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# Clinical implications of obesity and lipid-related parameters on cardiometabolic diseases

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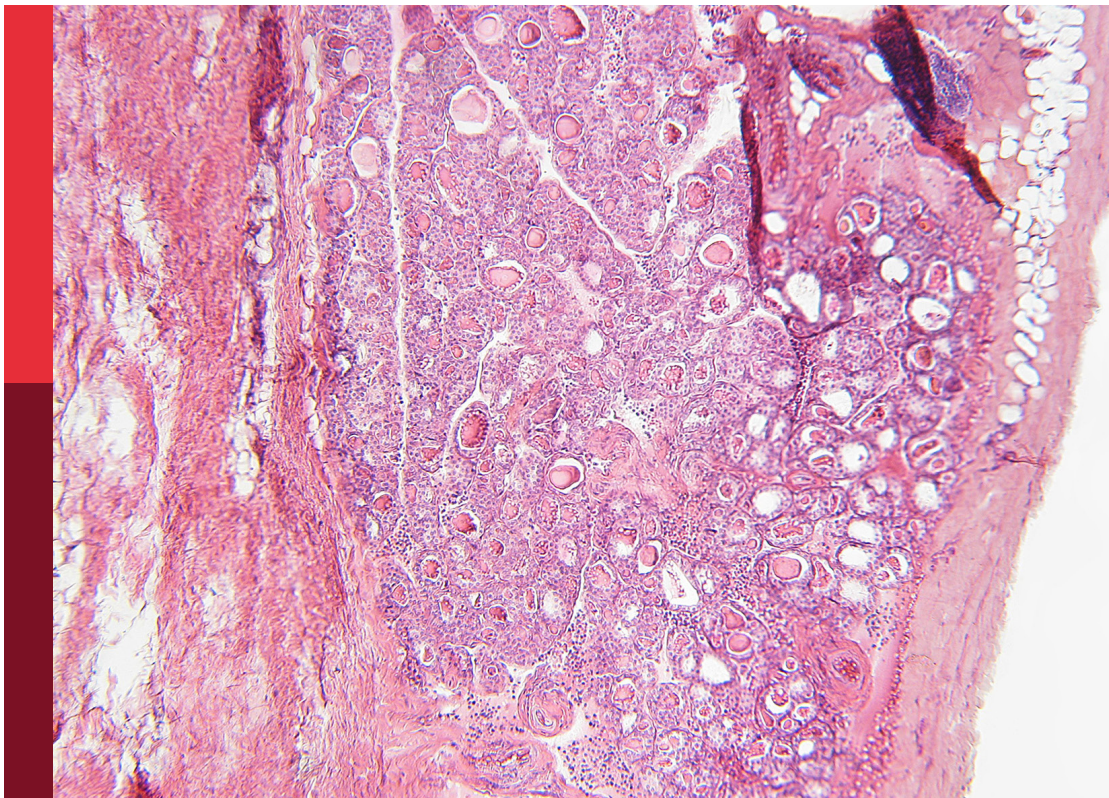

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# Clinical implications of obesity and lipid-related parameters on cardiometabolic diseases

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# Visceral adiposity measures are strongly associated with cardiovascular disease among female participants in Southwest China: A population-based prospective study

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**Background and aims:** Controversy remains regarding the prediction effects of different adiposity measure indicators for the risk of cardiovascular disease (CVD). Our study aimed to assess the associations of three traditional anthropometric indicators, namely, waist circumference (WC), waist-to-height ratio (WHtR), and body mass index (BMI) as well as three non-traditional anthropometric indicators, namely, the Chinese visceral adiposity index (CVAI), lipid accumulation product (LAP), and body shape index (ABSI), with the risk of CVD among Southwest Chinese population.

**Methods:** Our study was based on the Guizhou Population Health Cohort Study (GPHCS) conducted from 2010 to 2020. A total of 9,280 participants were recruited from 12 areas in Guizhou Province, China, from November 2010 to December 2012, and followed up for major chronic diseases until December 2020. A total of 7,837 individuals with valid data were included in this analysis. The gender-specific associations of WC, WHtR, BMI, CVAI, LAP, and ABSI with CVD were evaluated using Cox proportional hazards models. Receiver operating characteristic (ROC) curve analysis was used to estimate the prediction powers of different indicators for CVD.

**Results:** No association of six indicators with CVD was observed among male participants. Female participants with either WC-based central obesity (HR: 1.82, 95% CI: 1.12–2.97) or WHtR-based central obesity (HR: 1.68, 95% CI: 1.07–2.64) had a higher risk of CVD, after adjusted for age, area, ethnic group, smoking, alcohol drinking, MET, previous history of diabetes, hypertension and dyslipidemia, medication use, and nutraceutical intake. Compared with female participants in the lowest quartile (Q1), those in the highest quartile (Q4) of WHtR (HR: 2.24, 95% CI: 1.17–4.27), CVAI (HR: 3.98, 95% CI: 1.87–8.49), and ABSI (HR: 1.94, 95% CI: 1.06–3.52) had an increased risk for incident CVD. CAVI

showed the maximum predictive power of CVD with the biggest AUC of 0.687 (95% CI: 0.654–0.720) compared to other indicators in female participants.

**Conclusions:** Visceral adiposity measures, especially CVAI, are stronger predictive indicators of CVD among female and not male participants in Southwest China. Different anthropometric indexes need to be combined to comprehensively assess health risks.

KEYWORDS

visceral adiposity, anthropometric, cardiovascular disease, southwest China, cohort study

## Introduction

Cardiovascular disease (CVD) remains the leading cause of mortality and morbidity worldwide, with ischemic heart disease (IHD) and stroke as the main contributors (1). It was estimated that CVD caused 17.8 million deaths in 2017 worldwide (2), and 4 million deaths in 2016 in China (3). The burden of IHD and stroke in China has rapidly and substantially increased during the past two decades (4). IHD caused more than 1 million deaths per year, and the number of individuals with acute myocardial infarction (AMI) will increase to 23 million by 2030 (5). Unlike in Western countries, the epidemic profile of stroke in China surpasses that of IHD, with annual estimates of 11 million prevalent cases, 2.4 million new cases, and 1.1 million deaths (6). Adiposity is an imbalance between energy intake and metabolism expenditure resulting in abnormal fat accumulation. The prevalence of adiposity has reached nearly 33.3% worldwide, doubling since 1980, and is generally highest in developed countries and increasing in Asian countries (7). Two types of obesity, central (visceral) and general (peripheral) obesity, are often assessed by waist circumference (WC) and body mass index (BMI), respectively. Based on BMI criteria, the prevalence was 34.3% for overweight and 16.4% for obesity in Chinese adults (≥18 years) (8).

In fact, the distribution rather than the mount of adipose tissue may have a more critical effect on the development of CVD. Imaging-based assessments of subcutaneous adiposity tissue (SAT) and visceral adiposity tissue (VAT) by routine clinical practices, including computed tomography (CT), magnetic resonance imaging (MRI), dual-energy x-ray absorptiometry (DEXA), and dual bioelectrical impedance analysis (BIA), were largely limited due to their higher costs, related technical challenges, and potential radiation exposure risk. Previous research debated the prediction values of several common anthropometric indicators of adiposity for CVD (9–11); some suggested that waist-to-height ratio (WHtR) was superior to WC and BMI (12, 13). These results had significant heterogeneities as they covered different ethnic populations (14). Recently, visceral adiposity index (VAI), lipid accumulation product (LAP), and body shape index (ABSI), which are the products of WC, BMI, and blood lipids, have been proposed as reliable indexes of body fat accumulation, and they have been applied to the prediction of diabetes (15–17). With reference to VAI and considering the characteristic of body fat in the Asian population, the Chinese visceral adiposity index (CVAI) has been designed for the Chinese population; this surrogate indicator may be more sensitive than VAI, WC, and BMI to discriminate diabetes (18). However, studies investigating the associations between these novel indicators and CVD are limited.

Growing researches suggested that adiposity was significantly associated with CVD and CVD-related risk factors in East China (19–21). The disease burden and risk profiles for CVD vary geographically in China, with higher incidences but less healthcare services in southwestern provinces compared with eastern regions (22–24). However, limited knowledge is available on the effects of adiposity on CVD risk in Southwest China. Guizhou Province lies to the east of the Yunnan-Guizhou Plateau in Southwest China, with complex topography, poor transportation system, and undeveloped economic and educational level, leading to deficiency in medical resources. There are 56 ethnic groups in Guizhou Province, the majority of which are Han. The diet and living habits of different ethnic groups are different, and some prefer pickled food, which increases the risk of hypertension and stroke (25). In this study, we

**Abbreviations:** CVD, cardiovascular disease; SD, standard deviation; WC, waist circumference; WHtR, waist-to-height ratio; BMI, body mass index; CVAI, Chinese visceral adiposity index; LAP, lipid accumulation product; ABSI, body shape index; FPG, fasting plasma glucose; 2h-PG, 2-h postload glucose; TG, triglyceride; CHOL, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; MET, metabolic equivalent of task; ROC, receiver operator characteristic; AUC, area under the ROC curve.

aimed to provide an insight to explore the associations of several anthropometric indicators with cardiovascular onsets, by using data from a large cohort study in Guizhou Province, Southwest China.

# Methods

## Study population and data collection

The Guizhou Population Health Cohort Study (GPHCS) is a good representation of the geographic, socio-demographic, ethnic composition of the adult population in Guizhou Province in Southwest China, enrolling a total of 9,280 adults at baseline between November 2010 and December 2012, from 12 areas (5 urban districts and 7 rural counties) in Guizhou Province using the multistage proportional stratified cluster sampling method, considering population size, population stability, and local capacity. The inclusive criteria were as follows: (1) age of 18 years or above; (2) living locally for more than 6 months and having no plan to move out; (3) completing survey questionnaire, blood sampling, and physical examination; and (4) signing the written informed consent. All participants were followed up for major chronic diseases and vital status through a repeated investigation by trained investigators between 2016 and 2020, and record linkage to the Death Registration Information System and Basic Public Health Service System. Ethics approval was obtained from the ethics review board of Guizhou Province (No.S2017-02).

In this study, we excluded participants with a previous diagnosis of CVD, those with missing data of anthropometric measurements and CVD, or those lost to follow-up. Finally, 7,837 participants were included in the primary analyses (Figure 1).

## Outcomes of interest

The primary outcomes were the first occurrence of cardiovascular events, including stroke and IHD. The main types were ischemic stroke (I63), hemorrhagic stroke (I60–61), and myocardial infarction (I21), coded by International Classification of Diseases 10th revision (ICD-10). All reported CVD events were identified using imagological diagnosis by trained clinical staff. The person-year (PY) of follow-up was calculated from the date of baseline investigation to the date of the occurrence of CVD, death, or follow-up, whichever came first. Incidence rate was calculated as the number of incident cases divided by follow-up PYs.

## Anthropometric measurements and laboratory biochemical information

Anthropometric measurements, including standing height (cm), weight (kg), and WC (cm), were taken by trained health professionals according to standard protocols. Standing height and weight were measured with participants standing without shoes and in lightweight clothes. WC was measured on the midaxillary line between the lowest border of the rib cage and the top of the iliac crest. All parameters were recorded as the mean value of the twice measurements, and usually to the nearest 0.1 cm or 0.1 kg. Blood pressure was measured three

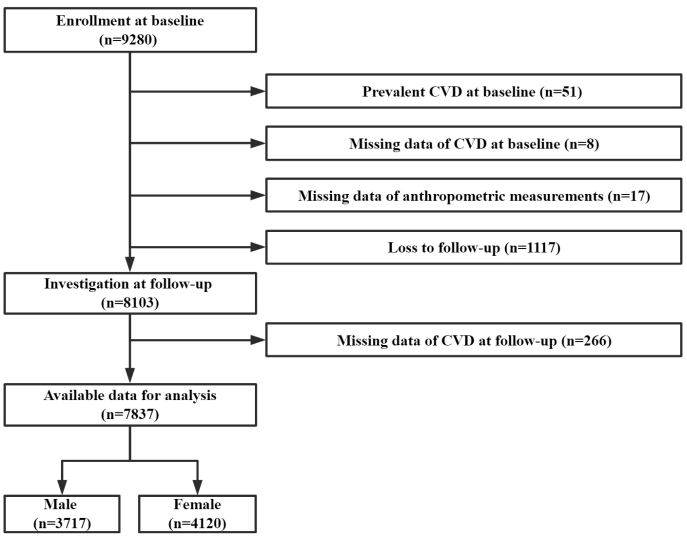


FIGURE 1
Flowchart of the study.

times in a 3-min interval from the left arm after the participant rests in a seated position; the recorded values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were calculated as the mean of the last two of three consecutive measurements.

All participants provided a 10-ml blood sample after an overnight fast of at least 10 h, they also undergo an oral glucose tolerance test (OGTT), and the plasma was obtained at 2 h during the test. Concentrations of fasting plasma glucose (FPG), 2-h postload glucose (2h-PG), and Hemoglobin A1c (HbA1c) were analyzed locally within 2 h after the blood sample collected using the glucose oxidase methods (Roche Diagnostics, Mannheim, Germany). Serum triglycerides (TG), total cholesterol (CHOL), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using enzymatic methods (Roche Diagnostics, Mannheim, Germany).

Traditional and non-traditional anthropometric indicators were calculated by the following formula:

$$\mathrm{WHtR} = \mathrm{WC} \div \mathrm{height}; \tag{1}$$

$$\mathrm{BMI}\left(\mathrm{kg/m^2}\right) = \mathrm{weight} \div (\mathrm{height} \div 100)^2; \tag{2}$$

$$\begin{aligned}\mathrm{CVAI} = {} & -267.93 + 0.68 \times \mathrm{Age} + 0.03 \times \mathrm{BMI} + 4.00 \\ & \times \mathrm{WC} + 22.00 \times \mathrm{LgTG} - 16.32 \\ & \times \mathrm{HDL(for\ male\ participants);\ CVAI} \\ = {} & -187.32 + 1.71 \times \mathrm{Age} + 4.23 \times \mathrm{BMI} + 1.12 \\ & \times \mathrm{WC} + 39.76 \times \mathrm{Lg\ TG} - 11.66 \\ & \times \mathrm{HDL(for\ female\ participants);}\end{aligned} \tag{3}$$

$$\begin{aligned}\mathrm{LAP} = {} & (\mathrm{WC} - 65) \times \mathrm{TG(for\ male\ participants);\ LAP} \\ = {} & (WC - 58) \times \mathrm{TG(for\ female\ participants);}\end{aligned} \tag{4}$$

$$\mathrm{ABSI} = \mathrm{WC}/\left(\mathrm{BMI}^{2/3} \times \mathrm{height}^{1/2}\right). \tag{5}$$

## Other data collections

Standardized in-person interviews using structured questionnaires were conducted for each participant to obtain the socio-demographic (age, gender, area, ethnic group, education level, marriage status, and occupation type), lifestyle (physical activity, tobacco smoking, and alcohol use), comorbidity status (diabetes, hypertension, and dyslipidemia), medication use, and nutraceutical consumption information.

Smoking was defined as smoking at least one cigarette a day for 12 months or more. Alcohol drinking was defined as drinking at least three times a week for 12 months or more. The physical activity level was calculated as the product of the duration and frequency of each activity, weighted by an estimate of the metabolic equivalent (MET) of that activity and summed for all activities performed, with the result expressed as the average MET hours per day. Diabetes was defined as those above the threshold of glycemia (FPG ≥ 6.1 mmol/L or 2h-PG ≥ 7.8 mmol/L), having a reported diabetes history, or experiencing anti-diabetes medications (26). Hypertension was defined as abnormal level of current blood pressure (SBP > 140 mmHg or DBP > 90 mmHg), having a reported hypertension history, or experiencing anti-hypertension medications (26). Dyslipidemia was defined as abnormal level of current blood lipids (TG ≥ 1.7 mmol/L, CHOL ≥ 5.2 mmol/L, LDL ≥ 3.4 mmol/L, HDL < 1.0 mmol/L), having a reported dyslipidemia history, or experiencing anti-dyslipidemia medications (26). Medication use was defined as regularly taking any medications for diabetes (including metformin, insulin, etc.), hypertension (including amlodipine, nifedipine, etc.), dyslipidemia (including atorvastatin, simvastatin, etc.), or obesity. Nutraceutical intake was defined as intaking common nutraceuticals (including vitamin, minerals, etc.) or foods with healthcare functions (including wine, tea, etc.) at least once a week for 12 months or more.

## Statistical analyses

Baseline characteristics for participants were presented according to the presence of incident CVD and compared using Student's *t*-test for continuous variables, or Chi-square test for categorical variables. Considering the body fat distribution and blood biochemical profiles are distinctly different in male and female participants, all analyses were conducted separately by sex.

The proportional hazard assumption was satisfied and then age-adjusted or multivariate-adjusted Cox proportional hazard models were used to assess the associations of incident CVD with adiposity categories and anthropometric indicators. The corresponding hazard ratios (HRs) and 95% confidence intervals (95% CIs) were calculated. Participants were categorized into different adiposity category groups according to gender-specific cutoffs for WC (normal weight, <85 cm for female participants and <90 cm for male participants; and central obesity, ≥85 cm for female participants and ≥90 cm for male participants) (27), WHtR (normal weight, <0.5; and central obesity, >0.5) (28), and BMI (lower weight, <18.5 kg/m$^2$; normal weight, 18.5–23.9 kg/m$^2$; overweight, 24.0–27.9 kg/m$^2$; and obesity, ≥28.0 kg/m$^2$) (27), based on Chinese guidelines. Participants were also categorized into four groups according to the quartiles of traditional (WC, WHtR, and BMI) and non-traditional (CVAI, LAP, and ABSI) anthropometric indicators, respectively. The restricted cubic splines (RCS) in Cox regression analyses were applied to

evaluate the potential dose–response relationships of these six anthropometric indicators with CVD events. Receiver operator characteristic (ROC) curve analyses were generated for multivariate-adjusted Cox proportional hazard models, and the predictive powers of six indicators for CVD were compared according to the area under the ROC curve (AUC). Similar analyses were conducted in subgroups stratified by baseline demographic (age, area, and ethnic group).

All analyses and figures were performed by using R program (version 4.1.0, R Foundation for Statistical Computing, Vienna, Austria).

