.91)**	344	31.73	**0.70 (0.56–0.88)**	238	29.46	**0.66 (0.51–0.85)**
High (>15)	1,357	32.02	1,102	34.76	0.85 (0.71–1.02)	390	35.98	**0.69 (0.54–0.88)**	260	32.18	0.76 (0.58–1.01)
Total Meat [a,b]											
Low (≤5)	1,326	31.29	826	26.06	1	249	22.97	1	180	22.28	1
Medium (>5–≤9)	1,470	34.69	1,121	35.36	**1.56 (1.29–1.88)**	405	37.36	**1.94 (1.51–2.50)**	300	37.13	**1.73 (1.29–2.32)**
High (>9)	1,442	34.03	1,223	38.58	**2.10 (1.58–2.79)**	430	39.67	**2.20 (1.50–3.24)**	328	40.59	**2.86 (1.84–4.47)**
Fish [c]											
Low (≤1.75)	1,400	33.03	917	28.93	1	279	25.74	1	230	28.47	1
Medium (>1.75–≤3.5)	1,456	34.36	1,263	39.84	**1.38 (1.16–1.64)**	451	41.61	**1.66 (1.31–2.11)**	327	40.47	1.28 (0.97–1.69)
High (>3.5)	1,382	32.61	990	31.23	**1.49 (1.20–1.85)**	354	32.66	**1.90 (1.41–2.57)**	251	31.06	**1.66 (1.55–3.18)**
Chicken or Poultry [c]											
Low (≤1.25)	1,527	36.03	1,479	46.66	1	435	40.13	1	346	42.82	1
Medium (>1.25–≤3)	1,507	35.56	1,159	36.56	1.06 (0.90–1.26)	442	40.77	**1.27 (1.01–1.60)**	314	38.86	1.09 (0.83–1.41)
High (>3)	1,204	28.41	532	16.78	0.96 (0.76–1.21)	207	19.10	**1.40 (1.01–1.94)**	148	18.32	1.14 (0.79–1.65)
Pork and other meat [c]											
Low (≤0.5)	1,989	46.93	867	27.35	1	266	24.54	1	220	27.23	1
Medium (>0.5–≤1.25)	905	21.35	655	20.66	0.84 (0.70–1.02)	217	20.02	1.07 (0.82–1.39)	136	16.83	0.80 (0.58–1.09)
High (>1.25)	1,344	31.71	1,648	51.99	**1.32 (1.08–1.62)**	601	55.44	**1.94 (1.46–2.56)**	452	55.94	**1.36 (1.00–1.89)**
Preserved meat [c,d]											
Non-consumer	2,437	57.50	1,200	37.85	1	364	33.58	1	257	31.81	1
Low (≤1)	1,030	24.30	1,333	42.05	**3.40 (2.88–4.01)**	515	47.51	**5.28 (4.23–6.59)**	384	47.52	**5.66 (4.34–7.36)**
High (>1)	771	18.19	637	20.09	**3.02 (2.46–3.70)**	205	18.91	**3.49 (2.63–4.65)**	167	20.67	**4.00 (2.89–5.54)**

OR, odds ratio; CI, confidence interval; EGFR, epidermal growth factor receptor.

[a] Adjusted for age, gender, education, ethnicity, BMI, smoking status and duration, family history of lung cancer, total energy intake, fruit, vegetable, and meat consumption.

[b] Summed weekly consumption of fish, chicken or poultry, pork and other meat, and preserved meat.

[c] Adjusted for age, gender, education, ethnicity, BMI, smoking status and duration, family history of lung cancer, total energy intake, fruit, vegetable, and fish, chicken or poultry, pork and other meat, and preserved meat consumption.

[d] As a large number of participants did not consume preserved meat, it was divided into non-consumer, consumed ≤1 standard serving, and consumed >1 standard serving per week.

Bold values refer to statistically significant results with $P < 0.05$.

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TABLE 3 Association between consumption of fruits, vegetables, and meat with risk of different histologic types of lung cancer.

Amount of food intake (Standard servings per week)	NSCLC ($N = 2,789$)		Adjusted OR (95% CI)[a]	Adenocarcinoma ($N = 2,242$)		Adjusted OR (95% CI)[a]	Squamous cell carcinoma ($N = 399$)		Adjusted OR (95% CI)[a]
	n	%		*n*	%		*n*	%	
Fresh fruits [a]									
Low (≤2.5)	1,324	47.47	1	997	44.47	1	246	61.65	1
Medium (>2.5–≤6.9)	533	19.11	**0.56 (0.47–0.67)**	432	19.27	**0.56 (0.47–0.68)**	71	17.79	**0.58 (0.37–0.89)**
High (>6.9)	932	33.42	**0.78 (0.66–0.93)**	813	36.26	**0.80 (0.67–0.96)**	82	20.55	**0.63 (0.40–0.98)**
Vegetables [a]									
Low (≤7.5)	969	34.74	1	743	33.14	1	182	45.61	1
Medium (>7.5–≤15)	851	30.51	**0.74 (0.62–0.88)**	690	30.78	**0.74 (0.62–0.89)**	115	28.82	**0.55 (0.37–0.83)**
High (>15)	969	34.74	**0.83 (0.69–0.99)**	809	36.08	**0.82 (0.67–0.99)**	102	25.56	0.66 (0.41–1.05)
Total Meat [a,b]									
Low (≤5)	717	25.71	1	557	24.84	1	119	29.82	1
Medium (>5–≤9)	992	35.57	**1.51 (1.24–1.83)**	801	35.73	**1.56 (1.27–1.90)**	140	35.09	1.09 (0.68–1.74)
High (>9)	1,080	38.72	**1.99 (1.49–2.67)**	884	39.43	**2.06 (1.52–2.79)**	140	35.09	1.21 (0.59–2.51)
Fish [c]									
Low (≤1.75)	797	28.58	1	621	27.70	1	131	32.83	1
Medium (>1.75–≤3.5)	1,116	40.01	**1.36 (1.14–1.63)**	887	39.56	**1.38 (1.15–1.66)**	171	42.86	1.43 (0.92–2.23)
High (>3.5)	876	31.41	**1.48 (1.18–1.85)**	734	32.74	**1.55 (1.23–1.95)**	97	24.31	1.10 (0.60–2.01)
Chicken or Poultry [c]									
Low (≤1.25)	1,281	45.93	1	1,007	44.92	1	200	50.13	1
Medium (>1.25–≤3)	1,040	37.29	1.08 (0.91–1.29)	837	37.33	1.06 (0.89–1.27)	145	36.34	1.08 (0.70–1.67)
High (>3)	468	16.78	0.95 (0.75–1.21)	398	17.75	0.97 (0.75–1.24)	54	13.53	1.29 (0.68–2.42)
Pork and other meat [c]									
Low (≤0.5)	764	27.39	1	624	27.83	1	96	24.06	1
Medium (>0.5–≤1.25)	567	20.33	0.83 (0.68–1.01)	451	20.12	0.83 (0.68–1.02)	79	19.80	0.82 (0.49–1.36)
High (>1.25)	1,458	52.28	**1.24 (1.01–1.54)**	1,167	52.05	**1.26 (1.01–1.57)**	224	56.14	1.23 (0.73–2.10)
Preserved meat [c,d]									
Non-consumer	1,040	37.29	1	828	36.93	1	140	35.09	1
Low (≤1)	1,182	42.38	**3.54 (2.98–4.20)**	968	43.18	**3.77 (3.16–4.50)**	174	43.61	**3.40 (2.16–5.34)**
High (>1)	567	20.33	**3.07 (2.48–3.79)**	446	19.89	**3.13 (2.51–3.90)**	85	21.30	**4.18 (2.50–7.00)**

OR, odds ratio; CI, confidence interval; NSCLC, non-small cell lung cancer.

[a] Adjusted for age, gender, education, ethnicity, BMI, smoking status and duration, family history of lung cancer, total energy intake, fruit, vegetable, and meat consumption.

[b] Summed weekly consumption of fish, chicken or poultry, pork and other meat, and preserved meat.

[c] Adjusted for age, gender, education, ethnicity, BMI, smoking status and duration, family history of lung cancer, total energy intake, fruit, vegetable, and fish, chicken or poultry, pork and other meat, and preserved meat consumption.

[d] As a large number of participants did not consume preserved meat, it was divided into non-consumer, consumed ≤1 standard serving, and consumed >1 standard serving per week.

Bold values refer to statistically significant results with $P < 0.05$.

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TABLE 4 Association between consumption of fruits, vegetables, and meat with risk of lung cancer among non-smokers.

Amount of food intake (Standard servings per week)	Controls		Cases		Adjusted Odds ratio (95% CI)	*EGFR* + Cases		Adjusted OR (95% CI)[a]	*EGFR* – Cases		Adjusted OR (95% CI)[a]
	n	%	*n*	%		*n*	%		*n*	%	
Never-smokers	N = 2,753		N = 1,632			N = 841			N = 305		
Fresh fruit [a]											
Low (≤2.5)	855	31.06	631	38.66	1	328	39.00	1	122	40.00	1
Medium (>2.5–≤6.9)	818	29.71	337	20.65	**0.56 (0.47–0.69)**	152	18.07	**0.48 (0.37–0.64)**	63	20.66	**0.57 (0.39–0.82)**
High (>6.9)	1,080	39.23	664	40.69	**0.80 (0.66–0.98)**	361	42.93	**0.78 (0.61–0.99)**	120	39.34	0.80 (0.57–1.13)
Vegetables [a]											
Low (≤8.5)	916	33.27	539	33.03	1	279	33.17	1	104	34.10	1
Medium (>8.5–≤16.5)	949	34.47	489	29.96	0.85 (0.70–1.05)	257	30.56	0.85 (0.66–1.09)	88	28.85	**0.68 (0.48–0.96)**
High (>16.5)	888	32.26	604	37.01	1.01 (0.82–1.26)	305	36.27	0.90 (0.68–1.18)	113	37.05	0.89 (0.62–1.29)
Total Meat [b]											
Low (≤5)	954	34.65	443	27.14	1	204	24.26	1	75	24.59	1
Medium (>5–≤9)	918	33.35	593	36.34	**1.95 (1.57–2.43)**	315	37.46	**1.75 (1.29–2.38)**	106	34.75	**2.39 (1.62–3.52)**
High (>9)	881	32.00	596	36.52	**2.64 (1.88–3.70)**	322	38.29	1.09 (0.69–1.71)	124	40.66	**4.56 (2.55–8.15)**
Never-smoking females	*N* = 2234	N = 1221		*N* = 600		*N* = 217					
Fresh fruit											
Low (≤2.5)	708	31.69	468	38.33	1	225	37.50	1	88	40.55	1
Medium (>2.5–≤6.9)	654	29.27	271	22.19	**0.60 (0.48–0.75)**	113	18.83	**0.50 (0.37–0.68)**	51	23.50	**0.66 (0.44–0.98)**
High (>6.9)	872	39.03	482	39.48	**0.80 (0.64–0.99)**	262	43.67	0.83 (0.63–1.10)	78	35.94	0.76 (0.51–1.13)
Vegetables											
Low (≤8.5)	747	33.44	403	33.01	1	194	32.33	1	79	36.41	1
Medium (>8.5–≤16.5)	754	33.75	362	29.65	0.81 (0.65–1.01)	185	30.83	0.82 (0.62–1.08)	56	25.81	**0.57 (0.38–0.84)**
High (>16.5)	733	32.81	456	37.35	0.99 (0.78–1.25)	221	36.83	0.87 (0.65–1.18)	82	37.79	0.88 (0.58–1.32)
Total Meat [b]											
Low (≤5)	813	36.39	374	30.63	1	167	27.83	1	61	28.11	1
Medium (>5–≤9)	739	33.08	450	36.86	**1.92 (1.52–2.44)**	228	38.00	**2.18 (1.61–2.95)**	75	34.56	**2.42 (1.58–3.72)**
High (>9)	682	30.53	397	32.51	**2.39 (1.66–3.45)**	205	34.17	**2.46 (1.53–3.94)**	81	37.33	**5.06 (2.64–9.72)**
Never-smoking Chinese females	*N* = 1112	*N* = 1081		*N* = 520		*N* = 171					
Fresh fruit											

(Continued)

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TABLE 4 (Continued)

Amount of food intake (Standard servings per week)	Controls		Cases		Adjusted Odds ratio (95% CI)	*EGFR* + Cases		Adjusted OR (95% CI)[a]	*EGFR* – Cases		Adjusted OR (95% CI)[a]
	n	%	*n*	%		*n*	%		*n*	%	
Low (≤2.75)	372	33.79	428	40.34	1	196	38.06	1	76	45.24	1
Medium (>2.75–≤6.9)	283	25.70	216	20.36	0.80 (0.62–1.02)	93	18.06	0.77 (0.56–1.06)	33	19.64	0.79 (0.49–1.25)
High (>6.9)	446	40.51	417	39.30	1.13 (0.90–1.42)	226	43.88	1.31 (0.98–1.73)	59	35.12	1.09 (0.71–1.66)
Vegetables											
Low (≤10)	365	33.15	397	37.42	1	182	35.34	1	70	41.67	1
Medium (>10–≤19)	377	34.24	349	32.89	0.97 (0.77–1.22)	189	36.70	1.08 (0.81–1.44)	49	29.17	0.77 (0.50–1.17)
High (>19)	359	32.61	315	29.69	1.11 (0.85–1.43)	144	27.96	1.03 (0.74–1.43)	49	29.17	1.08 (0.68–1.73)
Total Meat [b]											
Low (≤5.25)	371	33.70	336	31.67	1	142	27.57	1	45	26.79	1
Medium (>5.25–≤10)	398	36.15	457	43.07	**2.54 (1.95–3.31)**	228	44.27	**3.14 (2.23–4.43)**	73	43.45	**4.24 (2.51–7.15)**
High (>10)	332	30.15	268	25.26	**4.64 (2.95–7.30)**	145	28.16	**6.33 (3.56–11.25)**	50	29.76	**15.72 (6.46–38.26)**

OR, odds ratio; NSCLC, non-small cell lung cancer.

[a] Adjusted for age, gender, education, ethnicity, BMI, smoking status and duration, family history of lung cancer, total energy intake, fruit, vegetable, and meat consumption.

[b] Summed weekly consumption of fish, chicken/poultry, pork/meat, and preserved meat.

Bold values refer to statistically significant results with P-value < 0.05.

did not observe any huge differences between different lung cancer subtypes. Higher consumption of fruits and vegetables was less pronounced among adenocarcinoma cases as compared to squamous cell carcinoma cases. Few studies have analyzed the effect of fruits and vegetables among specific lung cancer subtypes, and the results were inconsistent: four previous studies demonstrated statistically insignificant associations for small-cell carcinoma, adenocarcinoma, squamous cell carcinoma, and large cell lung carcinoma (13, 14, 40, 52); whereas Voorrips et al. revealed a weaker protective effect for adenocarcinomas than for other types of tumors, which was consistent with our results (53).

Some potential mechanisms have been proposed but the conclusions from different studies remained inconsistent. The protective effect of fruits and vegetables was attributed to biologically active compounds, including flavonoids and carotenoids (54, 55). Flavonoids found in fruit modular cytochrome P450 enzyme systems are involved in the metabolism of carcinogens (56). However, another study indicated that the intake of carotene supplementation was not associated with a decreasing risk of lung cancer (57). Besides, the protective effect may likely result from a combination of each constituent in influences several pathways involved in lung carcinogenesis (43). Red meat and processed meat are sources of saturated fats and heme iron, and several mutagens when cooked at a high temperature, including polycyclic aromatic hydrocarbons (PHAs) and heterocyclic amines (HCAs). These chemicals and mutagens may contribute to an increased risk of lung cancer (16, 58–60). However, a cohort study demonstrated a non-significant association between cooking methods, intake of specific meat mutagens or heme iron, and the risk of lung cancer (19). Therefore, further studies may be needed to characterize the mechanisms in these associations.

Although the results of this study support the hypothesis that fruit consumption is inversely associated with the risk of lung cancer and the consumption of meat is positively associated with the risk of lung cancer, there are several caveats to consider. Firstly, the cases and controls were taken from three different studies, which were carried out over different periods and used different questionnaires. Consumption of fruits, vegetables, and meat was collected in different ways; interviewers might have been trained differently, eliciting different responses from subjects. Although we have tried our best to combine those datasets appropriately and harmonize the variables, these limitations may affect the robustness of our findings, which may attenuate the results. The three studies enrolled subjects from different time periods, although all the cohorts started recruitment in 2005–2007. To control for the potential effects of the different enrollment time periods, we adjusted for the enrollment period in our model, and we found that the overall ORs and 95% CIs remained similar. In addition, in these recent 10 years, although Singaporean diet format and categories did not change much, we cannot deny that some participants may tend to eat healthily or improve their diet quality during this

time (61, 62). Based on the Singapore National Nutrition Survey Report, from 2004 to 2018, the average daily intake increased a bit, from 2290 to 2470 kcal per day. The consumption of fruits and vegetables increased, but the percentage of protein remained stable at 14–15% of the total energy (63, 64). Overall, as more than half of our controls were recruited after 2008, this difference in the recruitment period may slightly overestimate our current results.

Secondly, recall bias is a major limitation for case-control studies. Although the food frequency questionnaire has been used previously and showed validity (18, 65), the participants may underreport or overreport some specific food items when asked to recall their past diet. Cases, especially among females and non-smokers, may be more likely than controls to report unhealthy diet habits and vice versa. We have made efforts to minimize this limitation by training the interviewers to limit investigator bias. Further research in a prospective cohort study is warranted to validate our findings.

Thirdly, we were unable to access the relative importance of each constituent and the effect of other food items, such as flavonoids, carotenoids rice, eggs, fast foods, soy, and dairy products due to the questionnaires. Furthermore, because the questionnaires of LCCS and MEC datasets were limited to each fruit and vegetable item portion size and frequency, we can only use the average energy intake to present each category to calculate the total energy intake (including fruits, green leafy, or other vegetables, and each kind of meat). Therefore, there are likely to be measurement errors. To avoid an under- or over-estimation of total energy intake, a total energy intake of $<$ 2.5th or higher than 97.5th percentiles was excluded. Despite the limitations, to our knowledge, this is the first study of dietary factors and the risk of lung cancer by *EGFR* +/- and histologic subtypes among Southeast Asians.

Conclusions

In summary, we found that higher vegetable consumption was significantly associated with a decreased risk of *EGFR*+ lung cancer. Consistent with prior studies, an inverse association between higher fruit consumption and lung cancer, and a positive association between higher meat consumption and lung cancer were identified. Both associations remained significant when stratified by different molecular and histological types of lung cancer. Further prospective studies are warranted to assess this association and characterize the underlying biological mechanisms.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: the data are not publicly available due to privacy or ethical restrictions. Requests to access these datasets should be directed to WS, ephswj@nus.edu.sg.

Ethics statement

This current study of using three datasets was approved by the National University of Singapore Institutional Review Board (NUS-IRB Ref: N-20-053E). The patients/participants provided their written informed consent to participate in this study.

Author contributions

XY: conceptualization, formal analysis, and writing–original draft. GL and DT: writing–review and editing. AS: resources and writing–review and editing. DL and WS: writing–review and editing and supervision. 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1079543/full#supplementary-material

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Influencing factors of serum magnesium in CKD5 patients: A multicenter study in southern China

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Introduction: Magnesium (Mg) disturbances are related to cardiac, bone, and renal patient mortality. In this study, we compared biochemical markers in hemodialysis (HD) and peritoneal dialysis (PD) patients and explored the influencing factors of serum Mg in stage 5 chronic kidney disease (CKD5) patients.

Material and methods: All 598 patients with CKD5 from three medical centers in South China were recruited into this prospective cohort study from March 1, 2018, to January 31, 2021. Our study recorded the clinical characteristics and laboratory data of the patients.

Results: Hemodialysis patients (0.99 ± 0.19 mmol/L) had a higher mean serum Mg level than PD patients (0.86 ± 0.20 mmol/L; $p < 0.01$). Regression analysis showed that only corrected calcium (Ca), phosphate (P), Ca/Mg, Ca × P, albumin (Alb), total protein and creatine (Cr) predicted Mg levels in CKD5 patients ($p < 0.01$). Ca/Mg predicts hypomagnesemia with 78% sensitivity and 85% specificity in CKD5 patients. The AUC value corresponding to Ca/Mg was 0.88.

Conclusions: This multicenter study in southern China showed that for all CKD5 patients, corrected Ca and Alb had a significant positive effect on serum Mg, while Ca/Mg had a significant negative effect on serum Mg. In 123 HD patients, Ca × P was positively associated with Mg while Ca/Mg and P were negatively associated with Mg. In 398 PD patients, Ca × P, Alb, and total protein were positively associated with Mg while Ca/Mg and P were negatively associated with Mg. In 77 non-dialysis patients, corrected Ca, Cr, and total protein were positively associated with Mg while Ca/Mg was negatively associated with Mg. Furthermore, Ca/Mg might be another useful technique to monitor blood Mg levels in CKD5 patients.

Clinical trial registration: ChiCTR1800014557.

KEYWORDS

magnesium, chronic kidney disease, hemodialysis (HD), peritoneal dialysis (PD), multicenter study

Introduction

Magnesium (Mg), the second most prevalent cation in cells after potassium, is involved in more than 300 enzymatic reactions and significantly impacts neurotransmitter release, oxidative stress prevention, bone metabolism, regulation of heart rhythm, and vascular tone (1). Mg ranges from 21 to 28 g in the human body. Around half of the total Mg in the human body is found in bones and teeth, with the remaining found in muscles or non-muscular soft tissue such as nerves and the brain (2). The intestines, bones, and kidneys of a healthy person maintain the homeostasis of Mg (3). Mg is obtained from the daily consumption of nuts, legumes, whole cereals, fruits, and so on (2). The excretion of serum Mg is significantly influenced by the kidney. In the kidney, 90%−95% of the filtered Mg is reabsorbed in the tubules, and 70%−80% of the ionized Mg is ultra-filterable (3).

In CKD patients, serum Mg abnormalities have been found (1). CKD patients may have hypomagnesemia or hypermagnesemia (1). Hypomagnesemia increases the risk of heart disease and a higher risk of hospitalization and death in dialysis patients (4, 5). Ventricular arrhythmias brought on by hypomagnesemia might be deadly (6). In addition, increased dietary Mg could reduce oxidative stress, proinflammatory response, and vascular calcification in the CKD animal model (7, 8). Furthermore, recent research has revealed that relatively high serum Mg concentrations may be beneficial for lowering cardiovascular risk, avoiding vascular calcification, managing hypertension, and controlling blood glucose in CKD patients (9, 10). Hypermagnesemia may reduce vascular calcification in dialysis patients. However, it can also cause pruritus and impair neuromuscular transmission, parathyroid gland function, and bone metabolism, resulting in bone mineralisation deficiency and renal osteodystrophy (11). Therefore, Mg is not an ion that should be neglected. It is necessary to have a deeper understanding of Mg abnormalities in CKD patients (10). Appropriate blood Mg monitoring is essential to keep its concentration within a reasonable range.

Abbreviations: Mg, magnesium; HD, hemodialysis; PD, peritoneal dialysis; CKD5, stage 5 chronic kidney disease; BMI, body mass index; Mg, magnesium; iPTH, immunoreactive parathyroid; Ca, calcium; P, phosphorus; K, kalium; Na, natrium; Cl, chlorine; Cr, creatinine; BUN, blood urea nitrogen; UA, uric acid; Alb, albumin; ALT, alanine aminotransferase; AST, aspartic transaminase; r-GT, r-glutamyl transferase; RBC, red blood cell; HB, hemoglobin; MCHC, mean corpuscular hemoglobin contentration; WBC, white blood cells; PLT, platelet; Ch, cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; hsCRP, hypersensitive C reactive protein; CK, creatine kinase; CK-MB, creatine kinase isoenzyme MB; Myo, myoglobin; CTnT, cardiac troponin T; ALP, alkaline phosphatase; ROC, receiver operating characteristic.

Patients with CKD may be able to compensate for the drop in Mg ultrafiltration brought on by the lower glomerular filtration rate by increasing their Mg urinary fractional excretion. However, fractional excretion cannot compensate for a substantial fall in estimated glomerular filtration rate (eGFR), particularly one below 30 ml/min. Thus, hypermagnesemia could be found in CKD patients. There are several reasons driving the development of hypomagnesemia in CKD. In CKD5 patients, dietary restriction is an important cause of hypomagnesemia. Limiting potassium consumption may decrease Mg intake as well, since potassium-rich foods are likewise high in Mg. Diabetes mellitus, proteinuria, and loop and thiazide diuretics all increase urinary Mg excretion. More significantly, due to reduced Mg reabsorption, tubular dysfunction or interstitial fibrosis may lead to urinary Mg loss (10). Serum Mg in dialysis patients has received increasing attention.

There are many reasons for abnormal blood Mg in CKD patients and exploring its influencing factors can help to understand the interrelationship between other laboratory indicators and serum Mg, clarify the clinical indicators that have a greater impact on serum Mg, help to speculate on the causes of serum Mg abnormalities, and finally provide a reference for correcting blood Mg abnormalities. However, only a few studies have been conducted in China (12–14). Reports of abnormal serum Mg levels and the factors affecting Mg in CKD patients have been inconsistent. Furthermore, there is no research on serum Mg levels in CKD5 patients in southern China. Clarifying the importance of Mg in CKD5 patients would improve outcomes and quality of life. Thus, we aimed to compare biochemical indicators in HD and PD patients and explore the factors influencing serum Mg in CKD5 patients.

Materials and methods

Study population

The protocol of this study was registered in the Chinese clinical trial registry (http://www.chictr.org.cn/showproj.aspx?proj=24882; No: ChiCTR1800014557). The inclusion criteria for participants were eGFR $<$ 15 ml/min/1.73 m^2 for at least 3 months with or without renal damage. Patients were excluded for the following reasons: eGFR $\geq$ 15 ml/min/1.73 m^2, eGFR $<$ 15 ml/min/1.73 m^2 for $<$3 months, and insufficient data. All 598 patients with CKD5 were recruited into this retrospective cohort study from the following three medical centers in South China from March 1, 2018, to January 31, 2021: the Second Affiliated Hospital of Shantou University Medical College, the Huadu District People's Hospital of Guangzhou of Southern Medical University, and the First Affiliated Hospital of Hainan Medical College. CKD5 was defined as an eGFR $<$ 15 ml/min/1.73 m^2. Demographic data, including sex, age, height, and weight, were

TABLE 1 Baseline data of characteristics of 598 patients.

Variables	Value	Variables	Value
Age (year)	56.22 ± 14.11	Indirect bilirubin (μmol/L)	6.6 ± 3.47
BMI (kg/m^2)	22.55 ± 3.57	RBC (10^{12})	3.35 ± 0.95
Mg (mmol/L)	0.89 ± 0.21	Hb (g/L)	95.12 ± 24.66
iPTH (pg/ml)	90.23 ± 213.13	MCHC (g/L)	49.11 ± 74.19
Ca (mmol/L)	2.26 ± 0.27	WBC (10^9)	9.25 ± 30.7
P (mmol/L)	1.81 ± 0.68	Plt (10^9)	226.96 ± 91.46
K (mmol/L)	4.11 ± 0.86	Serum iron (μmol/L)	13.56 ± 37
Na (mmol/L)	138.82 ± 3.83	Transferrin (μmol/L)	9.08 ± 54.51
Cl (mmol/L)	98.72 ± 5.76	Ferritin (ng/ml)	286.63 ± 327.02
Cr (μmol/L)	905.43 ± 350.54	Ch (mmol/L)	4.82 ± 1.46
BUN (mmol/L)	54.02 ± 173.6	TG (mmol/L)	1.84 ± 1.6
UA (μmol/L)	439.5 ± 118.27	HDL (mmol/L)	1.22 ± 0.44
Globulin (g/L)	29.76 ± 5.76	LDL (mmol/L)	2.93 ± 1.01
Alb (g/L)	33.63 ± 6.06	CRP (mg/L)	24.07 ± 37.66
Total protein (g/L)	63.39 ± 8.03	hsCRP (mg/L)	19.31 ± 41.04
ALT (U/L)	21.61 ± 57.5	CK (U/L)	233.48 ± 372.06
AST (U/L)	24.9 ± 61.15	CK-MB (U/L)	15.61 ± 9.8
r-GT (U/L)	51.84 ± 98.53	Myo (ng/ml)	242.77 ± 190.11
Total bilirubin (μmol/L)	8.98 ± 5.77	CTnT (ng/ml)	1.68 ± 7.54
Direct bilirubin (μmol/L)	2.38 ± 3.63	ALP (U/L)	105.96 ± 105.03

BMI, body mass index; Mg, magnesium; iPTH, immunoreactive parathyroid; Ca, calcium; P, phosphorus; K, kalium; Na, natrium; Cl, chlorine; Cr, creatinine; BUN, blood urea nitrogen; UA, uric acid; Alb, albumin; ALT, alanine aminotransferase; AST, aspartic transaminase; r-GT, r-glutamyl transferase; RBC, red blood cell; HB, hemoglobin; MCHC, mean corpuscular hemoglobin contentration; WBC, white blood cells; PLT, platelet; Ch, cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; hsCRP, hypersensitive C reactive protein; CK, creatine kinase; CK-MB, creatine kinase isoenzyme MB; Myo, myoglobin; CTnT, cardiac troponin T; ALP, alkaline phosphatase.

recorded, and the body mass index (BMI) was calculated. The study was approved by the institutions of the Second Affiliated Hospital of Shantou University Medical College, the Huadu District People's Hospital of Guangzhou of Southern Medical University, and the First Affiliated Hospital of Hainan Medical College, and written informed consent was obtained from all the included participants.

Parameters measurements

The clinical testing parameters were identified and extracted prospectively from three medical centers. The serum Mg level of three hospitals was detected by the xylidyl blue method; the normal reference range for Mg in this study was 0.70–1.1 mmol/L. Hypermagnesemia was defined as a serum Mg level of >1.1 mmol/L, and hypomagnesemia was defined as a serum Mg level of <0.7 mmol/L. Phosphorus (P), calcium (Ca), immunoreactive parathyroid (PTH), potassium (K), sodium (Na), chlorine (Cl), creatinine (Cr), blood urea nitrogen (BUN), uric acid (UA), total protein, albumin (Alb), globulin, alanine aminotransferase (ALT), aspartic transaminase (AST), r-glutamyl transferase (r-GT), total bilirubin, direct bilirubin, indirect bilirubin, red blood cells (RBC), hemoglobin (Hb), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), platelet (PLT), serum iron, transferrin, ferritin, cholesterol (Ch), triglyceride (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), C-reactive protein (CRP), high-sensitivity C reactive protein (hsCRP), creatine kinase (CK), creatine kinase isoenzyme MB (CK-MB), myoglobin, cardiac troponin T (CTnT), and alkaline phosphatase (ALP) were also determined. Corrected serum Ca was calculated as follows: measured serum Ca (mmol/L) + [40 – serum Alb (g/L)] × 0.02 (15, 16).

Statistical analysis

In this study, SPSS 25.0 statistical software was used for statistical analysis. The measurement data was compared between HD and PD patients. The measurement information is presented in the form of mean ± SD. Stepwise multiple regression analysis was performed using blood Mg levels as the dependent variable and factors that were significantly correlated with serum Mg in the correlation analysis as independent variables. Ridge regression analysis was used if covariance between independent variables was found

TABLE 2 Results of *t*-test analysis.

Variables	Treatment		*t*	*p*-Value	Variables	Treatment		*t*	*p*-Value
	HD	PD				HD	PD		
Age (year)	56.23 ± 13.59	56.01 ± 14.44	0.15	0.88	Indirect bilirubin (μmol/L)	6.91 ± 3.88	5.53 ± 2.72	2.98	0.00**
BMI (kg/m^2)	20.98 ± 3.43	22.85 ± 3.57	−4.86	0.00**	RBC (10^{12})	3.17 ± 0.87	3.54 ± 0.92	−3.93	0.00**
Mg (mmol/L)	0.99 ± 0.19	0.86 ± 0.20	6.36	0.00**	Hb (g/L)	90.32 ± 24.90	99.73 ± 23.27	−3.71	0.00**
iPTH (pg/ml)	235.53 ± 439.67	57.24 ± 50.18	4.26	0.00**	MCHC (g/L)	125.83 ± 137.73	28.89 ± 7.82	7.8	0.00**
Ca (mmol/L)	2.35 ± 0.31	2.26 ± 0.23	2.92	0.00**	WBC (10^9)	8.05 ± 4.12	9.87 ± 37.56	−0.54	0.59
Ca/Mg	2.28 ± 0.42	2.55 ± 0.49	−5.45	0.00**	Plt (10^9)	218.64 ± 104.10	233.06 ± 89.54	−1.5	0.13
P (mmol/L)	1.81 ± 0.63	1.77 ± 0.67	0.53	0.6	Serum iron (μmol/L)	12.72 ± 9.55	13.71 ± 39.43	−0.17	0.87
K (mmol/L)	4.52 ± 0.91	3.93 ± 0.82	6.75	0.00**	Transferrin (μmol/L)	2.11 ± 4.19	13.73 ± 70.12	−1.07	0.29
Na (mmol/L)	137.70 ± 3.52	138.97 ± 3.58	−3.45	0.00**	Ferritin (ng/ml)	397.65 ± 358.30	268.82 ± 319.23	2.38	0.02*
Cl (mmol/L)	98.30 ± 4.60	97.60 ± 5.06	1.38	0.17	Ch (mmol/L)	4.49 ± 1.43	4.93 ± 1.37	−2.96	0.00**
Cr (μmol/L)	759.77 ± 299.17	966.06 ± 347.96	−5.93	0.00**	TG (mmol/L)	1.79 ± 1.85	1.87 ± 1.55	−0.48	0.63
BUN (mmol/L)	90.76 ± 228.54	22.53 ± 57.01	3.27	0.00**	HDL (mmol/L)	1.18 ± 0.45	1.24 ± 0.44	−1.31	0.19
UA (μmol/L)	384.26 ± 124.37	439.54 ± 98.81	−4.29	0.00**	LDL (mmol/L)	2.68 ± 0.99	2.97 ± 0.92	−2.84	0.00**
Globulin (g/L)	30.86 ± 6.23	29.57 ± 5.39	2.04	0.04*	CRP (mg/L)	24.91 ± 34.25	15.32 ± 26.17	0.88	0.38
Alb (g/L)	33.90 ± 5.47	33.66 ± 6.14	0.38	0.71	hsCRP (mg/L)	52.61 ± 60.87	8.93 ± 21.64	5.92	0.00**
Total protein (g/L)	64.76 ± 7.46	63.23 ± 7.89	1.88	0.06	CK (U/L)	171.85 ± 358.16	222.99 ± 276.93	−0.94	0.35
ALT (U/L)	34.57 ± 117.76	17.83 ± 23.56	1.54	0.13	CK-MB (U/L)	14.23 ± 8.73	16.88 ± 9.93	−1.8	0.07
AST (U/L)	35.82 ± 122.18	22.02 ± 29.78	1.22	0.22	Myo (ng/ml)	238.66 ± 140.62	359.46 ± 310.76	−1.77	0.09
r-GT (U/L)	67.46 ± 127.50	37.59 ± 63.71	1.06	0.29	CTnT (ng/ml)	0.89 ± 5.12	3.13 ± 11.75	−1.05	0.3
Total bilirubin (μmol/L)	9.96 ± 7.47	7.65 ± 3.85	2.86	0.00**	ALP (U/L)	117.64 ± 97.33	117.81 ± 127.09	−0.01	0.99
Direct bilirubin (μmol/L)	3.04 ± 5.13	2.12 ± 1.92	1.56	0.12					

BMI, body mass index; Mg, magnesium; iPTH, immunoreactive parathyroid; Ca, calcium; P, phosphorus; K, kalium; Na, natrium; Cl, chlorine; Cr, creatinine; BUN, blood urea nitrogen; UA, uric acid; Alb, albumin; ALT, alanine aminotransferase; AST, aspartic transaminase; r-GT, r-glutamyl transferase; RBC, red blood cell; HB, hemoglobin; MCHC, mean corpuscular hemoglobin contentration; WBC, white blood cells; PLT, platelet; Ch, cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; hsCRP, hypersensitive C reactive protein; CK, creatine kinase; CK-MB, creatine kinase isoenzyme MB; Myo, myoglobin; CTnT, cardiac troponin T; ALP, alkaline phosphatase.

*$p < 0.05$.

**$p < 0.01$.

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TABLE 3 Results of stepwise multiple regression analysis in 598 CKD5 patients.

Variables	Unstandardized coefficients		Standardized coefficients	*t*	*p*-Value	VIF	R^2	Adjusted R^2	*F*
	B	SE	*β*						
(constant)	0.66	0.084	–	11.34	0.00**	–	0.98	0.98	$F_{(3,32)} = 520.08, p = 0.00$
Ca	0.47	0.02	0.58	20.03	0.00**	1.32			
Ca/Mg	−0.45	0.01	−1.05	−35.94	0.00**	1.36			
Alb	0.01	0	0.23	8.86	0.00**	1.04			

Dependent variable: Mg.
D-W value: 2.12.
**$p < 0.01$.

in the stepwise multiple regression analysis. The receiver operating characteristic (ROC) curve was used to analyse the indicators of Mg status. The significance level for all tests was $p < 0.05$.

Results

Baseline clinical data

A total of 598 stage 5 CKD patients were enrolled in this study. The sex distribution was 52.17% male and 47.83% female, and the mean age of the participants was 56.22 ± 14.11 years. The leading cause of CKD was glomerulonephritis (29.93%), followed by diabetic nephropathy (28.42%) and hypertensive nephropathy (14.38%). The CKD patient composition (HD/PD/non-dialysis) was 123/398/77. Most patients had normal serum Mg levels, whereas 13.47% had hypermagnesemia and 9.60% had hypomagnesemia. The baseline BMI was 22.55 ± 3.57. The serum Mg level was 0.89 ± 0.21 mmol/L. Other parameters are shown in Table 1.

Comparison of biochemical indexes between HD and PD patients

Compared to the mineral levels of HD and PD patients, the mean serum Mg levels of HD patients (0.99 ± 0.19 mmol/L) were higher than in PD patients (0.86 ± 0.20 mmol/L; $p < 0.01$). The mean PTH, corrected Ca, and K levels of HD patients were also higher than in PD patients (all $p < 0.01$). The mean Ca/Mg level of HD patients (2.28 ± 0.42) was lower than in PD patients (2.55 ± 0.49; $p < 0.01$). There were no significant differences in P and Ca × P levels between HD and PD patients.

Compared to the toxins and nutritional levels of HD and PD patients, the mean BUN, indirect bilirubin, total bilirubin, and MCHC levels of HD patients were higher than in PD patients ($p < 0.01$). The mean Cr, UA, RBC, Hb, Ch, and LDL levels of HD patients were lower than PD patients ($p < 0.01$). There were no significant differences between HD and PD patients in their levels of Alb, total protein, direct bilirubin, transferrin, TG, or HDL.

In comparison to the inflammatory biomarkers in HD and PD patients, HD patients had higher mean levels of ferritin, hsCRP, and globulin ($p < 0.05$; $p < 0.01$; $p < 0.05$). There were no significant differences in WBC and CRP levels between HD and PD patients.

There were no significant differences in age, BMI, ALT, AST, ALP, r-GT, CK, CK-MB, CTnT, and Myo levels between HD and PD patients (Table 2).

TABLE 4 Results of Ridge regression analysis in 123 HD patients.

Variables	Unstandardized coefficients		Standardized coefficients	t	p-Value	R^2	Adjusted R^2	F
	B	SE	β					
(constant)	1.236	0.419	–	2.951	0.005**	0.802	0.721	$F_{(15,37)} = 9.979$, $p = 0.000$
iPTH	0	0	−0.073	−0.869	0.39			
Ca/Mg	−0.274	0.045	−0.666	−6.035	0.000**			
P	−0.173	0.039	−0.716	−4.476	0.000**			
Ca × P	0.119	0.017	1.076	7.161	0.000**			
K	−0.01	0.016	−0.054	−0.633	0.53			
Cl	0.002	0.003	0.051	0.571	0.571			
Cr	0	0	0.001	0.011	0.991			
UA	0	0	−0.033	−0.335	0.739			
Total protein	0.003	0.002	0.144	1.386	0.174			
Indirect bilirubin	−0.005	0.004	−0.113	−1.349	0.186			
RBC	0.003	0.02	0.016	0.168	0.868			
Hb	0	0.001	−0.059	−0.623	0.537			
MCHC	−0.004	0.005	−0.07	−0.818	0.419			
Ch	0.015	0.009	0.145	1.76	0.087			
hsCRP	0	0	−0.071	−0.828	0.413			

Dependent variable: Mg.
**$p < 0.01$.

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TABLE 5 Results of Ridge regression analysis in 398 PD patients.

Variables	Unstandardized coefficients		Standardized coefficients	t	p-Value	R^2	Adjusted R^2	F
	B	SE	β					
(constant)	0.844	0.112	–	7.527	0.000**	0.828	0.768	$F_{(12,34)} = 13.658, p = 0.000$
iPTH	0	0	0.075	1.003	0.323			
Ca/Mg	−0.136	0.021	−0.507	−6.446	0.000**			
Ca × P	0.042	0.007	0.461	5.862	0.000**			
P	−0.038	0.017	−0.179	−2.281	0.029*			
K	0.012	0.018	0.054	0.695	0.492			
Cr	0	0	0.025	0.31	0.758			
UA	0	0	−0.136	−1.808	0.079			
Alb	0.005	0.001	0.207	3.314	0.002**			
Globulin	−0.001	0.002	−0.047	−0.758	0.454			
Total protein	0.002	0.001	0.112	3.387	0.002**			
Total bilirubin	0.002	0.004	0.042	0.575	0.569			
hsCRP	0	0	−0.051	−0.741	0.464			

Dependent variable: Mg.
*$p < 0.05$.
**$p < 0.01$.

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Influencing factors of serum Mg in all CKD5 patients

The stepwise multiple regression analysis demonstrated that only corrected Ca, Alb, and Ca/Mg predicted serum Mg levels in CKD5 patients ($p < 0.01$). Corrected Ca, Ca/Mg, and Alb could explain 98.0% of the variation in Mg ($R^2 = 0.98$). The model is valid ($F = 520.078$, $p = 0.000<0.01$) and equation of model is Mg (mmol/L) = 0.659 + 0.469*Corrected Ca (mmol/L) – 0.451*Ca/Mg + 0.008*Alb (g/L). In addition, there was no covariance problem (all VIF < 5) and no autocorrelation in the model (D-W = 2.12; Tables 3, 8).

Influencing factors of serum Mg in HD patients with CKD5

Ridge regression analysis with a k value of 0.030 demonstrated a significant positive correlation between Ca × P and Mg. There was a significant negative correlation between Ca/Mg, P and Mg (Tables 4, 8).

Influencing factors of serum Mg in PD patients with CKD5

A positive correlation was found between Ca × P, Alb, total protein, and Mg using ridge regression analysis with a k value of 0.100. A significant negative correlation was found between Ca/Mg, P, and Mg (Tables 5, 8).

Influencing factors of serum Mg in non-dialysis patients with CKD5

The stepwise multiple regression analysis demonstrated that only corrected Ca, Ca/Mg, and Cr predicted serum Mg levels in non-dialysis patients with CKD5 ($p < 0.01$). The model is valid ($F = 127.3732$, $p = 0.0000 < 0.01$) and the equation of the model is Mg (mmol/L) = 0.3656 + 0.5019*Corrected Ca (mmol/L) – 0.3958*Ca/Mg + 0.0001*Cr (μmol/L) + 0.005*Total protein (g/L). In addition, there was no covariance problem (all VIF < 5) and no autocorrelation in the model (D-W = 2.0054; Tables 6, 8).

ROC analysis for hypomagnesemia in all CKD5 patients

The ROC curves were applied to evaluate the predictors of hypomagnesemia further. As shown in Table 7, we found that the sensitivity and specificity of Ca/Mg to predict hypomagnesemia

TABLE 6 Results of stepwise multiple regression analysis in 77 non-dialysis patients.

Variables	Unstandardized coefficients		Standardized coefficients	t	p-Value	VIF	R^2	Adjusted R^2	F
	B	SE	β						
(Constant)	0.3656	0.1179	–	3.1021	0.0029**	–	0.89	0.883	$F_{(4,63)} = 127.3732$, $p = 0.0000$
Corrected Ca	0.5019	0.0377	0.6285	13.3298	0.0000**	1.2726			
Ca/Mg	−0.3958	0.0246	−0.8471	−16.0779	0.0000**	1.5893			
Cr	0.0001	0	0.1249	2.2613	0.0272*	1.7457			

Dependent variable: Mg.
D-W value: 2.0054.
*$p < 0.05$.
**$p < 0.01$.

were 78 and 85%, respectively (Table 7). The AUC value corresponding to Ca/Mg was 0.88, implying that Ca/Mg has a relatively high diagnostic value for hypomagnesemia. However, Ca and Alb are of less value for diagnosing hypomagnesemia. Figure 1 shows the ROC curves of Ca/Mg, Ca, and Alb for predicting hypomagnesemia in CKD5 patients, respectively (Figure 1).

Discussion

This multicentre study in southern China showed that in all CKD5 patients, corrected Ca and Alb had a significant positive effect on serum Mg; however, Ca/Mg had a significant negative effect on serum Mg. In HD patients, Ca × P was positively associated with Mg. However, Ca/Mg and P were negatively associated with Mg. In PD patients, Ca × P, Alb and total protein were positively correlated with Mg, while Ca/Mg and P were negatively correlated with Mg. In non-dialysis patients, Ca/Mg was inversely related to Mg, whereas corrected Ca, Cr, and total protein all had positive associations with Mg (Table 8). The ROC analysis for hypomagnesemia reported that Ca/Mg has a relatively high diagnostic value for hypomagnesemia. Similar studies have been conducted, but the findings of these studies are inconsistent. Cai et al. (12) found that serum Mg is positively correlated with dialysis duration, Alb, Hb, TG, K, Ca, and P but negatively correlated with Na in 253 PD patients in central China. Tsai et al. reported that serum Mg is positively correlated with P but negatively correlated with CRP and PTH in 180 PD patients in northern China (13). Ye et al. showed that Mg is negatively associated with hypertonic dialysate but positively associated with BMI, Alb, and P in 402 PD patients in southern China (14). A recent study reported that serum Mg concentrations were independently correlated with serum K concentrations in 60 PD patients (17).

This study showed that corrected Ca is positively associated with Mg in non-dialysis patients with CKD5, which means that patients with hypomagnesemia may also have hypocalcemia. This result is consistent with a study in Zhejiang, China (12). Mg is involved in the ion channels transporting Ca into and out of cells. Unsurprisingly, Mg deficiency may perturb Ca homeostasis (18). There are several factors that lead to hypocalcemia in patients with hypomagnesemia. The first one is hypoparathyroidism. Low serum Mg level inhibits hypocalcemia-induced PTH release. Immunoreactive PTH levels are normal or low in most hypomagnesemia-hypocalcemic individuals (18–20). The mechanism for this could include disruption of the phosphoinositol system and decreased adenylate cyclase activity, as both are Mg-dependent (18). The second factor is resistance to PTH (20, 21). Mg deficiency could interfere with PTH-induced cyclic AMP production and lead to resistance to PTH, which may alter Ca homeostasis (22, 23). PTH-induced calcium release from bone is inhibited when plasma Mg falls below 1 mg/dl (20). The third factor is vitamin D deficiency. Impaired PTH secretion and a direct effect of Mg depletion on the kidney could impair vitamin D metabolism (23, 24). Moreover, low plasma vitamin D could lower Ca levels. In summary, hypoparathyroidism, PTH resistance, and vitamin D insufficiency could cause hypocalcemia in hypomagnesemia individuals.

In our study, Ca/Mg significantly negatively affected serum Mg and had a relatively high diagnostic value for hypomagnesemia in CKD5 patients. Serum Mg levels do not necessarily indicate total body Mg status (25). A study reported that the blood Mg/Ca ratio might be a more useful and sensitive measure of Mg than the serum Mg level alone (26). Our study found that Ca/Mg has a high diagnostic value for serum Mg levels. Therefore, the Ca/Mg may also be another practical method to assess blood Mg levels in CKD patients.

This study showed that P is negatively associated with Mg in PD patients, suggesting that elevating serum Mg concentrations may be able to reduce blood P in PD patients. Several studies have been conducted to evaluate the association between blood P levels and renal prognosis and mortality. According to a meta-analysis of 12 cohort studies including a total of 25,546

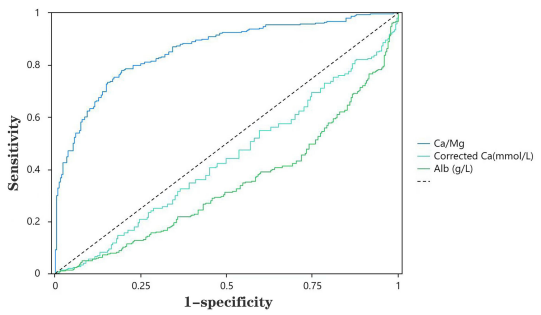

FIGURE 1
ROC curve of indicators for low magnesium.

TABLE 7 Results of ROC best bounds.

Variables	AUC	Optimum boundary	Sensitivity	Specificity	Cut-off
Ca/Mg	0.88	0.63	0.78	0.85	2.74
Corrected Ca	0.41	0.01	0.01	1	2.91
Alb	0.29	0	0	1	50

TABLE 8 Summary of influencing factors of serum Mg in different subgroups in this study.

	All CKD5 patients ($n = 598$)	HD patients ($n = 123$)	PD patients ($n = 398$)	Non-dialysis patients ($n = 77$)
Positive correlation	Corrected Ca, Alb	Ca × P	Ca × P, Alb, total protein	Corrected Ca, Cr, total protein
Negative correlation	Ca/Mg	Ca/Mg, P	Ca/Mg, P	Ca/Mg

individuals, every 1 mg/dl rise in blood P level was related to renal failure (hazard ratio, 1.36) and death (hazard ratio, 1.20) (27). Another meta-analysis also reported that every 1 mg/dl rise in blood P level raised the chance of death by 18% (relative risk, 1.18; 95% CI, 1.12–1.25), highlighting the importance of P in CKD patients (28). Additionally, when serum P levels were already high in patients with stage 4–5 CKD, renal function deteriorated faster (29). Therefore, lowering blood P is important for the prognosis of CKD patients, and raising serum Mg could be a viable way to lower blood P.

Our study also found a positive correlation between blood Mg and the Ca × P product in both HD and PD patients. In non-dialysis patients, blood Mg was positively correlated with Cr, which indicates that hypermagnesemia may be associated with elevated Ca × P product and Cr. The effects of hypermagnesemia are still debatable. More cohort studies are needed to define the dangerous range of hypermagnesemia and its effect on complications and patient survival.

In this study, serum Mg levels were positively correlated with serum Alb in PD patients and positively correlated with total protein in both PD and non-dialysis patients, suggesting that serum Mg is related to nutritional status. Protein-energy wasting is common among dialysis patients and has emerged as a significant risk factor for morbidity and mortality (30). Alb and total protein are indicators of nutritional status. According to the "Gibbs-Donnan Effect," Alb with an anionic charge during dialysis cannot pass through the semi-permeable membrane, producing an uneven charge and electric field, thus attracting positive ions, such as Mg ions, and preventing the Mg ions from moving across the semi-permeable membrane. Therefore, Alb decreases filterable Mg. In addition, serum Mg balance mainly depends on intestinal uptake and renal excretion, and patients with good nutritional status may have a high Mg intake. Therefore, the serum Mg level could reflect the Alb and total protein level to a certain extent.

The findings on the relationship between Mg and PTH have been conflicting in recent decades. In this study, no significant correlation was found in regression analysis between PTH and serum Mg, consistent with two retrospective studies, which enrolled 21,534 and 11,2017 HD patients (31, 32). However, some studies found that Mg had the opposite relationship with PTH (33).

This study was a multicenter study and included 598 CKD5 patients. The sample size of this study was relatively large. In this study, the mineral metabolism index, toxins and nutritional index, inflammatory index, and other indexes were included to explore the factors influencing serum Mg in CKD5 patients, and we also assessed the diagnostic value of several factors on serum Mg. Although this study was observational, this finding is important because few studies have investigated the importance of Mg. In clinical practice, serum Mg concentrations are not always routinely measured in all patients. This study highlights the importance of Mg and reminds us that proper monitoring of serum Mg concentrations is essential for CKD patients. In addition, this study found that Ca/Mg could be another practical way to assess blood Mg levels in CKD patients.

This study had several limitations. First, we did not investigate causality, as this was a cross-sectional and observational study. Second, we were unable to investigate the effect of Mg on all-cause mortality. Third, information on oral medications, such as Mg supplementation, was not collected. Therefore, the effect of oral medication on blood Mg concentrations was not explored in this study. Therefore, large-scale interventional studies are needed to clarify the importance of serum Mg and the effect of Mg supplementation in CKD patients.

Conclusions

In conclusion, this multicenter study in southern China revealed a significant correlation between the serum Mg and corrected Ca, Ca/Mg, P, Ca × P, Cr, Alb, and total protein in CKD5 patients. This study also emphasized the importance of Mg and discovered that Ca/Mg might be another helpful technique to monitor blood Mg levels in CKD5 patients. More multicenter studies with large sample sizes will be required to guide therapy.

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 study was approved by the institutions of the Second Affiliated Hospital of Shantou University Medical College, the Huadu District People's Hospital of Guangzhou of Southern Medical University, and the First Affiliated Hospital of Hainan

Medical College, and written informed consent was obtained from all the included participants. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

TZ contributed to the conception and design of the study and modified and polished the manuscript. JW, SL, H-YL, and WT were responsible for collection of data and performing the statistical analysis and manuscript preparation. YL and TZ were responsible for checking the data. JW wrote the manuscript. All authors were responsible for drafting the manuscript, read and approved the final version.

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Conflict of interest

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

Publisher's note

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Consistency between 3 days' dietary records and 24-h urine in estimating salt intake in children and adolescents

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Purpose: This study aimed to evaluate the salt intake in boarding school students and the consistency between salt intake measurements based on 24-h urine and weighed dietary records over 3 consecutive days in this population.

Methods: This was a school-based cross-sectional study. Overweight (including obesity) or hypertensive students aged 6–14 years and their normal counterparts were recruited for this study at three boarding schools in China. Three consecutive 24-h urine samples were collected from all participants. During the collection period of 24-h urine, the weighed diet records were collected in children who had all three meals at the school canteens on weekdays. Incomplete 24-h urine or dietary records were excluded from the analysis.

Results: The median salt excretion was 6,218 [4,636, 8,290] mg by 24-h urine and 120 (82.2%) consumed excess salt among the participants. The median salt intake was 8,132 [6,348, 9,370] mg by dietary records and 112 (97.4%) participants consumed excess salt than recommended in participants who have all three meals in the school canteens. In children with complete dietary records and 24-h urine, the level of salt intake estimated by 24-h urine accounted for 79.6% of the dietary records.

Conclusion: Our study showed that boarding school students consumed excessive salt from school canteens. Thus, policies or strategies targeting school canteens are urgently needed. Weighed dietary records are recommended if feasible.

KEYWORDS

sodium, 24-h urine, consistency, children and adolescents, weighed dietary record

1. Introduction

A high-salt diet is a major risk factor contributing to 44 million disability-adjusted life years (DALYs) and 1.8 million deaths worldwide in 2019 and was the third-most important risk factor of DALYs and deaths among Chinese adults in 2017 (1, 2). Health organizations recommend consuming <5 g of salt per day in adults (3, 4), although consumption by individuals in many countries far exceeds this level (5–7). High salt intake is also common among children and adolescents, similar to adults (8–10). To reduce the heavy health burden caused by high dietary salt intake, several large-scale intervention studies have been undertaken in China (11–13), with two studies conducted in urban children (14, 15). An important premise for these interventional studies is the accurate estimation of salt intake at the individual level.

Currently, the measurement of salt intake from 24-h urine samples is considered the gold standard for individual-level evaluations. Although studies conducted in strictly controlled environments have revealed that at least 3 days' 24-h urine records are needed and that a single 24-h urine result is no better than a coin flip in estimating salt intake at individual levels (16, 17), many studies were based on a single 24-h urine sample due to the heavy participant burden (11, 13). For boarding school students who have all three meals in school canteens on weekdays, weighed dietary records are a feasible and reliable approach to assess the level of salt intake. Although boarding schools are common in rural areas and there are over 26 million boarding school students (aged 7–15 years) in China (18), few studies have reported the level of salt intake in this young population using weighed dietary records or compared the differences between salt intake measurements obtained with 24-h urine and weighed dietary records in this convenient population. Therefore, this study aimed to evaluate the salt intake in boarding school students and the consistency between salt intake measurements based on 24-h urine and weighed dietary records over 3 consecutive days in this population.

2. Materials and methods

2.1. Participants

This cross-sectional study was conducted at three boarding schools, two primary schools (grades 1–6), and one junior high school (grades 7–8), located in the rural areas of Hunan Province, China, from September to October 2021. This survey aimed to explore the interactions of salt, overweight (including obesity) or hypertension, and gut microbiota and to evaluate the consistency between salt intake assessments based on dietary and 24-h urine records in children. Overweight or obese students from the three schools were recruited for this study. Students from grades 5 to 6 who were hypertensive after three separate blood pressure measurements were also recruited. For each overweight or hypertensive participant, a sex- and grade-matched control student with normal weight or blood pressure was included in this study. We excluded students with current diarrhea or fever and girls in menstruation from this study. After obtaining written informed consent from the participants and their guardians, a total of 180 students were recruited for the study. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Hunan Children's Hospital Review Board (KYSQ2021-212, HCHLL-2022-77).

2.2. 24-h urine collection

Three consecutive 24-h urine samples were collected from all participants. Before collection, detailed face-to-face instructions were provided to all participants. The start time of the 24-h urine collection was recorded after the participants emptied their bladders, and the participants were then provided with a 2-L wide-mouth container and a urine collection aid. The finish time was recorded at the same time on the second or third day after the participants had passed their last urine in the container. Another urine container was provided to the participants upon completion of the first or second 24-h urine collection. The 24-h urine samples were evenly mixed, and 2 ml aliquots were extracted within 2 h, temporarily stored at −20°C, and then transported to a centralized laboratory within 2 months.

Concentrations of urinary sodium, potassium, and creatinine were measured at the Laboratory Center of Hunan Children's Hospital using the ion-selective electrode method (sodium and potassium) and enzymatic method (creatinine) (Beckman AU-5800).

2.3. Weighed dietary records

3-day dietary records of the participants from grades 5 to 8 were obtained during the period of 24-h urine collection. The records included the weighed intake from the cafeteria and the participant-reported intake of snacks. During the preparation of each meal in the school canteens, the amounts of salt and other salty seasonings used were weighed and recorded. The sodium concentration in each food item was calculated as the amount of sodium divided by the net weight of the food item after cooking. For each participant, the researchers weighed each food item before the meal and the leftovers of each food item after the meal. The sodium intake of each food item was calculated as the sodium concentration (mg/100 g) multiplied by the net intake (g). The participants were also asked to record the details of snacks taken beside the meals (such as pickles, salty snacks, beverages, and biscuits), including the time of intake, snack name or brand, net weight, and source of the snacks (homemade

TABLE 1 Basic characteristics of all participants ($n = 180$).

Indicators	All	School 1 (G5–6)	School 2		School 3 (G7–8)
			G1–4	G5–6	
N	180	54	34	60	32
Sex, boy	128 (71.1)	36 (66.7)	28 (82.4)	32 (53.3)	32 (100)
Age, year	10.9 ± 1.5	11.1 ± 0.6	8.6 ± 1.1	11.1 ± 0.6	12.9 ± 0.6
Weight, kg	46.3 ± 14.2	48 ± 13.3	34.2 ± 10.3	47 ± 11.0	55.1 ± 16.6
Height, cm	146.8 ± 10.8	148.2 ± 8	132.8 ± 7.1	148.1 ± 7.4	156.7 ± 9.2
BMI, kg/m^2	21.1 ± 4.7	21.6 ± 4.8	19.1 ± 4.4	21.2 ± 4.2	22.1 ± 5.2
SBP, mmHg	109.7 ± 12.0	114.8 ± 11.1	101.9 ± 7.9	108.7 ± 11.0	111.2 ± 14.4
DBP, mmHg	64.6 ± 8.0	67.8 ± 9.4	61.1 ± 6.2	64.7 ± 7.3	62.9 ± 6.6
HR, beats/min	75.8 ± 16.9	73.2 ± 17.3	71.8 ± 16.4	78.2 ± 19.5	80.1 ± 8.0
BMI status					
Normal	80 (44.4)	22 (40.7)	17 (50.0)	24 (40.0)	17 (53.1)
Overweight	48 (26.7)	14 (25.9)	5 (14.7)	22 (36.7)	7 (21.9)
Obesity	52 (28.9)	18 (33.3)	12 (35.3)	14 (23.3)	8 (25.0)

School 1, FX; School 2, PX; School 3, PZ. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; BMI, body mass index.

or store-bought). If the net weights of the recorded snacks were missing, the net weight of the same or similar snacks sold in the school shop or shops around the school was used as a substitute. The sodium concentration of the snacks was determined from the values for the same or similar snacks sold in the school shop or shops around the school.

2.4. Statistical analysis

Incomplete 24-h urine was defined as follows: (1) urinary volume <300 mL; (2) >1 instance of leakage of urine; or (3) 24-h urine creatinine level <2.0 mmol for boys and <1.2 mmol for girls. Children with 2 or 3 days' complete 24-h urine were included in the urinary analysis. Incomplete dietary records were defined as follows: (1) missing one or more meals at the school cafeteria and (2) not handing in the snack-record sheet at the end of the collection period. Children with complete dietary records for 2 or 3 days were included in the dietary analysis. An average of 2 or 3 days' complete 24-h urine or dietary records was used for the analyses. The cutoffs for high salt and inadequate potassium intake were adjusted downward for children aged 6–14 years based on their energy requirements relative to adults. Absolute differences between salt intake measured using the averaged 24-h urine and dietary records, between salt intake measured using the complete 24-h urine record on day 2 and the complete dietary record on day 1, and between salt intake measured using the complete 24-h urine record on day 3 and the complete dietary record on day 2 were calculated and classified into three groups: ≤1, 1–3, and >3 g.

Weight and height were measured, and body mass index (BMI) was categorized as normal, overweight, or obese according to the International Obesity Task Force (IOTF) standards (19). Blood pressure (BP) was measured using validated devices (Omron HBP-1300) (20) and a standard procedure (21), and BP status was classified according to the reference values for Chinese children (22). Continuous variables were expressed as mean ± standard deviation or median and interquartile range. A *t*-test or rank-sum test was used to test the differences between the two groups. Categorical variables were presented as numbers and proportions. All analyses were conducted using SPSS 20.0, and a two-sided $P < 0.05$ was considered statistically significant.

3. Results

The participants' basic characteristics are listed in Table 1. Among the 180 participants (mean age, 10.9 ± 1.5 years; range: 6–14 years) who agreed to participate in this study, 128 (71.1%) were boys, 48 (26.7%) were overweight and 52 (28.9%) were obese, and 18 (10%) were hypertensive after BP measurement on three separate occasions.

Table 2 shows the results of the 3-day 24-h urine tests. A total of 173, 172, and 169 participants provided 24-h urine samples on the first, second, and third days, respectively, of which 137, 139, and 139 participants provided complete 24-h urine samples, respectively. Overall, 3- or 2-day complete 24-h urine records were available for 146 participants, and their average salt excretion was 6,218 [4,636, 8,290] mg, with the salt

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TABLE 2 Salt excretion from 24-h urine.

	All	School 1 (G5–6)	School 2		School 3 (G7–8)
			G1–4	G5–6	
The 1st 24 h urine					
N	137	41	25	43	28
Volume, ml	550 [428, 789]	595 [455, 848]	555 [420, 763]	540 [380, 715]	533 [456, 809]
Cna, mmol/L	161.1 [122.3, 202.3]	173 [126, 208]	137.8 [89.5, 190.4]	151 [95.3, 176.9]	182 [152, 214]
Ck, mmol/L	25.5 [19.9, 32.2]	29 [22, 38]	23.5 [18, 31.8]	25.9 [21.6, 33]	22 [16,17]
Ccr, umol/L	1,0450 [7,273, 14,262]	9,713 [6,435, 12,578]	7,326 [6,298, 9,334]	12,803 [9,343, 15,631]	13,795 [10,178, 18,329]
Sodium, mg	2,002 [1,408, 2,910]	2,422 [1,711, 3,460]	1,715 [1,333, 2,157]	1,572 [1,248, 2,775]	2,240 [1,725, 3,213]
Salt, mg	5,092 [3,581, 7,403]	6,160 [4,351, 8,799]	4,362 [3,390, 5,487]	3,998 [3,174, 7,057]	5,698 [4,388, 8,171]
K, mg	571 [428, 739]	737 [562, 919]	544 [459, 699]	549 [435, 712]	447 [353, 567]
Cr, mmol	5.91 [4.34, 7.96]	5.83 [4.77, 7.15]	3.90 [3.26, 4.63]	6.22 [5.13, 8.71]	8.24 [6.13, 9.16]
The 2nd 24 h urine					
N	139	49	21	40	29
Volume, ml	625 [465, 835]	670 [485, 893]	600 [515, 730]	534 [408, 802]	740 [497, 940]
Cna, mmol/L	167.3 [127.7, 220.7]	193 [147, 227]	151.6 [127.2, 183.2]	130.9 [90.6, 216.5]	194 [157, 230]
Ck, mmol/L	25.7 [17.8, 31.3]	27 [21,30]	30.7 [21.3, 38.1]	26.3 [16.6, 32.7]	21 [15,30]
Ccr, umol/L	9,311 [7,112, 13,374]	8,565 [6,851, 10,523]	7,550 [6,777, 11,057]	11,138 [7,952, 16,102]	12,502 [8,542, 18,661]
Sodium, mg	2,402 [1,847, 3,324]	2,788 [2,261, 3,738]	2,011 [1,669, 2,514]	1,837 [1,184, 2,281]	3,324 [2,411, 4,043]
Salt, mg	6,110 [4,698, 8,455]	7,091 [5,750, 9,509]	5,115 [4,244, 6,395]	4,671 [3,012, 5,802]	8,455 [6,132, 10,284]
K, mg	581 [465, 728]	705 [501, 842]	632 [584, 759]	548 [452, 633]	561 [446, 722]
Cr, mmol	6.10 [4.60, 7.95]	5.64 [4.09, 6.59]	4.72 [4.10, 5.51]	6.83 [4.75, 7.59]	9.05 [7.29, 11.18]
The 3rd 24 h urine					
N	139	49	21	40	29
Volume, ml	650 [475, 880]	650 [505, 838]	580 [425, 685]	530 [410, 765]	1,029 [685, 1,413]
Cna, mmol/L	179.5 [135.4, 241.2]	201 [144, 240]	166.4 [117.7, 209.2]	172.8 [110.7, 250.3]	178 [148, 242]
Ck, mmol/L	22.9 [17.2, 30.9]	24 [20, 34]	31.8 [23.1, 41]	24 [16.9, 29.9]	16 [12,21]
Ccr, umol/L	9,020 [6,738, 12,202]	8,501 [6,258, 11,712]	8,181 [6,215, 10,824]	10,319 [7,421, 16,214]	9,615 [7,308, 12,711]
Sodium, mg	2,488 [1,893, 3,693]	2,648 [2,045, 3,825]	1,961 [1,474, 2,493]	2,264 [1,693, 2,648]	4,495 [3,262, 5,762]
Salt, mg	6,327 [4,815, 9,394]	6,734 [5,201, 9,730]	4,988 [3,748, 6,342]	5,757 [4,306, 6,736]	11,432 [8,296, 14,654]
K, mg	596 [481, 743]	665 [531, 847]	621 [533, 893]	532 [411, 654]	535 [473, 721]
Cr, mmol	6.09 [4.65, 8.24]	5.48 [4.61, 6.83]	4.79 [3.79, 5.20]	6.55 [5.00, 7.67]	8.89 [7.58, 11.59]
Average					
N	146	51	23	41	31
Sodium, mg	2,445 [1,823, 3,259]	2,894 [2,147, 3,527]	1,858 [1,697, 2,541]	1,961 [1,474, 2,507]	3,335 [2,190, 4,218]
Salt, mg	6,218 [4,636, 8,290]	7,362 [5,461, 8,971]	4,726 [4,317, 6,464]	4,988 [3,749, 6,376]	8,483 [5,570, 10,729]
K, mg	589 [489, 714]	678 [579, 788]	616 [548, 736]	524 [449, 653]	517 [450, 698]
Excess salt	120 (82.2)	44 (86.3)	20 (87.0)	27 (65.9)	29 (93.5)
Inadequate K	146 (100)	51 (100)	23 (100)	41 (100)	31 (100)

School 1, FX; School 2, PX; School 3, PZ. Cna, concentration of sodium; Ck, concentration of potassium; Ccr, concentration of creatinine; K, potassium; Cr, creatinine.

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TABLE 3 Salt intake from weighed dietary records[a].