# Results

## Baseline characteristic descriptions

Of the 7,837 participants included, the mean (SD) age was 44.18 ± 14.97 years, and more than half were female (52.57%), ethnically Han (58.52%), and rural residents (67.02%). During the median 6.59 years of follow-up, 193 cases of first-onset CVD were identified (incident rate: 3.47 per 1,000 person-years), including 141 ischemic strokes (incident rate: 2.53 per 1,000 person-years), 46 hemorrhagic strokes (incident rate: 0.82 per 1,000 person-years), and 27 myocardial infarctions (incident rate: 0.48 per 1,000 person-years). Compared with female participants without CVD, those with CVD seemed to experience a higher level of WC, WHtR, CVAI, LAP, and ABSI at baseline ($p < 0.05$). However, there was no significant difference in these indicators between male participants with CVD and those without (Table 1).

## Major analyses

Cox proportional hazard models indicated no association between any obesity type and incident CVD among male participants (Tables 2, 3). Female participants with either WC-based central obesity (HR:1.83, 95% CI: 1.12–2.98) or WHtR-based central obesity (HR:1.69, 95% CI: 1.07–2.65) had a higher risk of CVD after adjusting for age, area, ethnic group, smoking, alcohol drinking, MET, previous history of diabetes, hypertension, and dyslipidemia (Table 2, Model 2). The effect sizes of these positive associations were slightly decreased when further adjusted for medication use and nutraceutical intake (WC-based, HR: 1.82, 95% CI: 1.12–2.97; WHtR-based, HR:1.68, 95% CI: 1.07–2.64; Table 2, Model 3).

The dose–response relationships of CVD with WC, WHtR, BMI, CVAI, LAP, and ABSI appeared to follow non-linear patterns among two gender groups (Figures S1, S2). Details regarding the associations of CVD risks and six anthropometric indicators are provided in Tables 3, 4. Similarly, these associations were seen only among female and not male participants. Compared with female participants in the lowest quartile (Q1), those in the highest quartile (Q4) of WHtR (HR: 2.24, 95% CI: 1.17–4.27), CVAI (HR: 3.98, 95% CI: 1.87–8.49), and ABSI (HR: 1.94, 95% CI: 1.06–3.52) had an increased risk of incident CVD (Table 4, Model 3). Additionally, per 1 SD increase in WHtR, CVAI, LAP, and ABSI increased 32%, 74%, 19%, and 26% risk of CVD, respectively (Table 4, Model 3). However, regardless of being evaluated in any form, BMI was unrelated to incident CVD.

Moreover, the HRs for ischemic stroke, hemorrhagic stroke, and myocardial infarction are presented in Tables S1–S6. Compared with female participants in the lowest quartile (Q1), those in the highest quartile (Q4) of CAVI (HR: 3.40, 95% CI: 1.56–7.44) had an elevated risk of ischemic stroke (Table S2, Model 3). In addition, CVAI and ABSI were positively associated with the risk of hemorrhagic stroke (Table S4, Model 3).

Figure 2 shows the ROC curves of six indicators in the prediction of CVD among male and female participants, respectively. Neither traditional nor non-traditional indicators predicted CVD in male participants ($p > 0.05$, Figures 2A, C). On the contrary, the area under the ROC curve (AUC) and 95% CI for each indicator were higher than 0.5 in female participants ($p < 0.05$, Figures 2B, D). CAVI showed the maximum predictive power of CVD with the biggest AUC of 0.687 (95% CI: 0.654–0.720) compared to other indicators in female participants.

## Sensitive analyses and stratified analyses

Sensitive analyses were conducted after excluding female participants with less than 1 year of follow-up, and the results were similar to those in major analyses (Figure S3).

The multiple-adjusted HRs for incident CVD among female participants predicted by six anthropometric indicators varied according to age, area, and ethnic group (Figures 3A–F). The associations of WC and CVD were only observed in female participants aged more than 45 years (HR: 2.51, 95% CI: 1.06–5.98, Q4 vs. Q1) and living in rural region (HR: 3.02, 95% CI: 1.27–7.14, Q4 vs. Q1) (Figure 3A). Similar patterns were also seen for WHtR (Figure 3B) and ABSI (Figure 3E). The most frequent and strongest associations with CVD were found for CVAI, with the HR exceeding 6 (HR: 6.80, 95% CI: 2.67–17.30) in rural residents (Figure 3D).

# Discussion

In this large population-based cohort study of 7,837 people with a median of 6.59 years of follow-up in Southwest China, we observed that visceral adiposity measures, especially CVAI, were

TABLE 1 Baseline characteristics for participants.

| Characteristics | Male participants | | | | Female participants | | | |
|---|---|---|---|---|---|---|---|---|
| | Total ($n$ = 3,717) | Non-CVD ($n$ = 3,621) | CVD ($n$ = 96) | $p$-value | Total ($n$ = 4120) | Non-CVD ($n$ = 4023) | CVD ($n$ = 97) | $p$-value |
| Age (years, mean ± SD) | 43.66 ±15.02 | 43.41 ± 14.99 | 53.18 ± 13.34 | <0.001 | 44.64 ± 14.90 | 44.41 ± 14.85 | 54.50 ± 13.78 | <0.001 |
| Area($n$,%) | | | | 0.305 | | | | 0.388 |
| Urban | 1,206 (32.4) | 1,180 (32.6) | 26 (27.1) | | 1,379 (33.5) | 1,351 (33.6) | 28 (28.9) | |
| Rural | 2,511 (67.6) | 2,441 (67.4) | 70 (72.9) | | 2,741 (66.5) | 2,672 (66.4) | 69 (71.1) | |
| Ethnic group($n$,%) | | | | 0.060 | | | | 0.092 |
| Ethnic Han | 2,190 (58.9) | 2,124 (58.7) | 66 (68.8) | | 2,396 (58.2) | 2,331 (57.9) | 65 (67.0) | |
| Minority | 1,527 (41.1) | 1,497 (41.3) | 30 (31.2) | | 1,724 (41.8) | 1,692 (42.1) | 32 (33.0) | |
| Education ($n$,%) | | | | 0.455 | | | | 0.016 |
| No formal education | 365 (9.8) | 352 (9.7) | 13 (13.5) | | 1,238 (30.0) | 1,196 (29.7) | 42 (43.3) | |
| Junior middle school and below | 2,772 (74.6) | 2,704 (74.7) | 68 (70.8) | | 2,421 (58.8) | 2,375 (59.0) | 46 (47.4) | |
| Senior high school and above | 580 (15.6) | 565 (15.6) | 15 (15.6) | | 461 (11.2) | 452 (11.2) | 9 (9.3) | |
| Marriage ($n$,%) | | | | 0.226 | | | | 0.327 |
| Married/Cohabit | 2,932 (78.9) | 2,851 (78.7) | 81 (84.4) | | 3,403 (82.6) | 3,327 (82.7) | 76 (78.4) | |
| Unmarried/Divorced/ Widowed/Separated | 785 (21.1) | 770 (21.3) | 15 (15.6) | | 717 (17.4) | 696 (17.3) | 21 (21.6) | |
| Occupation ($n$,%) | | | | 0.915 | | | | 0.045 |
| Farmers | 2,182 (58.7) | 2,127 (58.7) | 55 (57.3) | | 2,306 (56.0) | 2,247 (55.9) | 59 (60.8) | |
| Others | 1,118 (30.1) | 1,089 (30.1) | 29 (30.2) | | 973 (23.6) | 960 (23.9) | 13 (13.4) | |
| Unemployed/Retired | 417 (11.2) | 405 (11.2) | 12 (12.5) | | 841 (20.4) | 816 (20.3) | 25 (25.8) | |
| Smoking ($n$, %) | | | | 1.000 | | | | 0.018 |
| No | 1,779 (47.9) | 1,733 (47.9) | 46 (47.9) | | 4,074 (98.9) | 3,981 (99.0) | 93 (95.9) | |
| Yes | 1,938 (52.1) | 1,888 (52.1) | 50 (52.1) | | 46 (1.1) | 42 (1.0) | 4 (4.1) | |
| Alcohol drinking ($n$, %) | | | | 0.786 | | | | 0.447 |
| No | 2,371 (63.8) | 2,308 (63.7) | 63 (65.6) | | 3,660 (88.8) | 3,571 (88.8) | 89 (91.8) | |
| Yes | 1,346 (36.2) | 1,313 (36.3) | 33 (34.4) | | 460 (11.2) | 452 (11.2) | 8 (8.2) | |
| Diabetes ($n$, %) | | | | 0.015 | | | | 0.182 |
| No | 3,359 (90.7) | 3,281 (90.9) | 78 (83.0) | | 3,799 (92.6) | 3,714 (92.7) | 85 (88.5) | |
| Yes | 344 (9.3) | 328 (9.1) | 16 (17.0) | | 304 (7.4) | 293 (7.3) | 11 (11.5) | |
| Hypertension ($n$, %) | | | | 0.001 | | | | <0.001 |

*(Continued)*





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TABLE 1 Continued

| Characteristics | Male participants | | | | Female participants | | | |
|---|---|---|---|---|---|---|---|---|
| | Total (*n* = 3,717) | Non-CVD (*n* = 3,621) | CVD (*n* = 96) | *p*-value | Total (*n* = 4120) | Non-CVD (*n* = 4023) | CVD (*n* = 97) | *p*-value |
| No | 2,668 (71.8) | 2,614 (72.2) | 54 (56.2) | | 3,162 (76.7) | 3,108 (77.3) | 54 (55.7) | |
| Yes | 1,049 (28.2) | 1,007 (27.8) | 42 (43.8) | | 958 (23.3) | 915 (22.7) | 43 (44.3) | |
| Dyslipidemia (*n*, %) | | | | 0.322 | | | | 0.980 |
| No | 1,636 (44.0) | 1,599 (44.2) | 37 (38.5) | | 1,715 (41.6) | 1,674 (41.6) | 41 (42.3) | |
| Yes | 2,081 (56.0) | 2,022 (55.8) | 59 (61.5) | | 2,405 (58.4) | 2,349 (58.4) | 56 (57.7) | |
| Medication use (*n*, %) [a] | | | | 0.943 | | | | 0.760 |
| No | 3,263 (87.8) | 3,178 (87.8) | 85 (88.5) | | 3,589 (87.1) | 3,506 (87.1) | 83 (85.6) | |
| Yes | 454 (12.2) | 443 (12.2) | 11 (11.5) | | 531 (12.9) | 517 (12.9) | 14 (14.4) | |
| Nutraceutical intake (*n*, %) [a] | | | | 0.858 | | | | 1.000 |
| No | 3,282 (88.5) | 3,196 (88.5) | 86 (89.6) | | 3,647 (88.7) | 3,561 (88.7) | 86 (88.7) | |
| Yes | 427 (11.5) | 417 (11.5) | 10 (10.4) | | 465 (11.3) | 454 (11.3) | 11 (11.3) | |
| WC-based | | | | 1 | | | | <0.001 |
| Normal weight | 3,068 (87.3) | 2,992 (87.3) | 76 (87.4) | | 3,211 (83.8) | 3,149 (84.1) | 62 (69.7) | |
| Central obesity | 446 (12.7) | 435 (12.7) | 11 (12.6) | | 623 (16.2) | 596 (15.9) | 27 (30.3) | |
| WHtR-based | | | | 0.870 | | | | 0.001 |
| Normal weight | 2,334 (66.4) | 2,275 (66.4) | 59 (67.8) | | 2,168 (56.5) | 2,133 (57.0) | 35 (39.3) | |
| Central obesity | 1,180 (33.6) | 1,152 (33.6) | 28 (32.2) | | 1,666 (43.5) | 1,612 (43.0) | 54 (60.7) | |
| BMI-based | | | | 0.671 | | | | 0.064 |
| Underweight | 189 (5.1) | 182 (5.0) | 7 (7.3) | | 238 (5.8) | 236 (5.9) | 2 (2.1) | |
| Normal weight | 2,386 (64.2) | 2,329 (64.3) | 57 (59.4) | | 2,501 (60.7) | 2,438 (60.6) | 63 (64.9) | |
| Overweight | 915 (24.6) | 889 (24.6) | 26 (27.1) | | 1,044 (25.3) | 1,025 (25.5) | 19 (19.6) | |
| Obesity | 227 (6.1) | 221 (6.1) | 6 (6.2) | | 337 (8.2) | 324 (8.1) | 13 (13.4) | |
| WC (cm, mean ± SD) [a] | 77.92 ± 9.49 | 77.92 ± 9.48 | 77.74 ± 9.80 | 0.858 | 75.42 ± 9.30 | 75.35 ± 9.27 | 78.37 ± 9.91 | 0.002 |
| WHtR (mean ± SD) [a] | 0.48 ± 0.06 | 0.48 ± 0.06 | 0.48 ± 0.06 | 0.820 | 0.49 ± 0.06 | 0.49 ± 0.06 | 0.52 ± 0.07 | <0.001 |
| BMI ($kg/m^2$, mean ± SD) | 22.77 ± 3.15 | 22.77 ± 3.15 | 22.81 ± 3.10 | 0.911 | 22.99 ± 3.40 | 22.98 ± 3.40 | 23.55 ± 3.32 | 0.105 |
| CVAI (mean ± SD) | 53.52 ± 44.06 | 53.37 ± 44.03 | 59.39 ± 44.92 | 0.211 | 61.47 ± 42.57 | 60.86 ± 42.37 | 87.25 ± 43.32 | <0.001 |
| LAP (mean ± SD) | 28.49 ± 47.34 | 28.53 ± 47.48 | 26.66 ± 41.83 | 0.717 | 32.36 ± 39.63 | 32.07 ± 38.66 | 44.93 ± 68.26 | 0.003 |
| ABSI ($m^{11/6}/kg^{2/3,}$ mean ± SD) | 0.76 ± 0.06 | 0.76 ± 0.06 | 0.76 ± 0.05 | 0.801 | 0.76 ± 0.06 | 0.76 ± 0.06 | 0.78 ± 0.06 | 0.006 |
| FPG (mmol/L, mean ± SD) [a] | 5.32 ± 1.38 | 5.32 ± 1.37 | 5.46 ± 1.70 | 0.331 | 5.19 ± 1.13 | 5.19 ± 1.13 | 5.33 ± 1.41 | 0.205 |
| 2h-PG (mmol/L, mean ± SD) [a] | 5.87 ± 2.41 | 5.86 ± 2.40 | 6.42 ± 2.78 | 0.026 | 5.72 ± 2.10 | 5.71 ± 2.09 | 5.91 ± 2.20 | 0.350 |

*(Continued)*





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TABLE 1 Continued

| Characteristics | Male participants | | | | Female participants | | | |
|---|---|---|---|---|---|---|---|---|
| | Total ($n$ = 3,717) | Non-CVD ($n$ = 3,621) | CVD ($n$ = 96) | $p$-value | Total ($n$ = 4120) | Non-CVD ($n$ = 4023) | CVD ($n$ = 97) | $p$-value |
| SBP (mmHg, mean ± SD) [a] | 126.99 ± 20.36 | 126.78 ± 20.24 | 134.93 ± 23.24 | <0.001 | 123.37 ± 21.18 | 123.11 ± 20.93 | 134.32 ± 27.45 | <0.001 |
| DBP (mmHg, mean ± SD) [a] | 79.30 ± 11.83 | 79.19 ± 11.76 | 83.50 ± 13.85 | <0.001 | 77.28 ± 11.88 | 77.17 ± 11.80 | 82.02 ± 14.33 | <0.001 |
| TG (mmol/L, mean ± SD) [a] | 1.84 ± 1.81 | 1.84 ± 1.80 | 1.95 ± 2.04 | 0.552 | 1.68 ± 1.31 | 1.67 ± 1.28 | 1.94 ± 2.02 | 0.050 |
| CHOL (mmol/L, mean ± SD) [a] | 4.78 ± 1.26 | 4.77 ± 1.24 | 4.96 ± 1.83 | 0.157 | 4.81 ± 1.37 | 4.80 ± 1.36 | 5.01 ± 1.65 | 0.134 |
| HDL-C (mmol/L, mean ± SD) | 1.45 ± 0.54 | 1.45 ± 0.54 | 1.44 ± 0.55 | 0.817 | 1.45 ± 0.57 | 1.45 ± 0.56 | 1.41 ± 0.65 | 0.452 |
| LDL-C (mmol/L, mean ± SD) [a] | 2.64 ± 1.13 | 2.64 ± 1.13 | 2.51 ± 1.22 | 0.271 | 2.68 ± 1.23 | 2.68 ± 1.23 | 2.69 ± 1.48 | 0.921 |
| MET (per day, $n$, %) [a] | 117.18 ± 127.77 | 117.05 ± 127.92 | 121.79 ± 122.73 | 0.720 | 103.11 ± 117.32 | 103.13 ± 117.37 | 102.33 ± 115.77 | 0.947 |

CVD, cardiovascular diseases; SD, standard deviation; WC, waist circumference; WHtR, waist-to-height ratio; BMI, body mass index; CVAI, Chinese visceral adiposity index; LAP, lipid accumulation product; ABSI, body shape index; FPG, fasting plasma glucose; 2h-PG, 2-h postload glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; CHOL, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MET, metabolic equivalent of task.

[a]With missing value.