	All	School 1 (G5–6)	School 2 (G5–6)	School 3 (G7–8)
Day 1				
N	107	38	47	22
Salt from cafeteria, mg	7,047 [5,440, 9,129]	6,014 [5,088, 7,669]	8,537 [6,066, 9,874]	6,888 [5,691, 9,038]
Salt from snacks, mg	368 [0, 1,092]	879 [96, 1,945]	0 [0, 509]	388 [0, 993]
Total salt intake, mg	8,124 [6,200, 9,874]	7,385 [6,143, 9,406]	9,030 [6756, 10,221]	7,907 [6,025, 9,349]
Ratio[b]	0.95 [0.87, 1.00]	0.88 [0.74, 0.99]	1.00 [0.94, 1.00]	0.95 [0.87, 1.00]
Day 2				
n	117	40	49	28
Salt from cafeteria, mg	7,150 [5,520, 9,133]	9,217 [6,456, 10,560]	6,034 [4,242, 7,037]	8,187 [6,812, 9,445]
Salt from snacks, mg	137 [0, 749]	660 [0, 1,064]	0 [0, 388]	0 [0, 792]
Total salt intake, mg	7391 [6,001, 9,772]	10,114 [6,936, 11,467]	6,202 [4,478, 7,275]	8,615 [7,483, 9,735]
Ratio[b]	0.98 [0.90, 1.00]	0.93 [0.86, 1.00]	1.00 [0.94, 1.00]	1.00 [0.91, 1.00]
Day 3				
n	105	32	47	26
Salt from cafeteria, mg	7,789 [6,037, 9,907]	11,349 [8,241, 14,402]	6,277 [4,716, 7,642]	8,799 [7,638, 9,947]
Salt from snacks, mg	0 [0, 406]	0 [0, 708]	0 [0, 407]	0 [0, 378]
Total salt intake, mg	8,049 [6,427, 10,437]	11,585 [9,301, 14,481]	6,482 [4,716, 7,689]	9,044 [7,853, 10,385]
Ratio [b]	1.00 [0.95, 1.00]	1.00 [0.95, 1.00]	1.00 [0.94, 1.00]	1.00 [0.96, 1.00]
Average				
n	115	39	49	27
Total salt intake, mg	8,132 [6,348, 9,370]	9,357 [7,707, 11,629]	6,828 [5,724, 8,390]	8,590 [7,678, 9,271]
Excess salt intake	112 (97.4)	39 (100)	46 (93.9)	27 (100)
Ratio[b]	0.95 [0.91, 1.00]	0.93 [0.83, 0.96]	0.98 [0.92, 1.00]	0.96 [0.92, 1.00]

[a]Data are presented as median [IQR, interquartile range] or *n* (%); [b]ratio = salt intake from cafeteria/total salt intake. School 1, FX; School 2, PX; School 3, PZ.

levels exceeding the recommended level in 120 (82.2%) students (cutoff values are provided in Supplementary Table 1).

3-day dietary records were collected from 146 participants in grades 5–8. Four participants did not hand in the snack-record sheets at the end of the collection period and were excluded from the dietary analysis. On the 1st day of dietary recording, 35 students missed one or more meals in the school cafeteria; thus, 107 1st-day records were analyzed. Similarly, 117 and 105 dietary records for the second and third days, respectively, were analyzed. The salt intake from the school cafeteria was 7,047 [5,440, 9,129] mg, 7,150 [5,520, 9,133] mg, and 7,789 [6,037, 9,907] mg on the first, second, and third days, respectively. Salt intake from snacks was 368 [0, 1,092] mg, 137 [0, 749] mg, and 0 [0, 406] mg on the first, second, and third days, respectively. For the 115 participants with complete dietary records, the total salt intake was 8,132 [6,348, 9,370] mg, and 95% [IQR: 91%-100%] of the salt was consumed in school canteens (Table 3). Three-day records of the salt and salty seasonings used in each food item in the three schools' cafeterias are provided in Supplementary Table 2, and the net intake and sodium intake for each food item in the three schools are provided in Supplementary Table 3.

For the 100 participants with complete 24-h urine collection and dietary records, the average salt intake was 8,392 [6,733, 9,471] mg from dietary records and 6,362 [4,731, 9,153] mg from 24-h urine records, with a median ratio (salt assessed by 24-h urine records divided by salt assessed by dietary records) of 0.796 [IQR: 0.652–1.046]. In the three schools, the median differences in salt intake between the dietary and 24-h urine records were 1,955 [714, 3,423] mg, 1,521 [566, 2,776] mg, and −1,485 [−1,946, 1,923] mg, respectively (Table 4). The proportions of absolute differences in the average salt estimation between the 24-h urine and dietary records within 1 g, 1–3 g, and >3 g were 17, 61, and 22%, respectively (Table 5).

TABLE 4 Consistency between averaged dietary record and 24-h urine in estimating salt intake, mg[a].

	n	Dietary record	24-h urine	Difference		Ratio[b]
				Median [IQR]	Mean [95%CI]	
Averaged	100	8,392 [6,733, 9,471]	6,362 [4,731, 9,153]	1,435 [−441, 2,648]*	1,388 [906, 1,869]*	0.80 [0.65, 1.05]
Day 1	85	8,158 [6,628, 9,979]	5,377 [3,689, 7,537]	2,163 [194, 4,938]*	2,407 [1,634, 3,179]*	0.70 [0.46, 0.96]
Day 2	97	8,094 [6,300, 10,114]	6,444 [4,837, 9,044]	1,471 [−325, 3,102]*	1,372 [860, 1,884]*	0.82 [0.61, 1.04]
Day 3	88	8,479 [6,476, 11,048]	6,764 [5,324, 10,652]	879 [−2,009, 2,966]	718 [−167, 1,603]	0.87 [0.68, 1.24]
School 1	38	9,408 [7,665, 11,753]	7,064 [5,641, 9,305]	1,955 [714, 3,423]*	2,199 [1,347, 3,052]*	0.77 [0.64, 0.92]
School 2	36	6,799 [5,721, 8,437]	4,922 [3,901, 6,348]	1,521 [566, 2,776]*	1,671 [1059, 2282]*	0.75 [0.61, 0.90]
School 3	26	8,601 [7,871, 9,324]	9,494 [5,700, 11,185]	−1,485 [−1,946, 1,923]	−191 [−1,102, 721]	1.16 [0.73, 1.22]

[a]Data are presented as median [IQR] unless specified, [b]Ratio = salt from 24-h urine/salt from dietary record, *$P < 0.05$. School 1, FX; School 2, PX; School 3, PZ.

TABLE 5 Absolute difference distribution of salt estimation between dietary records and 24-h urine[a].

	≤1 g	(1, 3] g	>3 g
Total	17 (17.0)	61 (61.0)	22 (22.0)
2nd 24-h urine-−1st dietary records[b]	16 (16.7)	43 (43.9)	37(38.5)
3rd 24-h urine-−2nd dietary records[c]	26 (26.5)	40 (40.8)	32 (32.7)

[a]Data are presented as *n* (%); [b]$n = 96$; [c]$n = 98$.

4. Discussion

Our study is the first to explore salt intake in children and adolescents by using 3-day 24-h urine and dietary records. The average salt excretion assessed by 24-h urine records was 6218 [4636, 8290] mg in 146 participants with ≥2 days of complete 24-h urine records. The average salt intake assessed by dietary records was 8,132 [6,348, 9,370] mg in 115 participants from Grades 5–8 with ≥2 days of complete dietary records. In the 100 participants with both complete 24-h urine and dietary records, the level of salt intake estimated by the 24-h urine records accounted for 79.6% of the level estimated by dietary records.

The median salt excretion based on the 24-h urine records was similar between this study and our previous study conducted in another school in 2017 (8) (Grade 5–6: 6,146 vs. 6,192 mg; Grade 7–8: 8,483 vs. 7,236 mg). The level of salt intake in our population was also similar to the baseline salt intake in a randomized trial conducted on Chinese urban children in 2018 (5.3 ± 1.8 g vs. 5.5 ± 2.0 g) (9). A systematic review and meta-analysis showed that the mean salt excretion in the 24-h urine record was 8,839 mg in Chinese children aged 6–16 years (23), which was higher than that in our results. However, most studies included in that meta-analysis were based on single 24-h urine samples (23), with only one study based on two consecutive 24-h urine samples (24), which may have resulted in a high risk of random errors. Children from other countries also consumed much more salt than recommended (10, 25, 26), which indicated that attention and effective interventions are urgently warranted in this young population.

Assessment of individual-level salt intake using an accurate and reliable method is vital, especially in interventional studies. Assessments based on 24-h urine records are widely acknowledged as the gold standard, and over 92% of the salt can be recovered in the urine. One study conducted on 84 children in New Zealand also showed that 24-h urine records can provide an adequate measurement of sodium intake (27). The similar levels of salt intake estimated by the 3 days' 24-h urine collection records in our population and by 1 or 2 days' 24-h urine collection in other studies suggest that salt intake at the population level can be accurately estimated by single or multiple 24-h urine collections. However, studies conducted in adults have shown that seven 24-h urine collections are needed to achieve 90% accuracy in estimates of salt intake (16, 17), and for individuals on a high-salt diet, at least 10 repeated 24-h urine collections are required to reach 75% reliability (28). In comparison with the average of three complete 24-h urine records, an absolute difference of ≤1 g in salt intake measured by 1 day's 24-h urine record was only present in ∼50% of the participants in our study, suggesting a high risk of random errors and indicating that a single 24-h urine sample was not enough to estimate salt intake at individual levels.

Another problem identified in this study is the major source of salt and the development of intervention strategies to reduce salt intake. Processed food is a major source of salt intake in developed countries (10, 29, 30), and interventions targeted at food industries have been shown to reduce salt intake and BP (31). Approximately 67–75% of salt, however, is added by the consumer during home cooking in China (32, 33). Our results showed that up to 95% of the salt was consumed by boarding school students in the school canteen during weekdays. Two large-scale intervention studies targeted Chinese urban children and focused on health education (14, 24). To the best of our knowledge, there are no studies or policies that regulate salt usage or limit salt concentration in school canteens. Our results showed that salt concentrations varied greatly among school

canteens (Supplementary Table 1), which directly contributed to the high salt intake by schoolchildren. Over 26 million young children live in boarding schools and have meals in canteens in rural China; thus, interventions or policies targeted at salt usage in school canteens are urgently required and would make a significant contribution to salt reduction in children.

The strengths of our study included the analysis based on 3 days' 24-h urine collection and weighed dietary records, with 3 days' complete 24-h urine records obtained for 103 (70.5%) participants, 2 days' complete 24-h urine records obtained for 43 (29.5%) participants, 3 days' complete dietary records available for 91 (79.1%) participants, and 2 days' complete dietary records available for 24 (20.9%) participants. Moreover, quality control was performed by the same researcher during the entire collection period, and 10% of duplicate urine samples were tested in parallel, with the biochemist who performed the measurements blinded to the identity of the duplicate samples.

Our study had some limitations, particularly in relation to the representativeness of the study population. About half of our participants were overweight or obese and about 10.0% were hypertensive based on three separate BP measurements, and the proportions of boys in our population for assessment of the estimated salt intake may have been higher than that in the general population. However, the differences in salt intake estimated by dietary records in different weight/BP/sex groups were not statistically significant for our population (data not presented). Another limitation was that food items consumed outside the school cafeteria (including snacks, processed vegetables, or homemade salted vegetables) were not weighed. We recorded the salt density and net weight of snacks of the same brand name sold in stores near the school. However, the salt content in sugars, fruits, and sugar-sweetened beverages was not calculated, and the lowest salt density and net weight were calculated if there were several snacks with similar names. Therefore, the salt intake from snacks may have been underestimated.

In conclusion, our study showed that boarding school students consumed excessive salt from school canteens. Thus, policies or strategies targeting school canteens are urgently needed. Salt excreted from 24-h urine accounted for 79.6% of the salt assessed by dietary records over 3 consecutive days in children, and a single 24-h urine sample was insufficient for individual-level estimations of salt intake. Thus, weighed dietary records are recommended if feasible.

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 Hunan Children's Hospital Review Boards. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Conceptualization, writing, reviewing, and editing: JD and YY. Methodology: XY, SX, and XL. Formal analysis and writing the original draft preparation: JD. Investigation: JD, YQ, SZ, and JZ. Resources: YQ, SZ, and JZ. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1071473/full#supplementary-material

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Association of coffee consumption pattern and metabolic syndrome among middle-aged and older adults: A cross-sectional study

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Objectives: The association between coffee consumption and the risk of metabolic syndrome (MetS) remains inconsistent. The aim of this study was to evaluate the association between coffee intake and components of MetS.

Method: A cross-sectional survey including 1,719 adults was conducted in Guangdong, China. Data on age, gender, education level, marriage status, body mass index (BMI), current smoking and drinking status and breakfast habit, coffee consumption type, and daily servings were derived based on 2-day, 24-h recall. MetS were assessed according to the International Diabetes Federation definition. Multivariable logistic regression was conducted to examine the association between the coffee consumption type, daily servings, and the components of MetS.

Results: Regardless of the coffee type, compared with non-coffee consumers, coffee consumers had higher odds ratios (ORs) of the elevated fasting blood glucose (FBG) in both men [OR: 3.590; 95% confidence intervals (CI): 2.891–4.457] and women (OR: 3.590; 95% CI: 2.891–4.457). In women, the risk of elevated blood pressure (BP) was 0.553 times (OR: 0.553; 95% CI: 0.372–0.821, $P = 0.004$) for people who drank total coffee > 1 serving/day than for non-coffee drinkers.

Conclusion: In conclusion, regardless of type, coffee intake is associated with an increased prevalence of FBG in both men and women, but has a protective effect on hypertension only in women.

KEYWORDS

coffee consumption, black coffee, instant coffee, fasting blood glucose, blood pressure

Introduction

Metabolic syndrome (MetS), defined as the presence of physiologically related cardiovascular risk factors, including dyslipidemia, abdominal obesity, hyperglycemia, and hypertension, is closely correlated with increased cardiovascular risk and common cancers (1–3). The prevalence of MetS has considerably increased over recent decades and is now at epidemic proportions worldwide (4, 5). Insulin resistance is a key hallmark feature of MetS and a critical risk factor for diabetes and other cardiovascular diseases (CVD) (6). Recently, accumulating epidemiological and experimental evidence point out that MetS is affected by genetic (7–9) and lifestyle factors (10, 11), including smoking, alcohol consumption, sugar-sweetened beverage consumption physical activity, and sedentary behaviors. Indeed, MetS have been inversely affected by dietary intakes, such as vegetables, fruits, red wine, and green tea (12). Therefore, experts emphasize dietary intakes for the primary interventions on MetS prevention (13, 14).

Coffee, which has antioxidant properties and a distinctive smell and taste, is now one of the world's most popular beverages (15). With the far-reaching development of industry and rapid changes in dietary lifestyles, coffee consumption has been considerably increasing in Shenzhen. The constituents in coffee, including polyphenols, antioxidant properties, caffeine, potassium, niacin, vitamin E, and magnesium, have been proposed to be beneficial for potential health. Experimental studies revealed that caffeine might protect against type 2 diabetes mellitus (T2DM) by stimulating free fatty acid and fat oxidation release from peripheral tissues, increasing metabolic rate and thermogenesis, and mobilizing glycogen in muscles (16). Therefore, epidemiologic studies reported a significant association between higher coffee consumption and decreased incidence of new-onset hypertension (17, 18), arterial stiffness (19, 20), T2DM (21), and promote weight loss (22). However, another study conducted in the Japanese setting demonstrated that certain types of coffee led to an increase in all-cause mortality (23). Also, other investigations reported that the intake of coffee with creamer or sugar was significantly associated with increased abdominal obesity (24) and risk of MetS (25).

The above inconsistent findings might be caused by different research designs, that is, some focused on the effect of daily coffee consumption volume, while others focused on the habitual coffee pattern. However, daily consumption patterns of coffee containing both quantitative and qualitative information are still lacking. For this reason, we performed a cross-sectional study to examine the association between coffee consumption patterns and MetS components among middle-aged and older adults.

Materials and methods

Study population

This cross-sectional survey was based on a large-scale, community-based routine health examination for the middle-aged and elderly. In total, 2,200 participants aged 40 years and above were recruited from January 2021 to March 2022 in Guangdong province. All individuals received a routine health check-up, including venous blood sampling and anthropometry. Among these, a subset of the individuals ($n = 2{,}066$) completed the 24-h food recall. Furthermore, we excluded individuals with a history of ischemic heart disease ($n = 12$) or stroke ($n = 21$), and those who take drugs to treat hyperlipidemia, diabetes, or hypertension ($n = 314$). Finally, 1,719 participants (800 men and 919 women) were included in the present study. The study was approved by the Ethics Committee of the Health Science Centre, Shenzhen University. All individuals signed written informed consent before participation.

Diagnosis of mets

According to the guidance of the updated National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) (26), individuals who met at least three of the following criteria were diagnosed with MetS: (1) waist circumference (WC) $\geq$ 90 cm in men, $\geq$ 80 cm in women; (2) systolic blood pressure (SBP) $\geq$ 130 mmHg or diastolic blood pressure (DBP) $\geq$ 85 mmHg; (3) fasting blood glucose (FBG) $\geq$ 5.60 mmol/L; (4) blood high-density lipoprotein cholesterol (HDL-C) level $<$ 40 mg/dL in men, $<$ 50 mg/dL in women; (5) blood triglyceride (TG) $\geq$ 1.70 mmol/L.

Anthropometric and biochemical measurement

At the mobile physical examination centers, anthropometric variables including weight, height, and blood pressure (BP) were measured using standardized calibrated equipment under the guidance of professional medical staff. Body mass index (BMI, kg/m^2) was calculated as weight divided by the height squared. WC was measured at the narrowest between the iliac crest and the bottom of the ribs. BP was measured by a sphygmomanometer (Yu yue, YJ100002, Jiangsu, China) on the right arm after the individual had been supine for at least 20 min, and the mean value of three times record was used. Biochemical assessment variables including FBG, HDL-C, TG, and 2 h post-load glucose (2hPG) were assessed using a semi-automated analyzer (Sysmex 100 XN-3000, Tokyo, Japan) enzymatically after fasting for at least 8 h. Furthermore, a detailed data collection process was reported elsewhere (27).

Coffee consumption measurement

Information regarding coffee consumption was obtained based on a 2-day, 24-h recall. Individuals who drank coffee at least three times per week were described as coffee drinkers (28). The habitual coffee consumption questionnaire included habitual coffee type and daily coffee serving frequency. Black coffee was described as coffee powder or extracts without other ingredients. Coffee with creamer, milk, or sugar was defined as instant coffee. Other coffee is a collective name, covering a series of other types of coffee, excluding black coffee, and instant coffee. Based on the type of coffee they consumed, individuals were classified into the following five categories: non-coffee consumers, black coffee consumers, instant coffee consumers, other coffee consumers, and coffee consumers. If only black coffee or instant coffee was in a person's 2-day, 24-h food recall, the individual was determined as a black coffee or instant coffee consumer, respectively. Meanwhile, individuals who consumed any coffee type that appeared at least once were classified as coffee consumers. Other coffee consumers referred to participants who consumed other type's coffee.

Demographic measurement

Demographic information of participants including age, gender, education level, marital status, current smoking and drinking status, breakfast habits, physical activity, and sitting time was collected through questionnaires. The level of education was categorized as up to junior high school, high school or secondary specialized school, and college and above. Marital status categories included unmarried, married or cohabiting, and others (divorced, separated, or widowed). Current smoking and drinking status were categorized as yes or no. Breakfast categories included none, 1–3 times/week, 4–5 times/week, and every day. Physical activity divided into four categories: $<$ 0.5,

0.5–1, 1–2, and > 2 h/day. Sitting time divided into four categories: < 6, 6–8, 8–10, and > 10 h/day.

Statistical analysis

All data are presented as mean (standard deviation) for continuous data and as percentages for categorical data according to the Shapiro–Wilk test of normality. Participants' demographic characteristics including age, education level, marital status, BMI, body weight status, current smoking and drinking status, breakfast habit, physical activity, sitting time, and Mets parameters according to coffee consumption type by gender, were compared using the Chi-square test for categorical variables and generalized linear model for continuous variables. A multivariate-adjust logistic regression model was conducted to explore the association between coffee consumption patterns and MetS components. We assigned the median of daily servings of coffee as a continuous variable and performed stratified analysis across coffee consumption categories. We adjusted covariates including BMI, education level, alcohol status, and physical activity for all the regression models, and the 95% confidence intervals (CIs) of odds ratios (ORs) were estimated. A two-sided *P*-value of < 0.05 was considered statistical significance, and SAS software (version 9.4) was used to conduct all analyses.

Results

Table 1 presented the participants' demographic characteristics according to coffee consumption categories by gender. In men, the proportion of participants in high school or secondary specialized school was the largest for all coffee consumption categories ($P < 0.05$). In men, mean BMI and FBG levels were significantly higher in instant coffee consumers than in other groups (all $P < 0.05$). In women, mean BMI, SBP, and FBG levels were significantly higher in other coffee consumers than in other groups (all $P < 0.05$). In both men and women, the proportion of participants with normal weight status was the largest for all coffee consumption categories (all $P < 0.05$). In men, non-alcohol drinkers were more likely to be non-coffee consumers, while compared with non-alcohol drinkers, the proportion of alcohol consumers was higher than nondrinkers in the other three types of coffee pattern groups (all $P < 0.05$). In men, the duration of physical activity was higher in black coffee consumers than in the other three types of coffee pattern groups ($P < 0.05$).

Table 2 summarized the multivariable-adjusted OR and 95% CI of MetS components across the type of coffee by gender. Regardless of the coffee type, compared with non-coffee consumers, coffee consumers had higher ORs of the elevated FBG in both men (OR: 3.590; 95% CI: 2.891–4.457) and women (OR: 3.590; 95% CI: 2.891–4.457). In women, the prevalence of elevated blood pressure (OR: 0.661; 95% CI: 0.454–0.963) was significantly lower in black coffee consumers than in non-coffee consumers. The same inverse association can be also found in other types of coffee consumption.

We further conducted stratified analyses to explore multivariable-adjusted OR and 95% CI for MetS according to daily servings of coffee by gender, as presented in Table 3. In male black coffee drinkers, there was a linear trend between the increase of TG and the decrease in coffee consumption ($\boldsymbol{P} < 0.05$). In addition, men who drank coffee > 1 serving/day had an increased risk of elevated FBG (OR: 4.112; 95% CI: 2.537–6.666; $P < 0.05$). The same results were also observed in men who drank black coffee (OR: 3.835; 95% CI: 2.009–7.319; $P < 0.001$) and instant coffee (OR: 3.651; 95% CI: 1.329–10.031; $\boldsymbol{P} < 0.001$). In women, there was a positive correlation between coffee consumption and elevated FBG. The risk of elevated FBG in people who drink > 1 serving/day is 3.798 times higher than that in people who drink ≤ 1 serving/day (OR: 3.798; 95% CI: 2.555–5.647, $\boldsymbol{P} < 0.001$), and the same results can be found in women who drink black coffee and instant coffee. The risk of elevated FBG was 3.109 times (OR: 3.109; 95% CI: 1.799–5.371, $P < 0.001$) in women who drank black coffee and 3.514 times (OR: 3.109; 95% CI: 1.503–8.218, $P < 0.001$) in women who drank instant coffee. In women, compared with non-coffee consumers, there was a negative correlation between coffee consumption and elevated BP. The risk of elevated BP was 0.553 times (OR: 0.553; 95% CI: 0.372–0.821, $P = 0.004$) for people who drank coffee > 1 serving/day than for non-coffee drinkers in total coffee. The risk of elevated BP was 0.516 times that of non-coffee drinkers (OR: 0.516; 95% CI: 0.296–0.898, $P = 0.005$) in black coffee. Among instant coffee drinkers, the risk of elevated BP was 0.276 times that of non-coffee drinkers (OR: 0.276; 95% CI: 0.112–0.68, $P = 0.037$).

Discussion

The current study examined the associations between coffee consumption patterns and MetS components among middle-aged and older adults in Guangdong. We found that both black coffee and instant coffee had positive associations with elevated FBG. Furthermore, these positive associations were robust in the stratified analyses among participants who consumed ≤ 1 vs. >1 Serving daily. In addition, according to gender-stratified analysis, regardless of the coffee type, women who drank a good amount of coffee were significantly associated with a lower prevalence of elevated BP than non-coffee consumers. Whereas, the same results could not be found in men. Our results revealed that habitual coffee drinking could prevent women from hypertension in a certain sense.

Our study suggested that most of the participants were of normal weight regardless of the coffee consumption type. But, in both men and women, compared with non-coffee consumers, coffee consumers were more likely to have a higher BMI. These findings are not in line with previous epidemiology studies. In a national wide cross-sectional study conducted in 2003–2004, coffee consumption was not significantly associated with BMI or waist circumference in either men or women (29). However, another cross-sectional study in Poland revealed that lower coffee consumption was significantly associated with a higher risk of obesity (30). The inconsistency may be caused by the differences in diet assessment measures and study population. The previous survey collected the data by the means of a validated food frequency questionnaire (FFQ) to measure coffee consumption, which may cause non-differential misclassification, leading to biased study results (31). Furthermore, the response categories of FFQ were close-ended, which may lead to an underestimation of coffee consumption (32). In the current survey, we adapted a 2-day, 24-h food recall to assess participants' habitual coffee consumption, which could avoid the mentioned above biases.

Unexpectedly, we found that compared to non-coffee consumers, participants who consumed ≤ 1 serving/day or >1 serving/day

TABLE 1 General characteristics of the cross-sectional study population according to coffee consumption pattern by gender.

Variables	Men (n = 800)					Women (n = 919)				
	Non-Coffee Consumer (n = 475)	Black-Coffee Consumer (*n* = 117)	Instant-Coffee Consumer (*n* = 66)	Other Coffee Consumer (*n* = 142)	*P*	Non-Coffee Consumer (n = 549)	Black-Coffee Consumer (*n* = 143)	Instant-Coffee Consumer (*n* = 80)	Other Coffee Consumer (*n* = 147)	*P*
Age (years)	62.34 ± 15.62	61.24 ± 15.53	63.00 ± 16.28	62.44 ± 15.95	0.881	61.32 ± 14.83	62.16 ± 14.75	61.31 ± 15.42	62.46 ± 14.32	0.813
Educational level										
~Junior high school	90 (18.95)	16 (13.68)	8 (12.12)	10 (7.04)	0.016	94 (17.12)	20 (13.99)	13 (16.25)	26 (17.69)	0.943
High school/Secondary specialized school	262 (55.16)	62 (52.99)	39 (59.09)	94 (66.20)		322 (58.65)	83 (58.04)	49 (61.25)	85 (57.82)	
College~	123 (25.89)	39 (33.33)	19 (28.79)	38 (26.76)		133 (24.23)	40 (27.97)	18 (22.50)	36 (24.49)	
Marriage										
Unmarried	155 (32.63)	41 (35.04)	25 (37.88)	59 (41.55)	0.233	193 (35.15)	55 (38.46)	26 (32.50)	55 (37.41)	0.359
Married/cohabiting	289 (60.84)	63 (53.85)	35 (53.03)	74 (52.11)		319 (58.11)	85 (59.44)	51 (63.75)	82 (55.78)	
Divorced/Separated/ Widowed	31 (6.53)	13 (11.11)	6 (9.09)	9 (6.34)		37 (6.74)	3 (2.10)	3 (3.75)	10 (6.80)	
Height (cm)	163.77 ± 8.11	164.38 ± 7.59	162.91 ± 7.96	163.96 ± 7.99	0.669	158.37 ± 6.39	157.68 ± 5.89	158.31 ± 6.08	157.80 ± 6.85	0.569
Weight (kg)	61.03 ± 11.05	63.90 ± 11.11	63.74 ± 10.41	62.08 ± 9.53	0.034	55.69 ± 10.29	56.85 ± 8.47	56.36 ± 9.22	57.47 ± 8.76	0.167
Body mass index	22.68 ± 3.37	23.59 ± 3.40	24.01 ± 3.35	23.07 ± 2.97	0.004	22.18 ± 3.82	22.89 ± 3.43	22.45 ± 3.18	23.09 ± 3.43	0.020
Body weight status										
Underweight	36 (7.58)	8 (6.84)	2 (3.03)	11 (7.75)	0.024	86 (15.66)	12 (8.39)	4 (5.00)	3 (2.05)	<0.001
Normal	234 (49.26)	45 (38.46)	23 (34.85)	61 (42.96)		265 (48.27)	66 (46.15)	44 (55.00)	86 (58.90)	
Overweight	117 (24.63)	31 (26.50)	16 (24.24)	33 (23.24)		102 (18.58)	34 (23.78)	15 (18.75)	24 (16.44)	
Obese	88 (18.53)	33 (28.21)	25 (37.88)	37 (26.06)		96 (17.49)	31 (21.68)	17 (21.25)	33 (22.60)	
Current smoking status										
Yes	223 (46.95)	59 (50.43)	26 (39.39)	70 (49.30)	0.500	236 (42.99)	67 (46.85)	34 (42.50)	71 (48.30)	0.612
No	252 (53.05)	58 (49.57)	40 (60.61)	72 (50.70)		313 (57.01)	76 (53.15)	46 (57.50)	76 (51.70)	
Current alcohol status										
Yes	216 (45.47)	69 (58.97)	37 (56.06)	74 (52.11)	0.032	247 (44.99)	71 (49.65)	35 (43.75)	72 (48.98)	0.647
No	259 (54.53)	48 (41.03)	29 (43.94)	68 (47.89)		302 (55.01)	72 (50.35)	45 (56.25)	75 (51.02)	

(Continued)

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TABLE 1 (Continued)

Variables	Men (n = 800)					Women (n = 919)				
	Non-Coffee Consumer (n = 475)	Black-Coffee Consumer (*n* = 117)	Instant-Coffee Consumer (*n* = 66)	Other Coffee Consumer (*n* = 142)	*P*	Non-Coffee Consumer (n = 549)	Black-Coffee Consumer (*n* = 143)	Instant-Coffee Consumer (*n* = 80)	Other Coffee Consumer (*n* = 147)	*P*
Breakfast habit										
No	24 (5.05)	6 (5.13)	1 (1.52)	8 (5.63)	0.661	29 (5.28)	6 (4.20)	3 (3.75)	1 (0.68)	0.334
1-3 times/week	146 (30.74)	46 (39.32)	20 (30.30)	44 (30.99)		166 (30.24)	44 (30.77)	27 (33.75)	45 (30.61)	
4-5 times/week	159 (33.47)	32 (27.35)	25 (37.88)	42 (29.58)		179 (32.60)	53 (37.06)	20 (25.00)	51 (34.69)	
Every day	146 (30.74)	33 (28.21)	20 (30.30)	48 (33.80)		175 (31.88)	40 (27.97)	30 (37.50)	50 (34.01)	
Physical activity										
<0.5 h/day	185 (38.95)	44 (37.61)	23 (34.85)	66 (46.48)	0.025	195 (35.52)	57 (39.86)	25 (31.25)	55 (37.41)	0.258
0.5-1 h/day	139 (29.26)	46 (39.32)	19 (28.79)	50 (35.21)		186 (33.88)	36 (25.17)	36 (45.00)	54 (36.73)	
1-2 h/day	96 (20.21)	19 (16.24)	18 (27.27)	13 (9.15)		101 (18.40)	31 (21.68)	12 (15.00)	21 (14.29)	
≥ 2 h/day	55 (11.58)	8 (6.84)	6 (9.09)	13 (9.15)		67 (12.20)	19 (13.29)	7 (8.75)	17 (11.56)	
Sitting time										
<6 h/day	127 (26.74)	31 (26.50)	14 (21.21)	32 (22.54)	0.679	134 (24.41)	40 (27.97)	17 (21.25)	37 (25.17)	0.650
6-8 h/day	182 (38.32)	50 (42.74)	28 (42.42)	48 (33.80)		205 (37.34)	48 (33.57)	33 (41.25)	51 (34.69)	
8-10 h/day	98 (20.63)	20 (17.09)	14 (21.21)	38 (26.76)		144 (26.23)	32 (22.38)	22 (27.50)	34 (23.13)	
≥ 10 h/day	68 (14.32)	16 (13.68)	10 (15.15)	24 (16.90)		66 (12.02)	23 (16.08)	8 (10.00)	25 (17.01)	
Systolic blood pressure (mmHg)	134.89 ± 20.89	137.80 ± 19.39	131.80 ± 20.51	135.68 ± 20.19	0.257	134.92 ± 22.24	128.54 ± 20.29	131.69 ± 22.81	135.09 ± 22.47	0.008
Diastolic blood pressure (mmHg)	86.32 ± 13.35	87.13 ± 12.80	85.58 ± 14.68	85.51 ± 11.77	0.742	85.13 ± 13.51	82.17 ± 14.12	82.96 ± 14.67	84.62 ± 13.64	0.119
Triglycerides (mmol/L)	1.64 ± 0.51	1.53 ± 0.49	1.69 ± 0.47	1.64 ± 0.50	0.141	1.65 ± 0.51	1.71 ± 0.50	1.58 ± 0.51	1.63 ± 0.51	0.288
High-density lipoprotein cholesterol (mmol/L)	1.25 ± 0.24	1.29 ± 0.30	1.21 ± 0.23	1.26 ± 0.27	0.304	1.30 ± 0.28	1.28 ± 0.24	1.29 ± 0.26	1.29 ± 0.25	0.811
Fasting blood glucose (mmol/L)	5.72 ± 0.46	6.01 ± 0.47	6.02 ± 0.45	5.99 ± 0.47	<0.001	5.73 ± 0.48	6.02 ± 0.42	6.00 ± 0.51	6.12 ± 0.53	<0.001
Blood glucose two hours after meals (mmol/L)	9.11 ± 0.64	9.10 ± 0.66	8.92 ± 0.63	9.07 ± 0.64	0.146	9.05 ± 0.68	9.09 ± 0.67	9.06 ± 0.67	9.20 ± 0.69	0.115

P values were calculated by generalized linear model for continuous variables and χ^2 test for categorical variables. Underweight (BMI < 18.5 kg/m^2); normal (BMI ≥ 18.5 and < 23 kg/m^2); overweight (BMI ≥ 23 and < 25 kg/m^2); obese (BMI ≥ 25 kg/m^2).

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TABLE 2 Multivariable-adjusted odds ratios and 95% CIs for metabolic components according to the type of coffee consumed (by gender).

Variable	Non-coffee consumer (reference)	Black-coffee consumer	Instant-coffee consumer	Other coffee consumer	Coffee consumer
Men					
Elevated TG					
Model 1	Ref	0.878 (0.582, 1.324)	0.782 (0.451, 1.355)	0.988 (0.674, 1.447)	0.903 (0.678, 1.203)
Model 2	1.139 (0.755, 1.717)	Ref	0.891 (0.471, 1.686)	1.125 (0.682, 1.857)	-
Reduced HDL-C					
Model 1	Ref	0.933 (0.706, 1.234)	1.211 (0.854, 1.716)	0.927 (0.709, 1.211)	0.984 (0.809, 1.198)
Model 2	1.071 (0.811, 1.416)	Ref	1.297 (0.861, 1.955)	0.993 (0.704, 1.401)	-
Elevated BP					
Model 1	Ref	0.867 (0.651, 1.155)	0.729 (0.510, 1.043)	0.984 (0.744, 1.302)	0.880 (0.717, 1.079)
Model 2	1.153 (0.865, 1.536)	Ref	0.841 (0.553, 1.278)	1.135 (0.796, 1.618)	-
Elevated FBG					
Model 1	Ref	3.523 (2.635, 4.709)	3.268 (2.273, 4.699)	3.827 (2.895, 5.059)	3.590 (2.891, 4.457)
Model 2	0.284 (0.212, 0.379)	Ref	0.928 (0.617, 1.396)	1.086 (0.776, 1.521)	-
Women					
Elevated TG					
Model 1	Ref	1.317 (0.795, 2.179)	0.736 (0.340, 1.591)	0.874 (0.497, 1.535)	1.009 (0.683, 1.490)
Model 2	0.760 (0.459, 1.257)	Ref	0.559 (0.238, 1.310)	0.664 (0.340, 1.297)	-
Reduced HDL-C					
Model 1	Ref	0.983 (0.676, 1.430)	0.932 (0.579, 1.501)	0.911 (0.628, 1.321)	0.943 (0.721, 1.233)
Model 2	1.017 (0.699, 1.478)	Ref	0.948 (0.543, 1.653)	0.926 (0.579, 1.482)	-
Elevated BP					
Model 1	Ref	0.661 (0.454, 0.963)	0.738 (0.456, 1.194)	0.984 (0.668, 1.448)	0.790 (0.600, 1.040)
Model 2	1.512 (1.038, 2.203)	Ref	1.116 (0.640, 1.947)	1.488 (0.924, 2.397)	-
Elevated FBG					
Model 1	Ref	2.987 (2.021, 4.413)	3.192 (1.962, 5.195)	4.177 (2.844, 6.134)	3.464 (2.589, 4.634)
Model 2	0.335 (0.227, 0.495)	Ref	1.069 (0.616, 1.854)	1.398 (0.880, 2.221)	-

CIs, confidence intervals; TG, triglyceride; HDL-C, HDL cholesterol; BP, blood pressure; FBG, fasting blood glucose; Model 1: Reference = non-coffee consumer. Adjusted for BMI, education level, alcohol status, Physical activity. Model 2: Reference = black-coffee consumer. Adjusted for BMI, education level, alcohol status, and physical activity.

of any coffee were more likely to have increased FBG levels in both men and women. The results are not consistent with findings from previous epidemiologic surveys. In a cross-sectional prospective study in Dutch, higher coffee consumption tended to be significantly associated with a lower risk of T2DM (33). While numerous prospective cohort studies indicated the inverse relationship between habitual coffee consumption and the incidence of T2DM (34). This inconsistency could be attributed to various factors, such as coffee consumption type, dose, and other constitutional and environmental factors.

The mechanism of the association between coffee consumption and plasma glucose remains unclear yet. Caffeine, one of the main bioactive compounds in coffee, has numerous biological impacts on all aspects of human health (35). A previous experimental study indicated that short-term coffee consumption could impair glucose tolerance and reduce insulin sensitivity due to the A1 attenuating aortic dissection affected by the caffeine-blocking; however, this effect will not last long (36). Long-term coffee consumption could prevent the incidence of T2DM by affecting post-load rather than fasting glucose metabolism (37). On the other hand, the effect of caffeine on plasma glucose is determined by the glycemic index of food (38). From a genetic point of view, Robertson et al. revealed that the plasma glucose level might be affected by the plasma glucose level, such as rs762551 single-nucleotide polymorphism in the CYP1A2 gene, which can directly affect the rate of the body's metabolism of caffeine (39). In this aspect, CYP1A2 activity can be effected by numerous environmental factors, including race, gender, smoking, and drinking status. Another experimental study suggested that compared to baseline, fasting glucose concentrations were higher after consuming 1 L of coffee daily for 2 weeks, but not after 4 weeks, indicating that caffeine is substantially influenced by the development of tolerance (40). Therefore, the inconsistent results in the current

TABLE 3 Multivariable-adjusted odds ratios and 95% CIs for metabolic components according to daily servings of coffee (by gender).

Variable	Non-coffee consumer (reference)	Total coffee			Black-coffee			Instant-coffee		
		≤ 1 Serving/day	> 1 Serving/day	*P*	≤ 1 Serving/day	> 1 Serving/day	*P*	≤ 1 Serving/day	> 1 Serving/day	*P*
Men										
n	475	232	93		70	47		49	17	
Elevated TG	1	0.876 (0.545, 1.408)	0.486 (0.213, 1.108)	0.103	0.400 (0.152, 1.053)	0.348 (0.103, 1.174)	0.021	1.012 (0.443, 2.312)	0 (0, Inf)	0.240
Reduced HDL-C	1	1.032 (0.743, 1.435)	1.071 (0.678, 1.693)	0.751	0.770 (0.448, 1.323)	1.025 (0.551, 1.908)	0.731	1.588 (0.869, 2.901)	1.728 (0.650, 4.594)	0.084
Elevated BP	1	1.004 (0.704, 1.431)	1.182 (0.716, 1.953)	0.593	1.144 (0.638, 2.054)	1.637 (0.793, 3.378)	0.180	0.609 (0.323, 1.148)	0.777 (0.274, 2.208)	0.204
Elevated FBG	1	3.928 (2.726, 5.659)	4.112 (2.537, 6.666)	< 0.001	4.937 (2.863, 8.513)	3.835 (2.009, 7.319)	< 0.001	3.658 (1.925, 6.951)	3.651 (1.329, 10.031)	< 0.001
Women										
n	549	231	139		80	63		57	23	
Elevated TG	1	0.873 (0.543, 1.404)	1.044 (0.610, 1.786)	0.958	1.490 (0.793, 2.799)	0.917 (0.425, 1.980)	0.767	0.753 (0.305, 1.856)	0.603 (0.135, 2.698)	0.380
Reduced HDL-C	1	0.895 (0.652, 1.228)	0.934 (0.636, 1.371)	0.587	0.860 (0.526, 1.406)	1.061 (0.622, 1.812)	0.958	0.922 (0.526, 1.617)	0.886 (0.373, 2.102)	0.704
Elevated BP	1	0.839 (0.601, 1.171)	0.553 (0.372, 0.821)	0.004	0.602 (0.366, 0.992)	0.516 (0.296, 0.898)	0.005	1.134 (0.614, 2.092)	0.276 (0.112, 0.680)	0.037
Elevated FBG	1	3.316 (2.376, 4.627)	3.798 (2.555, 5.647)	< 0.001	2.964 (1.807, 4.863)	3.109 (1.799, 5.371)	< 0.001	3.077 (1.745, 5.425)	3.514 (1.503, 8.218)	< 0.001

[a]P values across increasing categories of coffee consumption were tested by assigning the median of each category and as a continuous variable in the logistic regression model after adjusting for BMI, education level, alcohol status, Physical activity.

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study may be attributed to the ignorance of caffeine dose, and the participants were old, so as to affect the metabolism of caffeine.

In the current study, we found that the protective effect of habitual coffee drinking on BP was significant only in women. In line with this finding, Grosso et al. (41) reported that higher coffee consumption was associated with a decreased risk of hypertension appeared to be significant only in women (41). Actually, numerous epidemiological studies on the influence of coffee or caffeine on the incidence of CVD system have provided controversial and inconsistent findings. A systematic review and meta-analysis of randomized controlled clinical trials indicated that habitual coffee consumption can slightly increases SBP and DBP (42). In this regard, some previous studies reported a negative association between habitual coffee consumption and the risk of CVD (43, 44), while others revealed a positive association (45), or no significant association (46). Another recent meta-analysis revealed that BP elevations tended to be associated only with caffeine but not coffee (47). Thus, these conflicting findings may be due to the different types of brewing coffee, various confounding dietary factors, and the daily consuming amount.

Overall, the caffeine acute effects on BP are well-known, but the mechanism underlying the effect of chronic coffee consumption remains unclear (48). There is experimental evidence that an acute raise in BP due to coffee intake develops increasing tolerance, and intravenous caffeine led to a rise in muscle sympathetic activity and increased BP among both non-habitual and habitual coffee consumers, while coffee dietary consumption led to elevated BP on existed in non-habitual coffee consumers (49). This may be the reason that, compared to non-coffee consumers, habitual coffee consumers are less likely to show an average BP response after coffee intake. Moreover, phenolic, the main compound of coffee, can play a key role in regulating the cellular processes that lead to inflammatory responses (50). Oxidative stress has a great impact on the process that causes metabolism impairment and chronic conditions development, including hypertension (51). In this aspect, women have more antioxidants than men in natural differences (52), this may explain the gender difference in coffee consumption effect. From the point of view of genetics, lifestyle habits (such as drinking or smoking status) or genetics may influence the activity of enzymes so as to affect metabolize caffeine and BP levels. Taking into account all variables mentioned earlier, it may explain the significant protective effect of coffee intake for women but not men.

To the best of our knowledge, the current study is the first to discuss the association between coffee consumption patterns and MetS among middle-aged and older adults in Shenzhen. We assessed individuals' coffee consumption patterns upon 2-day, 24-h recall data, which can relatively obtain accurate information about habitual coffee consumption. Moreover, we estimated both coffee consumption type and daily serving times of each type, so as to provide not only qualitative but also quantitative information regarding coffee consumption patterns. However, several limitations should be noted. First, the causal associations between coffee consumption patterns and MetS could not be confirmed due to the cross-sectional nature. Second, we did not include actual consumption volumes, brewing method, sugar in coffee or other coffee ingredient consumption, total energy intake, and presence of caffeine were not obtained. Third, the study only included healthy residents, it may be potential for residual confounding factors and other lifestyle factors. Multilateral studies considering coffee consumption timing, volumes, frequency, ingredients, and other behavioral factors by gender are needed to address the association of coffee consumption patterns on MetS in a more expanded population.

Conclusion

In conclusion, a significant positive association between coffee consumption patterns and elevated FBG in both men and women was found, whereas consumption was inversely associated with elevated BP only in women. Our findings reinforce the hypothesis on the possible benefits of hypertension for women. Due to methodological limitations, further research prospective studies or well-designed randomized controlled trials are needed to confirm the causal association.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Health Science Centre, Shenzhen University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

RN and ZY conceived the study. RN, HL, LQ, GH, SL, and ZY collected data. RN and GH provided the recruitment resources. RN completed the original draft preparation. ZY reviewed, edited the final draft, and received the funding. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

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The relationship between serum albumin and prostate-specific antigen: A analysis of the National Health and Nutrition Examination Survey, 2003–2010

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Background: Previous studies have shown that serum albumin is associated with prostate cancer (PCa), but not with prostate-specific antigen (PSA) levels in populations without PCa history. Therefore, we analyzed secondary data provided by the National Health and Nutrition Examination Survey (NHANES) (2003–2010).

Methods: In total, 5,469 participants were selected from the NHANES database (2003–2010). Serum albumin and PSA levels were serially considered independent and dependent variables, serially. A number of covariates were included in this study, including demographic, dietary, physical examination, and comorbidity data. Using weighted linear regression model and smooth curve fitting, the linear and non-linear relationship between serum albumin and PSA was investigated.

Results: After modulating underlying interference factors, the weighted multivariate linear regression analysis revealed that serum albumin did not independently predict PSA levels ($\beta = -0.009$ 95%CI: −0.020, 0.002). Nevertheless, a non-linear relationship was found between serum albumin and PSA, with a point of 41 g/L. Left of the inflection point, the effect size, 95%CI, and *P*-value were 0.019 (log2 transformation) (−0.006, 0.043) and 0.1335, respectively. We found a negative association between serum albumin and PSA on the right side of the inflection point, with effect size, 95%CI, and a *P*-value of −0.022 (log2 transformation) (−0.037, −0.007), 0.0036.

Conclusion: In summary, serum albumin and PSA levels are not linearly related. When serum albumin levels exceed 41 g, serum albumin levels are negatively associated with PSA levels.

KEYWORDS

serum albumin, prostate-specific antigen (PSA), NHANES database, non-linear relationship, inflection point

Introduction

Prostate cancer (PCa) remains to be the most diagnosed cancer in men and the second leading cause of cancer-related death worldwide (1). In the United States, the estimated number of new cancer cases and deaths caused by PCa was 268,490 and 34,500 in 2022 (2). Different countries have varying rates of PCa. For example, the incidence rate of Chinese and African American men is 0.8 and 102.1 per 100,000, severally (3). Nevertheless, the rising economic growth, longer life expectancy, and cultural exchange

have significantly contributed to the increase in incidence among the Chinese (4, 5). A growing body of evidence suggests that protein-limitation diets are associated with lower PCa rates and sufficient lycopene intake could be protective against the high risk of PCa in the Non-Hispanic White men (6, 7).

As the global incidence of PCa increases, it is important to improve its early detection and diagnosis to reduce the death rates. For the first time in 1980, Papsidero et al. quantified PSA levels in human blood, signaling the clinical application of PSA, which has since become a common screening tool for PCa today (8–10). Bergengren et al. revealed that using PSA testing resulted in a 15% decrease in prostate cancer deaths from 1996 to 2016 (11) and it is crucial to clarify the factors that affect PSA in order to ensure quality screening and prevent missed diagnoses. According to recent studies, a higher level of folate may reduce PSA levels and sugar consumption is positively and independently linked to PSA levels in adult American males without prostate cancer (12, 13). The dietary protein intake positively correlated with increased PSA levels when it exceeded the threshold of 181.8 g and over 1,151 mg of dietary phosphorus per day may increase PSA levels (14, 15). However, the relationship between PSA levels and serum albumin remains unknown. Therefore, we performed a secondary analysis aimed at investigating the relationship between serum albumin and PSA levels using NHANES data. Furthermore, we assessed whether PSA levels would decrease or increase in response to serum albumin changes.

Methods

Data source

The NHANES, headed by the National Centers for Disease Control (CDC) and Prevention National Health Statistics Center, was a research program projected to assess the health and nutrition of adults and children in America. Research ethics review board approval was granted to the NHANES protocol by National Center for Health Statistics research ethics review board. Informed consent was acquired from all participants. The comprehensive introduction can be download from the CDC official website (www.cdc.gov/nchs/nhanes/tutorials/default.asp). In our study, we collected data from the 2003–2010 NHANES datasets (Including five circles).

Study population

Throughout this study, 41,156 participants initially participated in NHANES between 2003 and 2010. Several elimination screenings were conducted, out of which 5,469 men were eventually enrolled for data analysis. The filter criteria were as follows: (1) Female (n =20,371); (2) Males < 40 years old (n = 7,140); (3) Male tumor patients (n = 6,508); (4) Influencing PSA drugs: Men who used 5ARI or other forms of hormone therapy and drugs (n = 6,174); (5) Affecting PSA factors: Men who had prostatitis or recent prostate operations (i.e., rectal examination within 1 week); surgery, cystoscopy within 1 month; Prostate biopsy (n = 6,049); (6) PSA data was missing (n = 5,479); (7) Albumin data was missing (n = 5,469). Finally, 5,469 research objects were included in the research (the flow chart was comprehensively presented in Figure 1). Informed consent was obtained from all participants before the interview and inspection took place.

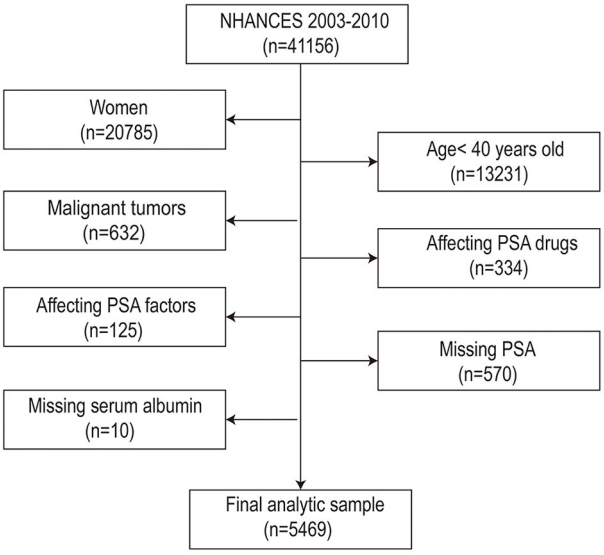

FIGURE 1
Flow chart of procedures from identification of eligible patients to final inclusions.

Variables

The dependent variable was serum albumin (g/L), whereas independent variable was serum PSA (ng/ml). A description of serum albumin and PSA measurement is available on NHANES official website (https://www.cdc.gov/nchs/nhanes). We selected covariates that had been confirmed to be associated with serum albumin and/or PSA levels in previous studies. The covariates were as follows: categorical variables included race, education level, diabetes-history, failing kidney-history, emphysema-history, stroke-history, marital status-history, coronary heart disease-history, hypertension-history and asthma-history. The continuous variables included Age (year), Poverty income ratio, Serum glucose (mmol/L), Body mass index (kg/m^2), Creatinine (umol/L), Aspartate aminotransferase (U/L), Alanine aminotransferase (U/L), Cholesterol (mmol/L), Blood urea nitrogen (mmol/L), Lactic dehydrogenase (U/L), Serum uric acid (umol/L), and Triglycerides (mmol/L). Generally, in the NHANES database, covariates originated from demographic, dietary, examination, laboratory, and questionnaire data, allowing us to comprehensively examine the variables.

Statistical analysis

All estimates took into account NHANES sample weights. Because PSA was skewed, we transformed it using the log2

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TABLE 1 Baseline characteristics of selected participants.

Serum albumin (g/L)	Q1 (19.00–40.00)	Q2 (41.00–42.00)	Q3 (43.00–44.00)	Q4 (45.00–53.00)	*P*-value
Total prostate specific antigen (ng/mL). log2 transform	0.01 ± 1.39	−0.03 ± 1.25	−0.06 ± 1.16	−0.19 ± 1.11	<0.0001
Age	58.63 ± 12.01	55.77 ± 11.28	54.13 ± 10.76	52.55 ± 9.60	<0.0001
Ratio of family income to poverty	2.91 ± 1.56	3.19 ± 1.53	3.41 ± 1.53	3.46 ± 1.50	<0.0001
Race/ethnicity (%)					<0.0001
Mexican American	6.66	7.18	7.28	5.58	
Other Hispanic	3.53	3.43	3.97	2.95	
Non-Hispanic White	68.80	72.76	76.06	79.01	
Non-Hispanic Black	16.27	11.25	8.15	6.11	
Other race—including multi-racial	4.74	5.38	4.54	6.34	
Education level (%)					<0.0001
Less than high school	25.29	20.74	17.07	15.16	
High school	28.03	26.31	23.77	24.56	
More than high school	46.19	52.92	59.16	60.28	
Marital status (%)					0.0002
Married	65.93	70.07	72.35	73.77	
Single	27.96	23.60	22.62	21.41	
Living with partner	5.78	6.34	4.92	4.38	
Serum glucose (mmol/L).	6.28 ± 2.70	5.94 ± 2.24	5.65 ± 1.93	5.48 ± 1.43	<0.0001
Body mass index (kg/m^2)	30.74 ± 7.51	29.64 ± 5.61	28.84 ± 4.96	27.84 ± 4.50	<0.0001
Creatinine (ummol/L)	97.71 ± 61.69	90.01 ± 36.50	89.23 ± 20.42	88.71 ± 22.01	<0.0001
Aspartate aminotransferase (U/L)	28.71 ± 21.27	27.72 ± 14.12	27.33 ± 11.89	28.58 ± 11.65	0.0338
Alanine aminotransferase (U/L)	28.84 ± 29.33	29.67 ± 18.86	29.63 ± 15.23	31.41 ± 17.00	0.0049
Cholesterol (mmol/L)	4.88 ± 1.15	5.17 ± 1.13	5.27 ± 1.03	5.42 ± 1.07	<0.0001
Blood urea nitrogen (mmol/L)	5.36 ± 2.65	5.01 ± 1.87	5.08 ± 1.73	5.04 ± 1.74	<0.0001
Lactic dehydrogenase (U/L)	135.47 ± 32.11	130.76 ± 26.61	129.57 ± 24.38	131.60 ± 25.63	<0.0001
Serum uric acid (umol/L)	365.12 ± 85.96	359.22 ± 74.96	365.96 ± 74.20	362.70 ± 71.69	0.1068
Triglycerides (mmol/L)	1.95 ± 1.91	2.06 ± 1.77	2.01 ± 1.55	2.09 ± 1.65	0.2043
Hypertension history (%)					<0.0001
Yes	46.39	41.00	37.61	33.90	
No	53.36	58.90	62.39	65.72	
Failing kidney history (%)					<0.0001
Yes	4.86	1.99	1.07	1.11	
No	94.77	97.85	98.88	98.83	
Diabetes history (%)					<0.0001
Yes	18.33	13.22	9.52	7.64	
No	78.96	84.65	88.48	89.89	
Borderline	2.64	2.10	1.95	2.45	
Stroke history (%)					<0.0001
Yes	5.61	3.74	2.14	2.14	
No	94.00	96.07	97.56	97.64	

(Continued)

TABLE 1 (Continued)

Serum albumin (g/L)	Q1 (19.00–40.00)	Q2 (41.00–42.00)	Q3 (43.00–44.00)	Q4 (45.00–53.00)	*P*-value
Asthma history (%)					0.0051
Yes	13.10	9.42	9.39	9.18	
No	86.61	90.32	90.28	90.82	
Emphysema history (%)					<0.0001
Yes	4.73	1.82	2.00	1.59	
No	94.78	97.69	97.94	98.41	
Coronary heart disease history (%)					<0.0001
Yes	10.71	6.73	5.88	5.30	
No	88.72	93.06	93.67	93.91	

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.

TABLE 2 Univariate and multivariate analysis by weighted linear regression model.

Exposure	Non-adjusted	Adjust I	Adjust II
Serum albumin (g/L)	−0.023 (−0.034, −0.013) 0.00002	0.006 (−0.004, 0.017) 0.21719	−0.009 (−0.020, 0.002) 0.09643
Serum albumin (g/L)			
Q1	0	0	0
Q2	−0.05 (−0.15, 0.05) 0.3587	0.07 (−0.02, 0.17) 0.1472	0.01 (−0.08, 0.11) 0.7590
Q3	−0.08 (−0.17, 0.02) 0.1130	0.10 (0.01, 0.20) 0.0279	0.01 (−0.09, 0.10) 0.8946
Q4	−0.21 (−0.30, −0.11) <0.0001	0.04 (−0.05, 0.13) 0.4004	−0.08 (−0.18, 0.01) 0.0780
P for trend	<0.001	0.489	0.226

Non-adjusted model adjust for: None.
Adjust I model adjust for: Age; Race/Hispanic; Education Level; Marital status; Poverty income ratio.
Adjust II model adjust for: Race, Education level, Diabetes history, Failing kidney history, Emphysema history, Stroke history, Marital status history, Hypertension history, Asthma history, Coronary heart disease history; Age (year), Poverty income ratio (PIR), Serum glucose (mmol/L), Body mass index (kg/m^2), Creatinine (umol/L), Aspartate aminotransferase (U/L), Alanine aminotransferase (U/L), Cholesterol (mmol/L), Blood urea nitrogen (mmol/L), Lactic dehydrogenase (U/L), Serum uric acid (umol/L), Triglycerides (mmol/L).

function. Our statistical analyses included three steps. First, four groups were established based on serum albumin levels (quartiles). Frequencies or percentages were used to express categorical variables, whereas means ± standard deviations were applied to express continuous variables. Secondly, the weighted univariate and multivariate linear regression model was employed: model I was run without adjusting for covariates; model II was run with only socio-demographic data adjusted; and model III was run with model 2 plus other covariates as shown in Table 1. As a third step in the data analysis, smooth curve fitting was used to examine the non-linearity between serum albumin and PSA levels. Additionally, the threshold effect of serum albumin and PSA levels was examined using two-piecewise linear regression models. Statistical software R (http://www.r-project.org, The R Foundation) and EmpowerStats (http://www.empower-stats.com, X&Y Solutions, Inc., Boston, MA) were used to perform the analysis. Statistical significance was defined as a p-value < 0.05 (two-sided).

Results

Baseline characteristics of participants

Table 1 shows the baseline characteristics of participants chosen from NHANES 2003 to 2010 by quartile of serum albumin. Variates including aspartate aminotransferase, uric acid, and triglycerides had no statistically significant differences between different groups. Participants with lower serum albumin were older and had lower cholesterol, poverty-to-income ratio, alanine aminotransferase, higher serum glucose, body mass index, and creatinine compared with the Q4 group. Moreover, participants with decreased serum albumin were more likely to have hypertension, failing kidneys, diabetes, stroke, asthma, emphysema, and coronary heart diseases when compared to the Q4 group. Most of the participants were Non-Hispanic White with a degree above that of high education.

Univariate and multivariate analysis

Table 2 shows the univariate and multivariate linear regression results. According to the non-adjusted model, the PSA levels decreased with serum albumin levels, decreasing −0.023 (−0.034, −0.013). The relationship between serum albumin and PSA levels was not significant after adjusting for demographic variables (minimally-adjusted) ($p = 0.21719$) and adjusting for all covariates in Table 2 ($p = 0.09643$).

To investigate whether there is a non-linear relationship between serum albumin and PSA, we translated serum albumin into categorical variables by quartile, and trend estimation for

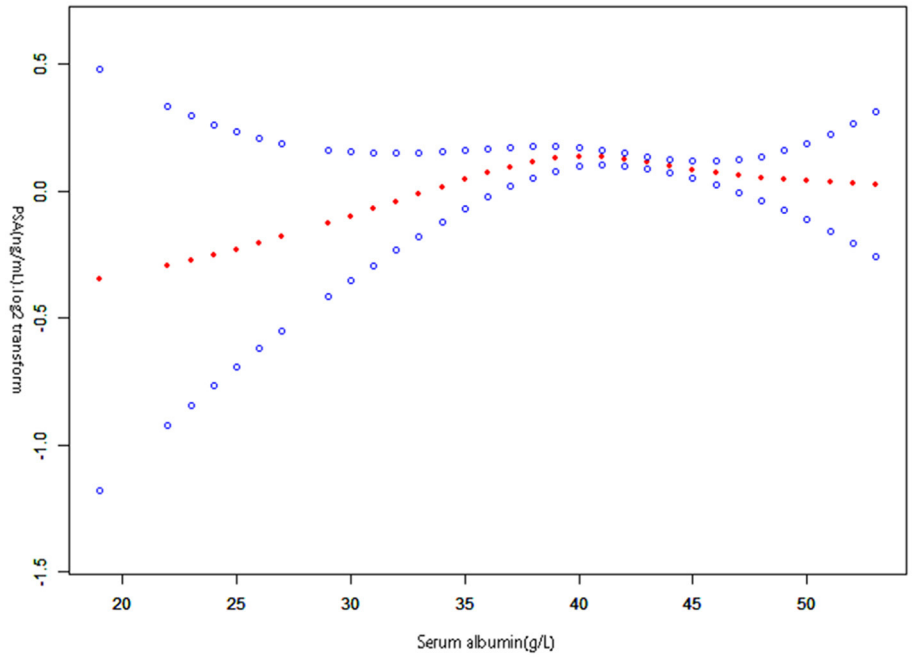

FIGURE 2
The relationship between serum albumin and prostate-specific antigen. A non-linear relationship between them was detected after adjusting for Race, Education level, Diabetes history, Failing kidney history, Emphysema history, Stroke history, Marital status history, Hypertension history, Asthma history, Coronary heart disease history; Age (year), Poverty income ratio (PIR), Serum glucose (mmol/L), Body mass index (kg/m^2), Creatinine (umol/L), Aspartate aminotransferase (U/L), Alanine aminotransferase (U/L), Cholesterol (mmol/L), Blood urea nitrogen (mmol/L), Lactic dehydrogenase (U/L), Serum uric acid (umol/L), Triglycerides (mmol/L).

TABLE 3 Non-linearity addressing by weighted two-piecewise linear model.

Outcome	PSA(ng/ml) log2 transform β (95% CI)
Fitting by weighted linear regression model	−0.009 (−0.020, 0.002) 0.0964
Fitting by weighted two-piecewise linear regression model	
Inflection point	41
<41	0.019 (−0.006, 0.043) 0.1335
>41	−0.022 (−0.037, −0.007) 0.0036
Log likelihood ratio test	0.013

PSA, prostate-specific antigen; Independent variable is serum albumin(g/L) and dependent variable is PSA (ng/mL log2 transform).
Covariates involved in this model was the same as Adjust II model presented in Table 2.

serum albumin was performed in a sensitivity analysis (Table 2). Consequently, we found that the trend of effect values was non-isometric among the serum albumin groups. These findings indicate the possibility of non-linearity in serum albumin and PSA levels.

Identification of non-linear relationship

This study examined the non-linear relationship between serum albumin and PSA (Figure 2). Using the smooth curve fitting, we found a non-linear relationship between serum albumin and PSA. We compared a linear regression model with a two-piecewise linear regression model and found P equal to 0.013 for the log-likelihood ratio test. This implies that the two-piecewise linear regression model should be applied to the model. By a two-piecewise linear regression model and recursive algorithm, the inflection point was calculated as 41 g /L (Table 3). Left of the inflection point, the effect size, 95%CI, and P-value were 0.019 (log2 transformation) (−0.006, 0.043) and 0.1335, respectively. A negative association was noted between serum albumin and PSA on the right side of the inflection point, with effect size, 95%CI, and a P-value of −0.022 (log2 transformation) (−0.037, −0.007), 0.0036. Serum albumin and PSA levels demonstrated a somewhat U-shaped relationship with a serum albumin threshold of 41 g/L. These results indicate that serum albumin and PSA levels had a threshold effect.

Discussion

The work investigated the relationship between serum albumin and PSA in a non-PCa population aged over 40 years in the United States. Despite the lack of a linear relationship, our findings revealed that serum albumin negatively correlated with PSA levels when it exceeded 41 g/L.

Several studies have reported relationships between serum albumin and PCa. For instance, Takehiro Sejima et al. discovered that low levels of serum albumin preoperatively may indicate extensive disease in clinically localized prostate cancer and may be linked to biochemical disease recurrence. A low serum albumin

level was thought to induce a decrease in albumin-binding testosterone, increasing free testosterone, which was thought to be important for hormone-sensitive PCa (16). Elsewhere, Wang et al. discovered that an increased fibrinogen level and decreased albumin level might contribute to cancer progression and poor outcomes (17). Low albumin levels were indicative of malnutrition and inflammation within the body. There was a high prevalence of malnutrition among cancer patients, with many negative effects including impaired immune function, lessened response to the treatment of cancer, and shorter survival time (18, 19). In inflammatory responses to the tumor or from the tumor itself, several inflammatory mediators were discharged, containing interleukin-1(IL-1), IL-6, necrosis factor, and acute phase reactants, which might promote albumin escape from capillaries and modulate the production of albumin in the liver (20, 21). Consequently, the albumin level may be an indicator of cancer prognosis.

In the US, PSA screening for early prostate cancer detection has become popular over the past 25 years (22). Early detection by PSA testing and appropriate treatment reduces the risk of death for men with aggressive prostate cancer (23). Nonetheless, PSA levels are affected by numerous factors, which have received significant research attention. Wang et al. noted that men with higher liver fibrosis scores had lower serum PSA. Liver dysfunction on a chronic basis can reduce testosterone levels, causing reduced PSA production (24). Song et al. investigated the correlation between PSA and dietary protein intake and found a non-linear correlation. A positive association was observed between dietary protein intake and elevated PSA levels as dietary protein intake exceeded 181.8 g (14). IGF-1 might be the important factor in the relationship between dietary protein intake and PSA concentrations by modifying IGF-1 levels and inhibiting PI3K/AKT/mTOR pathways. Additionally, dietary protein might decrease the sensitivity of insulin and boost the growth of prostate cancer cells in animal models, which in turn affects PSA levels (6, 25, 26). However, the relationship between serum albumin and PSA remains unknown; thus this secondary analysis based on the NHANES data aims to explore it. In our research, we found a non-linear relationship between serum albumin and PSA levels. Moreover, a negative correlation was present between serum albumin and PSA when serum albumin exceeded 41 g/L. The possible mechanism is that increased serum albumin level increased albumin-binding testosterone, resulting in a decrease in free testosterone, which was converted to dihydrotestosterone and regulated prostate development (16). Further studies are necessary to investigate the possible relevant mechanisms.

This study has a number of strengths. First, it has a large sample size, i.e., 5,469 participants, which provides a high level of statistical power to quantify the relationship between PSA and serum albumin. Secondly, we performed both linear and non-linear regression models to increase comparability, and the results indicated a possible non-linear relationship. Finally, a recursive algorithm was used to determine the inflection point, and a two-piecewise linear regression was used to determine the saturation effect of serum albumin and PSA.

On the other hand, this work also has limitations. First, we could not obtain a causal link between PSA and serum albumin due to its cross-sectional design. Secondly, the findings of this work are generally limited since it only focused on the American population. Thirdly, being a secondary analysis of published data, variables not contained in the dataset cannot be regulated.

Conclusion

In conclusion, serum albumin and PSA have a non-linear relationship. Serum albumin negatively correlates with PSA levels when serum albumin exceeds 41 g/L. However, our findings should be validated through a methodologically robust and large prospective clinical trial.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

NHANES protocol was approved by the NCHS Research Ethics Review Board. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

KX, CC, and JZh conceived and designed the manuscript, performed statistical analysis, and had primary responsibility for final content. JZe and YL wrote and revised the paper and re-analysis the data for the revised version. ZC drafted the manuscript. KX and SL constructed and cleared data. All authors read and approved the final manuscript.

Conflict of interest

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

Publisher's note

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Low carbohydrate intake correlates with trends of insulin resistance and metabolic acidosis in healthy lean individuals

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Introduction: Both obesity and a poor diet are considered major risk factors for triggering insulin resistance syndrome (IRS) and the development of type 2 diabetes mellitus (T2DM). Owing to the impact of low-carbohydrate diets, such as the keto diet and the Atkins diet, on weight loss in individuals with obesity, these diets have become an effective strategy for a healthy lifestyle. However, the impact of the ketogenic diet on IRS in healthy individuals of a normal weight has been less well researched. This study presents a cross-sectional observational study that aimed to investigate the effect of low carbohydrate intake in healthy individuals of a normal weight with regard to glucose homeostasis, inflammatory, and metabolic parameters.

Methods: The study included 120 participants who were healthy, had a normal weight (BMI 25 kg/m^2), and had no history of a major medical condition. Self-reported dietary intake and objective physical activity measured by accelerometry were tracked for 7 days. The participants were divided into three groups according to their dietary intake of carbohydrates: the low-carbohydrate (LC) group (those consuming <45% of their daily energy intake from carbohydrates), the recommended range of carbohydrate (RC) group (those consuming 45–65% of their daily energy intake from carbohydrates), and the high-carbohydrate (HC) group (those consuming more than 65% of their daily energy intake from carbohydrates). Blood samples were collected for the analysis of metabolic markers. HOMA of insulin resistance (HOMA-IR) and HOMA of β-cell function (HOMA-β), as well as C-peptide levels, were used for the evaluation of glucose homeostasis.