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TABLE 2 Hazard ratios (HRs) and 95% confidence intervals (95% CIs) for overall CVD associated with adiposity category among male (*n* = 3,717) and female (*n* = 4,120) participants according to Cox regression models.

| Anthropometric indexes | No (*n*) | Cases (*n*) | Incident density (cases per 1,000 PYs) | HR (95% CI) [a] | | |
|---|---|---|---|---|---|---|
| | | | | Model 1 | Model 2 | Model 3 |
| **Male participants** | | | | | | |
| **WC-based** | | | | | | |
| Normal weight | 3,068 | 76 | 3.45 | 1.00 | 1.00 | 1.00 |
| Central obesity | 446 | 11 | 3.50 | 1.04 (0.55–1.96) | 0.84 (0.44–1.63) | 0.85 (0.44–1.63) |
| **WHtR-based** | | | | | | |
| Normal weight | 2,334 | 59 | 3.52 | 1.00 | 1.00 | 1.00 |
| Central obesity | 1,180 | 28 | 3.34 | 0.97 (0.62–1.52) | 0.82 (0.51–1.32) | 0.82 (0.51–1.32) |
| **BMI-based** | | | | | | |
| Underweight | 189 | 7 | 5.24 | 1.59 (0.73–3.49) | 1.57 (0.67–3.66) | 1.58 (0.68–3.68) |
| Normal weight | 2,386 | 57 | 3.36 | 1.00 | 1.00 | 1.00 |
| Overweight | 915 | 26 | 3.99 | 1.18 (0.74–1.87) | 1.04 (0.65–1.68) | 1.04 (0.65–1.68) |
| Obesity | 227 | 6 | 3.81 | 1.15 (0.50–2.67) | 0.84 (0.35–2.01) | 0.84 (0.35–2.01) |
| **Female participants** | | | | | | |
| **WC-based** | | | | | | |
| Normal weight | 3,211 | 62 | 1.37 | 1.00 | 1.00 | 1.00 |
| Central obesity | 623 | 27 | 3.59 | 2.35 (1.50–3.70)*** | 1.83 (1.12–2.98)* | 1.82 (1.12–2.97)* |
| **WHtR-based** | | | | | | |
| Normal weight | 2,168 | 35 | 1.08 | 1.00 | 1.00 | 1.00 |
| Central obesity | 1,666 | 54 | 2.68 | 2.09 (1.37–3.20)*** | 1.69 (1.07–2.65)* | 1.68 (1.07–2.64)* |
| **BMI-based** | | | | | | |
| Underweight | 238 | 2 | 0.65 | 0.31 (0.08–1.28) | 0.32 (0.08–1.31) | 0.33 (0.08–1.33) |
| Normal weight | 2,501 | 63 | 1.81 | 1.00 | 1.00 | 1.00 |
| Overweight | 1,044 | 19 | 1.37 | 0.73 (0.43–1.21) | 0.56 (0.33–0.96) | 0.56 (0.32–0.96) |
| Obesity | 337 | 13 | 3.32 | 1.58 (0.87–2.87) | 1.05 (0.56–1.99) | 1.06 (0.56–2.00) |

CVD, cardiovascular and cerebrovascular diseases; WC, waist circumference; WHtR, waist-to-height ratio; BMI, body mass index; PYs, person-years; MET, metabolic equivalent of task. [a]**Model 1:** Adjusted for age only; **Model 2:** Model 1 + additionally adjusted for area, ethnic group, smoking, alcohol drinking, MET, diabetes, hypertension, and dyslipidemia,; **Model 3**: Model 2 + additionally adjusted for medication use and nutraceutical intake.
*0.01 < p < 0.05; ***p <0.001.

TABLE 3 Hazard ratios (HRs) and 95% confidence intervals (95% CIs) for overall CVDs associated with traditional and non-traditional anthropometric indicators among male participants (*n* = 3,717) according to Cox regression models.

| Anthropometric indicators | No (*n*) | Cases (*n*) | Incident density (cases per 1,000 PYs) | HR (95% CI) [a] | | |
|---|---|---|---|---|---|---|
| | | | | Model 1 | Model 2 | Model 3 |
| **Traditional** | | | | | | |
| **WC (cm)** [b] | | | | | | |
| Quartile 1 (Q1) | 843 | 23 | 3.80 | 1.00 | 1.00 | 1.00 |
| Quartile 2 (Q2) | 914 | 17 | 2.59 | 0.68 (0.36–1.27) | 0.62 (0.32–1.17) | 0.61 (0.32–1.17) |
| Quartile 3 (Q3) | 857 | 23 | 3.73 | 1.00 (0.56–1.78) | 0.92 (0.51–1.67) | 0.92 (0.51–1.68) |
| Quartile 4 (Q4) | 900 | 24 | 3.77 | 1.00 (0.57–1.78) | 0.80 (0.43–1.48) | 0.80 (0.43–1.48) |
| *p* trend | – | – | – | 0.687 | 0.783 | 0.788 |
| Per 1 SD | – | – | – | 1.00 (0.81–1.23) | 0.91 (0.73–1.14) | 0.91 (0.73–1.14) |
| **WHtR** [c] | | | | | | |
| Quartile 1 (Q1) | 878 | 23 | 3.64 | 1.00 | 1.00 | 1.00 |
| Quartile 2 (Q2) | 879 | 21 | 3.31 | 0.93 (0.51–1.67) | 0.86 (0.47–1.57) | 0.85 (0.46–1.57) |
| Quartile 3 (Q3) | 878 | 18 | 2.87 | 0.82 (0.44–1.52) | 0.74 (0.40–1.40) | 0.74 (0.40–1.40) |

*(Continued)*

TABLE 3 Continued

| Anthropometric indicators | No (*n*) | Cases (*n*) | Incident density (cases per 1,000 PYs) | HR (95% CI) [a] | | |
|---|---|---|---|---|---|---|
| | | | | Model 1 | Model 2 | Model 3 |
| Quartile 4 (Q4) | 879 | 25 | 4.02 | 1.15 (0.65–2.02) | 0.92 (0.50–1.69) | 0.92 (0.50–1.70) |
| *p* trend | – | – | – | 0.733 | 0.720 | 0.730 |
| Per 1 SD | – | – | – | 1.00 (0.81–1.24) | 0.92 (0.73–1.15) | 0.92 (0.73–1.15) |
| **BMI (kg/m$^2$) [d]** | | | | | | |
| Quartile 1 (Q1) | 929 | 24 | 3.62 | 1.00 | 1.00 | 1.00 |
| Quartile 2 (Q2) | 929 | 21 | 3.17 | 0.87 (0.48–1.55) | 0.81 (0.45–1.49) | 0.81 (0.45–1.48) |
| Quartile 3 (Q3) | 925 | 27 | 4.12 | 1.12 (0.65–1.94) | 1.03 (0.58–1.80) | 1.02 (0.58–1.80) |
| Quartile 4 (Q4) | 934 | 24 | 3.64 | 1.00 (0.57–1.76) | 0.81 (0.44–1.48) | 0.81 (0.44–1.48) |
| *p* trend | – | – | – | 0.776 | 0.675 | 0.673 |
| Per 1 SD | – | – | – | 1.02 (0.83–1.24) | 0.93 (0.75–1.16) | 0.93 (0.75–1.16) |
| **Non-traditional CAVI [e]** | | | | | | |
| Quartile 1 (Q1) | 865 | 17 | 2.74 | 1.00 | 1.00 | 1.00 |
| Quartile 2 (Q2) | 865 | 18 | 2.89 | 1.04 (0.54–2.02) | 1.00 (0.51–2.00) | 1.00 (0.51–1.99) |
| Quartile 3 (Q3) | 865 | 23 | 3.70 | 1.36 (0.73–2.55) | 1.27 (0.66–2.46) | 1.27 (0.66–2.45) |
| Quartile 4 (Q4) | 866 | 28 | 4.59 | 1.68 (0.92–3.06) | 1.41 (0.72–2.77) | 1.42 (0.72–2.78) |
| *p* trend | – | – | – | 0.056 | 0.239 | 0.235 |
| Per 1 SD | – | – | – | 1.15 (0.94–1.41) | 1.04 (0.83–1.31) | 1.04 (0.83–1.31) |
| **LAP [f]** | | | | | | |
| Quartile 1 (Q1) | 870 | 20 | 3.20 | 1.00 | 1.00 | 1.00 |
| Quartile 2 (Q2) | 868 | 18 | 2.91 | 0.91 (0.48–1.71) | 0.88 (0.46–1.69) | 0.88 (0.46–1.68) |
| Quartile 3 (Q3) | 869 | 30 | 4.83 | 1.54 (0.88–2.71) | 1.34 (0.74–2.43) | 1.33 (0.74–2.41) |
| Quartile 4 (Q4) | 872 | 18 | 2.88 | 0.90 (0.48–1.70) | 0.70 (0.35–1.41) | 0.70 (0.35–1.40) |
| *p* trend | – | – | – | 0.762 | 0.639 | 0.630 |
| Per 1 SD | – | – | – | 0.97 (0.76–1.22) | 0.87 (0.66–1.15) | 0.88 (0.66–1.16) |
| **ABSI [g]** | | | | | | |
| Quartile 1 (Q1) | 879 | 21 | 3.33 | 1.00 | 1.00 | 1.00 |
| Quartile 2 (Q2) | 878 | 25 | 4.00 | 1.23 (0.69–2.20) | 1.31 (0.72–2.38) | 1.31 (0.72–2.38) |
| Quartile 3 (Q3) | 878 | 22 | 3.47 | 1.08 (0.59–1.97) | 1.09 (0.59–2.03) | 1.10 (0.59–2.04) |
| Quartile 4 (Q4) | 879 | 19 | 3.04 | 0.97 (0.52–1.81) | 0.90 (0.47–1.71) | 0.90 (0.47–1.72) |
| *p* trend | – | – | – | 0.835 | 0.605 | 0.614 |
| Per 1 SD | – | – | – | 1.00 (0.81–1.24) | 0.97 (0.77–1.20) | 0.97 (0.78–1.20) |

CVD, cardiovascular and cerebrovascular diseases; WC, waist circumference; WHtR, waist-to-height ratio; BMI, body mass index; CVAI, Chinese visceral adiposity index; LAP, lipid accumulation product; ABSI, body shape index; PYs, person-years; MET, metabolic equivalent of task.

[a]**Model 1:** Adjusted for age only; **Model 2:** Model 1 + additionally adjusted for area, ethnic group, smoking, alcohol drinking, MET, diabetes, hypertension and dyslipidemia; **Model 3**: Model 2 + additionally adjusted for medication use and nutraceutical intake.

[b]**WC (cm):** Quartile levels as Q1, <71.00 cm; Q2, 71.00–76.54 cm; Q3, 76.55–84.39 cm; Q4, ≥84.00 cm

[c]**WHtR:** Quartile levels as Q1, <0.44; Q2, 0.44–0.47; Q3, 0.48–0.51; Q4, ≥0.52.

[d]**BMI (kg/m$^2$):** Quartile levels as Q1, <20.47 kg/m$^2$; Q2, 20.47–22.34 kg/m$^2$; Q3, 22.35–24.56 kg/m$^2$; Q4, ≥24.57 kg/m$^2$.

[e]**CAVI:** Quartile levels as Q1, <21.30; Q2, 21.30–47.73; Q3, 47.73–82.16; Q4, ≥82.15.

[f]**LAP:** Quartile levels as Q1, <6.29; Q2, 6.29–14.60; Q3, 14.60–33.60; Q4, ≥33.60.

[g]**ABSI:** Quartile levels as Q1, <0.73; Q2, 0.73– 0.76; Q3, 0.76–0.80; Q4, ≥0.80.

positively associated with overall CVD and ischemic stroke among female and not male participants. On the contrary, BMI, as a general obesity indicator, performed less predictive power for CVD.

The results of adiposity increasing the risks of CVD in this study are in accordance with those on previous studies (29–31). Adipose tissues release cytokines and chemokines into the vasculature, promoting systemic and vascular inflammation (32). Consistent with our results, obvious sex-related disparities in the associations of adiposity with CVD risk have also been proposed before (29), which may arise not only from differences in body fat distribution and metabolic profiles, but

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TABLE 4 Hazard ratios (HRs) and 95% confidence intervals (95% CIs) for overall CVDs associated with traditional and non-traditional anthropometric indicators among female participants ($n$ = 4,120) according to Cox regression models.

| Anthropometric indicators | No ($n$) | Cases ($n$) | Incident density (cases per 1,000 PYs) | HR (95% CI) [a] | | |
|---|---|---|---|---|---|---|
| | | | | Model 1 | Model 2 | Model 3 |
| **Traditional** | | | | | | |
| **WC (cm)** [b] | | | | | | |
| Quartile 1 (Q1) | 935 | 15 | 2.20 | 1.00 | 1.00 | 1.00 |
| Quartile 2 (Q2) | 974 | 19 | 2.70 | 1.24 (0.63–2.43) | 1.18 (0.60–2.32) | 1.18 (0.60–2.32) |
| Quartile 3 (Q3) | 962 | 20 | 2.91 | 1.34 (0.69–2.63) | 1.14 (0.58–2.25) | 1.14 (0.58–2.25) |
| Quartile 4 (Q4) | 963 | 35 | 5.16 | 2.39 (1.30–4.37)** | 1.74 (0.92–3.30) | 1.74 (0.92–3.29) |
| $p$ trend | – | – | – | 0.003 | 0.088 | 0.090 |
| Per 1 SD | – | – | – | 1.37 (1.13–1.66)** | 1.22 (0.99–1.50) | 1.22 (0.99–1.50) |
| **WHtR** [c] | | | | | | |
| Quartile 1 (Q1) | 959 | 14 | 1.99 | 1.00 | 1.00 | 1.00 |
| Quartile 2 (Q2) | 957 | 19 | 2.75 | 1.41 (0.71–2.81) | 1.35 (0.68–2.70) | 1.36 (0.68–2.71) |
| Quartile 3 (Q3) | 959 | 17 | 2.49 | 1.28 (0.63–2.60) | 1.14 (0.56–2.33) | 1.14 (0.56–2.32) |
| Quartile 4 (Q4) | 959 | 39 | 5.78 | 2.98 (1.62–5.48)*** | 2.23 (1.17–4.26)* | 2.24 (1.17–4.27)* |
| $p$ trend | – | – | – | <0.001 | 0.017 | 0.017 |
| Per 1 SD | – | – | – | 1.47 (1.21–1.78)*** | 1.32 (1.07–1.63)** | 1.32 (1.07–1.63)** |
| **BMI (kg/m$^2$)** [d] | | | | | | |
| Quartile 1 (Q1) | 1030 | 22 | 2.97 | 1.00 | 1.00 | 1.00 |
| Quartile 2 (Q2) | 1030 | 18 | 2.44 | 0.83 (0.44–1.54) | 0.82 (0.44–1.53) | 0.82 (0.44–1.53) |
| Quartile 3 (Q3) | 1030 | 27 | 3.71 | 1.25 (0.71–2.20) | 1.10 (0.62–1.94) | 1.10 (0.62–1.95) |
| Quartile 4 (Q4) | 1030 | 30 | 4.17 | 1.41 (0.81–2.44) | 1.00 (0.55–1.81) | 1.00 (0.55–1.81) |
| $p$ trend | – | – | – | 0.107 | 0.778 | 0.779 |
| Per 1 SD | – | – | – | 1.18 (0.98–1.42) | 1.05 (0.85–1.28) | 1.05 (0.85–1.28) |
| **Non-traditional** | | | | | | |
| **CAVI** [e] | | | | | | |
| Quartile 1 (Q1) | 941 | 10 | 1.46 | 1.00 | 1.00 | 1.00 |
| Quartile 2 (Q2) | 940 | 12 | 1.75 | 1.19 (0.52–2.76) | 1.19 (0.51–2.76) | 1.19 (0.51–2.76) |
| Quartile 3 (Q3) | 941 | 22 | 3.29 | 2.25 (1.07–4.75)* | 2.27 (1.06–4.87)* | 2.26 (1.05–4.84)* |
| Quartile 4 (Q4) | 941 | 43 | 6.51 | 4.44 (2.23–8.84)*** | 4.01 (1.88–8.54)*** | 3.98 (1.87–8.49)*** |
| $p$ trend | – | – | – | <0.001 | <0.001 | <0.001 |
| Per 1 SD | – | – | – | 1.79 (1.47–2.19)*** | 1.75 (1.38–2.21)*** | 1.74 (1.37–2.21)*** |
| **LAP** [f] | | | | | | |
| Quartile 1 (Q1) | 945 | 15 | 2.20 | 1.00 | 1.00 | 1.00 |
| Quartile 2 (Q2) | 947 | 19 | 2.81 | 1.27 (0.65–2.50) | 1.23 (0.62–2.42) | 1.23 (0.63–2.43) |
| Quartile 3 (Q3) | 945 | 21 | 3.10 | 1.41 (0.73–2.74) | 1.24 (0.63–2.44) | 1.23 (0.62–2.42) |
| Quartile 4 (Q4) | 952 | 32 | 4.69 | 2.10 (1.14–3.88)* | 1.75 (0.89–3.42) | 1.74 (0.89–3.40) |
| $p$ trend | – | – | – | 0.014 | 0.111 | 0.116 |
| Per 1 SD | – | – | – | 1.21 (1.08–1.37)** | 1.18 (1.03–1.36)* | 1.19 (1.03–1.37)* |

*(Continued)*

TABLE 4 Continued

| Anthropometric indicators | No (*n*) | Cases (*n*) | Incident density (cases per 1,000 PYs) | HR (95% CI) [a] | | |
|---|---|---|---|---|---|---|
| | | | | Model 1 | Model 2 | Model 3 |
| **ABSI** [g] | | | | | | |
| Quartile 1 (Q1) | 959 | 16 | 2.31 | 1.00 | 1.00 | 1.00 |
| Quartile 2 (Q2) | 958 | 18 | 2.60 | 1.13 (0.58–2.22) | 1.11 (0.57–2.19) | 1.12 (0.57–2.20) |
| Quartile 3 (Q3) | 958 | 19 | 2.78 | 1.23 (0.63–2.39) | 1.14 (0.58–2.22) | 1.15 (0.59–2.25) |
| Quartile 4 (Q4) | 959 | 36 | 5.30 | 2.37 (1.32–4.28)** | 1.92 (1.06–3.49)* | 1.94 (1.06–3.52)* |
| *p* trend | – | – | – | 0.002 | 0.025 | 0.023 |
| Per 1 SD | – | – | – | 1.35 (1.11–1.63)** | 1.26 (1.03–1.54)* | 1.26 (1.03–1.54)* |

CVD, cardiovascular and cerebrovascular diseases; WC, waist circumference; WHtR, waist-to-height ratio; BMI, body mass index; CVAI, Chinese visceral adiposity index; LAP, lipid accumulation product; ABSI, body shape index; PYs, person-years; MET, metabolic equivalent of task.

[a]**Model 1:** Adjusted for age only; **Model 2:** Model 1 + additionally adjusted for area, ethnic group, smoking, alcohol drinking, MET, diabetes, hypertension and dyslipidemia; **Model 3**: Model 2 + additionally adjusted for medication use and nutraceutical intake.

[b]**WC (cm):** Quartile levels as Q1, <69.00 cm; Q2, 69.00–74.19 cm; Q3, 74.20–80.99 cm; Q4, ≥81.00 cm.

[c]**WHtR:** Quartile levels as Q1, <0.45; Q2, 0.45–0.48; Q3, 0.49–0.52; Q4, ≥0.53.

[d]**BMI (kg/m$^2$):** Quartile levels as Q1, <20.59 kg/m$^2$; Q2, 20.59–22.47 kg/m$^2$; Q3, 22.48–24.95 kg/m$^2$; Q4, ≥24.96 kg/m$^2$.

[e]**CAVI:** Quartile levels as Q1, <30.46; Q2, 30.46–58.53; Q3, 58.53–91.25; Q4, ≥91.25.

[f]**LAP:** Quartile levels as Q1, <11.20; Q2, 11.20–20.93; Q3, 20.93–40.00; Q4, ≥40.00.

[g]**ABSI:** Quartile levels as Q1, <0.72; Q2, 0.72–0.76; Q3, 0.76–0.79; Q4, ≥0.79.

*p < 0.05; **0.05 < p < 0.01; ***0.01 < p < 0.001.

also from the differences in vascular anatomy and physiology, with female participants having smaller arterial diameter than male participants seen after normalizing for body size (33).

WC and WHtR are the most common indicators to measure visceral obesity. WHtR was designed to incorporate the effects of WC and height, namely, WC adjusted for height. There were more frequent associations of CVD with WHtR than WC in this study, suggesting that the distribution of body fat is important in discriminating CVD risk (34). We observed that BMI showed less prediction information for CVD as compared to WC or WHtR. The detrimental vascular effects of adiposity may be masked when using BMI as a measure of adiposity, which has been termed the "obesity paradox", due to methodological deficiencies such as BMI failing to distinguish between fat tissue and skeletal muscle. In fact, an increase in total fat tissue percent or a decrease in skeletal muscle accelerates the occurrence of CVD (15, 35).

Moreover, the effects of visceral adipose tissue (VAT) on cardio-metabolic outcomes have been proved to be more deleterious than subcutaneous adipose tissue (SAT) (36). CVAI, which is estimated by synergistically integrating information of age, BMI, WC, and lipid profiles (HDL-C and TG), has outperformed traditional anthropometric measures as a useful surrogate for visceral adiposity in a Chinese population (18). As expected, CVAI showed the maximum predictive power of CVD, with a maximum HR of 3.98 (95% CI: 1.87–8.49) and the biggest AUC of 0.687 (95% CI: 0.654–0.720), compared to other indicators in female participants. Previous studies reported that CVAI was superior to BMI, WC, WHtR, LAP, or VAI for the diagnosis of diabetes and related complications (18, 20, 37). Additionally, after combining multiple measurements, ABSI (given the metrics of WC, height, and weight) and LAP (given the metrics of WC and TG) have also been considered as applicable indicators of some chronic diseases in adults (38, 39). In general, several measure indicators need to be combined to comprehensively assess health risks.

In the stratified analyses, the positive associations between visceral adiposity indicators of CVD were stronger in female participants aged more than 45 years or living in rural regions. The mechanism through which adiposity leads to cardiovascular risk is also discrepant in female participants between their pre-menopausal, pregnancy, and post-menopausal phases of life (40). Middle-aged female participants were more likely to accumulate fat due to declines in basal metabolic rate, and the estrogen deprivation secondary to menopause may lead to adverse cardiovascular consequences (41). Area and ethnic variations could be partly explained by regional environmental, socioeconomic characteristics, diet cultures, and local customs (22).

To our knowledge, this is one of few population-based cohort studies to assess CVD risk by a series of adiposity measure indicators. The strengths of our study include covering standardized methods for anthropometric measurements and local residents from various ethnic groups. There were several limitations to this study. First, assessments of some factors in this study, including physical activities, tobacco smoking, alcohol drinking, medications, and nutraceutical consumption, rely on self-reports from questionnaires, which

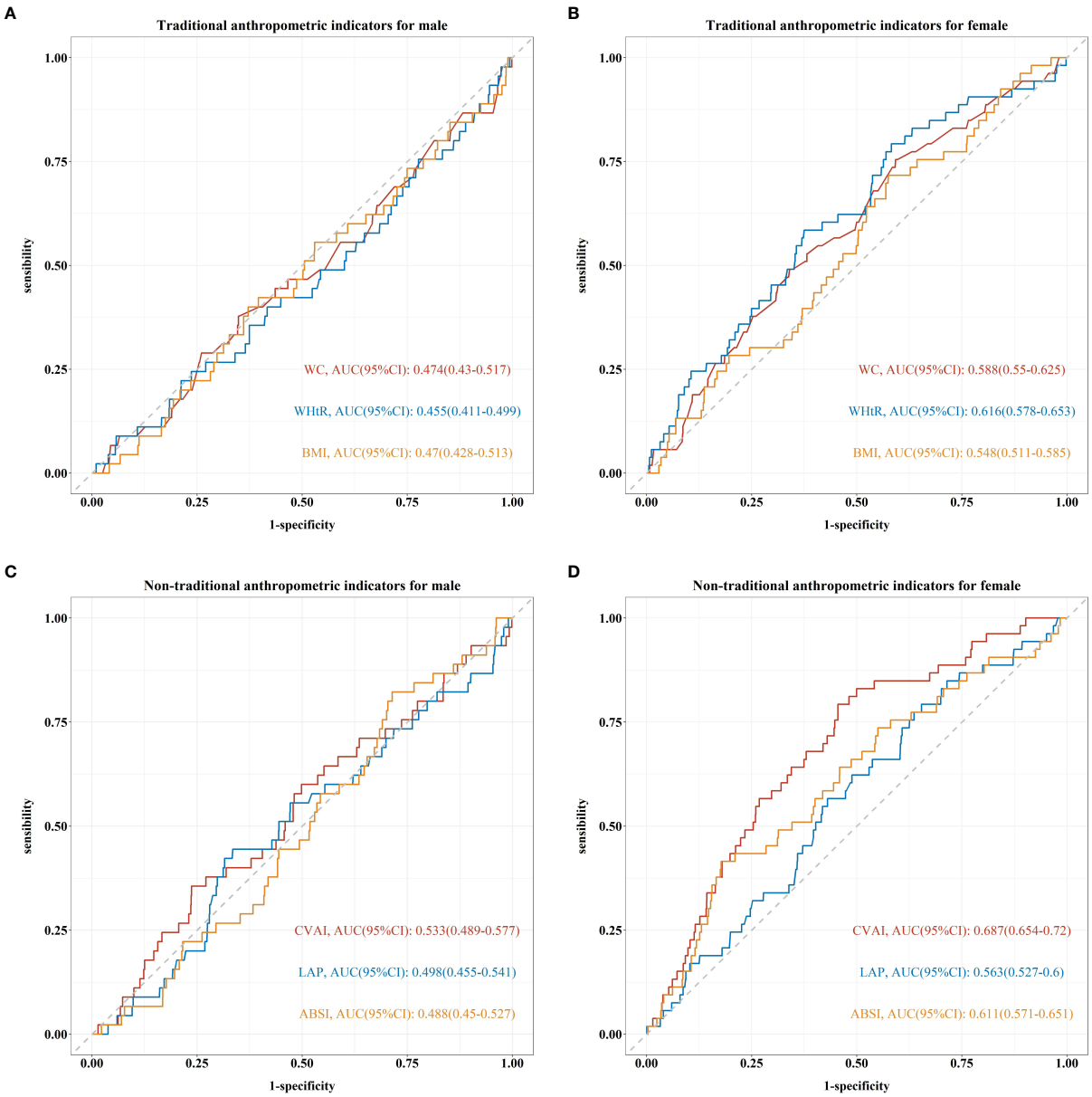


FIGURE 2
Receiver operating characteristic (ROC) curve and area under ROC curve (AUC) of traditional and non-traditional anthropometric indicators for predicting CVD among male and female participants based on the adjusted Cox regression model (Model 3). **(A, B)** For waist circumference (WC), waist-to-height ratio (WHtR), and body mass index (BMI), respectively. **(C, D)** For Chinese visceral adiposity index (CVAI), lipid accumulation product (LAP), and body shape index (ABSI).

might be influenced by recall bias. Second, we failed to collect any information of medication use for CVD, which have possible beneficial impact on CVD. Third, although the rate of loss to follow-up is above 10%, this rate is relatively low in all studies in Southwest China, given their poor traffic accessibility. Another thing to note is that our study population was from Southeast China, and CVAI was applicable to Chinese people; thus, the findings from this study should be generalized to other populations with caution.

In summary, our study contributes to a new knowledge about the associations of adiposity with incident CVD among Southwest Chinese across a variety of anthropometric indicators. Although visceral adiposity measure indicators are not diagnostic tools for cardiovascular events, the simplicity of

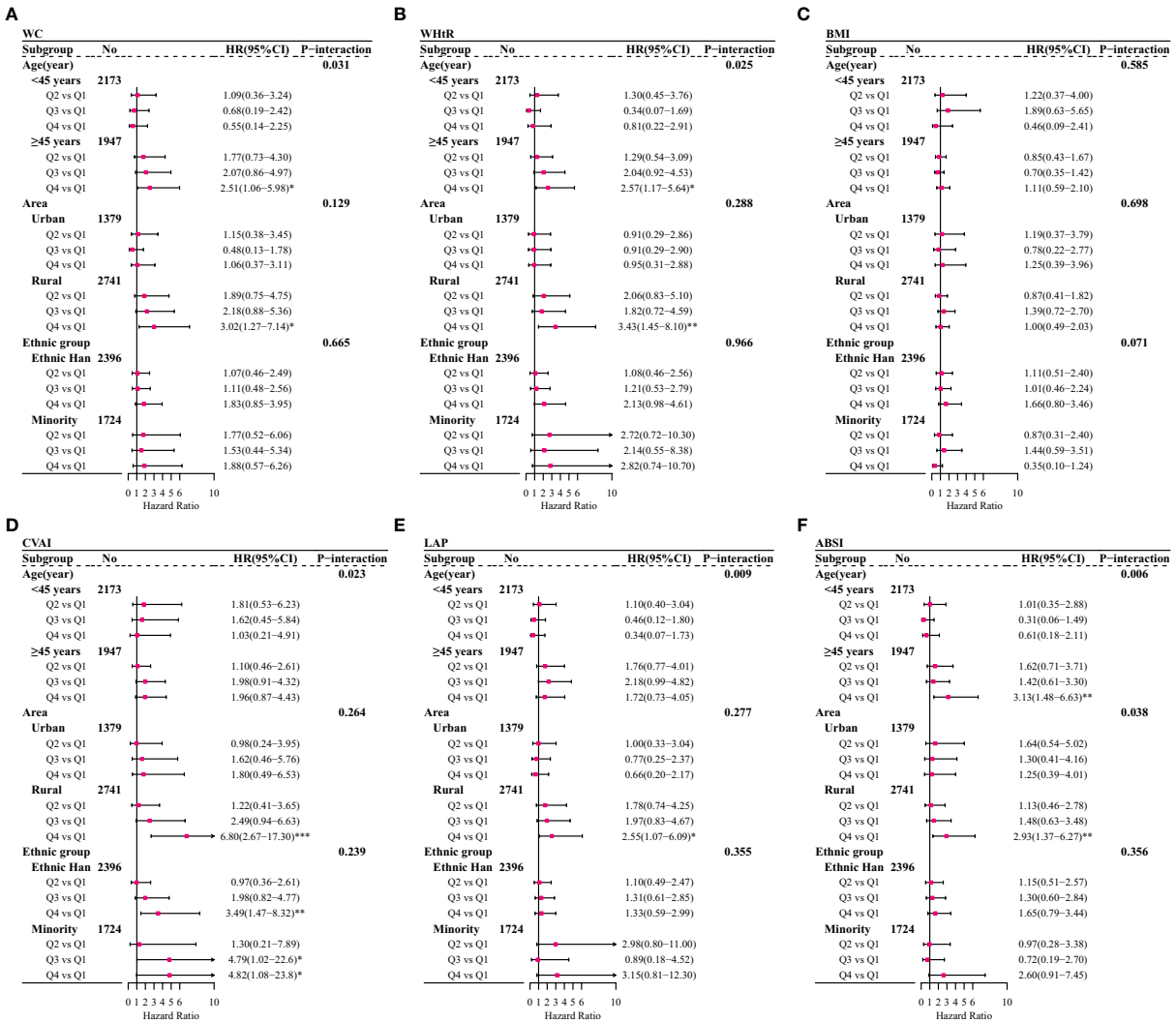


FIGURE 3
Adjusted hazard ratios (HRs) and 95% confidence intervals (95% CIs) for overall CVD or overall CVD associated with traditional and non-traditional anthropometric indicators among female participants after stratified by age, area, and ethnic group based on adjusted Cox regression model (Model 3). **(A)** For waist circumference (WC); **(B)** for waist-to-height ratio (WHtR); **(C)** for body mass index (BMI); **(D)** for Chinese visceral adiposity index (CVAI); **(E)** for lipid accumulation product (LAP); **(F)** for body shape index (ABSI); *$p < 0.05$; **$0.05 < p < 0.01$;***$0.01 < p < 0.001$.

anthropometric measurements (WC and BMI) and blood biochemical tests (TG and HDL) might therefore make them well applicable indicators for assessing CVD risk in clinical practice.

## Conclusions

Visceral adiposity measures, especially CVAI, are stronger indicators of CVD among female not male participants in Southwest China. Different anthropometric indicators need to be combined to comprehensively assess health risks.

## Data availability statement

The datasets for this manuscript will be made available upon request, further inquiries can be directed to the corresponding author TL, liutaombs@163.com and NW, na.wang@fudan.edu.cn.

## Ethics statement

The studies involving human participants were reviewed and approved by the ethics review board of Guizhou Province (No. S2017-02). The patients/participants provided their written informed consent to participate in this study.

## Author contributions

Conceptualization: NW, TL, and CF. Data curation: XZ and YC. Investigation: XZ, YY, and YZ. Methodology and formal analysis: YW and NW. Writing—original draft: YW and XZ. Writing—review and editing: NW, TL, and CF. All authors contributed to the preparation of the final document, read, and approved the final manuscript.

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## Conflict of interest

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

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.969753/full#supplementary-material

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# Is non-high-density lipoprotein associated with metabolic syndrome? A systematic review and meta-analysis

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**Introduction:** Novel atherogenic lipid indices, including non-high-density lipoprotein cholesterol (non-HDL-C) which is calculated by subtracting the HDL-C value from the total cholesterol level, atherogenic index (ratio between triglycerides (TG) and HDL-C concentrations (TG/HDL-C)), and Diff-C (calculated by subtracting low-density lipoprotein (LDL-C) from non-HDL-C), have been known as valuable predictors of dyslipidemia and subsequent cardiovascular diseases. Previous studies have reported the potential association of novel atherogenic lipid indices with metabolic syndrome (MetS). This meta-analysis aimed to assess the pooled association of novel atherogenic lipid indices with MetS or its components.

**Methods:** A systematic search was conducted through PubMed, Scopus, and Web of Science (WoS) databases from January 2000 until March 2021 to evaluate the association of novel atherogenic lipid indices, including non-HDL-C, atherogenic index, and the difference between non-HDL-C and LDL-C (Diff-C) with MetS. Observational studies were included without any language restriction. As exclusive studies evaluating the association of non-HDL-C with metabolic syndrome (MetS) were eligible to be included in quantitative analyses, a random-effect meta-analysis was performed to pool the odds ratios (ORs). A stratified meta-analysis was performed based on the definition of MetS [Adult Treatment Panel (ATP) and International Diabetes Federation (IDF)] and the studied population.

**Results:** Overall, 318 studies were retrieved from an initial systematic search. After screening, 18 and five studies were included in the qualitative and quantitative syntheses, respectively. Qualitative synthesis revealed an association between non-HDL-C, Diff-C, and atherogenic index with MetS and its components. Stratified meta-analysis showed that an increased non-HDL-C level was associated with an increased odds of MetS based on ATP criteria (OR: 3.77, 95% CI: 2.14-5.39) and IDF criteria (OR: 2.71, 95% CI: 1.98-3.44) in adults (OR: 3.53, 95% CI: 2.29-4.78) and in children (OR: 2.27, 95% CI: 1.65-2.90).

**Conclusion:** Novel atherogenic lipid indices, including atherogenic index, Diff-c, and non-HDL-C, are strongly associated with increased odds of MetS and its components. The indices could be considered as potential predictors of MetS and its components in clinical practice.

KEYWORDS

**dyslipidemia, non-high-density lipoprotein cholesterol, metabolic syndrome, cardiovascular disease, cardiometabolic, cholesterol**

## Introduction

Metabolic syndrome (MetS) is a well-established risk factor which increases the likelihood of experiencing cardiovascular events (1). The current study considers diagnoses of metabolic syndrome (MetS) and other cardiometabolic risk factors such as hypertension, central obesity, insulin resistance, hyperinsulinemia, diabetes, and hyperlipidemia as outcomes. It is estimated that the prevalence of these risk factors has risen remarkably (2, 3). Five of these risk factors comprise a syndrome called MetS. Although several definitions of MetS have been introduced, the five parameters serum glucose levels, high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), obesity, and blood pressure have generally been the defining factors of the syndrome (4). Among all MetS components, hyperlipidemia has been recognized as an independent and significant risk factor for cardiovascular disease (CVD) (5). According to the Framingham Heart Study, among the parameters measured in the lipid profile, a low level of HDL-C and a high level of low-density lipoprotein cholesterol (LDL-C) are strongly associated with the increased risk of CVDs (6). This information reveals that the incident risk of CVD is increased by 2%–3% with each mg/dL decrease in HDL-C levels (7). The Framingham Heart Study's findings on LDL-C have been repeatedly confirmed by other studies (8–10) to the point that controlling LDL-C levels is currently recognized as the primary target in treating hyperlipidemia (11).