Results: Low carbohydrate intake (<45% of total energy) was found to significantly correlate with dysregulated glucose homeostasis as measured by elevations in HOMA-IR, HOMA-β% assessment, and C-peptide levels. Low carbohydrate intake was also found to be coupled with lower serum bicarbonate and serum albumin levels, with an increased anion gap indicating metabolic acidosis. The elevation in C-peptide under low carbohydrate intake was found to be positively correlated with the secretion of IRS-related inflammatory markers, including FGF2, IP-10, IL-6, IL-17A, and MDC, but negatively correlated with IL-3.

Discussion: Overall, the findings of the study showed that, for the first time, low-carbohydrate intake in healthy individuals of a normal weight

might lead to dysfunctional glucose homeostasis, increased metabolic acidosis, and the possibility of triggering inflammation by C-peptide elevation in plasma.

KEYWORDS

HOMA-IR, C-peptide, low carbohydrate, insulin resistance, anion gap, inflammation

1. Introduction

Insulin resistance syndrome (IRS) is a modern-day epidemic. With the increase in research endeavors and on the focus on IRS, it has become evident that IRS can drive the disease pathogenesis of several clinical syndromes, such as T2DM and cardiovascular diseases (1, 2). Interestingly, while IRS is often regarded as the primary underlying mechanism for T2DM, several reports from sub-Saharan Africa and South Asian populations indicate that pancreatic beta-cell secretory dysfunction is the driving factor of the lean T2DM phenotype (3, 4). The current recommended dietary guidelines for treating obesity and obesity-related complications revolve around reducing daily energy intake, improving portion control, and improving the quality of the diet to achieve a calorie deficit status (5). However, over the past decade, further research has unraveled the benefits of redirecting the weight loss strategy toward readjusting levels of macronutrients, such as consuming fewer carbohydrates and a larger quantity of proteins in daily meals (5–7). The three macronutrients found in food include carbohydrates (4 kcal/g), proteins (4 kcal/g), and fat (9 kcal/g). A daily intake of <10% or 20–50 g of carbohydrates is considered a very low carbohydrate intake, <26% or <130 g is considered a low carbohydrate intake, 26–44% is considered a moderate carbohydrate intake, and ≥45% is regarded as a high carbohydrate intake (8). There are more than a dozen types of low-carbohydrate diets, of which the ketogenic or keto, Atkins, and paleo diets are relatively more widely known. Keto diets are characterized by reduced carbohydrate content (<50 g per day) and relatively increased fat and protein content. Keto diets are further categorized as follows: (i) standard keto diet (SKD), which contains very low carbohydrate (10%), moderate protein (20%), and high fat (70%) content; (ii) cyclical keto diet (CKD), which involves periods of high-carbohydrate diet in between keto diets, e.g., 5 keto days followed by two high-carbohydrate days as a dietary cycle; (iii) targeted keto diet (TKD), which allows for adding additional carbohydrates around periods of intensive physical workout; and (iv) high-protein keto diet (HPKD), which has a relatively high-protein content (35%) with a low carbohydrate content (5%) but still a high fat (60%) content (9).

With carbohydrates being the macronutrient with the greatest impact on postprandial blood glucose response, low-carbohydrate diets, such as the Atkins and keto diets, have become an effective strategy for weight loss (10, 11). We assumed that, during low carbohydrate intake, the body undergoes ketogenesis, a process that switches the utilization of glucose from the carbohydrate as an energy source to the use of ketone bodies in the mitochondria for ATP synthesis, shifting the metabolic process to the ketosis-favoring pathways (12). This metabolic shift following low-carbohydrate dietary interventions is the cornerstone of weight loss mechanisms. Nevertheless, the use of a low-carbohydrate diet in healthy individuals of a normal weight and in children has been associated with unwanted diet-induced ketoacidosis (13–15). The influence of a low-carbohydrate diet and the activation of the ketogenesis pathway in individuals with obesity, especially those with metabolic complications, such as T2DM, have been proven to be quite effective in reducing body weight (11); however, the significance and adaptation of this lifestyle in individuals of a normal weight in the absence of a family history of diabetes or in those with no history of a major ailment remain unclear. Therefore, the present study aimed to investigate the effect of low carbohydrate intake in healthy, normal weight individuals with regard to glucose homeostasis and inflammatory and metabolic parameters. Herein, we identified that low carbohydrate intake in healthy individuals of a normal weight correlates with dysfunctional glucose homeostasis, increased metabolic acidosis, and the risk of inflammation suggested to be triggered by the elevation in plasma C-peptide levels.

2. Materials and methods

2.1. Anthropometric, clinical, and dietary characteristics of the study participants

This is a cross-sectional observational study that involved healthy men and women of a normal weight aged 21–65 years. Data were collected between January 2016 and December 2019 and were processed at the Dasman Diabetes Institute, Kuwait. The sample size was determined using the ClinCalc tool software (www.clincalc.com). The incident rate of T2DM onset in individuals of a normal weight is estimated to be 7.7–21% worldwide. Considering 21% as the guiding reference, we achieved a statistical power (1-β) of 95% and a level of significance (α) of 0.05, which yielded the minimum sample size required for this study as 52 individuals. Taking into account a fair margin for possible dropouts, we thus aimed to recruit 100 participants. A total of 138 adult (>18 years) Kuwaiti individuals were reached out to randomly by word of mouth, through flyers, or through social media contacts and were invited to participate in the study. Out of these 138 participants, 120 of them (57 men and 63 women) with a mean age of 31.9 ± 5.7 years and BMI of ≤25 kg/m^2, were found to be eligible for the final analysis. The study was conducted in accordance with the Helsinki Declaration and the institutional review board ethics of the Kuwait Ministry of Health (MOH) Ethics Board (2017/542) (16). Each participant was required to complete a full-length health screening questionnaire that tracked their past and current health status and history. The health screening questionnaire also asked participants about the

health of their immediate family members and inquired about any family-related disease problems. The exclusion criteria were as follows: patients who were physically diagnosed with diabetes, hypertension (>160/90 mmHg), and anti-hypertensive therapy, those with a previous history of established coronary heart disease, e.g., myocardial infarction, coronary artery bypass graft surgery, coronary angioplasty, or a family history of diabetes or early cardiac death (<40 years), those with a history of cancer within the past 2 years, those diagnosed with depression, and those under medications that could influence body weight due to effects on the lipid or carbohydrate metabolism, as well as those who were pregnant or lactating women. A flow chart of participant recruitment is summarized in Supplementary Figure 1A. None of the participants had physical disabilities that would prevent or severely limit physical mobility or physical activity. The characteristics of male and female participants are summarized below in Supplementary Table 1. The presented study follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations (17).

2.2. Physical evaluations

In the physical activity laboratory, a standard protocol was used to carry out all anthropometric assessments for all participants wearing tight-fitting clothes and using the same equipment throughout the study. Measurements were made to the nearest 0.1 unit. Height (cm) was taken by instructing the volunteer to stand with their feet together and back and heels against the upright bar of the height scale. The volunteer was asked to position their head upright against the backboard. The volunteer was requested to take a deep breath as the investigator applied gentle, upward pressure under the angle of the mandible. Other investigators slid down the horizontal bar attached to the scale so that it rested snugly on the examinee's head, and measurements were taken. Body weight (kg) was measured using a beam balance, and BMI was calculated as follows: BMI = weight (kg)/height (m^2). Waist and hip circumferences (cm) were measured in duplicate using non-elastic tape. Waist circumference was measured at the minimum circumference horizontally between the iliac crest and the rib cage, while hip circumference was measured at the maximum protuberance of the buttocks, and the waist-to-hip ratio was calculated. The same investigator performed these measurements for all volunteers on every occasion. Whole body composition, including body fat percentage, soft lean mass, and total body water, were measured using an IOI 353 Body Composition Analyzer (Jawon Medical, South Korea).

2.3. Physical activity measurements

All participants in this study were given an electronic accelerometer (ActiGraph GT3X; ActiGraph LLC, Pensacola, FL, USA) to measure daily physical activity (PA) levels. Subjects were advised to maintain their normal daily habitual PA levels during the study period. The accelerometers were attached to an elasticized belt and worn on the right hip for 7 consecutive days (except when bathing and during water activity). The accelerometer provided PA measurements that included activity counts, vector magnitude, energy expenditure, step counts, PA intensity levels, and metabolic equivalents of tasks (METs). A 1-min epoch was used in this study with activity counts assessed at 1-min intervals to ensure that the data quality for the participants included at least 4 days in which the accelerometer was worn for at least 60% of the time of the day. A non-wear time was taken as any block of time ≥60 min wherein the activity count was equal to zero (18). Individuals that did not meet those criteria were excluded from the study, and their collected data were removed from the data pool.

Freedson's cutoffs (14) were used to differentiate between PA intensity levels, including light-intensity activity (100–1,951 counts/min), moderate-intensity activity (1,952–5,724 counts/min), and high-intensity activity (>5,725 counts/min). All counts ≤99 counts/min were considered sedentary. The data were also expressed as the mean intensity for each activity during the monitoring time (total accelerometer counts per total monitoring time).

2.4. Measurement of metabolic and inflammatory markers

Volunteers were asked for a second visit after an overnight fast of at least 10 h. Blood pressure and heart rate were measured for each participant using a semiautomatic Omron portable monitor. In brief, the cuff was placed on the upper arm to ensure uniform compression of the brachial artery, and three consecutive readings were collected (19). Blood samples were collected in 10 mL Ethylenediaminetetraacetic acid (EDTA) tubes (BD Vacutainer system, Plymouth, UK). Plasma was separated and frozen immediately at−80°C for further analysis. Total blood glucose, fasting plasma insulin, cholesterol, HDL-cholesterol, and triglycerides were determined by biochemical analysis using a single assay upon the completion of the sampling (refer to Supplementary Table 2 for information regarding normal ranges). Quality control sera were used to monitor the accuracy and precision of the assays.

Quantitative insulin sensitivity indices, HOMA-IR and HOMA-β, were calculated as follows:

$$\begin{aligned}\text{HOMA}-\text{IR} &= \text{fasting insulin}\ (\mu\text{U/L}) \times \text{fasting glucose}\ (mmol/L)/22.5\\ \text{HOMA}-\text{beta}-\text{cell function}(\text{HOMA}-\beta)\% &= 360\times \text{fasting insulin}\ (\mu\text{U/mL})\\ &/(\text{fasting glucose}\ (\text{mg/dL})\ -\ 63)\end{aligned}$$

The anion gap was calculated according to the following equation:

$$\begin{aligned}\text{Anion gap} &= \text{serum sodium (mmol/l)}\\ &- \text{[serum chloride (mmol/l)}\\ &+ \text{serum bicarbonate (mmol/l)]}\\ \text{Albumin corrected anion gap} &= \text{anion gap} + [2.5 \times (4\\ &- albumin,\ g/dL)]\end{aligned}$$

2.5. Dietary monitoring and analysis

All participants were given food diaries and were instructed to weigh and record their daily intake of food and drinks on electronic scales for the length of the study (7 days) (Salter Housewares, Kent, United Kingdom). A visual demonstration of how to use scales and diaries was given to each individual prior to the start of the study. All individuals were advised to maintain their normal dietary intake. Diaries were completed prior to the second visit. Food diary data were analyzed using CompEat pro (Nutrition systems, Banbury, United Kingdom), and an average of the daily nutrient intake was calculated. According to the international health guidelines established by the Food and Nutrition Board of the National Academies of Sciences, Engineering, and Medicine for the Recommended Dietary Allowance (RDA) for carbohydrates, the recommended daily energy intake from carbohydrates is set between 45 and 65% of daily calorie intake since this amount has been linked to a lower risk of chronic illnesses (15, 20). Based on these criteria, study participants were divided into three groups as follows: the low-carbohydrate (LC) group (those consuming <45% of daily energy intake from carbohydrates), the recommended range of carbohydrate (RC) group (those consuming 45–65% of daily energy intake from carbohydrates), and the high-carbohydrate (HC) group (those consuming higher than 65% of daily energy intake from carbohydrates).

2.6. Enzyme-linked immunosorbent assay

Commercially available ELISA kits were used for the detection of plasma levels of fasting insulin and C-peptide (Mercodia, Uppsala, Sweden), following instructions from the manufacturers.

2.7. Determination of plasma cytokines/chemokines

A total of 41 cytokines and chemokines were measured using the MILLIPLEX MAP Human Cytokine/Chemokine panel with Magnetic Bead Panel-Premixed 41 Plex-Immunology Multiplex Assay (Milliplex map kit, HCYTMAG-60 K-PX41; Millipore, USA), following the manufacturer's instructions. Data from the reactions were acquired by Luminex using a MILLIPLEX analyzer, while a digital processor managed the data output. MILLIPLEX Analyst software was used to determine the mean fluorescence intensity (MFI) and analyte concentration (pmol/mL).

2.8. Statistical analysis

Data were analyzed using SPSS version 25 (SPSS, Inc., Chicago, IL) and GraphPad Prism 7.01 (version 6.05; San Diego, CA, USA) and expressed as the mean ± standard deviation (SD). The data were tested for normality using the Shapiro–Wilk normality test. For comparing the means between two groups, two-tailed *t*-tests and Wilcoxon–Mann–Whitney U tests were used to assess the differences between means of parametric and non-parametric data, respectively. For comparing the means between the three groups, a one-way ANOVA and exact Kruskal–Wallis tests were used when comparing the differences between the means of parametric and non-parametric data, respectively. Multiple linear regression analysis was conducted to examine the correlation between the calculated anion gap and blood electrolyte levels that were found to be associated with LC intake. Exact chi-squared tests of independence were performed to evaluate differences in immune–metabolic parameters. The correlation between energy intake from carbohydrates and immune–metabolic parameters was evaluated with Spearman's correlation coefficients. All p-values of ≤ 0.05 were considered statistically significant.

3. Results

3.1. Participants' characteristics

A total of 138 people were invited to participate in the ActiGraph track assessments. Following a comprehensive health screening, only 134 individuals were found to meet the inclusion health criteria. Of those 138 individuals, only 120 of them (57 men and 63 women) had sufficient data from the ActiGraph that included at least 4 days in which the accelerometer was worn for at least 60% of the time. The general characteristics of the study participants and dietary and energy intake data are summarized in Supplementary Table 3. Based on the WHO chart for age and sex, all participants were within the normal range of BMI, with an average BMI of 22.7 ± 2.4 kg/m^2. In our study, 52.5% of participants were women, and the mean age of all participants was 32.2 ± 5.7. The mean systolic and diastolic blood pressure measurements were normal (109.9 ± 11.3 and 67.15 ± 9.8, respectively), and the average heart rate per min (HR) was 71 ± 10. The study participants had normal levels of serum triglycerides (TG) (0.87 ± 0.38 mmol/L), total cholesterol (4.6 ± 0.8 mmol/L), and HDL-C (1.49 ± 0.34 mmol/l). All participants also showed normal glucose homeostasis, with an average fasting glucose of 4.9 ± 0.64 mmol/L and a serum insulin level of 3.7 ± 2.11 U/ml. All individuals were within the normal range of HOMA-β (%) (74.8 ± 58.9) and HOMA-IR (<2.5) indices. The participants also had normal fasting blood C-peptide levels (1.4 ± 0.37 ng/mL). The average total calorie intake per day for all participants was 2143.8 ± 571.9 kcal, with most energy consumed from carbohydrates at 49.5 ± 12.5% of daily energy.

To explore the contribution of the level of carbohydrate energy intake on the general health of the participants, we decided to pool both the male and female data. Based on the international health guidelines of daily RDA, study participants were divided into three groups as follows: the low-carbohydrate (LC) group (those consuming <45% of daily energy intake from carbohydrates), the recommended range of the carbohydrate (RC) group (those consuming 45–65% of daily energy intake from carbohydrate); and the high-carbohydrate (HC) group (those consuming higher than 65% of daily energy intake from carbohydrate). Group characteristics are summarized in Table 1, which shows significant cross-group differences with regard to lean weight (LC vs. RC and HC), HOMA-beta-cell function (HOMA-β%) (LC vs. RC), fasting glucose, insulin, C-peptide, and the homeostasis model assessment

TABLE 1 Differences between groups based on daily calorie intake (%) from carbohydrates.

Physical characteristics of subjects	<45% (LC)		45–65% (RC)		>65% (HC)		*p*-value
	(*n* = 38) 20 M/18 F	Median-IQR	(*n* = 62) 30 M/32 F	Median-IQR	(*n* = 20) 7 M/ 13 F	Median-IQR	
Age (years)	32 ± 4		31 ± 4		33 ± 6		0.551
Weight (kg)	64.1 ± 10.1		66.1 ± 12.8		63.0 ± 13.7		0.538
Height (cm)	167.8 ± 11.4		170.1 ± 11.6		163.4 ± 12.5		0.090
BMI (kg/m^2)	22.7 ± 2.9	22.9–43	22.9 ± 2.4	22.6–2.9	23.2 ± 2.6	22.4–3.2	0.796
Waist circumference (cm)	75.9 ± 9.1		80.5 ± 7.8		79.5 ± 13.7		0.079
Hip circumference (cm)	105.1 ± 30.7	39.2–4.6	100.5 ± 10.1	39.7–5.2	100.5 ± 9.6	40–3.2	0.483
Fat weight (kg)	20.3 ± 11.2		23.4 ± 12.1		27.8 ± 11.6		0.074
Lean weight (kg)	49.5 ± 11.6	51–23.1	43.4 ± 11.4	45.2–10.5	42.2 ± 9.3	43.1–13	**0.0075**
Fat %	21.3 ± 10.3		25.9± 10.0		26.8 ± 7.3		0.092
Total calorie intake (Kcal)	2104.7 ± 581		2124 ± 588		2277 ± 504		0.514
BP/systolic (mmHg)	110.6 ± 11.7	110–14	109.3 ± 11.1	108.5–14	110.9 ± 11.6	110–10.5	0.800
BP/diastolic (mmHg)	67.7 ± 10.4	70–18.2	66.5 ± 10.2	68–16.2	67.8 ± 7.1	67–4	0.810
Heart rate	70 ± 11	71–20.5	70 ± 10	73–15	74 ± 9	76–12.1	0.381
Fasting glucose (mmol/l)	5.2 ± 0.7	5.1–0.86	4.7 ± 0.5	4.7–0.60	5.5 ± 0.5	5.2–0.85	**0.0008**
Triglycerides (mmol/l)	0.8 ± 0.3	0.9–0.43	0.8 ± 0.4	0.7–0.48	0.8 ± 0.3	0.9–0.47	0.602
Total cholesterol (mmol/l)	4.5 ± 0.7	4.5–0.86	4.6 ± 0.7	4.5–0.77	4.9 ± 1.0	4.83–0.96	0.469
HDL cholesterol (mmol/l)	1.5 ± 0.32	1.3–0.53	1.45 ± 0.37	1.3–0.63	1.6 ± 0.3	1.5–0.54	0.064
Insulin Con. (mU/l)	4.5 ± 1.4	4.4–2.0	3.2 ± 2.3	2.7–4.1	4.6 ± 2.2	5.0–3.0	**0.001**
HOMA-IR	1.0 ± 0.37	1.0–0.42	0.70 ± 0.47	0.6–0.7	1.0± 0.55	1.0–0.57	**0.013**
HOMA-β	56.3 ± 30.3	52.2–42.9	80.5 ± 59.4	45.7–68.8	58.9 ± 37.0	51.8–56.8	**0.016**
C-Peptide (pg/ml)	1.5 ± 0.36	1.4–0.64	1.3 ± 0.34	1.3–0.37	1.56 ± 0.36	1.6–0.62	**0.019**

Results are presented as mean ± SD. BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance. The bold values indicate significant values with $p < 0.05$ for easier identification.

of insulin resistance (HOMA-IR) index (LC vs. RC and RC vs. HC). Concurrently, no significant differences were found regarding anthropometric characteristics, lipid profile, and total calorie intake per day.

We also found no significant differences in the level of objectively measured physical activity, as indicated in Table 2. However, individuals consuming RC were found to have significantly lower HOMA-IR than those consuming LC ($p \leq 0.05$) and HC ($p \leq 0.05$) and significantly higher HOMA-β (%) than those consuming LC ($p \leq 0.05$) (Figures 1A, B). It was also observed that participants consuming RC had significantly lower C-peptides in their serum than in the LC and HC groups. However, only LC was found to be significantly higher than RC ($p < 0.05$) (Figure 1C).

To further investigate these findings, a Spearman correlation test was performed to determine the correlation between carbohydrate energy % and surrogate markers of insulin resistance, β-cell function, and insulin secretion [HOMA-IR, HOMA-β (%), and c-peptide levels, respectively]. No significant correlation was found between HOMA-IR and carbohydrate energy % across all groups (Figure 2A). However, unlike the RC and HC groups, a clear trend of negative HOMA-IR associated with carbohydrate energy % was observed in the LC group. Interestingly, fasting serum C-peptide levels in the LC group had a significant negative correlation ($p \leq 0.05$) with carbohydrate energy %, while C-peptide levels in the RC and HC groups tended to have a direct correlation with carbohydrate energy % (Figure 2B). In terms of β-cell function assessment, the HOMA-β% index was associated positively with levels of carbohydrate energy % in both LC and RC groups, whereas HOMA-β% tended to have a negative correlation with carbohydrate energy % in the HC group (Figure 2C). On the whole, these data clearly indicate that low carbohydrate intake might be correlated with insulin resistance and that the consumption of 45–65% of energy intake from carbohydrates is important to maintain normal glucose hemostasis in individuals of a normal weight.

3.2. Association of the percentage of energy intake from dietary carbohydrate with the serum anion gap marker for metabolic acidosis

Because of the role of ketone bodies in causing acid-base disruption, measuring plasma electrolytes and calculating anion gap became standard clinical practice for the evaluation of

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TABLE 2 Comparison of physical activity levels across groups.

Physical activity level	>45% (LC)		45–65% (RC)		<65% (HC)		*p*-value
	(*n* = 38) 20 M/18 F	Median-IQR	(*n* = 62) 30 M/32 F	Median-IQR	(*n* = 20) 7 M/ 13 F	Median-IQR	
Overall activity (%)	29.2 ± 6.2	70–15.5	30.3 ± 7.5	69.1–7.3	32.4 ± 6.3	66.9–11.4	0.244
Light intensity (%)	22.5 ± 4.5	22.5–6.0	23.9 ± 5.2	23.1–6.4	25.4 ± 5.8	25.6–9.8	0.107
Moderate intensity (%)	5.6 ± 2.3	5.3–2.8	5.9 ± 2.4	5.3–3.0	5.7 ± 1.2	5.48–1.9	0.807
Vigorous intensity (%)	1.0 ± 0.9	0.7–0.96	0.8 ± 0.7	0.6–0.5	1.1 ± 0.6	0.9–1.0	0.372
Average MET rate/day	1.5 ± 0.1	1.5–0.18	1.5 ± 0.2	1.5–0.13	1.5± 0.1	1.6–0.17	0.350
Average step count/day	9,541 ± 3,608	9087–6693.5	1,1781 ± 17,031	9,207–5573.5	10,134 ± 2,174	10,867–4,009	0.666

Results are presented as mean ± SD, MET, metabolic equivalent of task.

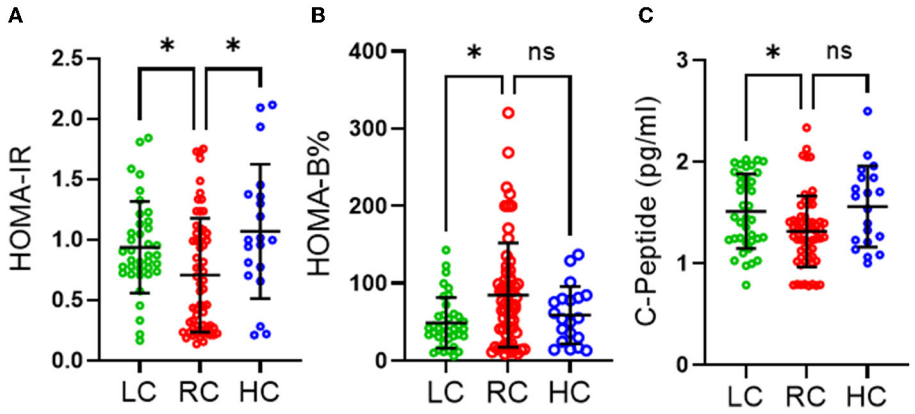

FIGURE 1
Effects of different levels of carbohydrate intake on glucose homeostasis. **(A)** HOMA-IR homeostasis model assessment of insulin resistance, **(B)** HOMA-beta homeostasis model assessment of β-cell function **(C)** C-peptide section. Data are expressed as mean ± SD. Data analyzed by exact Kruskal-Wallis test. ns, non-significant, *$P \leq 0.05$. Low carbohydrate (LC) (those consuming <45% of daily energy percentage) represented in green, recommended range of carbohydrate (RC) (those consuming 45–65% of daily energy percentage) represented in red and high carbohydrate (HC) (those consuming higher than 65% of daily percentage) represented in blue.

metabolic acidosis. Similar to other metabolic blood markers, the mean values of serum bicarbonate (24 ± 1.7 mmol/l), serum albumin (64.3 ± 2.8), serum sodium (Na) (136.5 ± 3.9 mmol/l), serum chloride (Cl) (98.3 ± 2.9), and calculated anion gap (10.5 ± 3.2) were all within ranges considered normal. However, the LC group displayed significantly lower serum bicarbonate and serum albumin levels (Figures 3A, B) than the RC group. The HC group was also found to have significantly upregulated serum sodium levels compared to the RC group only (Figure 3C), while no significance was found in the level of serum chloride across all groups (Figure 3D). Both disturbances of serum bicarbonate and albumin are considered signs of metabolic acidosis. Indeed, the calculated anion gap further reflected a significant upregulation in metabolic acidosis in the LC group compared to the RC group, with trends of upregulation in the HC group being found to not be significant (Figure 3E). Through the use of the Spearmen *r* coefficient, it was observed that the serum anion gap was inversely associated with the percentage of energy intake consumed from carbohydrates (Figure 3F); we also found a negative correlation between the anion gap and C-peptide (Figure 3G). As indicated by multilinear regression analyses, both serum albumin levels as well bicarbonates were found to be associated independently with the calculated anion gap (Table 3). Together these observations indicate an increase in high anion gap metabolic acidosis triggered by imbalanced serum bicarbonate and albumin under the consumption of a low-carbohydrate diet.

3.3. Association of the C-peptide levels with circulatory inflammatory markers

C-peptide is a biologically active short polypeptide (31 amino acids) that serves as a diagnostic biomarker to distinguish between type 1 and type 2 diabetes and is a strong indicator of insulin biosynthesis and insulin resistance syndrome (IRS) (21–23). Over the past decade, several studies demonstrated a biological effect of plasma circulating C-peptide on activating inflammatory signaling pathways (24, 25). Thus, we questioned the possible association of elevated levels of C-peptide under low carbohydrate intake with insulin resistance-related inflammatory cytokines. A multiplex cytokine assay was conducted to investigate the secretion of these cytokines, known to be involved in several metabolic disorders, such as diabetes and insulin resistance syndrome. Out of the 41

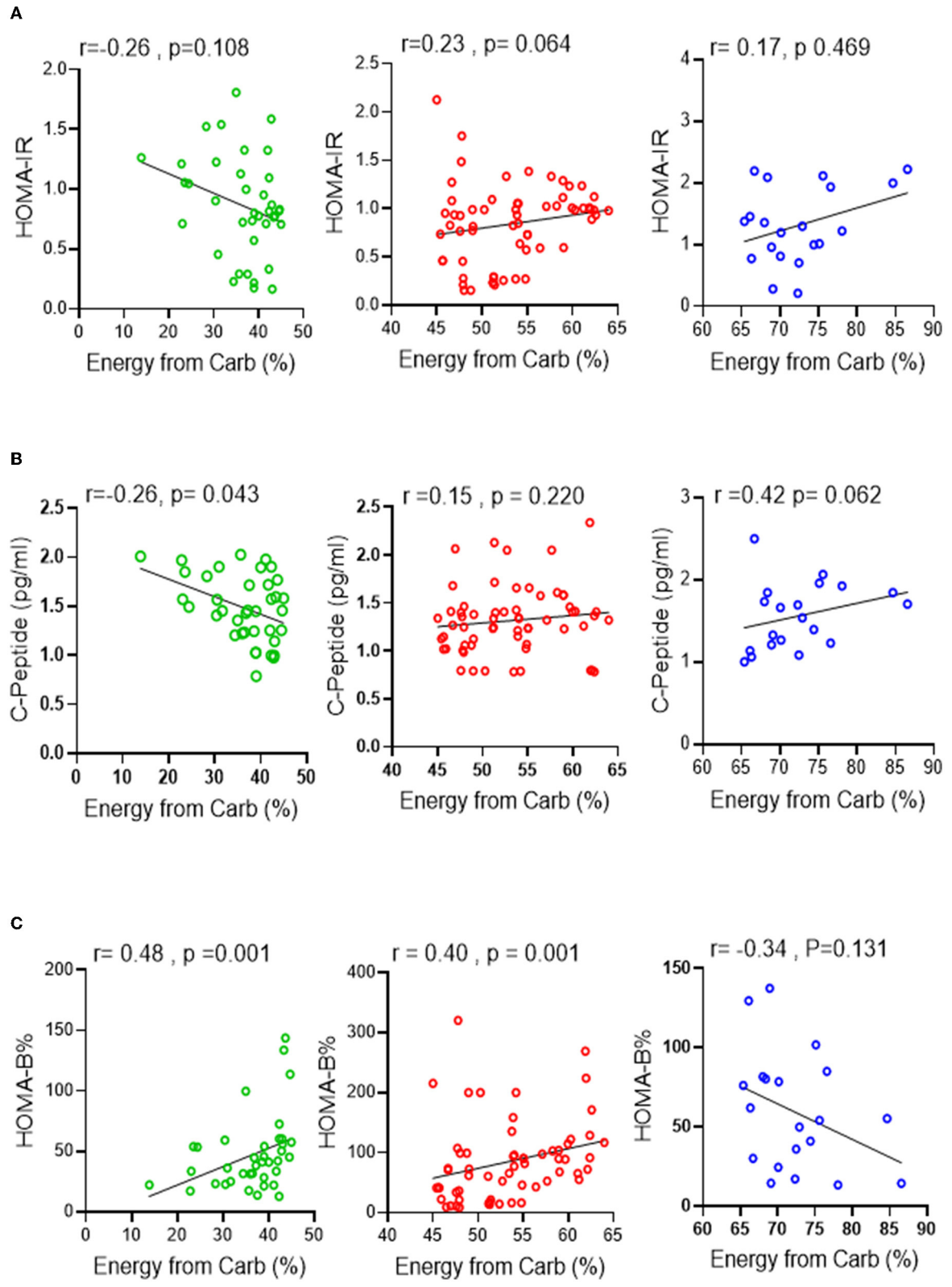

FIGURE 2
Association between carbohydrate intake levels and glucose homeostasis. Spearman correlation test was conducted to investigate the association between **(A)** HOMA-IR, **(B)** C-peptide secretion and **(C)** HOMA-β% and the level of carbohydrate intake % of energy in all three groups. All data are expressed as mean ± SD. $P \leq 0.05$ was considered statistically significant. Low Carbohydrate intake (<45% energy from carb; green), Recommended range of carbohydrate intake (45–65% energy from carb; red), High carbohydrate intake (>65% energy from carb; blue).

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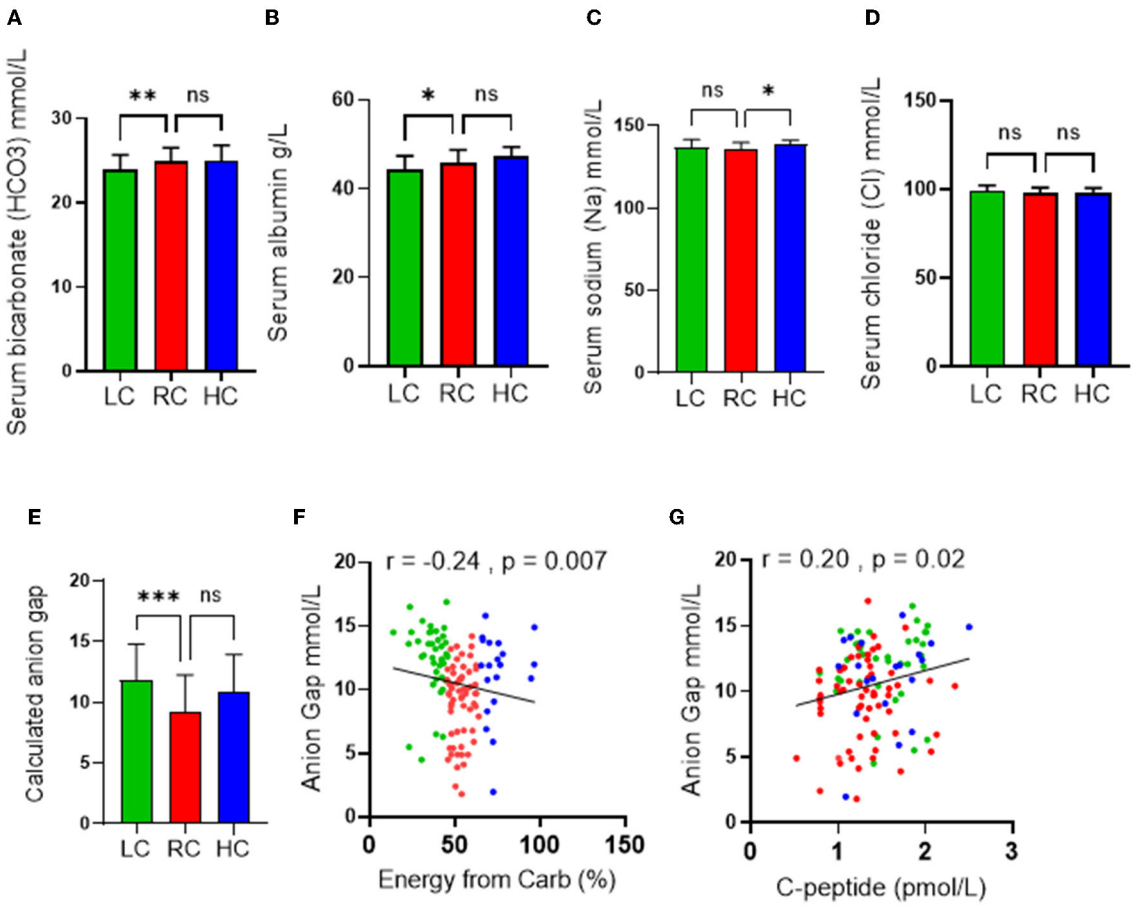

FIGURE 3
Effects of the levels of carbohydrate intake on metabolic acidosis. Serum levels of **(A)** Bicarbonate; **(B)** Albumin; **(C)** Chloride; and **(D)** Sodium are shown in individuals with low-carbohydrate (LC group: <45% of daily energy from carbohydrate), recommended range of carbohydrate (RC group: 45–65% of daily energy from carbohydrate) and high-carbohydrate (HC group: >65% of daily energy from carbohydrate) intake. **(E)** Calculated anion gap is shown for individuals in LC, RC, and HC dietary groups. **(F)** Association (Spearman r correlation test) between anion gap and carbohydrate intake. **(G)** Association (Spearman r correlation test) between anion gap and C-peptide. Data are expressed as mean ± SD. ns, non-significant; *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.005$.