Based on the National Cholesterol Education Program Adult Treatment Panel (NCEP ATP III) suggestion, the secondary target in treating hyperlipidemia in patients with a triglyceride higher than 200 is non-high-density lipoprotein cholesterol (non-HDL-C) (11). Non-HDL-C measures LDL-C, VLDL-C, chylomicrons, lipoprotein (a), IDL, and chylomicron remnant. Non-HDL cholesterol (non-HDL-C) is calculated by subtracting the HDL-C value from the total cholesterol level. Although several components make up non-HDL, this index mainly comprises atherogenic lipoproteins such as LDL, very low-density lipoprotein (VLDL-C), and intermediate-density lipoprotein (IDL-C). Different studies have shown that even after a significant decrease in LDL-C levels, a considerable amount of residual risk for CVD incidence remains. It was concluded that other lipids (other than LDL-C) are also involved in increasing the risk of CVD (12, 13). One of the indices measuring these lipids is non-HDL-C. Non-HDL-C measures different components such as LDL-C, VLDL-C, chylomicrons, lipoprotein(a), IDL, and chylomicron remnants. Moreover, the atherogenic index (ratio between TG and HDL-C concentrations (TG/HDL-C) and Diff-C (calculated by subtracting LDL-C from non-HDL-C)) measures the cumulative effects of these lipids on the CVD risk increment. The data extracted from Framingham's study show that some of these components, such as VLDL-C, even further increase the risk of CVD incidence compared to LDL-C; the importance of this result is so significant that a study demonstrated that after multivariate adjustment for the non-HDL-C level, LDL-C would not increase the risk of CVD independently (14).

The accompanying of high non-HDL-C and other metabolic syndrome parameters showed a cumulative increment in CVD mortality risk. In other words, the risk of developing CVD is 200 times higher in diabetic patients than in non-diabetic patients (15). If diabetes is accompanied by dyslipidemia, the risk of CVD

is further increased in the patients. Prior studies have searched for lipid targets to help decrease this added risk (16–18), and they conclude that compared to LDL-C, non-HDL-C is a stronger predictor for CVD fatality in diabetic patients (19). This study aims to evaluate the association of non-HDL-C, atherogenic index, and Diff-C with MetS and its components.

# Methods

This systematic review and meta-analysis were performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.

## Study question

- Are novel atherogenic lipid indices associated with metabolic syndrome?

## Information sources and search strategy

A systematic search was independently carried out through PubMed, Scopus, and Web of Science (WoS) databases (from January 2000 until March 2021) by two reviewers (ES and FA) on the link of MetS and the atherogenic index, Diff-c, or non-HDL-C. The search strategy is demonstrated in Supplementary Table 1. Moreover, other resources, related gray literature, publications' reference lists, and related key journals were searched for additional publications.

## Study selection

EndNote reference management software was used for the study selection process so as to manage the papers. After removing duplicate papers, the title and abstract of the articles were evaluated based on the inclusion criteria. Eventually, the full texts were screened in detail. The selection process was independently conducted by two authors (PM and MQ).

## Eligibility criteria

The following criteria were considered for screening the included articles: 1) observational studies which include participants' novel atherogenic lipid indices including atherogenic index, Diff-C, or non-HDL-C level; 2) articles must include data on patients' MetS or its components' diagnosis, including hypertension, obesity, insulin resistance, hyperinsulinemia, diabetes, hyperlipidemia, and coronary heart disease; 3) articles must demonstrate a link between MetS or its components' diagnosis and the atherogenic lipid indices; 4) articles can be published in any language.

## Data collection process and data items

The data extraction form has been filled by two researchers independently. Another researcher resolved conflicts.

## Quality assessment

Quality assessment was conducted by the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement. This statement provides general reporting recommendations for descriptive observational studies and studies which investigate the associations between exposures and health outcomes. Both of these guidelines consist of 25 subitems. Each of these subitems was rated yes (1 point) or no (0 points); the final quality assessment score is the sum of these subitem points. The quality assessment was carried out by two researchers independently based on the guidelines' items.

## Data synthesis

Results are presented as odds ratio (OR) and its 95% confidence interval (95% CI). STATA version 11.2 (StataCorp, College Station, TX) software was used to conduct the meta-analysis. We conducted a meta-analysis when two or more than two studies report the association between an atherogenic lipid index with MetS or its components. The pooled estimate of ORs and their 95%CI were calculated based on extracted data from the studies which were included in quantitative analysis. The heterogeneity was evaluated based on the $I^2$ statistic and the chi-square-based Q test. Lack of heterogeneity was defined when the p-value was more than 0.10. Random or fixed effect models were used to pool the association of non-HDL-C-C with MetS. Subgroup analysis was used based on the study population (adults/children) and criteria (ATP III/IDF). Publication bias was assessed by using Begg's test. We considered a substantial publication bias whenever the p-value was calculated less than 0.1. Sensitivity analysis was performed to assess the effect of exclusion of studies which did not adjust the potential confounders.

# Results

## Study and patient characteristics

Our searches revealed 269 studies from PubMed, 317 studies from Scopus, and 205 studies from the Web of Science. In addition, our manual search for gray literature yielded 456

studies. After the rejection of duplicates, we screened 415 studies, followed by a full-text assessment for eligibility for 222 papers. Finally, 19 (20–38) and 5 (20, 25, 27, 29, 31) studies were included in the qualitative and quantitative syntheses, respectively. The detailed flow diagram is demonstrated in Figure 1. Four of the included studies were cohorts, while 14 of them were cross-sectional studies. Seven studies were originated from the United States, followed by three papers that originated from Iran. The largest sample size was for Miyazaki et al.'s study with 5,853 participants, and the smallest sample size was for Dharuni et al.'s study with 100 participants. Studies' provenance, sample size, target population, and their patients' characteristics are summarized in Table 1.

## Qualitative synthesis

### Diagnostic values of Diff-C, non-HDL-C, and atherogenic index

Ten of included papers reported diagnostic values, including sensitivity, specificity, the area under the ROC curve (AUC) of Diff-C, non-HDL-C, and atherogenic index to predict metabolic syndrome or one of its diagnostic components. The highest sensitivity, specificity, and AUC for Diff-C reported in the literature were 0.86 (0.78-0.93) (20), 89.1 (25), and 0.828 (0.770-0.887) (25), respectively. Similarly, non-HDL-C showed a sensitivity ranging from 0.22 in Liu et al.'s (33) study to predict insulin resistance to 75.7 in Ghodsi et al.'s (25) study to predict MetS diagnoses by ATP III criteria. While the highest specificity for non-HDL-C was 89% for patients diagnosed with MetS by Harmonious criteria (33), the lowest specificity was reported 57.1 in Ghodsi et al.'s (25) study for patients diagnosed with MetS IDF criteria. Likewise, the atherogenic index showed AUC, ranging from 0.625 (32) to 0.872 (24). Table 2 demonstrates diagnostic values of Diff-C, non-HDL-C, and atherogenic index to predict MetS or its components.

### Association of Diff-C, non-HDL-C, and atherogenic index and MetS or its components

Our search yielded 14 articles measuring OR, correlation coefficient, risk ratio, Spearman correlation, Pearson correlation, multiple linear regression, t-test, and Poisson regression analysis to evaluate the association of Diff-C, non-HDL-C, and atherogenic index with MetS or its components. Regarding the association of MetS and Diff-C, the highest adjusted OR was 26.29 (17.71-39.05) in patients

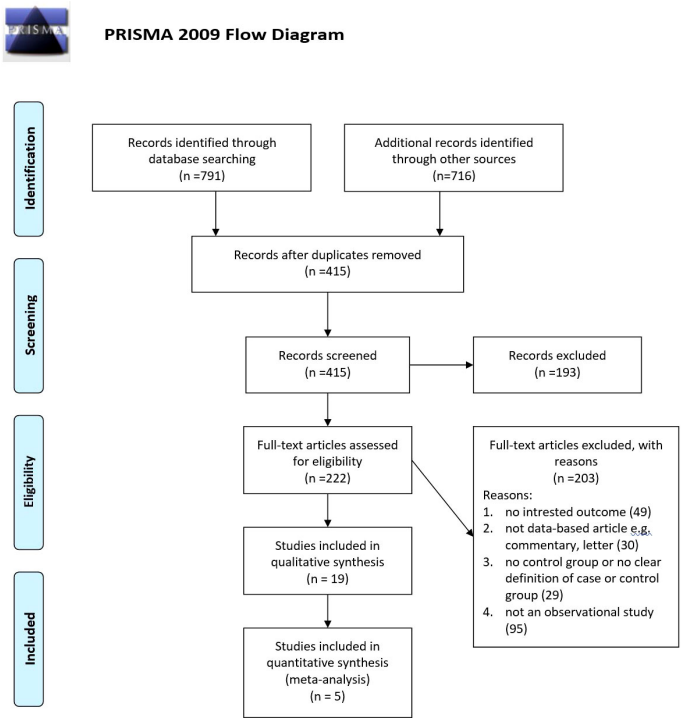


FIGURE 1
PRISMA diagram for searching resources.

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TABLE 1 Characteristics of the included studies.

| Author (ref) | Year | Study type | Country | Target population | Sample size | Sex ratio (M/F) | Age (year) | Quality score |
|---|---|---|---|---|---|---|---|---|
| Angoorani (20) | 2018 | Cross-sectional | Iran | Healthy children and adolescents | 3,843 | 2,010/1,833 | 7-18 | 22* |
| Dharuni (21) | 2016 | Cross-sectional | India | Metabolic syndrome | 100 | 35/65 | Case: 50.4 ± 9.7 Control: 50.2 ± 9 | 20* |
| Frontini (23) | 2007 | Cohort | USA | Asymptomatic younger adult | 1,203 | 43% man, 71% white | 24-34 | 18** |
| Frontini (22) | 2008 | Cohort | USA | Children | 437 | 40% male, 70% white | 5-19 | 19** |
| Gasevic (24) | 2014 | Cross-sectional | Aboriginal, Chinese, European, and South Asian origin | Healthy adult | 797 | 380/417 | 35-60 | 20* |
| Ghodsi (25) | 2017 | Cross-sectional | Iran | Adults | 2,125 | 957/1,168 | 25-64 | 21* |
| Huang (26) | 2008 | Cross-sectional | United States | Adults | 928 | 297/631 | 53 | 21* |
| Kazemi (27) | 2010 | Cross-sectional | Iran | Healthy adults | 3,277 | 1,578/1,699 | 15< | 16* |
| Khan (28) | 2018 | Cross-sectional | Pakistan | Asymptomatic subjects referred for CVD risk evaluation | 229 | 109/120 | Male:47.98 ± 11.30 females: 45.27 ± 12.42 | 19* |
| Lee (29) | 2007 | Cross-sectional | Korean | Women | 511 | – | 48.36 ± 5.29 | 18* |
| Li (30) | 2008 | Cross-sectional | US | Non-diabetic adults | 2,652 | 1,358/1,294 | ≥20 | 17* |
| Li (31) | 2011 | Cross-sectional | US | Healthy children and adolescents | 2,734 | 1,444/1,290 | 12-19 | 20* |
| Liang (32) | 2015 | Cross-sectional | China | Obese children | 976 | 690/286 | 6-16 | 19* |
| Liu (33) | 2013 | Cross-sectional | US | Healthy adult | 366 | 143/223 | 22-70 | 19* |
| Miyazaki (34) | 2016 | Cross-sectional | Japan | schoolchildren | 5,853 | 2,963/2,890 | 6-15 | 20* |
| Onat (35) | 2010 | Cohort | Turkey | Middle-age adult 7.8-year follow-up | 2,676 | 1,294/1,382 | 28-80 | 21** |
| Park (36) | 2015 | Cross-sectional | Korea | Adult males who visited the Health Promotion Center and underwent medical examination and abdominal CT | 372 | 1 | Mean: 52 | 18* |
| Srinivasan (37) | 2002 | Cross-sectional | US | Healthy children | 2,843 | 1,422/1,421 | 5-17 | 20* |
| Srinivasan (38) | 2006 | Cohort | US | Healthy children | 1,163 | 519/644 | Children: 5-14-year adults: 27< | 19** |

*Quality assessed by STROBE for cross-sectional studies. **Quality assessed by STROBE for cohort studies.

diagnosed by ATP III, followed by 10.71 (7.47–15.35) in patients diagnosed by IDF, both reported in Ghodsi et al.'s study (25). Non-HDL-C showed a relatively strong correlation with MetS with ORs as high as 5.87 (3.92-8.80) (25) and Spearman correlation results as high as 0.95 p < 0.0001 (37). Similarly, ORs reported for atherogenic index and MetS range from 1.00 (0.92 to 1.09) in Angoorani et al.'s (20) study per one-unit increment of the atherogenic index to predict high blood pressure to 40.26 to predict high triglyceride level (20) (Table 3).

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TABLE 2 Characteristics of the included studies which assessed the diagnostic value of Diff-C, non-HDL, and atherogenic index to predict CMRFs.

| Author, year | Outcome | Diagnostic criteria | | Cutoff value | SE %(95% CI) | SP %(95% CI) | AUC (95% CI) |
|---|---|---|---|---|---|---|---|
| Diff-C (mg/dL) | | | | | | | |
| Angoorani, 2018 (20) | MetS | ATP III for pediatrics | M | 19.9 (19.26-20.33) | 0.84 (0.76-0.91) | 0.76 (0.73-0.79) | 0.80 |
| | | | F | 19.9 (19.37-20.22) | 0.86 (0.78-0.93) | 0.74 (0.70-0.78) | 0.80 |
| Ghodsi, 2017 (25) | Mets | ATP III | | 29.55 | 73.3 | 82.9 | 0.819 (0.801,0.838) |
| | | ATP III in DM (−) | | 30 | 72.4 | 88.3 | 0.817 (0.797, 0.834) |
| | | ATP III in DM (+) | | 30 | 70.3 | 89.1 | 0.828 (0.770, 0.887) |
| | | IDF | | 29.50 | 65.9 | 80.4 | 0.777 (0.757, 0.797) |
| | | IDF in DM (−) | | 29.45 | 67.5 | 79.6 | 0.786 (0.765, 0.807) |
| | | IDF in DM (+) | | 30 | 68.2 | 59 | 0.627 (0.549, 0.705) |
| Non-HDL-C | | | | | | | |
| Angoorani, 2018 (20) | MetS | ATP III for pediatrics | M | 119.5 (103.37,134.62) | 0.49(0.26-0.71) | 0.73 (0.50-0.95) | 0.61 |
| | | | F | 115.5 (88.58,141.4) | 0.49 (0.18-0.78) | 0.64 (0.25-1.01) | 0.56 |
| Liu, 2013 (33) | MetS | "Harmonious" criteria | | 160 | 0.46 | 0.72 | – |
| | | | | 190 | 0.24 | 0.89 | – |
| | Insulin resistance | SSPG ≥10.3 mmol/l | | 160 | 0.44 | 0.69 | – |
| | | | | 190 | 0.22 | 0.87 | – |
| Li, 2011 (31) | MetS | ATP III for pediatrics | | 120 | 0.75 | 0.69 | 0.77 (0.73-0.81) |
| | | ATPIII for adults | | 120 | 0.73 | 0.75 | 0.81 (0.76-0.86) |
| | | IDF for pediatric | | 120 | 0.67 | 0.75 | 0.79 (0.74-0.84) |
| | | IDF for adult | | 125 | 0.68 | 0.75 | 0.78 (0.73-0.83) |
| Ghodsi, 2017 (25) | Mets | ATP III | | 153.5 | 0.75 | 0.57.2 | 0.719 (0.697, 0.740) |
| | | ATP III in DM (−) | | 161.5 | 0.67 | 0.64.1 | 0.717 (0.693, 0.740) |
| | | ATP III in DM (+) | | 175.5 | 0.55 | 0.84.8 | 0.733 (0.659, 0.807) |
| | | IDF | | 153.5 | 0.73 | 0.57.1 | 0.693 (0.670, 0.715) |
| | | IDF in DM (−) | | 160 | 0.67 | 0.63.4 | 0.698 (0.674, 0.722) |
| | | IDF in DM (+) | | 175.8 | 0.54 | 0.65.3 | 0.608 (0.534, 0.683) |
| Frontini, 2008 (22) | Excess carotid IMT in children | Top 10th percentile | | – | – | – | 0.65 (0.56-0.70) |
| Frontini, 2007 (23) | Increased carotid intima-media thickness in adults | Top 10th percentile | | – | – | – | 0.73 (0.68-0.78) |
| Miyazaki, 2016 (34) | Cardiovascular disease/MetS | Takaoka/nationwide | | 152 mg/dL (97th percentile) | 0.98 | – | – |
| Atherogenic index | | | | | | | |
| Angoorani, 2018 (20) | MetS | ATP III for pediatrics | M | 2.53 (2.35,2.71) | 0.80 (0.71-0.88) | 0.80 (0.76-0.83) | 0.80 |
| | | | F | 2.54 (2.19,2.89) | | | 0.83 |

*(Continued)*

TABLE 2 Continued

| Author, year | Outcome | Diagnostic criteria | Cutoff value | | SE %(95% CI) | SP %(95% CI) | AUC (95% CI) |
|---|---|---|---|---|---|---|---|
| | | | | | 0.86 (0.77-0.94) | 0.79 (0.71-0.86) | |
| Gasevic, 2014 (24) | Mets | Number of Mets components | M | 1.62 | 0.84 | 0.80 | 0.869 (0.830, 0.908) |
| | | | F | 1.18 | 0.70 | 0.88 | 0.872 (0.832, 0.912) |
| Li, 2008 (22) | Hyperinsulinemia | FSI of 13.13 μU/ml (the 75th percentile) | NHW | 1.2 | 0.70 | 0.71 | 0.77 (0.74 to 0.79) |
| | | | NHB | 0.9 | 0.61 | 0.77 | 0.75 (0.69 to 0.77) |
| | | | MA | 1.2 | 0.64 | 0.71 | 0.74 (0.69 to 0.76) |
| Liang, 2015 (32) | Mets | MS-CHN2012 | | 1.25 | 0.80 | 0.75 | 0.843 |
| | Insulin resistance | HOMA1-IR | | 4.59 | 0.59 | 0.66 | 0.640 |
| | | HOMA2-IR | | 2.76 | 0.53 | 0.70 | 0.625 |

MetS, metabolic syndrome; ATP III, Adult Treatment Panel III; IDF, International Diabetes Federation; M, male; F, female; MA, Mexican American; NHW, non-Hispanic white; NHB, non-Hispanic black.

## Quantitative synthesis

Data were from 17,860 participants of the five papers included in quantitative analysis which revealed that metabolic syndrome is linked with non-HDL-C in both adults (OR 3.53, 95% CI: 2.29-4.78) and children (OR 2.27, 95% CI: 1.65-2.90). Concerning the two different definitions used for metabolic syndrome in studies, the current meta-analysis demonstrated that the non-HDL-C level is correlated with metabolic syndrome using either ATP III diagnostic criteria (OR 3.77, 95% CI: 2.14-5.39) or IDF diagnostic criteria (OR 2.71, 95% CI: 1.98-3.44). The meta-analysis results are summarized in Table 4. Also, Figure 2 illustrates the forest plot of included studies.

### Publication bias

Begg's (p = 0.567) showed no evidence of significant publication bias between non-HDL-C level and odds of being diagnosed with MetS. None of the included study population dramatically influenced the overall pooled OR. The funnel plot is demonstrated in Figure 3.

# Discussion

This study demonstrated that not merely can atherogenic lipid indices predict the diagnosis of MetS or its components but also these indices are correlated with higher odds of being diagnosed with these risk factors, including Mets, obesity, hypertriglyceridemia, reduced HDL cholesterol, diabetes, and hypertension.

In other words, our findings revealed that the odds of being diagnosed with MetS are nearly three times higher in patients with high non-HDL-C levels. Our data revealed that not only is non-HDL-C a reliable test to predict the MetS diagnosis in adults but also there are higher odds of being diagnosed with MetS in children with increased non-HDL-C levels. As pediatric MetS is a strong predictor of adulthood MetS (40), these patients are at a higher risk of type 2 diabetes and cardiovascular events (41). Moreover, the previous studies proved that interventions in pediatric MetS patients in early life could prevent MetS complications (42, 43). Therefore, this study proposes non-HDL-C as a marker to predict the odds of being diagnosed with MetS in pediatric patients.

In this study, we compared the criteria with which the MetS is diagnosed. It should be noted that in the same population the number of patients diagnosed by ATP III criteria is lower compared to patients diagnosed by IDF criteria (44). That is to say, ATP III has higher sensitivity, while IDF has higher specificity to diagnose MetS, and on average, patients diagnosed by ATP III are at a higher risk of cardiovascular events in comparison to patients diagnosed by IDF (45). Nevertheless, as confidence intervals regarding ATPIII and IDF overlap, this study demonstrated that the correlation of MetS and non-HDL is regardless of the MetS criteria.

Regardless of the criteria in which MetS is defined, it consists of five components, including obesity, hypertriglyceridemia, reduced HDL cholesterol, diabetes, and hypertension. The current study indicated a notable link between non-HDL-C and atherogenic index. Likewise, Sheth et al.'s study showed a

TABLE 3 Characteristics of the included studies which assessed relationship between Diff-C, non-HDL, and atherogenic index and CMRFs.

| Author, year | Outcome | Definition of outcome | Cutoff for Diff-C, non HDL, and atherogenic index | Type of effect size | effect size | Confounder |
|---|---|---|---|---|---|---|
| Diff-C | | | | | | |
| Angoorani, 2018 (20) | High TC (mg/dl) | More than 200 | Per 1-mg/dl increment. | Adjusted odds ratio (95 % CI) | 1.07(1.06-1.09)* | Adjusted for age, sex, living area, screen time, SES and physical activity and adjusted for BMI except for overweight, obesity and abdominal obesity. |
| | High LDL(mg/dl) | More than 110 | | | 1.02(1.01-1.03)* | |
| | MetS | ATP III | | | 1.08(1.07-1.10)* | |
| | Low HDL (mg/dl) | Less than 40 mg/dl, except for boys between 15 and 19 years old; which is less than 45 mg/dl | | | 1.04(1.04,1.05)* | |
| | Overweight (Kg/m2) | 85th < BMI < 95th | | | 1.01 (1.00-1.02)* | |
| | Abdominal Obesity | Waist to height ratio more than 0.5 | | | 1.00 (0.99-1.01) | |
| | Obesity (Kg/m2) | BMI more than 95th | | | 1.00 (0.99-1.01) | |
| | High FBS (mg/dl) | More than 100 | | | 1.03 (1.02-1.05)* | |
| | High TG (mg/dl) | More than 100 | | | 1.02 (1.01-1.03)* | |
| | Hypertension (mmHg) | More than 90th | | | 1.00 (0.98-1.01) | |
| Ghodsi, 2017 (25) | Mets (IDF) | ATP III | 30 mg/dl | Adjusted odds ratio (95% CI) | 26.29 (17.71-39.05) | Age, sex, residential area, Hypertension, total physical activity, waist circumference, FBS, Insulin resistance (HOMA.IR), and BMI |
| | | IDF | | | 10.71 (7.47-15.35) | |
| Non-HDL-C | | | | | | |
| Angoorani, 2018 (25) | High TC(mg/dl) | More than 200 mg/dl | Per 1-mg/dl increment | Adjusted odds ratio (95% CI) | 1.19 (1.16,1.22)* | Age, sex, living area, screen time, SES and physical activity; additionally for BMI except for BMI, and WC outcomes. |
| | High LDL (mg/dl) | More than 110 | | | 1.19 (1.17,1.21)* | |
| | MetS | ATP III | | | 1.01 (1.00, 1.01) | |
| | Low HDL (mg/dl) | Less than 40 mg/dl, except for boys between 15 and 19 years old; which is less than 45 mg/dl | | | 0.99(0.99,0.99) | |
| | Overweight (Kg/m2) | 85th < BMI < 95th | | | 1.00 (0.99,1.00) | |
| | Abdominal Obesity | Waist to height ratio more than 0.5 | | | 1.00(0.99,1.00) | |
| | Obesity (Kg/m2) | BMI more than 95th | | | 1.00(0.99,1.00) | |
| | High FBS (mg/dl) | More than 100 mg/dl | | | 1.00(.99,1.01) | |
| | High TG (mg/dl) | More than 100 | | | 1.03(1.02,1.03)* | |
| | High BP (mmHg) | More than 90th percentile | | | 0.99 (0.99,1.01) | |

*(Continued)*



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TABLE 3 Continued

| Author, year | Outcome | Definition of outcome | Cutoff for Diff-C, non HDL, and atherogenic index | Type of effect size | effect size | Confounder |
|---|---|---|---|---|---|---|
| Huang, 2008 (26) | MetS | Diagnosed with MetS by ATP III | Reporting non-HDL value in each group | T-test (mean ± SD) | M:174±64<br>F:165±50 | None |
| | | Not diagnosed with MetS by ATP III | | | M:156±57<br>F:147±41 | |
| Liu, 2013 (33) | Waist circumference (cm) | As a continuous variable | As a continuous variable | Correlation coefficient (r) | 0.25* | Age, sex, BMI |
| | SBP (mmHg) | | | | 0.24* | |
| | DBP (mmHg) | | | | 0.21* | |
| | FBS (mg/dl) | | | | 0.13 | |
| | HDL(mg/dl) | | | | −0.19* | |
| | TG (mg/dl) | | | | 0.46* | |
| Li, 2011 (31) | MetS | ATP III for pediatrics | 120 mg/dl | Adjusted odds ratio (95% CI) | 2.8 (1.7-4.8)* | Sex, age, race/ethnicity, and poverty-to-income ratio, cotinine, C-reactive protein, fasting insulin, BMI |
| | | | 145 mg/dl | | 4.0 (2.4-6.9)* | |
| | | ATP III for adult | 120 mg/dl | | 3.5 (1.8-6.9)* | |
| | | | 145 mg/dl | | 5.6 (2.6-12.3)* | |
| | | IDF for pediatric | 120 mg/dl | | 3.2 (1.6-6.5)* | |
| | | | 145 mg/dl | | 4.5 (2.1-9.6)* | |
| | | IDF for adult | 120 mg/dl | | 3.0 (1.6-5.6)* | |
| | | | 145 mg/dl | | 3.9 (1.9-7.9)* | |
| Srinivasan, 2006 (38) | Dyslipidemia | Receiving medication for dyslipidemia | More than144 mg/dl versus less than 123 mg/dl | Adjusted odds ratio (95% CI) | 4.49 (2.51 – 8.04)* | Baseline BMI and change after 27 years. |
| | Obesity | BMI greater than or equal to 30 kg/m2 | | Prevalence odds ratio (95% CI) | 1.9438 (1.0866 - 3.4773)* | |
| | High LDL(mg/dl) | LDL greater than or equal to 160 | | | 4.6885 (2.2713 - 9.6782)* | |
| | High TG(mg/dl) | TG greater than or equal to 150 | | | 3.1441 (1.7000 - 5.8148)* | |
| | Low HDL(mg/dl) | HDL less than 40 | | | 1.8387 (1.0025 - 3.3725)* | |
| | High FBS(mg/dl) | FPG greater than or equal to 126 | | | 2.8116 (0.7236 - 10.9243) | |
| | High Insulin (μU/mL) | Insulin more than 18 | | | 1.8446 (0.9190 - 3.7026) | |
| | Hypertension | SBP more than 140 mm Hg in addition to DBP more than 90 mm Hg | | | 1.8434 (0.7989 - 4.2534) | |

*(Continued)*





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TABLE 3 Continued

| Author, year | Outcome | Definition of outcome | Cutoff for Diff-C, non HDL, and atherogenic index | Type of effect size | effect size | Confounder |
|---|---|---|---|---|---|---|
| Ghodsi, 2017 (25) | Mets | ATP III | 160 mg/dl | Adjusted odds ratio (95% CI) | 2.75 (2.10, 3.61)* | Age, sex, residential area, hypertension, total physical activity, waist circumference, FBS, Insulin resistance (HOMA.IR), and BMI |
| | | | 190 mg/dl | | 3.61 (2.67, 4.88)* | |
| | | | Q2 versus Q1 | | 1.78(1.18, 2.70)* | |
| | | | Q3 versus Q1 | | 2.62(1.74, 3.95)* | |
| | | | Q4 versus. Q1 | | 5.87(3.92, 8.80)* | |
| | | IDF | 160 mg/dl | | 3.14(2.30, 4.29)* | |
| | | | 190 mg/dl | | 2.70(2.03, 3.59)* | |
| | | | Q2 versus Q1 | | 1.43(0.85, 2.44) | |
| | | | Q3 versus Q1 | | 3.08(1.83, 5.19)* | |
| | | | Q4 versus. Q1 | | 4.90(3.00, 8.16)* | |
| Srinivasan, 2002 (27) | BMI(Kg/m2) | As a continuous variable | As a continuous variable | Spearman correlation | 0.13* | Age, race, gender, cigarettes/week, and alcohol (mL/week). |
| | WC (cm) | | | | 0.09* | |
| | TC(mg/dl) | | | | 0.9* | |
| | TG(mg/dl) | | | | 0.42* | |
| | LDL (mg/dl) | | | | 0.95* | |
| | HDL (mg/dl) | | | | -0.12* | |
| Onat, 2010 (35) | Diabetes | AHA criteria | Per 40-mg/dl increment | Risk ratio (95% CI) | M;1.27 (1.00–1.60) | Age, BP, smoking, BMI, atherogenic index |
| | | | | | F; 1.13(0.85–1.49) | |
| | Coronary heart disease | The presence of angina pectoris, of a history of myocardial infarction with or without accompanying Minnesota codes of the electrocardiogram | | | M; 1.49 (1.22–1.81)* | |
| | | | | | F; 1. 32(1.04–1.61)* | |
| Lee, 2007 (39) | Mets | ATP III | T3 vs. T1 | Adjusted odds ratio (95% CI) | 4.005 (1.151-13.939)* | BMI, age, BP,FBS, atherogenic index |
| | | IDF | T3 vs. T1 | | 1.772 (0.510-6.161) | |
| Khan, 2018 (28) | BMI (Kg/m2) | As a continuous variable | As a continuous variable | Pearson correlation (r) | 0.139* | BMI, age, BP, WHpR, fasting plasma glucose, A1c, insulin, HOMA-IR, urine albumin creatinine ratio |
| | SBP (mmHg) | | | | 0.078 | |
| | DBP (mmHg) | | | | 0.110 | |
| | WHpR | | | | 0.191* | |
| | FBS(mg/dl) | | | | 0.071 | |
| | HbA1c (mg/dl) | | | | -0.040 | |
| | Insulin | | | | 0.109 | |
| | HOMA-IR | | | | 0.125 | |

*(Continued)*





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TABLE 3 Continued

| Author, year | Outcome | Definition of outcome | Cutoff for Diff-C, non HDL, and atherogenic index | Type of effect size | effect size | Confounder |
|---|---|---|---|---|---|---|
| Kazemi, 2010 (27) | Mets | ATP III | 190 mg/dl | Adjusted odds ratio (95% CI) | 5.1 (4.1-6.2)* | BMI, waist circumstance, BP,LDL, cholesterol, triglycerides, HDL-C, VLDL, LDL, non-HDL-C, HDL-C |
| Atherogenic index | | | | | | |
| Angoorani, 2018 (20) | High TC (mg/dL) | More than 200 | Per 1 increment | Adjusted odds ratio (95% CI) | 1.35 (1.24,1.47) | age, sex, living area, screen time, SES and physical activity; additionally for BMI except for BMI and WC outcomes. |
| | High LDL (mg/dL) | More than 110 | | | 1.03 (0.96,1.10) | |
| | MetS | ATP III | | | 1.9(1.80- 2.19)* | |
| | Low HDL (mg/dl) | Less than 40 mg/dl, except for boys between 15 and 19 years old; which is less than 45 mg/dl | | | 2.50(2.30-2.72)* | |
| | Overweight (kg/m2) | 85th < BMI < 95th | | | 1.07(0.98-1.15) | |
| | Abdominal obesity | Waist-to-height ratio more than 0.5 | | | 1.01(0.95-1.08) | |
| | Obesity (kg/m2) | BMI more than 95th percentile | | | 1.03(0.95-1.12) | |
| | High FBS (mg/dL) | More than 100 mg/dl | | | 1.28 (1.18-1.40)* | |
| | High TG (mg/dL) | More than 100 | | | 40.26(30.36-53.40)* | |
| | High BP(mg/dL) | More than 90th | | | 1.00 (0.92-1.09) | |
| Li, 2008 (30) | Fasting serum insulin | As a continuous variable | As a continuous variable | Multiple linear regression βm (SE) | Men, NHW; 0.19 (0.02) | age, education attainment, poverty-income ratio, smoking, systolic blood pressure, C-reactive protein, and waist circumference |
| | | | | | M, NHB; 0.24 (0.04) | |
| | | | | | M, MA; 0.22 (0.04) | |
| | | | | | F, NHW; 0.24 (0.05) | |
| | | | | | F, NHB: 0.21 (0.05) | |
| | | | | | F, MA: 0.34 (0.03) | |
| | Hyperinsulinemia | More than 78.77 pmol/l (or 13.131 μU/ml) | 3.5 | Prevalence ratio (95% CI) | NHW: 2.3(1.7-3.1)* | |
| | | | 3.0 | | NHW: 2.3(1.8 – 3.0)* | |
| | | | 3.5 | | NHB: 1.9(1.5 – 2.5)* | |

*(Continued)*





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TABLE 3 Continued

| Author, year | Outcome | Definition of outcome | Cutoff for Diff-C, non HDL, and atherogenic index | Type of effect size | effect size | | Confounder |
|---|---|---|---|---|---|---|---|
| | | | 2.0 | | NHB: 2.1(1.5-2.9)* | | |
| | | | 3.5 | | MA: 1.8(1.5 – 2.2)* | | |
| | | | 3.0 | | MA: 2.0(1.6 – 2.5)* | | |
| Onat, 2010 (35) | Fasting insulin | Per 1 mIU/l | As a continuous variable | Spearman correlation results in first column and multiple linear regression results in second column β (SE) | M: 0.28* | 1.26 (1.11) | age, BP, Smoking, BMI, atherogenic |
| | | | | | F: 0.20* | 1.02 (1.10) | |
| | BMI (kg/m2) | Per 5 kg/m2 | | | M: 0.34* | 1.08 (0.02) | |
| | | | | | F: 0.29* | 1.04 (0.01) | |
| | Waist circumference (cm), | Per 11 cm | | | M: 0.32* | | |
| | | | | | F: 0.29* | | |
| | TC (mg/dL) | Per 1.03-mmol/l increment | | | M: 0.32* | 1.15 (0.04) | |
| | | | | | F: 0.31* | 1.07 (0.04) | |
| | LDL-cholesterol (mg/dL) | Per 0.93-mmol/l increment | | | M: 0.12* | 0.90 (0.04) | |
| | | | | | F: 0.22* | 0.96 (0.04) | |
| | FBS (mg/dL) | Per 1.39-mmol/l increment | | | M: 0.06* | 1.05 (0.008) | |
| | | | | | F: 0.11* | 1.03 (0.008) | |
| | SBP (mmHg) | Per 25-mmHg increment | | | M: 0.11* | 1.004 (0.025) | |
| | | | | | F: 0.20* | 1.016 (0.025) | |
| | DBP (mmHg) | Per 25-mmHg increment | | | M: 0.16* | | |
| | | | | | F: 0.19* | | |
| | | 140,90 | | | | | |

*(Continued)*





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TABLE 3 Continued

| Author, year | Outcome | Definition of outcome | Cutoff for Diff-C, non HDL, and atherogenic index | Type of effect size | effect size | Confounder |
|---|---|---|---|---|---|---|
| | Hypertension | | Q4 versus Q1 (Q4 for men = 2.26 woman = 2.99 and Q1 = 1 for both genders) | Risk ratio (95% CI) | M: 1.35 (0.87–2.09)<br>F: 1.47(0.94–2.29) | systolic BP, smoking status, BMI, and total and LDL-cholesterol |
| | Diabetes | AHA | Per 0.3 increment | | M:1.15 (0.90–1.47)<br>F: 1.09 (0.83–1.44) | |
| | MetS | ATP III | Q4 versus Q1 (Q4 for men = 2.26 woman = 2.99 and Q1 = 1 for both genders) | | M: 7.81 (3.90–15.6)*<br>F: 6.72 (3.22–14.0)* | |
| | Coronary heart disease | The presence of angina pectoris, of a history of myocardial infarction with or without accompanying Minnesota codes of the electrocardiogram | Per 0.3 increment | | M: 1.28 ( 1.05 -1.57)*<br>F: 1.26 ( 1.01–1.56)* | |
| Gasevic, 2014 (24) | Number of Mets components | As a continuous variable | As a continuous variable | Poisson regression analyses | M:1.26 (1.19, 1.33)*<br>F:1.29 (1.20, 1.36)* | age, ethnicity, smoking, alcohol consumption, physical activity, family history of cardiovascular disease, BMI.for women: all + menopause status |
| Park, 2015 (36) | BMI (kg/m2) | As a continuous variable | As a continuous variable | Multiple linear regression β (95% CI) | 0.440( 0.293–0.588)* | age, smoking behavior, the frequency of alcohol intake/wk, and the frequency of exercis- ing/wk. |
| | Waist circumference (cm) | | | | 0.951( 0.547–1.355)* | |
| | SBP (mmHg) | | | | 0.419( -0.207–1.045) | |
| | DBP (mmHg) | | | | 0.225( -0.215–0.664) | |
| | A1c (mg/dL) | | | | 0.100( 0.051–0.150)* | |
| | FBS (mg/dL) | | | | 2.849( 1.698–4.001)* | |
| | Subcutaneous fat | | | | 1.270( -2.100–4.639) | |
| | Visceral fat | | | | 0.048( 0.027–0.068)* | |

*(Continued)*





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TABLE 3 Continued

| Author, year | Outcome | Definition of outcome | Cutoff for Diff-C, non HDL, and atherogenic index | Type of effect size | effect size | Confounder |
|---|---|---|---|---|---|---|
| | Visceral-subcutaneous fat ratio (based on CT scan findings) | | | | 0.048( 0.027–0.068)* | |
| | BMI (kg/m2) | | | Greater than or equal to 25 | 3.0 | |
| | Adjusted odds ratio (95% CI) | | 5.566(2.759–11.187)* | | | |
| | | Waist circumference (cm) | Greater than or equal to 90 | 2.723(1.393-5.321)* | | |
| | | Visceral fat | Greater than or equal to 100 | 2.584(1.493-4.472)* | | |
| | | Hypertension | SBP more than 140 mm Hg in addition to DBP more than 90 mm Hg | 1.204(0.572-2.535) | | |
| Diabetes mellitus | | NR | | 2.746(1.447-5.212)* | | |

MetS, metabolic syndrome; ATP III, Adult Treatment Panel III; IDF, International Diabetes Federation; SBP, systolic blood pressure; DBP, diastolic blood pressure; BP, blood pressure; TG, triglycerides; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; BMI, body mass index; ICC, intraclass (within-observer) correlation coefficients; ICR, intraclass coefficients of reliability; F, female; M, male. Quartiles of non-HDL-c defined as: Q1: non-HDL-c <132, Q2: 132–160, Q3:160–188, Q4: non-HDL-c > 188; tertile CC, correlation coefficient; CR, coefficients of reliability; OR, odds ratio; POR, prevalence odds ratio; PC, Pearson correlation; RC, regression coefficients; SC, Spearman coefficient; *Statistically significant.