TABLE 3 **Multiple regression analysis.**

	Blood electrolytes levels	Standardized coefficient β	95% confidence interval	*p*-value
Calculated anion gap	Albumin g/L	0.3984	"0.9181 to 2.496"	**<0.0001**
	Sodium (Na) mmol/L	0.2998	"-0.3829 to 0.8046"	0.4832
	Chloride (Cl) mmol/L	0.4092	"-0.1723 to 1.449"	0.1216
	Bicarbonate	0.3984	"1.029 to 3.768"	**0.0007**

The bold values indicate significant values with $p < 0.05$ for easier identification.

inflammatory mediators investigated, only seven of them (IP-10; $p = 0.045$, VEGF; $p = 0.049$, IL-6; 0.049, IL-17A; $p = <0.0001$, FGF-2; $p = 0.025$, MDC; $p = 0.019$, and GRO; $p = 0.035$) were found to be significantly elevated in the LC group when compared with the RC group, and only one cytokine (IL-3; $p = 0.036$) was found to be significantly reduced in the LC group when compared with HC group, as depicted in Table 4. The Spearmen correlation analysis further showed that, out of those eight cytokines/bioactive factors, five were found to be positively correlated with C-peptide expression (FGF-2; $r = 0.52$, $p = 0.001$, IP-10; $r = 0.33$, $p = 0.04$, IL-6; $r = 0.31$, $p = 0.05$, IL-17A; $r = 0.39$, $p = 0.015$, and MDC; $r = 0.36$, $p = 0.025$) (Figures 4A–E), one was negatively correlated with C-peptide expression (IL-3; $r = 0.45$, $p = 0.005$) (Figure 4F), and two (VEGF and GRO) had no correlation with C-peptide levels (Figures 4G, H). Together, the presented data suggest that, under the condition of low dietary carbohydrate intake, a correlation is found between the plasma C-peptide levels and IRS-related cytokine/mediator expression, supporting the active role of C-peptide as a bioactive molecule and its significance as an IRS biomarker.

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TABLE 4 Group-based comparison of plasma inflammatory markers.

Plasma inflammatory markers	<45% (LC)		45–65% (RC)		>65% (HC)		*p*-value
	(*n* = 38) 20 M/18 F	Median-IQR	(*n* = 62) 30 M/32 F	Median-IQR	(*n* = 20) 7 M/ 13 F	Median-IQR	
EGF (pmol/L)	211.5 ± 229.7	158.5–330.3	139.7 ± 156.0	146.5–231	95.0 ± 102.9	75–173.1	0.198
Eotoxin (pmol/L)	112.3 ± 110.5	74.2–215.8	101.0 ± 105.2	51.8–204.5	68.6 ± 69.1	63.8–142	0.512
FGF-2 (pmol/L)	723.2 ± 350.8	492–686.6	494.0 ± 425.4	297–829.0	457.3 ± 365.8	464.5–767.5	**0.025**$^{\Psi}$
Fit-3L (pmol/L)	13.9 ± 24.7	1.4–4.23	8.3 ± 19.7	1.4–1.0	13.6 ± 38.5	1.4–1.0	0.716
Fractalkine (pmol/L)	244.4 ± 695.7	0.3–139.9	144.0 ± 238.6	0.3–137.6	206.4 ± 624.6	0.3–72.2	0.779
G-CSF (pmol/L)	126.2 ± 205.6	35.8–295.1	239.6 ± 210.2	32.7–359.4	71.7 ± 118.6	13.5–118	0.474
GM-CSF (pmol/L)	13.9 ± 29.8	8.3–8.87	17.9 ± 24.8	9.4–17.2	25.9 ± 62.6	6.1–3.7	0.678
GRO (pmol/L)	1282.5 ± 1687.8	51.2–1390.3	1051.4 ± 2279.2	64.9–1324	1530.1 ± 1930.6	654.3–1581.8	**0.035**$^{\Psi}$
IFN-α2 (pmol/L)	112.6 ± 119.4	72.1–215.1	143.3 ± 173.7	102.1–170.9	169.7 ± 179.6	151–255	0.637
IFN-γ (pmol/L)	1196.5 ± 2417.1	642–1069.5	1054.3 ± 1857.7	599–1569.7	1059.3 ± 1700.3	441–1331.2	0.970
IL-10 (pmol/L)	29.3 ± 56.4	1.4–38.5	41.5 ± 56.3	1.8–88.4	28.9 ± 42.8	2.5–63.6	0.680
IL-13 (pmol/L)	36.0 ± 98.2	8.1–15.6	18.8 ± 65.8	1.6–3.5	37.3 ± 44.3	1.6–0.1	0.586
IL-12P40 (pmol/L)	5.2 ± 6.99	1.6–97.3	7.22 ± 13.5	1.6–4.3	21.2 ± 66.0	1.6–2.8	0.351
IL-12P70 (pmol/L)	105.1 ± 241.0	1.6–97.3	65.6 ± 170.9	1.6–26.4	37.7 ± 90.7	1.6–20.8	0.603
IL-15 (pmol/L)	4.77 ± 10.9	1.6–3.1	3.7 ± 4.6	1.6–5.3	4.1 ± 4.3	1.6–7	0.769
IL-17A (pmol/L)	55.6 ± 30.6	37.7–35.7	27.4 ± 21.2	28.4–25.4	48.5 ± 29.2	41.9–64.9	**<0.0001**$^{\Psi\Delta}$
IL-1RA (pmol/L)	134.1 ± 228.5	73.3–166.6	118.2 ± 170.5	50.6–204.6	101.9 ± 114.0	92.5–148.1	0.893
IL-1α (pmol/L)	50.1 ± 90.4	8.4–50.6	51.8 ± 123.1	9.1–31.1	72.4 ± 107.8	7.6–146.4	0.839
IL-1β (pmol/L)	6.9 ± 6.2	5.0–7.5	7.8 ± 11.6	4.7–7	7.1 ± 8.3	3–8.6	0.902
IL-2 (pmol/L)	2.2 ± 1.0	1.7–0.94	2.9 ± 2.0	1.7–1.6	6.8 ± 6.9	7.2–23.7	0.087
IL-3 (pmol/L)	2.3 ± 1.5	1.7–0.6	9.2 ± 13.5	1.3–0.07	2.9 ± 4.7	1.7–0.6	**0.0367**$^{\Psi}$
IL-4 (pmol/L)	1.2 ± 2.4	1.1–0	2.3 ± 1.9	1.1–1.2	1.1 ± 2.3	1.1–0	0.149
IL-5 (pmol/L)	13.6 ± 29.6	2.43–7.2	7.5 ± 15.9	1.4–2.1	12.9 ± 13.9	4.6–27.2	0.743
IL-6 (pmol/L)	7.8 ± 6.1	6.5–11.4	5.5 ± 5.1	3.2–7.9	6.2 ± 7.3	3.7–8.3	**0.0495**$^{\Psi}$
IL-8 (pmol/L)	24.6 ± 27.2	15.6–31.1	16.8 ± 19.0	9.9–16	15.0 ± 21.0	6.5–32.3	0.169
IL-9 (pmol/L)	240 ± 382.7	14.6–641.0	244.9 ± 342.0	26.5–519.3	155.5 ± 250.1	13.1–263.5	0.721
IP-10 (pmol/L)	441.9 ± 215.6	262–303	375.2 ± 283.5	283–351	413.6 ± 241.4	322–374.7	**0.045**$^{\Psi}$
MCP-1 (pmol/L)	318.7 ± 218.4	269.5–304.7	391.1 ± 231.0	239.5–260	272.6 ± 193.8	266.5–284.5	0.738
MCP-3 (pmol/L)	17.6 ± 20.7	1.9–36.9	26.4 ± 74.3	1.4–12.6	10.2 ± 26.8	1.4–6.3	0.676
MDC (pmol/L)	514.2 ± 285.6	217–778.3	489.5 ± 436.9	612–800	546.1 ± 482.7	358.8–909.6	**0.019**$^{\Psi}$
MIP-1α (pmol/L)	25.8 ± 24.2	15.2–36.7	22.6 ± 20.3	16.4–32.6	25.0 ± 21.7	19.9–27.4	0.765
MIP-1β (pmol/L)	43.7 ± 54.5	24.3–29.3	32.2 ± 34.4	20–41.3	40.9 ± 47.7	24.7–49.7	0.538
sCSD40L (pmol/L)	2286.0 ± 3223.5	124–4563.7	2419.0 ± 3347.7	114–3503.7	2559.3 ± 2898.6	1753.8–5167.9	0.975
TGF-α (pmol/L)	44.1 ± 79.9	9.2–686.6	35.3 ± 57.9	8.9–51.1	12.6 ± 20.7	5.8–4	0.368
TNF-α (pmol/L)	71.7 ± 74.7	56.9–59.7	64.8 ± 82.9	41–55.1	69.1 ± 26.4	51.7–45.3	0.901
TNF-β (pmol/L)	13.5 ± 12.1	12.1–17.7	11.1 ± 11.2	8.8–17.4	11.7 ± 11.1	4.8–11.5	0.774

Results are presented as mean ± SD. $^{\Psi}$LC vs. RC, and $^{\Delta}$RC vs. HC. The bold values indicate significant values with $p < 0.05$ for easier identification.

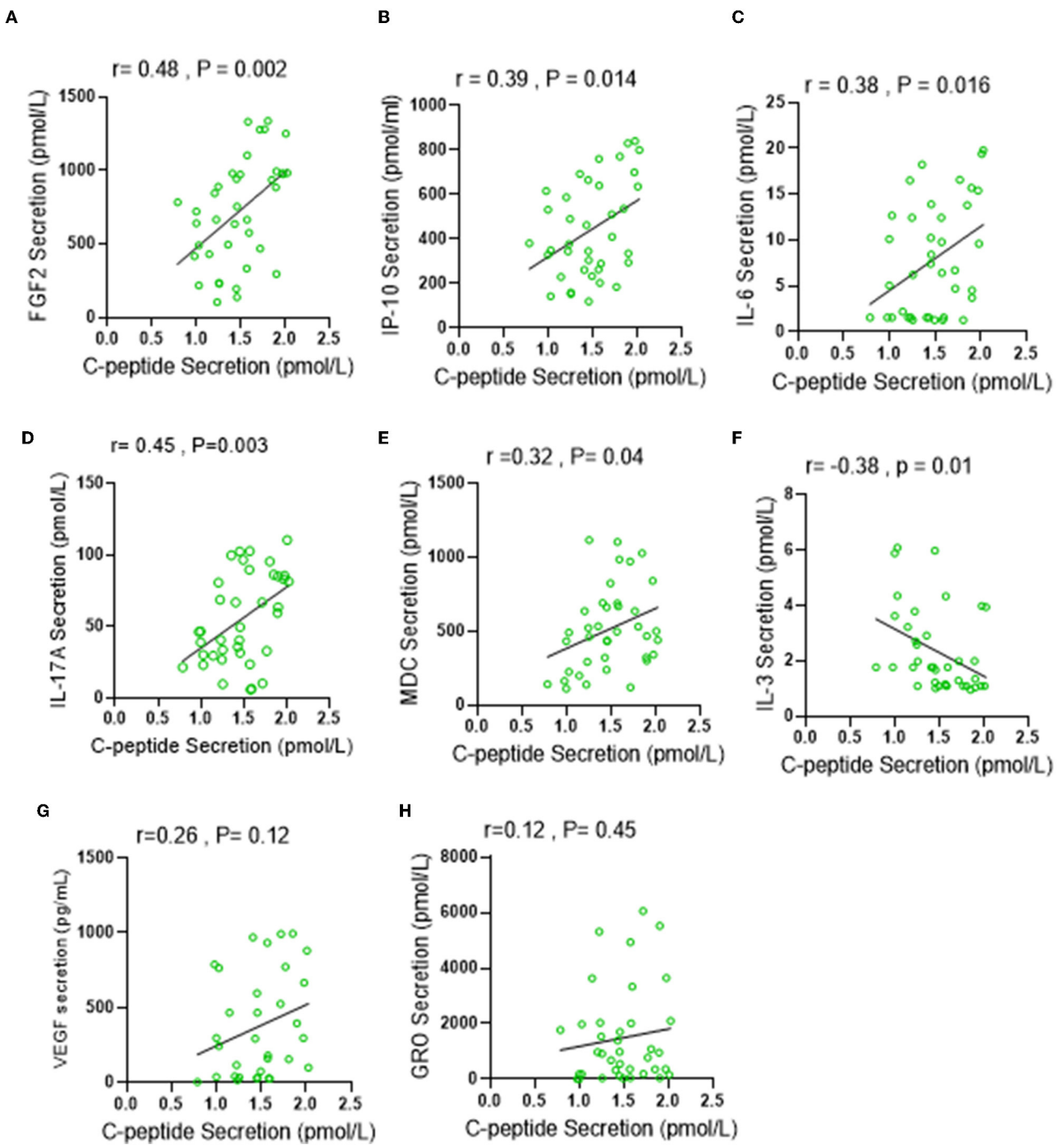

FIGURE 4
Low carbohydrate (LC) intake is associated with IRS-related inflammatory markers under low carbohydrate intake. Plasma levels of IRS related secretion of 41 cytokine and chemokine were determined by multiplex Assay. **(A–H)** Spearman correlation analysis was conducted to investigate the relationship between significantly elevated cytokines in LC group and C-peptide secretion levels. All data are expressed as mean ± SD. $P \leq 0.05$ was considered statistically significant.

4. Discussion

The combination of many mechanisms, including homoeostatic, environmental, and behavioral, regulates body weight. The hypothalamus is central to homoeostatic control because it integrates information about food intake, energy balance, and body weight. However, an "obesogenic" environment and behavioral patterns influence the amount and kind of food consumed and physical activity. Unfortunately, physiological weight loss adaptations have been found to favor weight recovery (26). These modifications include changes in the circulatory levels of hunger-related hormones, energy homeostasis, nutrition

metabolism, and subjective appetite (27). Notably, individuals need to adhere to behaviors that resist physiological adaptations and other variables that favor weight recovery to successfully sustain weight reduction. With the global rise of obesity and T2DM in humans, various dietary strategies that target the restriction of calorie intake have been used not only to promote weight loss but also to prevent and reduce the onset of T2DM (28, 29). Over the past decade, a low-carbohydrate diet has been centered on weight loss in individuals with obesity and those who are overweight, as well as in patients with or at risk of T2DM (30, 31). Even though the impact of a low-carbohydrate diet, especially the ketogenic diet, has been found to be very effective in the rapid induction of weight loss in both individuals with obesity and those who are overweight, the impact of such a diet remains to be well characterized in normal weight or lean counterparts.

In this study, to the best of our knowledge, we investigated, for the first time, the effect of different dietary carbohydrate intake levels on glucose hemostasis, blood electrolyte balance, and T2DM-related inflammatory markers in 120 individuals of a normal weight (BMI $\leq$ 25 kg/m^2). The data presented herein show that individuals with low carbohydrate intake, i.e., those consuming $\leq$45% of their daily calorie intake from carbohydrates, presented with the trends of IRS. We found that, under the condition of low carbohydrate intake, plasma insulin levels and, consequently, the HOMA-IR were both significantly elevated compared to weight-matched counterparts that consumed sufficient levels of carbohydrate for energy (45–56% of daily calorie intake), while the HOMA index representing beta-cell function (HOMA-β%) was found to be decreased under low carbohydrate intake diet. In this regard, we observed increased plasma insulin levels and HOMA-IR values in individuals of a normal weight who had low dietary carbohydrate intake, which may explain why proteolytic and lipolytic responses are enhanced under low dietary carbohydrate intake as part of alternate compensatory mechanisms to generate glucose from amino acids and glycerol (32). Such gluconeogenic responses following a carbohydrate-restricted diet could be helpful for maintaining glycemia in healthy individuals; however, exacerbated glucose production and ketogenesis remain the major concerns involved (33). Carbohydrate restriction to very low levels may also have deleterious effects on intestinal homeostasis and fiber-derived antioxidant phenolic acids compared with a moderate or high carbohydrate intake (34). Furthermore, a relative increase of ketone concentrations under low dietary carbohydrate intake may at first stimulate the pancreas to increase insulin release, which may accumulate metabolic stress over time (35). In addition, carbohydrate restriction induces lipolysis, releases free fatty acids, and increases citric acid cycle flux, all of which are known reasons to promote reactive oxygen species (ROS) production (36) and suppress the function of beta cells (37), which may be explained by the lower HOMA-β% values that we observed in individuals of a normal weight on low dietary carbohydrate intake.

Interestingly, plasma C-peptide levels were also found to be significantly elevated under low carbohydrate intake. A significant correlation was found between glucose homeostasis markers and low dietary carbohydrate intake, further supporting the effect of low-carbohydrate diet intake on glucose homeostasis. Carbohydrate metabolism is a fundamental biochemical process that ensures a constant supply of energy to living cells. With the prolonged consumption of low carbohydrate intake, the liver starts to produce ketone bodies as an alternative source of energy. Ketone bodies travel from the liver to extrahepatic tissues to provide energy to different organs by breaking down fatty acids and ketogenic amino acids (38, 39). In the studied cohort, an elevation in the anion gap was observed in the LC group. Through further multi-regression analysis, it was discovered that this elevation was caused by the reduction in both serum bicarbonate and serum albumin levels. An association was also observed between the level of anion gap and the level of energy from carbohydrate intake. It is irrefutable that any diet based on restrictions and exclusions of certain foods will induce a possible increase in the risk of mineral deficiencies and electrolyte imbalance. In fact, studies have shown that consuming a low-carbohydrate diet while maintaining a high intake of protein can lead to a disturbance in fluid and electrolytes, which can further cause kidney damage (40, 41).

Interestingly, in our cohort, we also highlight a correlation between the anion gap and C-peptide levels. C-peptide is the part of proinsulin that is cleaved from pancreatic beta cells prior to co-secretion with insulin. A 20-year follow-up study by Fung et al. (42) revealed that increased dietary intake of protein and high-fat dairy products is positively associated with higher plasma C-peptide levels and directly associated with the risk of colorectal cancer. While another study presented by Seidelmann et al. (43) concluded the presence of a U-shaped association between the percentage of energy consumed from carbohydrates and mortality, as they reported that both low-carbohydrate consumption (<40%) and high-carbohydrate consumption (>70%) presented a greater risk of mortality than moderate carbohydrate intake. Even though the role of C-peptide in the regulation of inflammation remains controversial, in our study, we observed a significant correlation between lower carbohydrate intake and higher plasma C-peptide. Multiplex analysis of several inflammatory cytokines further revealed that plasma C-peptide upregulation correlated positively with plasma FGF2, IP-10, IL-6, MDC, and IL-17A levels. Notably, these cytokines have been previously identified to induce the development of insulin resistance and cause the pathogenesis of T2DM (44–48). However, a negative correlation was observed between the C-peptide levels and the expression of the anti-inflammatory cytokine IL-3, a pleiotropic regulator of inflammation (49).

Nevertheless, the present study is limited by certain caveats. In this study, sample collection was achieved randomly and not systemically. Even though such an analysis provides a better approximation of the entire population, several limitations should be considered. For instance, the dietary intake in this study was assessed through self-reported diary logs and not by intervention. Even though adequate training was given to each participant along with a food scale, we could not possibly rule out false reporting. We also have no record of how long each individual would have maintained this dietary lifestyle beyond the 7-day follow-up period. Therefore, the effects of long-term vs. short-term dietary interventions involving carbohydrate intake may not be evaluated. Nevertheless, the most substantial limitation found in this study was the sample size in the HC group. Throughout

the investigation, it became clear that a U-shaped effect was observed, indicating that both LC and HC intake reflect unwanted outcomes. This observation falls in line with observations made by other groups (43). However, owing to the small number of participants at the higher end of carbohydrate intake (<70%), it was difficult to reach statistical significance in this group. It is also crucial to note that, although there is a significant correlation between low carbohydrate consumption and IRS risk indicators, this should not be interpreted as a direct impact but rather as a contributory behavioral factor that could enhance the probability of such an outcome if continued uninterrupted over time.

All in all, the presented data highlight, for the first time, the effect of low carbohydrate intake on factors related to IRS in the normal-weight population. We have shown that individuals of a normal weight consuming <45% of their daily energy intake from carbohydrates had trends of dysregulated glucose homeostasis with elevated plasma C-peptide levels and higher anion gap metabolic acidosis. Under the consumption of low carbohydrate intake, we also observed the upregulation of T2DM-related inflammatory markers that were found to significantly correlate with C-peptide levels.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Kuwait Ministry of Health (MOH) Ethics Board (2017/542). The patients/participants provided their written informed consent to participate in this study.

Author contributions

FA-R conceived the idea, guided the research study, provided material support, procured funds, collected and analyzed data, and wrote the manuscript. SS participated in performing some statistical analysis, writing, and reviewing the manuscript. AA participated in performing experiments and analyzing data. FB, HA, and NA participated in performing experiments and data collection. MM participated in performing statistical analysis and statistical methodology and contributed to writing and reviewing. FA participated in performing some statistical analysis and in writing and reviewing the manuscript. FA-M reviewed and critically commented on the manuscript. RA guided the research study, provided material support, procured funds, wrote, edited, and approved the manuscript for submission. 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/fpubh.2023.1115333/full#supplementary-material

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Is first pregnancy age associated with hypertension in the Chinese rural women population?

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Introduction: The purpose of this study was to investigate the relationship between first pregnancy age and hypertension later in the life of women from Chinese rural areas.

Methods: In total, 13,493 women were enrolled in the Henan Rural Cohort study. Logistic regression and linear regression were used to evaluate the association between first pregnancy age and hypertension and blood pressure indicators [including systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP)]. The restricted cubic spline was used to examine the dose–response relationship between the first pregnancy age and hypertension or blood pressure indicators.

Results: After adjusting for potential confounders, each 1-year increase in first pregnancy age was associated with a 0.221 mmHg increase in SBP values, a 0.153 mmHg increase in DBP values, and a 0.176 mmHg decrease in MAP values (all $P < 0.05$). The β of SBP, DBP, and MAP showed a trend of first increasing and then decreasing with increasing first pregnancy age and there was no statistical significance after first pregnancy age beyond 33 years on SBP, DBP, and MAP, respectively. A 1-year increment in first pregnancy age was associated with a 2.9% [OR (95% CI): 1.029 (1.010, 1.048)] higher odds of prevalent hypertension. The odds of hypertension increased sharply and then eventually leveled off with an increment of first pregnancy age after adjusting for potential confounders.

Conclusion : First pregnancy age might increase the risk of hypertension later in life and might be an independent risk factor for hypertension in women.

KEYWORDS

first pregnancy age, hypertension, blood pressure indicators, rural population, women

Introduction

Hypertension is a principal cause of the cardiovascular disease (CVD), which has become one of the most severe diseases in the world and China (1, 2). Nearly one-third of these deaths in 2019 were due to CVD. The leading risk factor is raised blood pressure (BP) or hypertension, which accounted for 10.8 million deaths (19.2% of all deaths in 2019) and 9.3% of disability-adjusted life-years lost globally (3). According to the latest data released by the "Report on Disease of Cardiovascular in China 2020," the standardized prevalence of hypertension was 23.20% in China (4). Sex differences in the prevalence of hypertension are ubiquitous. A woman's lifetime undergoes many dynamic changes, including menarche, pregnancy, lactation, and menopause (5).

Pregnancy represents a unique challenge for the mother's body, especially the first pregnancy. Studies have shown that hormonal, immunological, and microbiological changes occur in the maternal body during pregnancy (6). In addition, this was particularly true for the first pregnancy, various potential factors that exist in the first pregnancy might have an impact on health later in life. Ozdemir et al. (7) showed that a higher first pregnancy age will increase the risk of osteoporosis. Moreover, first pregnancy age is associated with cancer (8), metabolic syndrome (9), and diabetes (10) in future. Therefore, issues related to first pregnancy age and future health risks are attracting increased attention. However, most of the studies are from foreign populations or urban areas in China. According to the different populations, the research results are different, and there are few studies in rural areas. China is a large agricultural country with severe aging of the rural population and limited medical resources. Focusing on the future health status of rural women will help reduce the burden of the country and enhance social harmony.

Thus, the purpose of this study was to investigate the relationship between first pregnancy age and hypertension later in the life of women from Chinese rural areas.

Methods

Study design and participants

A cross-sectional study was implemented from the baseline data of the Henan Rural Cohort Study launched in Henan from July 2015 to September 2017. More details of the cohort have been described in the previous study (11). In summary, this rural-based study incorporated a total of 39,259 participants (23,769 were women) aged 18–79 years, and a 93.7% response rate was reported (11). In this study, 13,749 female participants with complete information on first pregnancy age were included. The participants were excluded who were without the information on hypertension ($n = 22$) or first pregnancy age <18 years ($n = 44$) or suffering from cancer ($n = 167$). Finally, 13,493 female participants were included in our study. All the participants provided informed consent. The study was approved by the "Life Science Ethics Committee of Zhengzhou University."

Assessment of first pregnancy age

The first pregnancy age was calculated from the date of delivery of the first baby and the date of birth of the mother (12). The information was obtained by self-reporting using a standard questionnaire. Participants were asked an open question: "when did you have your first pregnancy?" Details about the questionnaire have been introduced elsewhere (13).

Definition of hypertension

Hypertension was considered in patients who were with systolic blood pressure (SBP) of at least 140 mmHg or diastolic blood pressure (DBP) of at least 90 mmHg or self-reported hypertension diagnosed by a physician and patients who were taking current antihypertensive treatment during the last 2 weeks (14). A family history of hypertension was defined as at least one first-degree member with hypertension. Blood pressure was measured three times by electronic sphygmomanometer (Omron HEM-7071A, Japan) in the right arm in the sitting position after at least 5 min rest. There were 30 s intervals between the three measurements. Mean arterial pressure (MAP) was calculated using the formula, $MAP = DBP + 1/3(SBP - DBP)$. More detailed descriptions were previously published (15).

Other covariate variables

Participants' demographic characteristics (age, marital status, educational level, and family per capita annual income), lifestyle behaviors (smoking status, drinking status, and physical activity), dietary, and reproductive factors (age at menarche, menopause status, age at menopause, parity information, use of oral contraceptive pills, breastfeeding, and gestational hypertension, gestational diabetes mellitus, and age at last birth) were collected by a standard questionnaire. Education levels were divided into three groups: elementary school or below, middle school, and high school or above. Family per capita annual income (RMB) was divided into four groups: ≤10,000, 10,001∼, 20,001∼, and 50,001∼. Marital status was divided into single/widowed/separated/divorced and married/cohabiting. Smokers were defined as a person who smoked more than one cigarette per day in the past 6 months (16). Participants who consumed alcohol 12 or more times every year were viewed as drinkers (11). The Physical Activity Questionnaire (IPAQ 2001) was used to assess the levels of physical activity (17). Adequate intake of vegetables and fruits was defined as a person who consumed an average of more than 500 g of vegetables and fruits per day (18). High-fat diet was defined as a person who took an average of more than 75 g of livestock and poultry meat per day (19).

Height, weight, and blood pressure are measured by trained personnel according to standard instructions described elsewhere (20). Body mass index (BMI, kg/m^2) was calculated as weight (kg) divided by the square of height (m). The details of the equipment for anthropometric and clinical examinations have been introduced elsewhere (21, 22).

Statistical analysis

Categorical variables were presented as numbers (percentages), and the Chi-square test was used to compare the participants' baseline characteristics. Continuous variables were reported as mean ± standard deviation (SD), and the Student's *t*-test was used for comparative analysis. Five models were developed to assess associations of first pregnancy age and hypertension or blood pressure indicators (SBP, DBP, and MAP) by using the logistic regression model or linear regression. We produced five models as

follows: Model 1: adjusted for age, marital status, education level, family per capita yearly income, smoking, alcohol consumption, physical activity, adequate intake of vegetables and fruits, high-fat diet, family history of hypertension, and BMI; Model 2: adjusted as in model 1 plus age at menarche, menopause status, breastfeeding, and use of oral contraceptive pills; Model 3: adjusted as in model 2 plus gestational hypertension and gestational diabetes mellitus; Model 4: adjusted as in model 3 plus parity; Model 5: adjusted as in model 4 plus age at last birth. We examined the dose–response relationship between the first pregnancy age and hypertension or blood pressure indicators using the restricted cubic spline analysis. We furthermore performed subgroup analyses by several factors: age, education level, averaged yearly income, adequate intake of vegetables and fruits, high-fat diet, physical activity, age at menarche, menopause status, and parity ≤2. The interactions were performed to test the effect modification in subgroup analyses by a generalized linear model. In addition, according to the outcome of the first pregnancy, we further assessed associations of first pregnancy age and hypertension or blood pressure indicators (SBP, DBP, and MAP) by using a logistic regression model or linear regression. Considering that the associations of first pregnancy age with hypertension or blood pressure indicators might be attributable to their outliers, we conducted a sensitivity analysis to examine the linearity of associations of age at first pregnancy which was fixed at 2.5th−97.5th percentile ranges and hypertension or blood pressure indicators. In addition, to exclude the effect of taking antihypertensive drugs on blood pressure indices, we further excluded the participants who used antihypertensive medicine and performed sensitivity analysis. Moreover, we analyzed the association with hypertension using age at first birth rather than age at first pregnancy to test the credibility of the results.

All data were analyzed by SPSS software V.26.0 and R version 4.0.0. Statistical significance was set to a p-value of <0.05 at two tails.

Results

Basic characteristics of the study population

Table 1 shows the main demographic characteristics of 13,493 female participants aged from 19 to 79 years according to hypertension status. The mean ± SD age at recruitment was 54.99 ± 12.14 years, and the mean ± SD age of first pregnancy was 23.57 ± 2.47 years. Compared to those without hypertension, those with hypertension were likely to be older, with higher levels of BMI, first pregnancy age, age at menarche, age at menopause, parity, SBP, DBP, and MAP (all $P < 005$). In addition, those with hypertension were likely to be unmarried/divorced/widowed and postmenopausal; have lower levels of education, yearly income, and physical activity; have a not-so-high-fat diet and enough vegetables and fruits; and report a family history of hypertension. Distributions of the selected variables were statistically significantly different between hypertension and normotensive individuals groups (all $P < 0.05$), except for the distribution of smoking status ($P = 0.796$) and gestational hypertension ($P = 0.530$).

Association of first pregnancy age with blood pressure indicators

Figure 1 and Supplementary Table S1 present the results of multivariable analysis of first pregnancy age and blood pressure indicators (SBP, DBP, and MAP). Each 1-year increase in first pregnancy age was associated with a 0.109 mmHg increase in SBP values (95% CI: 0.025, 0.193), a 0.112 mmHg increase in DBP values (95% CI: 0.040, 0.184), and a 0.109 mmHg decrease in MAP values (95% CI: 0.025, 0.193) in model 2 adjusting for age, marital status, education level, family per capita yearly income, smoking, alcohol consumption, physical activity, adequate intake of vegetables and fruits, high-fat diet, family history of hypertension, BMI, age at menarche, menopause status, breastfeeding, and use of oral contraceptive pills (all $P < 0.05$). A significant positive association between first pregnancy age, DBP, and MAP was observed in Model 3 (further adjustment of gestational hypertension and gestational diabetes mellitus) and Model 4 (further adjustment of parity). The effect of first pregnancy age on SBP [β (95% CI): 0.221 (0.082, 0.359)], DBP [β (95% CI): 0.153 (0.072, 0.234)], and MAP [β (95% CI): 0.176 (0.081, 0.270)] was stronger after adjusting for age at last birth (Model 5) and remained statistically significant. However, no association was observed between first pregnancy age and SBP in Model 3 (further adjustment of gestational hypertension and gestational diabetes mellitus) and Model 4 (further adjustment of parity). To observe the trend between first pregnancy age and SBP, DBP, and MAP, multivariable restricted cubic regression splines were conducted (Figure 2). The spline analysis showed a significant non-linear relationship between first pregnancy age and SBP (P-overall association < 0.001; P-non-linear association = 0.001), DBP (P-overall association < 0.001; P-non-linear association < 0.001), and MAP (P-overall association < 0.011; P-non-linear association < 0.001). The β of SBP, DBP, and MAP showed a trend of first increasing and then leveling off or decreasing with increasing first pregnancy age and there was no statistical significance after first pregnancy age beyond 33 years on SBP, DBP, and MAP, respectively.

Association of first pregnancy age with and hypertension

The results of the multivariable analysis of first pregnancy age and hypertension are shown in Figure 1 and Supplementary Table S1. A 1-year increment in first pregnancy age was associated with a 2.1% (95% CI: 1.004, 1.038) higher odds of prevalent hypertension in Model 2. After further adjusting for confounding factors including gestational hypertension and gestational diabetes mellitus (Model 3), parity (Model 4), and age at last birth (Model 5), the associations were still significant and the magnitude of associations was slightly decreased in Model 5 [OR (95% CI): 1.029 (1.010, 1.048)]. Moreover, age at first pregnancy was classified as a categorical variable (18–21, 22, 23, 24, 25, 26, and ≥27 years) according to a seven-point scale, with the 18–21 years category as the reference category (Supplementary Table S2). Age at first pregnancy was positively associated with risk of hypertension with adjusted OR (95% CI) of 1.000, 1.235 (1.067,

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TABLE 1 Demographic characteristics of participants according to hypertension status.