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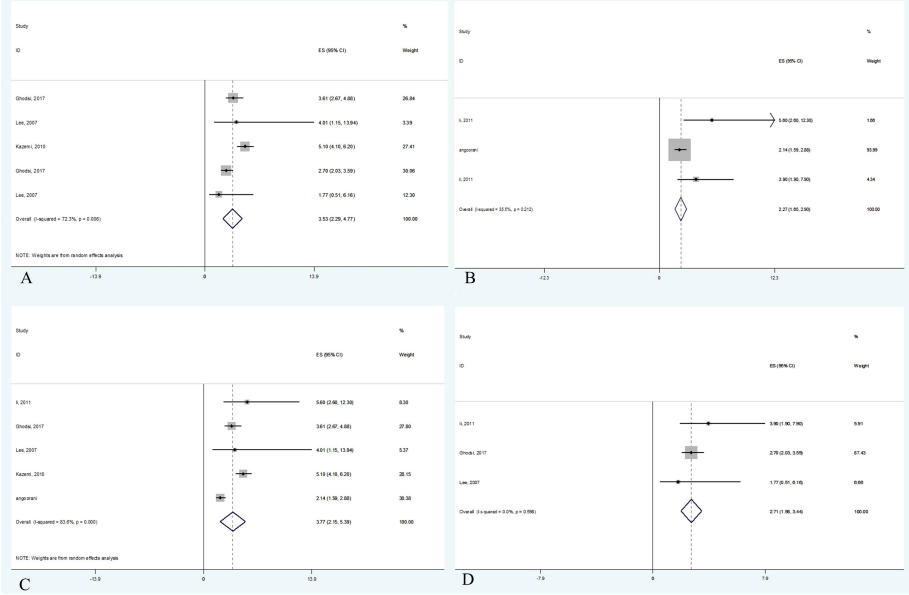


FIGURE 2
Forest plot of studies included in meta-analysis. **(A)** The association between non-HDL-C with metabolic syndrome in adults. **(B)** The association between non-HDL-C with metabolic syndrome in children. **(C)** The association between non-HDL-C with metabolic syndrome based on ATP III criteria. **(D)** The association between non-HDL-C with metabolic syndrome based on IDF criteria.

significant correlation between obesity and non-HDL-C. Besides, they concluded that non-HDL-C and obesity have a cumulative role, and both should be considered possible biomarkers for CVD (46).

Another cardiometabolic risk factor is hypertriglyceridemia. This systematic review identified a notable association between hypertriglyceridemia and increased non-HDL-C and atherogenic index levels. Genetic and epidemiologic studies confirmed a causal association between elevated triglyceride and atherosclerosis (47, 48).

In the comparison of hypertriglyceridemia, and non-HDL-C, on the one hand, Puri et al. showed that non-HDL-C is more closely connected with coronary atheroma progression compared to triglyceride. In other words, non-HDL-C is linearly related to plaque progression, while only patients with triglyceride levels higher than 200 mg/dl showed an increment in risk of progression of coronary atheroma (49).

On the other hand, Bonito et al.'s study stated that non-HDL-C level is a weaker predictor for CVD incidence compared to triglyceride. It should be noted that their data demonstrated that patients with increased non-HDL-C and triglyceride levels are at a much higher risk of CVD compared to patients who solely have one increased parameter. That is to say, their data suggests that non-HDL-C and hypertriglyceridemia have a cumulative effect (50). Our data revealed that patients with increased non-HDL-C

TABLE 4 Meta-analysis of the association between non-HDL-C with metabolic syndrome.

| | Sample size | Pooled OR (CI) | Heterogeneity | | | |
|---|---|---|---|---|---|---|
| | | | Chi-square | $I^2$ | p-value | model |
| By study population | | | | | | |
| Adults | 8549 | 3.53 (2.29-4.78) | 14.46 | 72.3 | 0.006 | Random |
| Children | 9311 | 2.27 (1.65-2.90) | 3.10 | 35.5 | 0.212 | Fixed |
| By MetS definition | | | | | | |
| ATP III | 12490 | 3.77 (2.14-5.39) | 24.36 | 83.6 | 0.001 | Random |
| IDF | 5370 | 2.71 (1.98-3.44) | 1.03 | 0.0 | 0.598 | Fixed |

MetS, metabolic syndrome; ATP III, Adult Treatment Panel III; IDF, The International Diabetes Federation.

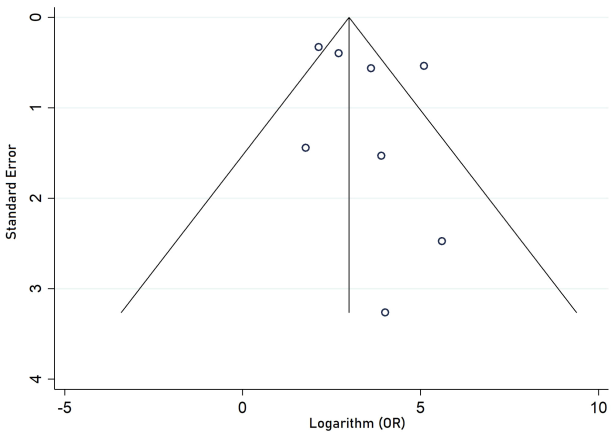


FIGURE 3
Funnel plot of studies included in quantitative analysis.

are at a higher risk of hypertriglyceridemia, and both are at a higher risk of MetS complications, especially CVD and atherosclerosis. For example, a cross-sectional article, which was conducted on 2,843 participants of the Bogalusa Heart Study, showed that non-HDL-C was related positively to triglycerides (Spearman correlation coefficient r = 0.42, p-value < 0.05) (37).

Another significant risk factor of CVD is diabetes. This study proved that diabetic patients have higher non-HDL-C levels compared to non-diabetic patients. Interestingly, diabetic patients with increased non-HDL-C levels are at a higher risk of severe coronary artery disease, regardless of their LDL-C level. In other words, in order to reduce CVD incidence in diabetic patients, not merely decreasing the LDL-C cholesterol is crucial but also reducing non-HDL-C levels should be considered (51).

This study showed that patients with increased non-HDL-C levels are at a higher risk of hypertension incidence. For example, Liu et al.'s article, a cross-sectional study conducted on 366 adult volunteers, showed that non-HDL-C levels and SBP and DBP are correlated (Spearman correlation coefficient r = 0.21, p-value < 0.05) (33). To justify this coincidence, Halperin et al. stated that dyslipidemia, especially high non-HDL-C level, is correlated with atherosclerosis, which may be an essential factor in the development of hypertension (52).

## Limitations

This study has some limitations which have to be addressed. First, the number of studies included was relatively low. In addition, some of these studies reported a small number of indices. Second, the heterogeneity of included studies, especially in terms of cutoff points of lipid, metabolic syndrome definition, and study population (children and adults), made the comparability of included articles challenging. Third, it should be noted that non-HDL comprises different lipoproteins, each of which affects the outcome differently. This study focused on overall effects of these lipoproteins, instead of assessing each of their effects, separately. Fourth, although we included adjusted studies in the meta-analysis, it should be considered that confounders may be different in included studies. Fifth, it should be considered that the Diff-C amount is alike the TG level; however, it also includes the remnant cholesterol (53).

## Conclusion

Although a limited number of studies were included in our study, non-HDL-C, Diff-C, and atherogenic index have shown to be associated with increased odds of being diagnosed with metabolic syndrome or its components. These findings were consistent in both adults and children and MetS diagnosed with both ATP III and IDF diagnostic criteria. Concerning the distinct designs and different diagnostic criteria, cohort studies with higher sample sizes should be conducted to more strongly evaluate the association between these lipid markers and MetS.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

## Author contributions

PM interpreted the data and drafted the manuscript. MQ and AG designed the study, interpreted the data, reviewed the

article critically, and revised it for important intellectual content. FA and AE participated in systematic search conduction. JH and ESh participated in data extraction, and quality assessment. PM, ES, and SD analyzed and interpreted the data. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

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

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.957136/full#supplementary-material

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# Triglyceride–glucose index in the prediction of major adverse cardiovascular events in patients with type 2 diabetes mellitus after coronary artery bypass surgery: A retrospective cohort study

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**Background:** Insulin resistance (IR) is a significant risk factor for cardiometabolic diseases and a defining feature of type 2 diabetes mellitus (T2DM). This study aimed to examine the potential value of triglyceride-glucose (TyG) index as a predictor of prognosis in coronary heart disease (CHD) patients with T2DM after coronary artery bypass grafting (CABG) surgery and to facilitate the identification of those at high risk of major adverse cardiovascular events (MACEs) for closer monitoring or possible early intervention.

**Methods:** This study enrolled 386 T2DM patients who underwent CABG surgery at Nanjing Drum Tower Hospital. Patients were separated into two groups according to the median preoperative TyG Index. The Kaplan-Meier plot was used to compare the rate of MACEs-free survival in T2DM patients after CABG. The independent risk factors for the occurrence of MACEs were investigated using multivariate analysis. Nomogram was used to depict the predictive model.

**Results:** Significantly more MACEs occurred in individuals with higher medians of the TyG index (65 (33.7%) vs. 39 (20.2%), p=0.003). TyG index [hazard ratio (HR) 12.926], LVEF [hazard ratio (HR) 0.916], and NYHA functional class III/IV [hazard ratio (HR) 4.331] were identified as independent predictors of MACEs incidence in post-CABG T2DM patients by multivariate analysis. The area under the curve (AUC) for predicting MACEs using the TyG index was 0.89 at five years. Combining the TyG index, LVEF, and NYHA functional class III/IV to build a novel risk assessment model for postoperative MACEs, the AUC climbed to 0.93 at five years. With AUCs, the nomogram comprised of the TyG index, LVEF,

and NYHA functional class III/IV demonstrated strong specificity in the training and test sets.

**Conclusions:** The incidence of MACEs is high among post-CABG T2DM patients with a high TyG index. TyG index improves the diagnostic accuracy of MACEs, especially at long-term follow-up. A high TyG index may serve as an early warning signal for individuals to undertake lifestyle adjustments that can reduce the progression or incidence of MACEs.

KEYWORDS

**triglyceride-glucose index, coronary artery bypass grafting, major adverse cardiovascular events, nomogram model, type 2 diabetes mellitus**

# Introduction

Coronary heart disease (CHD) continues to be the most significant worldwide cause of morbidity and mortality (1, 2). Patients with type 2 diabetes mellitus (T2DM) have a more significant risk of developing CHD (3–5). CHD death rates are twice higher among adults with DM than those without diagnosed DM, mainly due to an increased risk of stroke and myocardial infarction (MI) (6). Myocardial revascularization, including coronary artery bypass grafting (CABG) and percutaneous transluminal coronary intervention (PCI), is the primary therapeutic technique for CHD (7). However, despite the use of treatments currently indicated by guidelines, recurrent cardiovascular events (CVEs) in diabetic patients are higher than in non-diabetic patients (8).

Insulin resistance (IR) is a crucial risk factor for cardiometabolic illnesses and a defining characteristic of T2DM (9, 10). The triglyceride–glucose (TyG) index has become a reliable alternative diagnostic of IR with better performance than other IR markers such as homeostasis model assessment of IR (HOMA-IR) (11), glycosylated hemoglobin (HbA1c), triglyceride/high density lipoprotein (TG/HDL (12–15). In prior meta-analysis of high- and low-TyG patients without atherosclerotic cardiovascular

diseases (ASCVDs), a higher TyG index could be independently associated with a higher incidence of ASCVDs, CAD, and stroke in people without ASCVDs at baseline (16). TyG index is a reliable predictor of coronary artery disease prognosis.

CABG remains the treatment choice for diabetic individuals with severe coronary artery disease affecting multiple vessels over PCI (17). Even though several recent pieces of research have demonstrated the link between the TyG index and vascular disease, no studies have analyzed the prognosis of diabetic patients who underwent CABG.

This study aimed to examine the potential value of the TyG index as a predictor of prognosis in CHD patients with T2DM after CABG and to facilitate the identification of those at high risk of MACEs for closer monitoring or possible early intervention.

# Methods

## Patients

From January 2014 to December 2020, 1051 patients received isolated CABG surgery whether under cardiopulmonary bypass or off-pump at Nanjing Drum Tower Hospital. Four hundred twenty-five patients with T2DM were eligible for this study. The following factors determined exclusion: 1) Acute myocardial infarction; 2) New-onset diabetes untreated; 3) Absence of detailed baseline data; 4) Lost during follow-up. We separated 386 T2DM patients who underwent CABG surgery into two groups according to the median preoperative TyG Index. Medical assistants and nurses conducted telephone interviews monthly for patients' follow-ups and documented the resulting data. The study flow chart is provided in Figure 1.

This study was approved by the Ethics Committee of Nanjing Drum Tower Hospital (NO.2020-281-01). Informed consent was exempt because of the retrospective nature of the study. The present study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki (as revised in 2013).

## Data collection and definitions

All patient information was verified, rectified, and placed into a database at discharge time. Regular medical follow-up information was collected by phone and clinic visits.

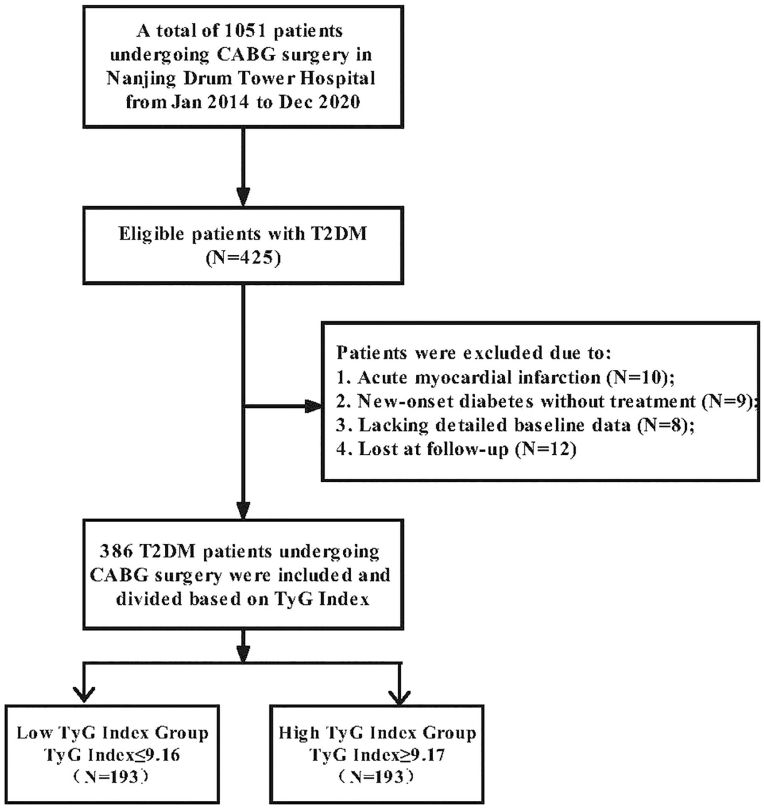


FIGURE 1
Flowchart of participant selection.

T2DM was defined on the fasting blood-glucose (FBG) ≥7.0 mmol/L according to the American Diabetes Association's standards of medical care (18) or self-reported physician diagnosis.

The TyG index was calculated as Ln [fasting triglycerides (mg/dL) × fasting glucose (mg/dL)/2] (19).

MACEs in this study included all-cause death, nonfatal myocardial infarction (MI), nonfatal stroke, revascularization (CABG or PCI) and rehospitalization for heart failure (HF).

The diagnosis of MI was according to the Fourth Universal Definition of Myocardial Infarction (20).

We used the New York Heart Association (NYHA) functional classification system to assess the severity of heart failure symptoms. Left ventricular function was expressed using ejection fraction of 2D echocardiography.