Variables	Total	Normotensive	Hypertension	P^a
N (%)	13,493 (100)	8,861 (65.67)	4,632 (34.33)	
Age (year)	54.99 ± 12.14	51.65 ± 12.09	61.38 ± 9.35	<0.001
Marital status				<0.001
Married/cohabitation	12,223 (90.59)	8,254 (93.15)	3,969 (85.69)	
Unmarried/divorced/ widowed	1,270 (9.41)	607 (6.85)	663 (14.31)	
Educational level				<0.001
Elementary school or below	6,883 (51.01)	3,864 (43.61)	3,019 (65.18)	
Middle school	4,646 (34.43)	3,423 (38.63)	1,223 (26.40)	
High school or above	1,964 (14.56)	1,574 (17.76)	390 (8.42)	
Income (RMB)[b]				<0.001
≤10,000	3,009 (22.33)	1,748 (19.74)	1,261 (27.30)	
10,001∼	2,382 (17.68)	1,525 (17.22)	857 (18.55)	
20,001∼	4,714 (34.98)	3,202 (36.16)	1,512 (32.73)	
50,001∼	3,370 (25.01)	2,381 (26.89)	989 (21.41)	
Physical activity				<0.001
Low	4,378 (32.45)	2,679 (30.23)	1,699 (36.68)	
Moderate	5,215 (38.65)	3,646 (41.15)	1,569 (33.87)	
High	3,900 (28.90)	2,536 (28.62)	1,364 (29.45)	
Current regular smokers	40 (0.30)	25 (0.28)	15 (0.32)	0.796
Current regular drinking	257 (1.90)	199 (2.25)	58 (1.25)	<0.001
Vegetable/fruit (yes)[c]	5,778 (42.82)	4,052 (45.73)	1,726 (37.26)	<0.001
High fat diet (yes)	1,627 (12.06)	1,239 (13.98)	388 (8.38)	<0.001
BMI (kg/m^2)	25.16 ± 3.64	24.54 ± 3.42	26.35 ± 3.75	<0.001
First pregnancy age (years)	23.57 ± 2.47	23.53 ± 2.43	23.66 ± 2.54	0.003
Age at last birth (years)	29.89 ± 4.43	29.66 ± 4.44	30.35 ± 4.38	<0.001
Age at menarche (years)	15.74 ± 2.16	15.49 ± 2.10	16.21 ± 2.20	0.004
Age at menopause (years)	48.97 ± 4.81	48.84 ± 4.77	49.13 ± 4.85	<0.001
Menopause status				<0.001
Premenopausal	4,682 (34.70)	4,024 (45.41)	658 (14.21)	
Postmenopausal	8,811 (65.30)	4,837 (54.59)	3,974 (85.79)	
Use of oral contraceptive pills	356 (2.64)	281 (3.17)	75 (1.62)	<0.001
Parity	2.53 ± 1.02	2.37 ± 0.93	2.84 ± 1.12	<0.001
Gestational hypertension	105 (0.78)	72 (0.81)	33 (0.71)	0.530
Gestational diabetes mellitus	17 (0.13)	15 (0.17)	2 (0.04)	0.050
Family history of hypertension	2,689 (19.93)	1,432 (16.16)	1,257 (27.14)	<0.001
SBP (mmHg)	126.44 ± 21.11	115.15 ± 12.24	148.05 ± 17.36	<0.001
DBP (mmHg)	77.22 ± 11.43	72.05 ± 8.03	87.11 ± 10.39	<0.001
MAP (mmHg)	93.62 ± 13.85	86.41 ± 8.68	107.42 ± 11.16	<0.001

[a]Chi-square test or t-tests; [b]per capita annual income (RMB); [c]Adequate intake of vegetables and fruits (yes). BMI, body mass index, weight (kg)/height $(m)^2$; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. Values are means and standard deviation for continuous variables, and numbers and percentages for categorical variables.

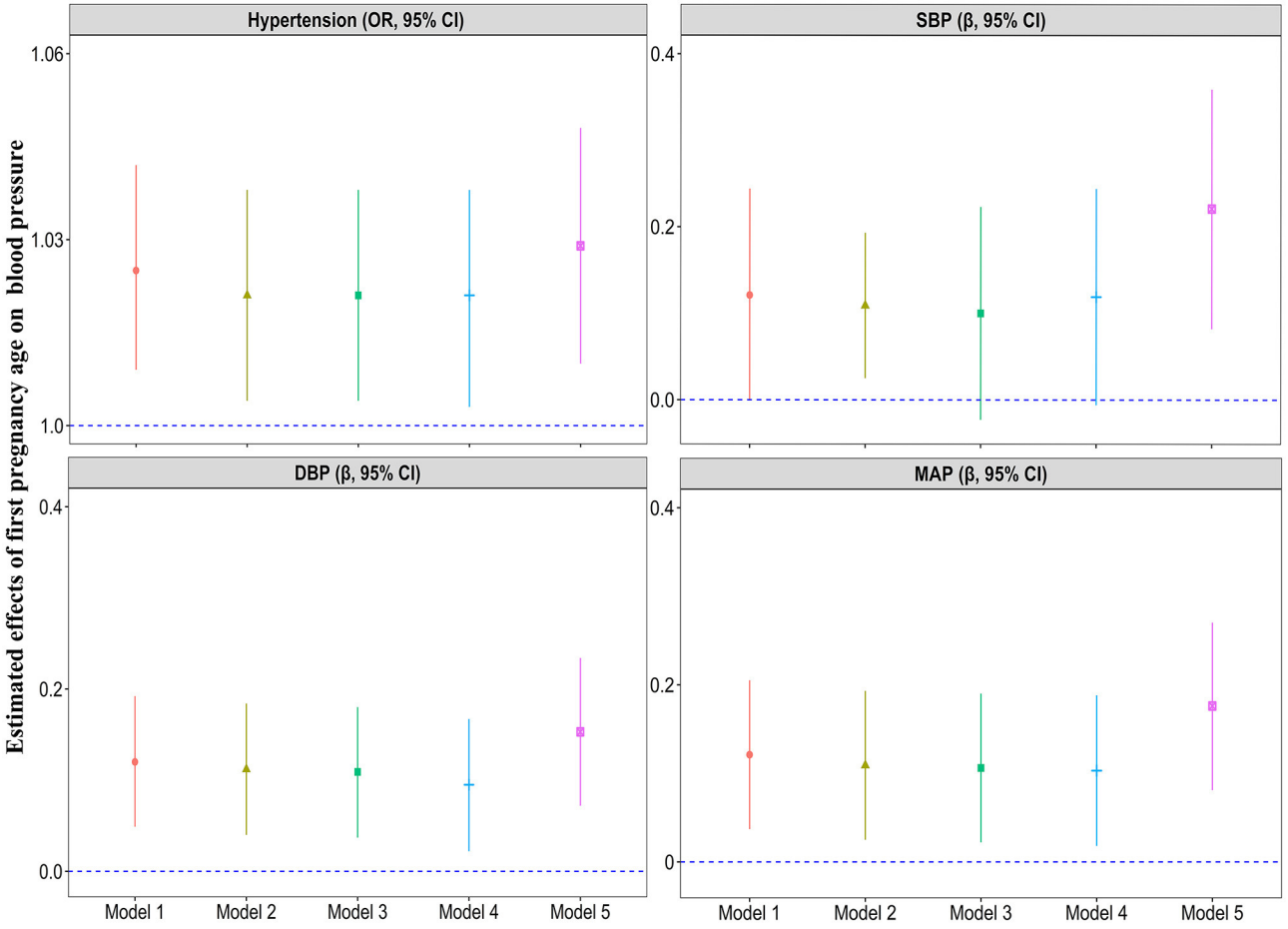

FIGURE 1
Multivariable analysis of first pregnancy age with hypertension, SBP, DBP, and MAP. Model 1: adjusted for age, marital status, education level, family per capita yearly income, smoking, alcohol consumption, physical activity, adequate intake of vegetables and fruits, high-fat diet, family history of hypertension, and BMI; Model 2: adjusted as in model 1 plus age at menarche, menopause status, breastfeeding, and use of oral contraceptive pills; Model 3: adjusted as in model 2 plus gestational hypertension and gestational diabetes mellitus; Model 4: adjusted as in model 3 plus parity; Model 5: adjusted as in model 4 plus age at last birth.

1.431), 1.223 (1.062, 1.408), 1.194 (1.037, 1.374), 1.212 (1.042, 1.410), 1.273 (1.063, 1.524), and 1.234 (1.038, 1.467) for those with age at first pregnancy 18–21 (reference), 22, 23, 24, 25, 26, and ≥27 years, respectively (P trend = 0.021). Figure 2 displays the dose–responsive relationship between first pregnancy age and hypertension from restricted cubic splines with three knots placed at the 5th, 50th, and 95th. The spline analysis showed a significant linear relationship between first pregnancy age and hypertension (P-overall association = 0.011; P-non-linear association = 0.169). The odds of hypertension increased sharply and then eventually leveled off with an increment of first pregnancy age after adjusting for potential confounders. However, there was no statistical significance after the first pregnancy age beyond 34 years.

Subgroup analyses and sensitivity analyses

The results of subgroup analyses of first pregnancy age and hypertension and blood pressure indicators are revealed in Supplementary Table S3 and Figure 3. After stratification by age, we found that the first pregnancy age was significantly associated with hypertension among younger female participants (<65 years). Statistically significant interactions were only detected between first pregnancy age and age (P interaction = 0.029). For the associations between first pregnancy age and SBP, first pregnancy age was significantly associated with higher SBP levels in subgroups including age <65 years, education levels ≤ primary school, no high-fat diet, high physical activity, age at menarche ≥14 years, and parity ≤2 subgroups. First pregnancy age was significantly associated with higher DBP levels in subgroups including the age of 65 years, education levels ≤ Primary school, income <10,000 Yuan, adequate intake of vegetables and fruits, no high-fat diet, high-fat diet, age at menarche ≥14 years, postmenopausal, and parity ≥2. First pregnancy age was significantly associated with higher MAP levels in subgroups including age < 65 years, education levels ≤ Primary school, income <10,000 Yuan, adequate intake of vegetables and fruits, no high-fat diet, high-fat diet, age at menarche ≥14 years, postmenopausal, and parity ≤2. In addition, statistically significant interactions were only detected between first pregnancy age and age on SBP ($P_{interaction}$ = 0.029). Furthermore,

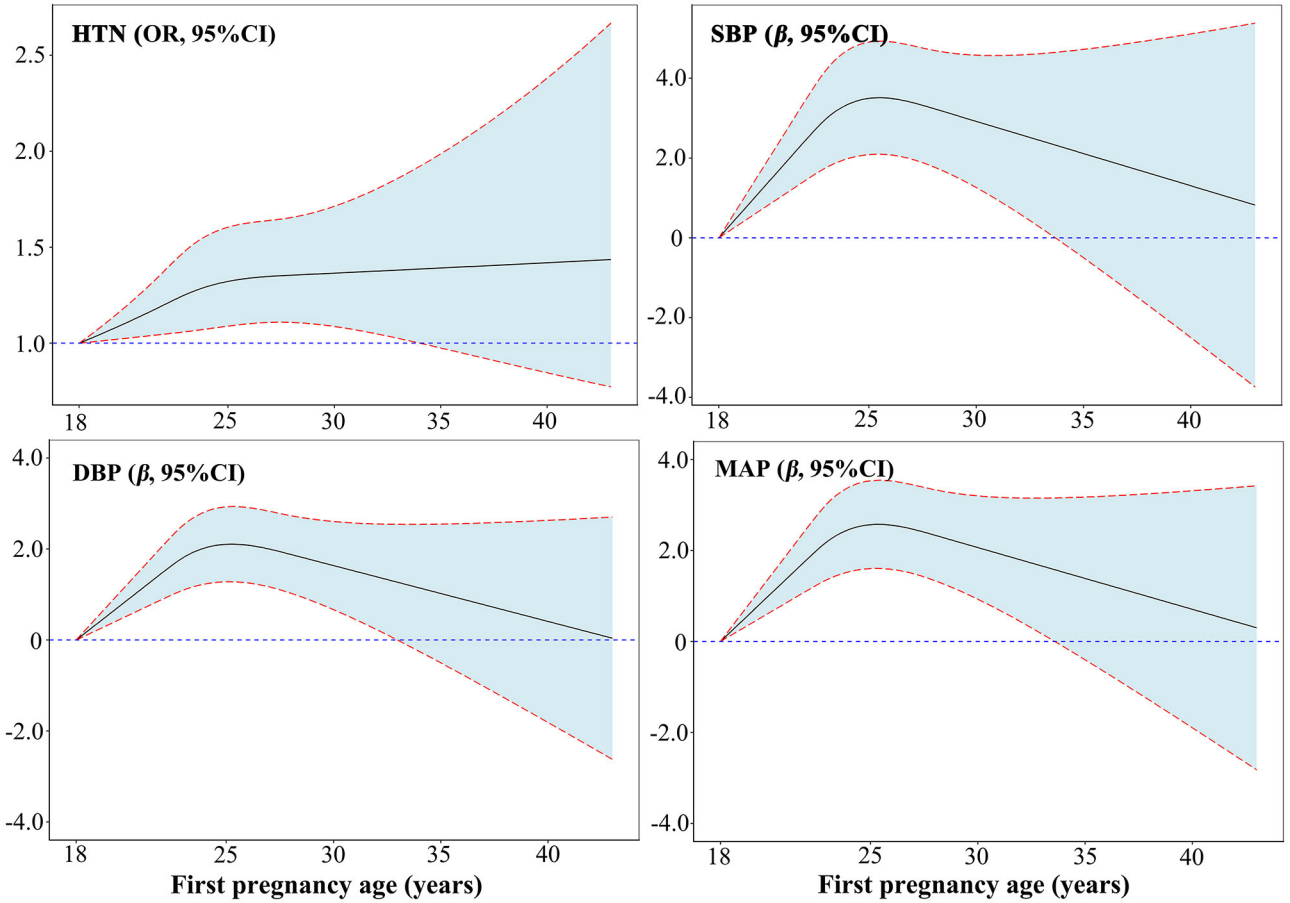

FIGURE 2
Dose–responsive relationship of first pregnancy age with hypertension, SBP, DBP, and MAP from restricted cubic splines. Adjusted for age, marital status, education level, family per capita yearly income, smoking, alcohol consumption, physical activity, adequate intake of vegetables and fruits, high-fat diet, family history of hypertension, BMI, age at menarche, menopause status, breastfeeding, use of oral contraceptive pills, gestational hypertension, gestational diabetes mellitus, parity, and age at last birth.

we did not observe significant interactions between first pregnancy age and age, education levels, economic conditions, adequate intake of vegetables and fruits, high-fat diet, physical activity, age at menarche, menopause status, or parity on SBP, DBP, and MAP (all $P_{interaction} > 0.05$). In addition, we divided the group into two groups based on the first pregnancy outcome: the first pregnancy with the delivery group and the first pregnancy with the abortion group (Supplementary Tables S4). A meaningful positive association between age at first pregnancy and both risks of hypertension and blood pressure indicators (SBP, DBP, and MAP) was found among women in the first pregnancy with normal delivery (all $P < 0.05$). No association was found between age at first pregnancy and both risk of hypertension and blood pressure indicators among women in the first pregnancy miscarriage. However, there were no significant between-group differences (all $P \geq 0.05$).

The results of the sensitivity analyses are shown in Supplementary Table S5. The estimated effects of the associations between first pregnancy age and hypertension, SBP, DBP, and MAP did not appreciably change the result after fixing the levels of first pregnancy age at the 2.5th and 97.5th ranges. In addition, a sensitivity analysis excluding the participants who used anti-hypertensive medicine yielded similar results (Supplementary Tables S6). Moreover, when we analyzed the association with hypertension using age at first birth rather than age at first pregnancy, the results were robust (Supplementary Table S7).

Discussion

In this study, we explored a higher likelihood of the relationship between first pregnancy age and hypertension later in life in depth. Our study showed that elevated first pregnancy age was associated with an increased risk of hypertension and increased levels of blood pressure parameters after adjusting for potential confounders including age, marital status, education level, family per capita yearly income, smoking, alcohol consumption, physical activity, adequate intake of vegetables and fruits, high-fat diet, family history of hypertension, BMI, age at menarche, menopause status, breastfeeding, use of oral contraceptive pills, gestational hypertension gestational diabetes mellitus, parity, and age at last birth. A 1-year increment in first pregnancy age was associated with a 2.9% higher risk of hypertension after adjusting for confounding

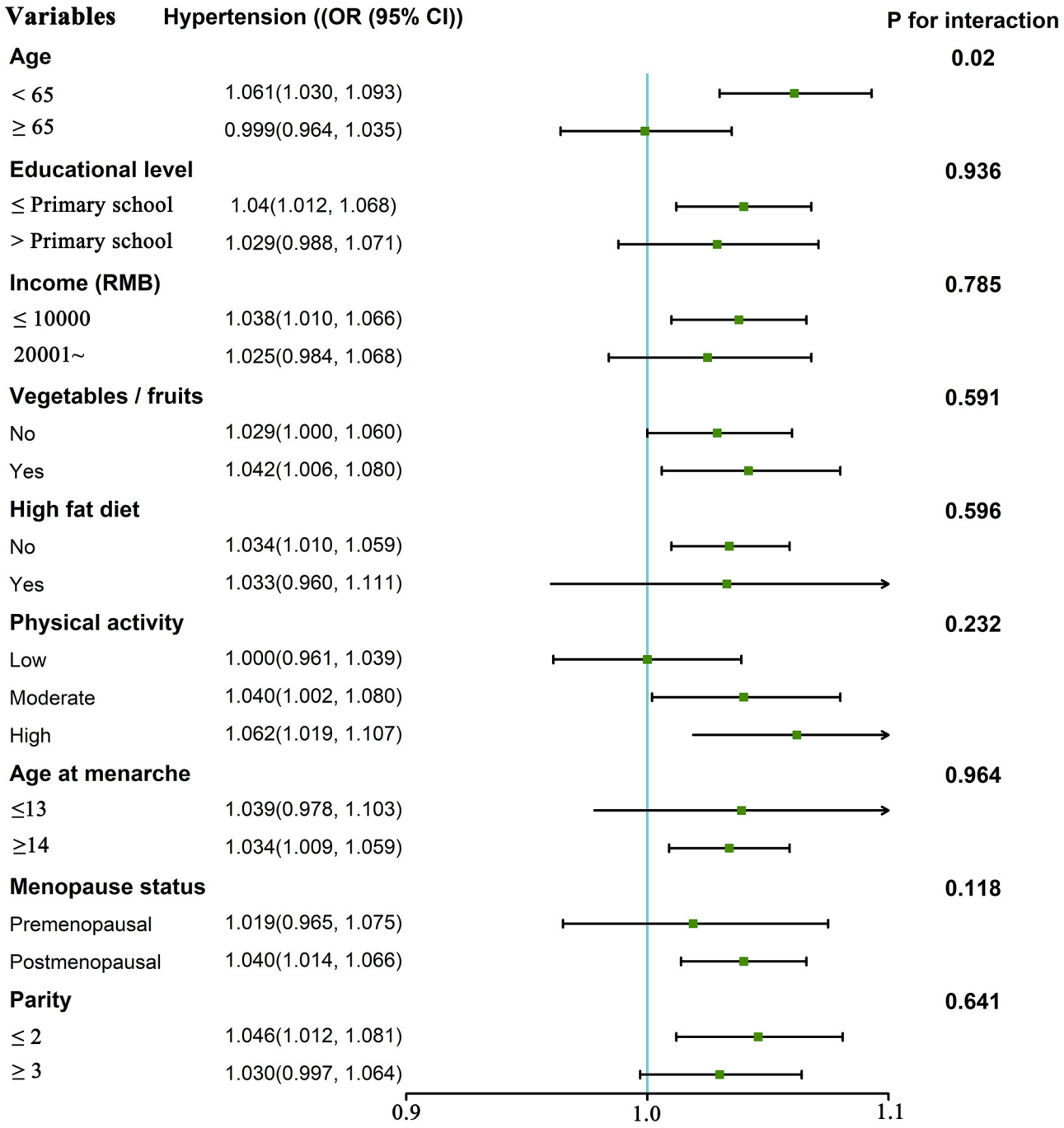

FIGURE 3
The odds ratio of hypertension (and 95% CI) associated with first pregnancy age according to potential modifiers. Adjusted for age, marital status, education level, family per capita yearly income, smoking, alcohol consumption, physical activity, adequate intake of vegetables and fruits, high-fat diet, family history of hypertension, BMI, age at menarche, menopause status, breastfeeding, use of oral contraceptive pills, gestational hypertension, gestational diabetes mellitus, parity, and age at last birth (unless stratified by the respective factor).

factors. However, there was no statistical significance after the first pregnancy age beyond 34 years.

Our study suggested that the risk of hypertension increased with the increasing age of the first pregnancy after adjusting for potential confounders in rural Chinese women. Previous studies have shown that reproductive factors were associated with many unfavorable outcomes, such as hypertension (23), metabolic syndrome (24), and cardiovascular disease (25). It is important to identify individuals at high risk for unfavorable outcomes in women attributed to reproductive factors, which would allow them to benefit from early intervention. Our results indicated that age at first pregnancy might be a significant predictor of a woman's health as well. However, there was inconsistency regarding the association between age at first pregnancy and adverse health

outcomes in later life. A study showed that women who have a first child at an earlier age were more likely to have cardiovascular disease (26). An observational cohort study of healthy aging in Australia found that women who were older when they gave their first birth had lower odds of treatment for high blood pressure compared with women who were younger when they gave birth to their first child (27). The age at first birth was used according to similar studies. Moreover, when we analyzed the association with hypertension using age at first birth rather than age at first pregnancy, the results were similar. We have demonstrated the plausibility of this association that later first pregnancy age might increase the risk of hypertension by adjusting the possible potential factors and stratifying the analysis as much as possible. Furthermore, a previous study showed that one in five rural Chinese postpartum women with a history of gestational diabetes mellitus were found to have elevated blood pressure (28). The likelihood of early ischemic heart disease and stroke is higher in women with a history of hypertension during pregnancy (29, 30). To exclude the effect of gestational diabetes mellitus and gestational hypertension, we further conducted a sensitivity analysis to examine the associations between age at first pregnancy and hypertension or blood pressure indicators after excluding gestational hypertension and gestational diabetes mellitus, the estimated effects of the associations between first pregnancy age and hypertension, SBP, DBP, and MAP did not appreciably change the result after excluding gestational hypertension and gestational diabetes mellitus, and the result further demonstrated the stability and feasibility of our results. A later age of first pregnancy might not only lead to some serious adverse reproductive outcomes (31) but also increase the risk of hypertension later in life. Therefore, an appropriate age at first pregnancy strategy is likely to help provide preventive strategies and improve the future health of women. Before the present study, there was no study carried out in China to investigate the impact of age at first pregnancy on the risk of hypertension, which was useful for the screening and intervention of hypertension among Chinese rural women. However, the conflicting findings from the limited body of previous studies were partly accounted for by the variations in study design, population, and sample size.

Notably, the association between first pregnancy age and hypertension was even stronger in younger women (age < 65 years), which reinforced the idea that age at first pregnancy is an independent influence on hypertension. In addition, we divided the group into two groups based on the first pregnancy outcome: the first pregnancy with the delivery group and the first pregnancy with the abortion group. A meaningful positive association between age at first pregnancy and the risk of hypertension and the levels of SBP, DBP, and MAP was found among women in the first pregnancy with normal delivery. No association was found between age at first pregnancy and both risk of hypertension and blood pressure indicators among women in the first pregnancy miscarriage. However, further analysis of subgroup differences was carried out and no differences were found. Therefore, we could not obtain similar results after stratified analysis that later first pregnancy age might increase the risk of hypertension according to the outcome of the first pregnancy. In addition, the number of women included in this study who had first pregnancy and miscarriage was small. Further exploration and validation in large samples and prospective cohort studies are still needed.

Strengths and limitations

Our study is based on the relatively large sample size of the rural population in China. Standardized investigation tools, training, and on-site implementation, as well as adjustments of a wide range of potential confounding factors, ensure the reliability of the analysis. Furthermore, this is the first description of the association of age at first pregnancy with hypertension for women in rural areas of China. Nevertheless, several limitations should also be considered in the current analysis. First, these findings came from a cross-sectional study, rather than a prospective cohort design, and thus do not accurately describe causality. Second, the participants are mainly rural women in China. Whether the observed association could be applied to other ethnic groups and areas warrants further investigation. Third, although we have comprehensively considered typical risk factors and potential confounders, residual confounding is inevitable owing to the observational study design.

Conclusion

Our study showed that the first pregnancy age might increase the risk of hypertension later in the life of women from Chinese rural areas. First pregnancy age might be an independent risk factor for hypertension in women. However, further prospective studies are anticipated to assess the causality and the specific mechanism of the association.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Henan Rural Cohort Study has been registered at Chinese Clinical Trial Register (Registration Number: ChiCTR-OOC-15006699). Date of registration: 2015-07-06. http://www.chictr.org.cn/showproj.aspx?proj=11375. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZD: data curation, formal analysis, visualization, and writing-original draft. XW: investigation, data curation, methodology, formal analysis, visualization, and writing-original draft. WL and ZH: investigation, validation, and writing-review and editing. JY, XD, HZ, and XL: investigation and writing-review and editing. CW: conceptualization,

methodology, investigation, validation, supervision, funding acquisition, project administration, and writing-original draft. BZ: investigation, data curation, and writing-review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1120732/full#supplementary-material

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Validation of the illustrated questionnaire on food consumption for Brazilian schoolchildren (QUACEB) for 6- to 10-year-old children

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Introduction: Evaluating the food consumption of school-aged children is crucial to monitor their dietary habits, promote targeted interventions, and contribute public policies that aimed healthy eating. In this context, our objective was to develop and validate the Illustrated Questionnaire on Food Consumption for Brazilian Schoolchildren (QUACEB) of 6 to 10 years old, which is a self-reported illustrated recall.

Methods: Validity was obtained in four stages as follows: selection of foods, validation of items, validation of illustrations, and pretest. Foods were selected by considering the data from the main surveys that have been conducted with the Brazilian population and schoolchildren in recent years, the degree of food processing, and the main foods from each of the country's five macroregions. The content of the items was validated by comparing the children's and their parent's responses. For this, the questionnaire was published in an online format, and 6- to 10-year-old elementary schoolchildren were recruited using the snowball technique. The first part of the questionnaire was answered by the parent after the child's lunch, and the second was completed by the child the following day. Thirty-two parent and child dyads participated. Sensitivity, specificity, area under the curve (AUC), and kappa (k) tests were performed.

Results: Of the 30 foods presented on the questionnaire, 15 were reported as consumed. High sensitivity (mean of 88.5%), high specificity (average of 92.0%), substantial agreement ($k = 0.78$), low disagreement (6.2%), and AUC of 0.90 were found. The illustrations were validated in a focus group with fourth-grade children from a school chosen for convenience. The food illustrations were designed for children, who were asked to name the food. Eighteen children participated and verified that the images were representative of the foods. In the pretest, three schools were chosen for convenience that announced the link to the online questionnaire in WhatsApp groups of parents with students from first to fifth grade. Fifteen children answered the questionnaire and 86.7% ($n = 13$) judged it excellent or good.

Conclusion: Thus, the food consumption questionnaire is valid for elementary schoolchildren of 6 to 10 years old and can be applied in research to assess the dietary patterns of children in Brazil.

KEYWORDS

validation study, feeding pattern, diet, food intake, surveys and questionnaires, child, chronic diseases

1. Introduction

Childhood obesity has reached epidemic levels and is a modern public health problem (1, 2). In Brazil, data from the Food and Nutrition Surveillance System (SISVAN in Portuguese) indicate an unfavorable trend toward obesity in Brazilian children between 5 and 10 years old, with a prevalence of 10.45% and 16.96% in a temporal variation between 2008 and 2021, respectively (3). Although the etiology of childhood obesity is complex, poor diet is an important independent risk factor for the development of non-communicable diseases (NCDs) and obesity (4).

The data are related to changes in the quality of the Brazilian diet in recent years, marked by an increase in the consumption of ultra-processed foods (5). In general, ultra-processed foods are highly palatable, are low in fiber, contain excess sugar and/or sodium, and have high levels of total and saturated fats, which add greater energy value to the diet and could increase the risk of chronic diseases (6–8).

The unfavorable dietary nutrient profile of ultra-processed foods impacts the quality of the diet negatively and has direct consequences on health, and their consumption should be continuously evaluated and monitored at this stage of life (9). The most common methods to monitor the food consumption of Brazilian children are the Food Record, the 24-h Dietary Recall (R24 h), and the Food Frequency Questionnaire (10). Structured food consumption questionnaires, such as the R24 h, are a good option for studies that assess student health, as they are simple, practical, and low-cost methods (11).

Most of the tools developed to assess food consumption are not validated and are not intended for school-aged children, or the food list is outdated (12–14). Most of the recent questionnaires validated for school-aged children are from other countries, including Poland (15), Japan (16), Turkey (16), Malaysia (17), Lebanon (18), England (19), Spain (20), Chile (21), and Europe (22). Specifically in Brazil, instruments validated for school-aged children are rare. Studies with children from São Paulo (23, 24), Salvador (25), and Western Amazon (26) stand out. However, all of these questionnaires are semi-quantitative, and none are illustrated. In addition, most respondents are parents (15, 16, 18, 19, 21, 23, 24, 26, 27), but a few have a questionnaire applied directly to children under parental or teacher guidance (17, 18, 20, 22). Burrows et al. (28) showed that the Food Frequency Questionnaire reported by children of 8 to 12 years of age was the closest to the gold standard measure (doubly labeled water method) when compared with the report of children's food consumption by their parents.

However, especially for children aged 6 to 10 years, few instruments are available to collect information on food consumption, especially when the objective is for the child to be the informant. This is explained by the fact that the assessment of food consumption is challenging, considering that children in this age group are not able to provide reliable information on usual intake and serving size, in addition to requiring memory, attention span, motivation, and cognition (29).

The instruments proposed to fulfill this objective are the Previous Day Food Questionnaire (PDFQ) and the Food Consumption and Physical Activity Questionnaire for schoolchildren (Web-CAAFE). The PDFQ is a questionnaire designed for schoolchildren that uses an illustrated recall to qualitatively analyze food consumption on the previous day (30). However, the instrument does not include regional Brazilian foods. The presence of these foods is important to enhance the culture, habits, and food traditions. Web-CAAFE is a software for the qualitative measurement of food consumption through the recall of the previous day. The instrument includes more food options than the PDFQ, including regional ones. However, access is restricted and is through a system with login and password (31).

The importance of research that evaluates the food consumption of schools for carrying out epidemiological studies is undeniable. However, this assessment must be carried out with adequate, updated, and validated instruments, which consider the cognitive limitations of each age. Thus, the objective of this study is to validate an accessible qualitative questionnaire on food consumption for Brazilian children of 6 to 10 years of age.

2. Methods

We developed and validated an illustrated questionnaire to investigate the food consumption of elementary schoolchildren between 6 and 10 years of age. This is a quantitative and qualitative study and was carried out in four stages as follows: (1) selection of foods to develop the questionnaire; (2) validity test of the chosen foods by comparing the children's self-report and their parents' observation; (3) two focus groups with children to validate the illustrations; and (4) pretest.

The project was approved by the Ethics Committee on Research with Humans of the Faculty of Health Sciences of the University of Brasilia (Protocol CAAE 25866919.4.0000.0030). The parents or guardians agreed with the free and informed consent form and the children with the free and informed consent form.

The proposed questionnaire was given the acronym QUACEB, corresponding to the initials of the name in Portuguese: "*Questionário de Consumo Alimentar para Crianças Escolares Brasileiras*" (Illustrated Questionnaire on Food Consumption for Brazilian Schoolchildren).

2.1. Stage 1: QUACEB development

The questionnaire was created according to the following criteria: (a) The most consumed foods were included according to data from the Family Budget Survey (POF in Portuguese) 2017–2018 (32) and the 2015 National School-based Health Survey (PeNSE in Portuguese) (33); (b) To choose the foods, the food groups and the degree of food processing were considered, according to the Dietary Guidelines for the Brazilian Population (7); (c) Later, representative foods from all Brazilian macroregions were inserted (34, 35); and (d) The name of the food was added in uppercase letters as a caption for the figures. The figures were designed by a graphic designer specializing in products for children. Initially, 30 foods or food groups were included, among those most consumed according to the national surveys (Table 2).

2.2. Stage 2: tests to validate the foods in the QUACEB

Evidence of the validity of the foods illustrated was obtained through comparison tests between the parent's account (father, mother, or guardian) who observed the food consumed by the child after lunch and the child's self-report on the following day of the food they had eaten for lunch. The parents' report was considered a gold standard. We chose only one meal to make the instrument faster, simpler, and accessible. Lunch was selected because it is the most consumed meal for Brazilians (32).

The sample was selected for convenience and consisted of dyads of Brazilian parents and children, between 6 and 10 years of age, who had access to the Internet and enrolled in elementary schools in Brazil. The sample size was calculated based on kappa for 2 raters' estimation with an expected kappa (k) equal to 0.75, expected precision of 0.3, the proportion of the outcome (p) of 0.5 (considering the same probability of having or not having the binary outcome), and the confidence level of 99%. The sample size was calculated at 33, and we added 10% to cover drop-outs, totaling 37 dyads of parents and children. We used an online calculator available at https://wnarifin.github.io/ssc/sskappa.html (36). Study participants were recruited using the snowball methodology (37). The snowball is a sampling technique, and the participants recommend the survey to another individual in his network (37). For this, a research poster containing the QRCode for accessing the questionnaire link was prepared. Moreover, the poster was publicized on the researcher's and the university's social networks. In addition, disclosure was carried out in groups of WhatsApp parents from some schools known to the researchers.

On the first day, parents completed their questionnaire immediately after the child's lunch. The questionnaire for the guardians included four screens as follows: (1) the free and informed consent form; (2) identification of data about the child (date of birth, sex, and initial name); (3) characterization of the parent (gender, marital status, age, education, family income in minimum wages, and state residing), education network of the child's school and the child's school year; and (4) information on child's food consumption. The included questions are as follows: whether the child had lunch that day; a list of 30 foods for the parent to mark which of these the child consumed at lunch; and an open-ended question to write down any foods that were consumed but were not on the list.

At the end of the questionnaire, the parent was instructed that the child should answer the questionnaire the next day, in the morning, without any interference.

The child's questionnaire contained three screens as follows: (1) data to identify the child (date of birth, gender, and initials); (2) term of consent of the minor; and (3) information on food consumption. This screen asked what the child had eaten for lunch the day before and contained 30 illustrations of food with captions for the child to select which ones were consumed at lunch the day before, for better understanding and adaptation to the age group.

The researchers used the collection of child identification data in both questionnaires to aggregate the responses obtained on the collection for 2 days and identify the respective parent–child dyads. The questionnaire was accessible for 31 days from September to October 2020.

The responses to the questionnaires automatically generated a database in Microsoft Office Excel format, which was exported to use for the analysis. The tests were performed using the MedCalc software, adopting a significance level of 5%. Data are presented in absolute (n) and relative (%) frequencies.

For the external validity of the questionnaire, the values of sensitivity (the ability to detect the consumption actually presented, i.e., true positives divided by the sum of true positives and negatives), specificity (the ability to indicate no consumption when there were actually none presented, i.e., true negatives divided by the sum of true negatives and false positives), the area under the curve (AUC), and their respective 95% confidence intervals (95% CI) were calculated using the parents' report as the gold standard. The closer the AUC value is to 1, the better the instrument performed (38). Kappa statistics (k) with its 95% CI were also calculated to assess the agreement between the responses of the parent and the child, considering $k = 0$ as an absence of agreement; k between 0.41 and 0.60 for moderate agreement; k between 0.61 and 0.80 for substantial agreement; k between 0.81 and 0.99 for almost perfect agreement; and $k = 1$ for perfect agreement (39).

2.3. Step 3: focus groups to substantiate the validity of QUACEB illustrations

To validate whether the illustrations were representative of the food, two focus groups were conducted in October 2021 with fourth-grade children (9 years old) from a public school in the Federal District, Brazil, chosen for convenience and the fourth-grade class was chosen by the school principal. Two sessions were held in the classroom, and each session lasted for ∼50 min. In the second session, reached information saturation. The participants included nine children in each session and were conducted by three researchers (one moderator and two observers). The sessions were audio-recorded with the children's permission.

The group sessions were organized according to the following steps: presentation of the researchers and the research; clarification of the dynamics of participatory discussion and request for consent for participation and audio recording; presentation of illustrations and individual active listening; and ending by thanking them. The group dynamics to assess students' understanding of the figures occurred through the projection on a classroom wall of 43 illustrations without captions (33 food illustrations after modifications based on the results of the earlier validity test and 10 more regional food groups in Brazil). The group moderator provided the following guidance: "Let's play a guessing game. I'm going to show you some pictures and I would like you to tell me the names of what you see." Then, each figure of food was presented separately. Next, they were asked: "Do you recognize this food? And what is the name of this food?". After the question, it was advised that the child lifts his hand to tell the name of the food being projected. To ensure that everyone participates, we randomly chose a few children to say what they observed. The participants were free to discuss among themselves and actively listen.

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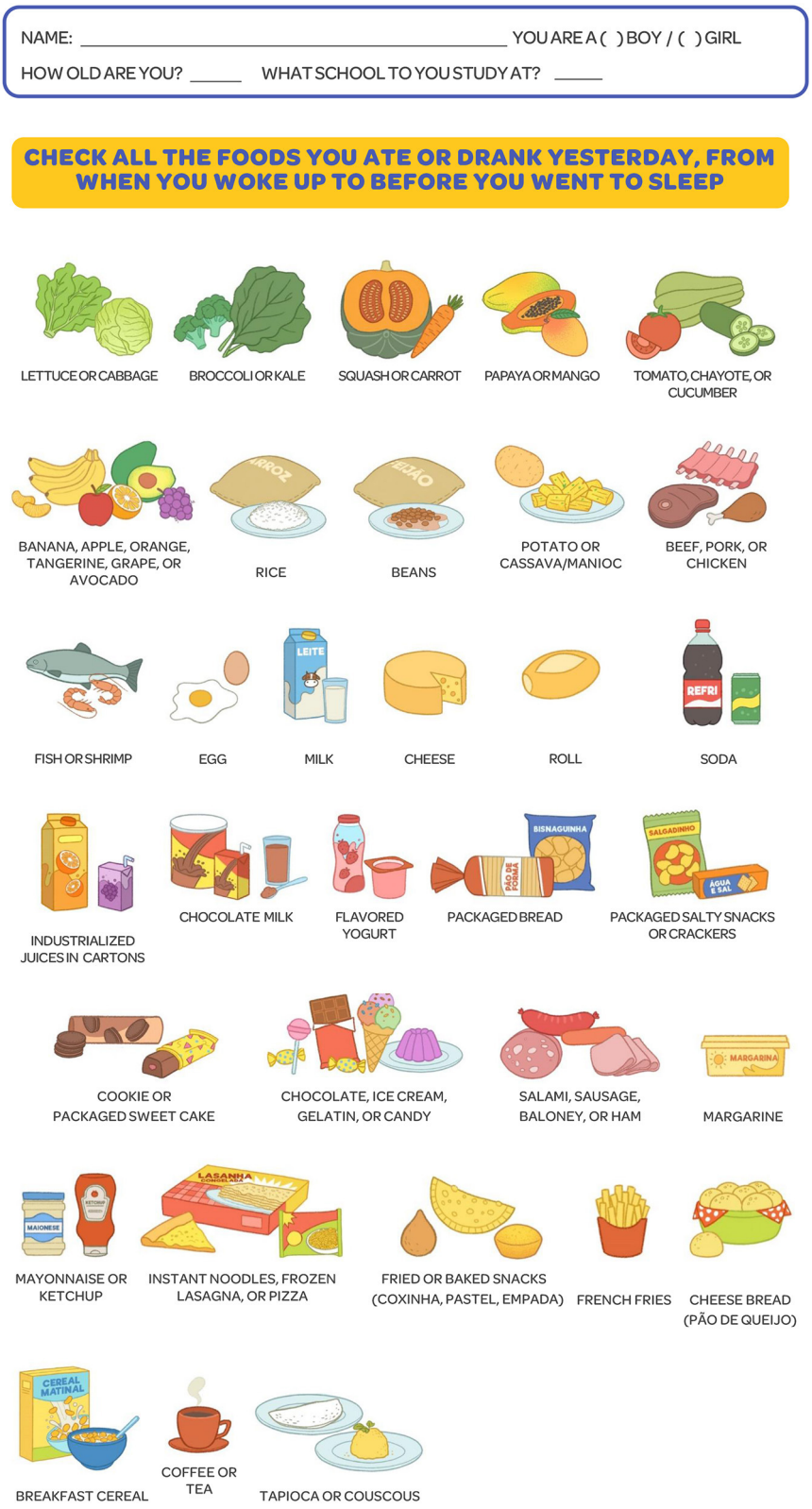

FIGURE 1
Illustrated Questionnaire on Food Consumption for Brazilian Schoolchildren (QUACEB), 2021.

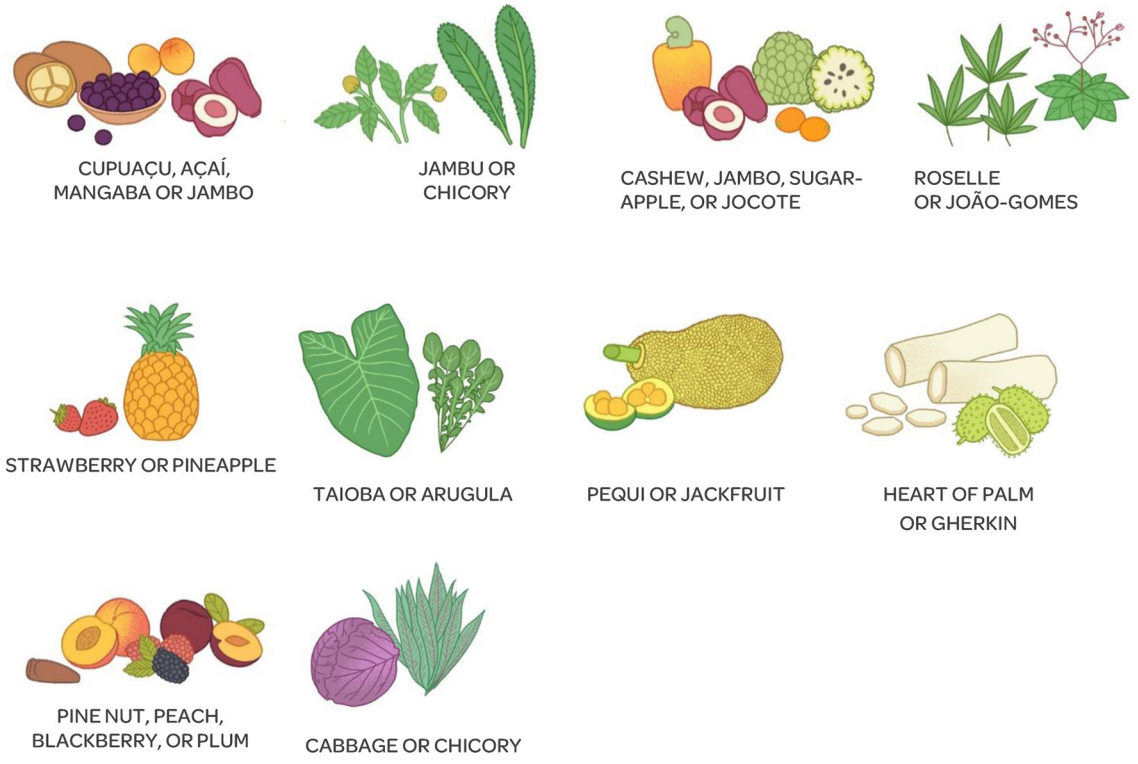

FIGURE 2
Additional module with figures of regional foods that can be inserted in the Illustrated Questionnaire on Food Consumption for Brazilian Schoolchildren (QUACEB).

2.4. Stage 4: QUACEB pretest

The Illustrated Questionnaire on Food Consumption for Brazilian Schoolchildren was built on the Google Forms platform, with items, writing, and illustrations modified according to the results of the previous stages. In addition, information on the age and gender of the child was included, as well as the number of meals eaten on a typical day and possible foods consumed the previous day which were not included in the questionnaire. Three schools were chosen for convenience, one public, located in the Federal District, Brazil, and two-thirds of the sample coming from private schools located in a small municipality in the State of Goiás, Brazil. The schools were contacted *via* telephone and agreed to publicize the research link in the WhatsApp groups of parents in the elementary school (from the first to the fifth school year). The questionnaire link along with a link to publicize it was provided to the schools. The link was available for 20 days in November 2021. The pretest was applied to test the application format, i.e., whether children could fill out the online questionnaire alone or under their guardian's supervision just to help read the questions. For this, an orientation was written for parents to deliver their cell phones or computers for children to fill out the questionnaire alone, and whether necessary adults can ask them the questions without interfering with the child's answers. At the end of the questionnaire, the children were asked what they thought of the questionnaire, with response options on a five-point Likert scale (ranging from 1 "great" to 5 "very bad"), and children were also asked to fill in an open question for suggestions to improve the questionnaire.

3. Results

As a result of the four stages, the instrument was developed for an illustrated self-reported recall, intended for 6- to 10-year-old Brazilian children. It contained a list of 33 groups of national food figures (Figure 1), with the option of adding 10 illustrations of regional fruits and vegetables (Figure 2).

In the online study to validate the content of QUACEB items (the second stage of the study), 32 parent–child dyads participated, most of them from the Federal District (59.38%). Most of the parents were women (93.75%), between 35 and 54 years old (71.87%), had a graduate degree or more (56.25%), were married/in a stable relationship (68.75%), and with a monthly family income above 10 minimum wages (equivalent to R$ 10,450.00 or U$ 2,061) (53.12%). Half of the children were girls (50.00%) and most studied in private schools (78.13%) (Table 1), with a mean age of 8 ± 0.85 years.

Of the 30 food groups initially listed in the instrument, stage 1 of the QUACEB development elucidated that only 15 had a minimum consumption frequency that would allow statistical tests for validation. Comparisons of the responses between children and their guardians indicated frequent consumption (more than 37.5%) of rice, beans, beef/pork/chicken,

TABLE 1 Research stage to validate the content of the Illustrated Questionnarie on Food Consumption for Brazilian Schoolchildren (QUACEB), 2021.

Study variables	*n*	%
Place of residence		
Federal District	19	59.38
Minas Gerais	2	6.25
Rio de Janeiro	1	3.13
São Paulo	1	3.13
Tocantins	9	28.13
Parent's gender		
Female	30	93.75
Male	2	6.25
Age of the parent		
19 to 34 years old	8	25.00
35 to 44 years old	15	46.87
45 to 64 years old	9	28.13
Education of the parent		
Incomplete higher education or less	5	15.63
Complete higher education	9	28.12
Postgraduate degree	18	56.25
Marital status of the parent		
Married/stable union	22	68.75
Single/divorced	10	31.25
Family income (in minimum wages*)		
up to 3	5	15.63
3 to 6	4	12.50
6 to 10	6	18.75
More than 10	17	53.12
Child's gender		
Female	16	50.00
Male	16	50.00
Child's age (Years)		
6	2	6.25
7	9	28.13
8	14	43.75
9	7	21.88
Child's school type		
Private	25	78.13
Public	7	21.87
Child's grade in school (year)		
1st	5	15.63
2nd	6	18.75
3rd	18	56.25
4th	3	9.38

*Minimum Wage at the time of the survey was R$1,045.00, equivalent to U$206.00.
Source: compiled by authors.

juice, and lettuce/tomato. Of the food groups that had low consumption (<3.13%), 10 were not mentioned by parents or children (nuggets/hamburger/pizza/instant noodles, coffee, milk, cookie/packaged sweet cake, breakfast cereal, packaged bread, rolls, couscous/tapioca, cheese bread/coxinha/pig in blanket, and snack chips); three groups were reported only by children (cheese, chocolate milk boxed or powdered/industrialized yogurt, and salami/sausage/baloney/ham); one group was reported only by parents (mango/papaya); and one group was reported by both parents and children (soup) (Table 2).

There was a low disagreement between the answers of the parents and children, with an average of 6.2% and a variation from 0 (broccoli/chayote/kale, egg, fish/shrimp, and soup) to a maximum of 25.0% (juice) (Table 2).

Sensitivity values, i.e., the probability that the children reported what they actually ate as presented by their parents, indicate an average of 88.5% for all food groups. The lowest sensitivity value (50.0%) was found in the soda group and maximum values (100.0%) occurred in the broccoli/chayote/kale, beef/pork/chicken, egg, fish/shrimp, sweets, apple/grape/banana/orange, and pasta groups (Table 2).

The specificity values (average of 92.02%) demonstrated that the questionnaire was able to detect foods that were not consumed when, in fact, there was no consumption. The beef/pork/chicken group had the lowest value for specificity (62.50%), while the beans, broccoli/chayote/kale, egg, fish/shrimp, and soda groups had the highest values (100%) (Table 2).

The indices of the area under the curve (AUC) were employed to verify the global accuracy of the questionnaire, as this parameter considers the simultaneous analysis of the specificity and sensitivity measures for each food item. As shown in Table 2, the egg and fish/shrimp food items had maximum values of sensitivity and specificity and, thus, the highest values for AUC (1.00). On the other hand, the soda and juice groups had the lowest value (0.75). The mean of the 15 groups analyzed was 0.90, indicating the good performance of the instrument for these food items.

The kappa test between the child's and the parent's reports was significant for all items that presented satisfactory consumption for validation (more than 6.25% consumption), with an average of $k = 0.78$ (38). Of the 15 food items analyzed, 7 groups (beans, broccoli/chayote/kale, egg, fish/shrimp, soup, sweets, and pasta) had an "almost perfect or perfect" agreement ($k \geq 0.81$) and only juice obtained a kappa value with moderate classification ($k =$ 0.41–0.60) (Table 2).

Based on the results found for the validity of the items, the following changes were made to the questionnaire. Ten illustrations of regional foods were added—five fruits and five vegetables from each Brazilian macroregion (northern region—cupuaçu/açaí/mangaba/jambo and jambu/chicory; northeast—cashew/jambo/sugar-apple/jocote and roselle/João-Gomes; southeast —strawberry/pineapple and taioba/arugula; central-west—pequi/jackfruit and heart of palm/herkin; and south—pine nut/peach/blackberry/plum and cabbage/chicory). This consequently caused the pequi to be removed from the squash and carrot group; reformulation of groups of raw vegetables, including tomato and chayote, in a specific grouping with cucumber; inclusion of groups of dark green vegetables, including

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TABLE 2 Analysis of disagreement, sensitivity, specificity, area under the curve, and kappa between the reports of children and their parents participating in the validation survey of the Illustrated Questionnarie on Food Consumption for Brazilian Schoolchildren, 2021.

Food	Report (%)		Disagreement (%)	Sensitivity $CI_{95\%}$	Specificity $CI_{95\%}$	AUC $CI_{95\%}$	Kappa $CI_{95\%}$
	Child	Parent					
Rice	81.25	81.25	6.16	96.15 (80.4–99.99)	83.33 (35.9–99.6)	0.90 (0.74–0.98)*	0.79 (0.52–1.00)*
Beans	75.00	71.88	3.13	95.83 (78.9–99.99)	100.00 (63.1–100.00)	0.98 (0.85–1.00)*	0.92 (0.77–1.00)*
Toasted manioc	9.38	9.38	6.16	66.67 (9.40–99.20)	96.55 (82.20–99.90)	0.82 (0.64–0.93)¥	0.63 (0.16–1.00)*
Lettuce/tomato	37.50	37.50	12.50	83.33 (51.60–97.90)	90.00 (68.30–98.80)	0.87 (0.70–0.96)*	0.73 (0.49–0.98)*
Broccoli/chayote/ kale	12.50	12.50	0	100.00 (39.8–100.00)	100.00 (87.70–100.00)	1.00 (0.89–1.00)*	1.00 (1.00–1.00)*
Squash/carrot/ pequi	18.75	25.00	12.50	83.33 (35.90–99.60)	88.46 (69.80–97.60)	0.86 (0.69–0.96)¥	0.63 (0.31–0.96)*
Beef/pork/ chicken	75.00	84.38	9.38	100.00 (85.80–100.00)	62.50 (24.50–91.50)	0.81 (0.64–0.93)*	0.71 (0.42–1.00)*
Egg	9.38	9.38	0	100.00 (29.20–100.00)	100.00 (88.10–100.00)	1.00 (0.89–1.00)*	1.00 (1.00–1.00)*
Fish/shrimp	6.25	6.25	0	100.00 (29.20–100.00)	100.00 (88.10–100.00)	1.00 (0.89–1.00)*	1.00 (1.00–1.00)*
Soup	3.13	3.13	0	BC	BC	BC	1.00 (1.00–1.00)*
Nuggets/ hamburger/ pizza/ instant noodles	0	0	0	BC	BC	BC	BC
Soda	6.25	3.13	3.13	50.00 (1.30–98.70)	100.00 (88.40–100.00)	0.75 (0.57–0.88)¥	0.65 (0.02–1.00)*
Juice	53.13	53.13	25.00	76.47 (50.10–93.20)	73.33 (44.90;92.20)	0.75 (0.56–0.88)**	0.50 (0.20–0.80)**
Coffee	0	0	0	BC	BC	BC	BC
Sweets	9.38	12.50	3.13	100.00 (29.20–100.00)	96.55 (82.20–99.90)	0.98 (0.86–1.00)*	0.84 (0.53–1.00)*
Milk	0	0	0	BC	BC	BC	BC
Cheese	3.13	0	3.13	BC	BC	BC	0.00 (0.00–0.00)€
Chocolate milk boxed or powdered/ industrialized yogurt	3.13	0	3.13	BC	BC	BC	0.00 (0.00–0.00)€
Cookie/packaged sweet cake	0	0	0	BC	BC	BC	BC
Breakfast cereal	0	0	0	BC	BC	BC	BC
Packaged bread	0	0	0	BC	BC	BC	BC
Roll	0	0	0	BC	BC	BC	BC
Apple/grape/ banana/orange	6.25	9.38	3.13	100.00 (15.80;100.00)	96.67 (82.80;99.90)	0.98 (0.86;1.00)*	0.78 (0.38–1.00)*
Pasta	9.38	12.50	3.13	100.00 (29.20–100.00)	96.55 (82.20–99.90)	0.98 (0.86–1.00)*	0.84 (0.53–1.00)*
Couscous/Tapioca	0	0	0	BC	BC	BC	BC
Cheese bread/coxinha/pig in blanket	0	0	0	BC	BC	BC	BC
Salami/sausage/ baloney/ham	3.13	0	3.13	BC	BC	BC	0.00 (0.00–0.00)€
mango/papaya	0	3.13	3.13	BC	BC	BC	0.00 (0.00–0.00)€

(Continued)

TABLE 2 (Continued)

Food	Report (%)		Disagreement (%)	Sensitivity $CI_{95\%}$	Specificity $CI_{95\%}$	AUC $CI_{95\%}$	Kappa $CI_{95\%}$
	Child	Parent					
Cassava/manioc/ potato	12.50	12.50	6.26	75.00 (19.40–99.40)	96.43 (81.70–99.90)	0.86 (0.69–0.95)**	0.71 (0.34;1.00)*
Snack chips	0	0	0	BC	BC	BC	BC
All			6.24	88.45	92.02	0.90	0.78

AUC, area under the curve; $CI_{95\%}$- 95% confidence interval; LC, low consumption reported and observed (statistical tests could not be performed).
*$p < 0.001$; **$p < 0.01$; and ¥ $p > 0.05$; €p-value not obtained due to insufficient sample.
Source: completed by authors.

broccoli and kale, and of light green vegetables, containing lettuce and cabbage. Toasted manioc, soup, nuggets, hamburgers, açaí preparation, popsicle, pasta, salami, and pigs in blanks were removed to make fewer items on the list, along with tangerine and avocado in the fruit grouping, potato chips, mayonnaise and ketchup, margarine, frozen lasagna, tea, fried pastry, and baked pastry. With these changes, QUACEB now contains 43 illustrations of food groups, 33 of which are national food groups and 10 are regional food groups.

In the focus group for the illustration validation stage, the 43 updated illustrations were presented to the children. The figures that were difficult to recognize were raw cassava/manioc, jocote, açaí, and mangaba, due to the disproportionate size of the food in the drawing and the group of pine nuts. Thus, the students gave suggestions to improve the images, which were accepted, and the drawings were redone. Therefore, in the final version of QUACEB, a bowl of boiled cassava/manioc was used; jocote, açaí, and mangaba were resized; and only one pine nut was captured. Some images of regional foods including the heart of palm, gherkin, taioba, jambu, chicory, roselle, and João-Gomes were not recognized because children were unaware of the food itself. During the focus groups, the children also presented suggestions for the captions. From this, the following changes were made to the caption: the description of the term "pão de sal" was included in the image of rolls; including the two terms "*biscoito*" and "*bolacha*" (both of which regional words for cookie) in the corresponding image; and the nomenclature of industrialized yogurt was changed to flavored yogurt and from boxed chocolate to chocolate milk. With the focus groups, we concluded that the students satisfactorily understood most of the images, and the need to change some figures and legends was raised, providing final improvements to the questionnaire.

In the pretest, 15 children who participated had a mean age of 9 years ± 1.13, mostly boys (66.7%), 8 of whom studied in the public school and 7 in the two private schools. Most reported an average consumption of 4 ± 0.80 meals per day, and all reported eating breakfast and lunch. Of the 33 national food groups listed, 29 had a frequency of consumption reported on the previous day. Among these, the most consumed were beef, pork, or chicken (86.67%); rice (80.00%); beans (66.67%); and milk (66.67%). The least consumed foods (6.67%) were broccoli or kale; egg; packaged salty snacks or crackers; instant noodles, frozen lasagna or pizza; fried or baked snacks (coxinha, pastel, and empada); French fries; and cheese bread. Regarding regional foods, only one child (6.67%) reported consumption of the following groups: cupuaçu, açai, mangaba, or jambo; and pine nuts, peaches, blackberries, or plums (Table 3). Four children recorded the consumption of other foods that were not on the list, namely, water, cotton candy, cheese cracker, and macaroni. Regarding the evaluation of the questionnaire, 86.7% of the participating children judged the questionnaire as excellent or good and did not register possible suggestions.

4. Discussion

The present study demonstrated that the illustrated Questionnaire on Food Consumption for Brazilian Schoolchildren (QUACEB) was valid for schoolchildren. Overall, the instrument achieved good performance, according to the sensitivity, specificity, kappa, and AUC indices, in addition to having a low discordance value. Furthermore, the graphic representations proved to be understandable and attractive to the children.

Currently, validated and illustrated questionnaires with children from southern Brazil in which the child is the respondent include the Previous Day Food Questionnaire (PDFQ) (31, 40, 41); the Typical Day of Physical Activity and Food Intake (DAFA) questionnaire (42) and its electronic version—the WEBDAFA (43); and the Food Intake and Physical Activity of School Children (Web-CAAFE) (31, 44). PDFQ and DAFA contain a list of foods that are repeated in the six daily meals (breakfast, morning snack, lunch, afternoon snack, dinner, and bedtime snack). The difference is that the QUADA assesses the consumption of the previous day and the DAFA of a regular day (30, 42). WEBDAFA has the same structure as the printed instrument but is hosted on a website; however, the interface is currently not available (43). Web-CAAFE has been enhanced from the PDFQ and DAFA experience, is a recall of the day before hosting, and is hosted on a website. However, it is necessary to register in the system to issue a password, and currently, the system only monitors schools in the municipal education network of Florianopolis (31).

Another aspect considered necessary for a food consumption assessment questionnaire for schoolchildren is that it allows the analysis of consumption according to the degree of food processing. The NOVA classification, adopted in the Dietary Guidelines for the Brazilian Population (7), has already been widely described in the literature as important for public health, considering that the consumption of ultra-processed foods has been associated with several chronic diseases at different stages of life (6, 45, 46). The

TABLE 3 Frequency of food consumption reported by children participating in the QUACEB pre-test of Brazil, 2021.

Food groups	Frequency	
	n	%
Lettuce or cabbage	3	20.00
Broccoli or kale	1	6.67
Squash or carrot	4	26.67
Papaya or mango	0	0.00
Tomato, chayote, or cucumber	4	26.67
Banana, apple, orange, tangerine, grape or avocado	7	46.67
Rice	12	80.00
Beans	10	66.67
Potato or cassava/manioc	2	13.33
Beef, pork, or chicken	13	86.67
Fish or shrimp	0	0.00
Egg	1	6.67
Milk	10	66.67
Cheese	4	26.67
Roll	8	53.33
Soda	6	40.00
Industrialized juices in cartons	3	20.00
Chocolate milk	8	53.33
Flavored yogurt	0	0.00
Packaged bread	2	13.33
Packaged salty snacks or crackers	1	6.67
Cookie or packaged sweet cake	4	26.67
Chocolate, ice cream, gelatin, or candy	7	46.67
Salami, sausage, baloney, or ham	6	40.00
Margarine	5	33.33
Mayonnaise or ketchup	4	26.67
Instant noodles, frozen lasagna, or pizza	1	6.67
Fried or baked snacks (coxinha, pastel, and empada)	1	6.67
French fries	1	6.67
Cheese bread	1	6.67
Breakfast cereal	3	20.00
Coffee or tea	5	33.33
Tapioca or couscous	0	0.00
Cupuaçu, açaí, mangaba, or jambo fruits	1	6.67
Jambu or chicory	0	0.00
Cashew, jambo, sugar-apple, or jocote	0	0.00
Roselle or João-Gomes	0	0.00
Strawberry or pineapple	0	0.00
Taioba or arugula	0	0.00
Pequi or jackfruit	0	0.00
Heart of palm or gherkin	0	0.00
Pine nut, peach, blackberry, or plum	1	6.67
Cabbage or chicory	0	0.00

(Continued)

TABLE 3 (Continued)

quantitative questionnaire considered the extent and purpose of food processing to assess the usual diet of schoolchildren of 9 to 10 years of age constructed by Amorim et al. (47). The results showed that the children in the study were able to respond without the support of their parents. However, the instrument has not yet been validated, is restricted to children in a very narrow age group, and is not illustrated (47).

Among the ultra-processed foods evaluated, the consumption of industrialized juices, sweets, and soda was observed at lunch. The other foods in this category that were initially included in the list of 30 food groups in the questionnaire had no consumption reported by the participants. A study that evaluated the intake of ultra-processed foods in 105 schoolchildren of 7 to 10 years of age from a public district located in Teresina, Piaui, highlights the participation of these three groups in the list of the ultra-processed foods most consumed by the public evaluated (45). This again reinforces the need for instruments such as the questionnaire developed and validated here, which can detect food consumption according to the degree of processing (30, 48).

Although there are no population studies in Brazil with children aged 5 to 9 years, other studies find similar patterns of food consumption among schoolchildren. For example, a study carried out in a Brazilian municipality in 2007, with children aged 7 to 10 years, found that the most consumed foods at lunch and dinner were rice, beef or poultry, beans, soft drinks, and pasta (49). In another study conducted in Brazil in 2017, with children aged 7 to 13 years, the foods that had the highest average daily frequency of consumption were rice, bread, beef/chicken, and beans (50). Furthermore, the foods most commonly consumed by the Brazilian population are rice, beans, beef or poultry, and bread (32). In this study, the most consumed foods were beef, pork or chicken, rice, beans, and milk.

It is important to emphasize that comparisons between the results for the validation of this questionnaire with other validated instruments are limited due to methodological differences, especially in the reference method used and the age group covered. Even so, the kappa values were similar to the validation results of the last version of PDFQ (30), obtaining the same value of the agreement test for the fruit group ($k = 0.78$). Other foods, such as the meat and pasta group, were also similar in both studies for this variable, with $k = 0.69$ and $k = 0.81$ being found in PDFQ, respectively, for the items mentioned and $k = 0.71$ and $k = 0.84$ obtained in this validation.

The kappa value was lower for the juice group, and it had the greatest disagreement in responses between the reports of the children and their parents. The data found highlight a

discrepancy, which could be the different interpretations of this drink for parents and children within the analyzed group. The caption of this group "juice" could be interpreted as encompassing various preparations, such as fresh juice, industrialized juice, and concentrated drink. The initial illustration only contained an image of a box of juice, which could be interpreted exclusively as this type of preparation. Thus, we understand that a more specific description of this item and the adequacy of the illustration were necessary to reduce different interpretations and produce better levels of agreement.

The developed questionnaire is inexpensive and easy to apply, which has been demonstrated in previous studies with similar questionnaires (30, 43). The computerized format saves application time, eliminates interviewer-related biases, and ensures automated storage of collected information. Few validated online instruments are available that assess food consumption, especially for school-aged children (29, 30, 43).

The access of Brazilian students to new information and communication technologies has increased in significant proportions (51), which makes online self-report instruments useful and promising to assess the eating habits of the public. Studies state that the application of an online questionnaire is a promising alternative that helps to keep children's attention on the research (52, 53).

Another advantage of the developed and validated questionnaire is that, unlike traditional paper questionnaires, the online format enables data collection from different Brazilian regions. This will allow the inclusion of multiple cultural spheres and facilitate the generalization of results to the general population in future studies with a larger sample size. The possibility of including fruits and vegetables typical of all Brazilian regions, such as pequi, jackfruit, avocado, the heart of palm, and gherkin from the central-west; cashew, jambo, jambu, sugar-apple, jocote, roselle, and João-Gomes from the northeast; cupuaçu, açaí, mangaba, jambo, jambu, and chicory from the north; strawberry, pineapple, avocado, taioba, and arugula from the southeast; and pine nut, peach, blackberry, plum, cabbage and chicory from the south, is also noteworthy to allow the regionalization of the questionnaire with the inclusion of regional foods (33, 34).

This work differs from other validation studies of children's questionnaires, as it proposes to evaluate children's food consumption when they are outside the school context, both in person and online, considering the classification of foods according to the degree of processing recommended by the Dietary Guidelines for the Brazilian Population (7), allows the inclusion of regional foods, and describes the food in the caption. Thus, the instrument can serve as support material for future epidemiological studies of health and nutrition and nutritional intervention programs for this age group.

When using QUACEB, food consumption can be analyzed according to different markers, for example by the NOVA score (46), the classification proposed by the Dietary Guidelines for the Brazilian Population (41), the food diversity score (54), markers of protective foods and risk of excess body fat (55–58), nutritional profile represented by nutrient sources in food groups (19), food-based classification of eating episodes (FBCE) (42, 59), identifying dietary patterns (60), or describing the consumption of regional foods.

The weaknesses of the developed and validated questionnaire include the lack of information about the portion size or the possibility of estimating the child's energy consumption. However, it allows a qualitative assessment of children's consumption, in a brief and straightforward way, in which the researcher can provide reliable data on food consumption, seeking to avoid biasing the child's memory. Other limitations of this work include the lack of presentation of internal validity, only external due to the small sample size and the presence of foods with infrequent consumption, but the most frequent foods were validated and this would possibly happen for other foods; the absence of sensitivity, specificity, AUC, and kappa analyzes by gender and age, due to the insufficient sample; and the lack of exploration of factors associated with disagreements due to the low prevalence and sample size. Furthermore, validation occurred only by testing a meal and a 1-day period. Thus, future studies should be carried out with a larger sample, all meals of the day (to assess consumption of items not reported at lunch), and also test–retest style analysis, whereby participants fill it in for a number of multiple days to see how accurate it is over 1 day. Despite acknowledging that children within the study's age group lack purchasing power and parents are conscious of their children's dietary consumption, we recommend that future validation studies incorporate direct observation throughout the entire day to assess the child's food consumption.

In conclusion, the illustrated online food consumption questionnaire demonstrated adequate concordance, sensitivity, specificity, and area under the curve values to assess the Illustrated Questionnaire on Food Consumption for Brazilian Schoolchildren of 6- to 10-year-old when compared with the parents' report. The QUACEB is a valid, simple, brief, practical, easy-to-apply questionnaire available on the Internet in any Brazilian region, which can be adopted for epidemiological research to assess the diet of that population. This tool is specifically designed to be appropriate to Brazil because it represents the foods most consumed by Brazilian schoolchildren.

Data availability statement

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

Ethics statement

The studies involving humans were approved by Ethics Committee on Research with Humans of the Faculty of Health Sciences of the University of Brasilia (Protocol CAAE 25866919.4.0000.0030). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

NT and MG worked on the project design. GO, MS, DB, and GA participated in data collection and analysis. RS performed the

statistical analyses. All authors helped with the data interpretation and writing of the article, reviewed and approved the final version, and verified that they were responsible for all aspects of the study in guaranteeing the accuracy and integrity of any part of the study.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1051499/full#supplementary-material

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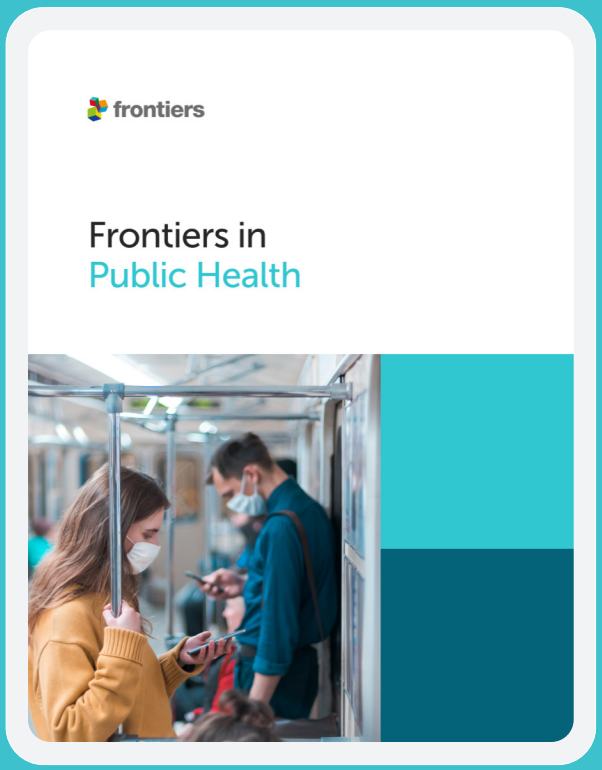

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