## Statistical analysis

Data were summarized using descriptive statistics, and numerical data were reported as means (SD). Using the Kolmogorov–Smirnov test, the distribution of continuous data variables was examined for normality. Using Fisher's exact test, differences in the frequencies of categorical variables were also evaluated. All outcome indicators were compared across all patient groups. Fisher's exact test was used to analyze variables that may have impacted the occurrence of MACEs in patients (univariate predictors). In order to identify independent predictors, the MACEs incidence predictors were subjected to multivariable logistic regression analysis. The final model includes the TyG index, NYHA functional class III/IV, and LVEF. In addition, the area under the curve (AUC) and ideal cut-off value were determined using receiver operating characteristic (ROC) curve analysis to evaluate the predictive efficacy of the TyG index, NYHA functional class III/IV, and LVEF for MACEs incidence. Survival was illustrated graphically using Kaplan–Meier curves. AUCs were utilized to determine the predictive value of the TyG index for MACEs.

The nomogram was constructed using the 'rms' R package and provides a risk score for each patient. The calibration curve was used to determine consistency between the predicted survival probability of the nomogram with bootstrap resamples. All statistical analyses were conducted with R (version 4.0.2) software, and a two-tailed P value of 0.05 was considered statistically significant.

# Results

## Baseline characteristics

The average age of the study participants was 66 years, and 276 (71.5%) were male. This study divided all patients into two groups based on the cut-off value of 9.17 for the TyG index. In two groups, the mean values of TyG index were 8.67 and 9.62, respectively. Table 1 presents the study patients' baseline clinical and laboratory characteristics according to the TyG index groups. Patients with a high TyG index were more likely to have had a stroke in the past. In proportion to the TyG index, BMI, TC level, LDL-C level, FPG level, and triglyceride level increased, whereas HDL-C level and LVEF declined. Furthermore, when the TyG index climbed, the proportion of three and above vascular disease and on-pump CABG also increased. Moreover, there was no significant change in the other variables, such as hospitalization duration and 30-day mortality.

## Association between the TyG index and the risk of MACEs

During the follow-up period, 104 (26.9%) cases of MACEs in total. 38 (9.8%) cases of all-cause death occurred. 12 (3.1%) and 16 (4.1%) patients experienced nonfatal MI and stroke. 7 (1.8%) and 67 (17.4%) patients were re-admitted to the hospital for further revascularization and HF treatment. The incidence of MACEs was significantly higher in patients with higher medians of the TyG index (65(33.7%) vs. 39(20.2%), p=0.003). 13 (6.7%) of the deceased patients were in the low TyG Index Group, and 25 (13%) were in the high TyG index group. 46 (33.1%) patients with high TyG index were re-admitted to the hospital for HF treatment, while only 21 (10.9%) patients with low TyG index were re-admitted. All-cause death and rehospitalization for HF were statistically different between the two groups of patients. Although not statistically significant, high TyG index patients had a higher tendency to experience myocardial infarction (9

TABLE 1 Baseline characteristics in T2DM patients with CABG surgery based on tertiles of TyG Index.

| Variables | All patients (n=386) | Low TyG Index Group (n=193) | High TyG Index Group (n=193) | P-value |
|---|---|---|---|---|
| Male (n, %) | 276 (71.5%) | 138 (71.5%) | 138 (71.5%) | 1.000 |
| Age (years) | 66 (60-72) | 66 (59-71.5) | 66 (60-72) | 0.806 |
| BMI (kg/m$^2$) | 24.85 (22.77-26.82) | 24.24 (22.49-26.00) | 25.34 (23.13-27.34) | 0.003 |
| Past history | | | | |
| Hypertension (n, %) | 288 (74.6%) | 146 (75.6%) | 142 (73.6%) | 0.640 |
| Cerebral infarction (n, %) | 59 (15.3%) | 36 (18.7%) | 23 (11.9%) | 0.066 |
| Atrial fibrillation (n, %) | 9 (2.3%) | 5 (2.6%) | 4 (2.1%) | 0.736 |
| Hyperthyroidism (n, %) | 1 (0.3%) | 0 (0.0%) | 1 (0.5%) | 0.317 |
| PCI (n, %) | 43 (11.1%) | 20 (10.4%) | 23 (11.9%) | 0.627 |
| LVEF, % | 50 (49,58) | 51 (48.5,59) | 50 (50,57) | 0.093 |
| NYHA class | | | | 0.223 |
| I-II | 269 (69.7%) | 129 (66.8%) | 140 (72.5%) | |
| III-IV | 117 (30.3%) | 64 (33.2%) | 53 (27.5%) | |
| Laboratory test | | | | |
| Triglyceride | 1.46 (1.10-1.93) | 1.15 (0.95-1.46) | 1.92 (1.46-2.56) | <0.001 |
| Glucose | 7.34 (6.08-10.10) | 6.18 (5.02-7.22) | 9.70 (7.50-12.90) | <0.001 |
| TyG Index | 9.17 (8.67-9.62) | 8.67 (8.45-8.96) | 9.62 (9.39-9.86) | <0.001 |
| LDL-C | 1.86 (1.42-2.41) | 1.77 (1.30-2.18) | 1.93 (1.49-2.42) | 0.010 |
| HDL-C | 0.93 (0.64-1.07) | 0.98 (0.82-1.09) | 0.88 (0.73-1.02) | 0.004 |
| TC | 3.42 (2.86-4.14) | 3.28 (2.66-4.02) | 3.60 (2.89-4.37) | <0.001 |
| Creatinine | 70.95 (58.53-89.00) | 71.00 (58.50-88.00) | 70.9 (58.35-89.80) | 0.865 |
| BUN | 6.20 (5.00-7.70) | 6.20 (4.90-7.60) | 6.30 (5.05-7.80) | 0.360 |
| Off-pump CABG (n, %) | 344 (89.1%) | 176 (91.2%) | 168 (87.0%) | 0.191 |
| Number of grafts | | | | |
| I-II | 32 (8.3%) | 21 (10.9%) | 11 (5.7%) | |
| III-IV | 300 (77.7%) | 146 (75.9%) | 154 (79.8%) | |
| V-VI | 54 (14.0%) | 26 (13.5%) | 28 (14.5%) | |
| In-hospital time (Day) | 21 (18-25) | 21 (17.5-25.5) | 21 (18-25) | 0.635 |
| Mortality (n, %) | 18 (4.7%) | 9 (4.7%) | 9 (4.7%) | 1.000 |

BMI, body mass index; PCI, percutaneous transluminal coronary intervention; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; BUN, blood urea nitrogen.

(4.7%) vs. 3(1.6%), p=0.078). Other outcomes, including nonfatal stroke and the need for revascularization, showed no significant differences (Table 2). Figure 2 depicts the Kaplan–Meier curves during the follow-up without a MACE survival curve according to the first occurrence of MACE for both TyG index groups. At five years, the incidence of MACE with a high TyG index was significantly higher (p<0.001) among T2DM patients.

TABLE 2 Clinical outcomes in T2DM patients with CABG surgery based on tertiles of TyG Index.

| CV Outcomes | All patients (n=386) | Low TyG Index Group (n=193) | High TyG Index Group (n=193) | P-value |
|---|---|---|---|---|
| MACE | 104 (26.9%) | 39 (20.2%) | 65 (33.7%) | 0.003 |
| All-cause death | 38 (9.8%) | 13 (6.7%) | 25 (13.0%) | 0.040 |
| Nonfatal MI | 12 (3.1%) | 3 (1.6%) | 9 (4.7%) | 0.078 |
| Nonfatal stroke | 16 (8.3%) | 6 (3.1%) | 10 (5.2%) | 0.307 |
| Revascularization | 7 (1.8%) | 3 (1.6%) | 4 (2.1%) | 0.703 |
| Rehospitalization for HF | 67 (17.4%) | 21 (10.9%) | 46 (33.1%) | 0.001 |

CV, cardiovascular; MI, myocardial infarction; HF, heart failure.

## TyG index, LVEF and NYHA functional class III/IV were predictors of MACEs occurrences

Table 3 presents univariate and multivariate Cox proportional hazards regression analysis and predictors for composite MACEs. LVEF, NYHA functional class III/IV, TGs, FPG, TyG index, HDL-C, creatinine, BUN, off-pump CABG, ≥three-vessel disease were identified as risk factors for MACEs by univariate analysis (p<0.1). Further multivariate analysis of the risk factors mentioned above showed that TyG index (HR = 12.926, 95% CI = 3.457-48.323, P<0.001), LVEF (HR = 0.916, 95% CI = 0.886-0.946, P<0.001) and NYHA functional class III/IV (HR = 4.331, 95% CI = 2.410-7.781, P<0.001) were identified as independent predictors of MACEs occurrences in patients with T2DM underwent CABG.

The predictive value of nomogram at 12/36/60 months postoperatively was evaluated by plotting time-dependent ROC curves for each of the three variables TyG Index, LVEF, and NYHA functional class III/IV. LVEF and NYHA functional class III/IV exhibited a better predictive value at 12 and 36 months postoperatively, whereas the TyG Index was more accurate at 60 months. The predictive value of the AUC of the TyG Index for the incidence of MACE was as high as 0.89. Then we combined TyG, LVEF, and NYHA functional class III/IV to build a novel risk assessment model for postoperative MACEs. At 12, 36, and 60 months, the AUC climbed to 0.89, 0.92, and 0.93, respectively. These findings indicate that the TyG index improves the diagnostic accuracy of this prediction model, especially at 60 months postoperatively (Figure 3).

To quantitative predict the incidence of MACEs in T2DM patients after CABG surgery, we established a prediction nomogram for MACEs was developed using the TyG index, NYHA functional class III/IV, and LVEF (Figure 4). All of these variables were assigned a score on the points scale. Summation over the variable points were summed, and a total point was obtained and located on the Total Points scale. A line was drawn straight down to the 12-/36-/60-months incidence of MACEs, and the estimated incidence at each time point is shown. Furthermore, the calibration plots showed good consistency between the nomogram predictions and actual observations for overall survival rate in each time point (Figure 5).

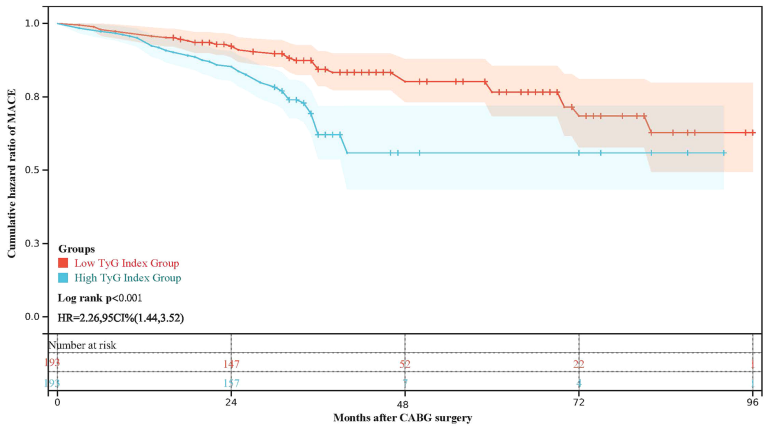


FIGURE 2
Kaplan-Meier survival curve for freedom from MACE in high TyG index group and low TyG index group.

TABLE 3 Univariate and multivariate analyses of MACE in T2DM patients with CABG surgery.

| Variables | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | HR value | 95% CI | P-value | HR value | 95% CI | P-value |
| Sexy (Male) | 1.325 | 0.839-2.092 | 0.227 | | | |
| Age | 1.001 | 0.980-1.021 | 0.954 | | | |
| BMI | 1.015 | 0.960-1.074 | 0.599 | | | |
| Hypertension | 1.144 | 0.725-1.805 | 0.564 | | | |
| Cerebral infarction | 1.173 | 0.697-1.973 | 0.548 | | | |
| Atrial fibrillation | 3.607 | 1.579-8.240 | 0.002 | | | |
| PCI | 0.508 | 0.223-1.160 | 0.108 | | | |
| LVEF | 0.885 | 0.867-0.904 | <0.001 | 0.916 | 0.886-0.946 | <0.001 |
| NYHA class (III-IV) | 7.967 | 5.238-12.117 | <0.001 | 4.331 | 2.410-7.781 | <0.001 |
| Triglyceride | 1.435 | 1.210-1.703 | <0.001 | 0.601 | 0.322-1.124 | 0.111 |
| Glucose | 1.118 | 1.077-1.160 | <0.001 | 0.911 | 0.806-1.031 | 0.141 |
| TyG Index | 2.886 | 2.117-3.934 | <0.001 | 12.926 | 3.457-48.323 | <0.001 |
| LDL-C | 1.129 | 0.916-1.392 | 0.254 | | | |
| HDL-C | 0.370 | 0.150-0.911 | 0.030 | 0.756 | 0.236-2.423 | 0.638 |
| | HR value | 95% CI | P-value | HR value | 95% CI | P-value |
| TC | 1.118 | 0.948-1.317 | 0.184 | | | |
| Creatinine | 1.002 | 1.001-1.004 | <0.001 | 1.001 | 0.998-1.003 | 0.583 |
| BUN | 1.078 | 1.033-1.125 | 0.001 | 0.990 | 0.916-1.069 | 0.798 |
| Off-pump CABG | 0.407 | 0.252-0.658 | <0.001 | 0.717 | 0.420-1.222 | 0.221 |
| I-II grafts | 1 | | | 1 | | |
| III-IV grafts | 3.099 | 0.978-9.814 | 0.054 | 0.770 | 0.234-2.527 | 0.666 |
| V-VI grafts | 4.563 | 1.361-15.299 | 0.014 | 0.979 | 0.280-3.420 | 0.974 |

BMI, body mass index; PCI, percutaneous transluminal coronary intervention; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; BUN, blood urea nitrogen.

## Discussion

Our study assessed the predictive effectiveness of the TyG index for the occurrence of MACEs in patients with T2DM who had CABG. We found that diabetic CABG patients with a low TyG index had a higher long-term MACE-free rate than those with a high TyG index at a cut-off value of 9.17. Combining TyG, LVEF, and NYHA functional class III/IV, we constructed an effective model for evaluating the occurrence of MACEs.

Diabetes is an independent risk factor for coronary heart disease (9, 10, 21). More than 90% of diabetic patients are diagnosed with T2DM (22). Insulin resistance is a defining property of metabolic syndrome, a critical trait of T2DM, and a risk factor for cardiovascular events (23). Few pieces of research have examined insulin resistance as a cardiovascular risk factor. Srinivasan et al. examined 61 T2DM patients who underwent coronary arteriography and discovered that insulin resistance was positively associated with coronary risk severity (24). The San Antonio Heart Study demonstrated a substantial relationship between insulin resistance and the risk of cardiovascular disease (25). In a large observational trial, Hedblad B et al. found that insulin-resistant patients' relative risk for cardiovascular events and death was twofold higher (26). Recent studies have demonstrated the close link between the TyG index and the homeostasis model for measuring insulin resistance (HOMA-IR) (27, 28). In addition, the predictive value of the TyG index for IR was superior to that of the HOMA-IR (11). Indicating TyG index, a hematological indicator of IR that is straightforward, practical, and stable, is a reliable predictor of coronary artery disease prognosis. Others have shown that a higher TyG index is associated with an increased risk of significant adverse cardiac and cerebrovascular events in patients having a percutaneous coronary intervention for ST-elevation myocardial infarction (STEMI) (29). Although multiple recent studies have proven the association between the TyG index and vascular disease (30, 31), no studies have examined the prognosis of diabetes patients with CABG. Therefore, we studied its role further in CABG patients with T2DM and discovered that it had potential prognostic value.

The effect of diabetes on post-CABG short-term mortality was minimal. Both diabetic and non-diabetic individuals have comparable in-hospital and 1-year mortality rates (32). Indeed, LVEF and NYHA functional classes significantly impact in-hospital and 1-year mortality rates in post-CABG patients. Following these findings, we found that LVEF and NYHA functional class III/IV did well predicting the occurrence of

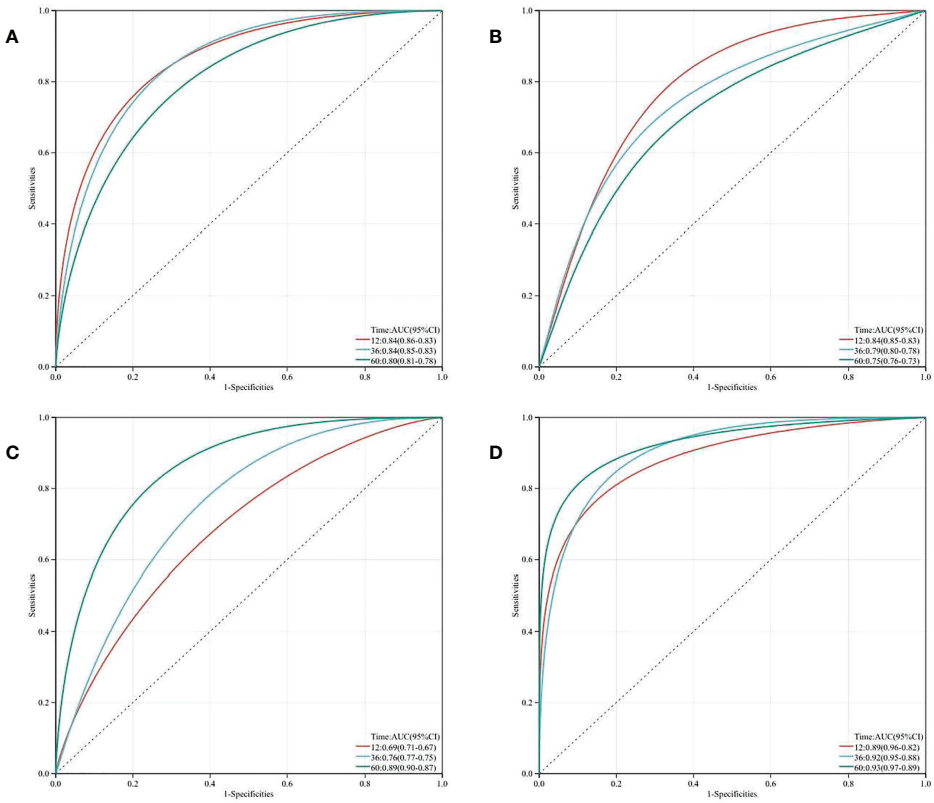


FIGURE 3
Time-dependent receiver operating characteristic curve (ROC) analysis with the area under the curve, sensitivity and specificity of LVEF **(A)**, NYHA class **(B)**, TyG Index **(C)** and LVEF + NYHA class + TyG Index **(D)** in predicting MACEs of T2DM patients after CABG surgery.

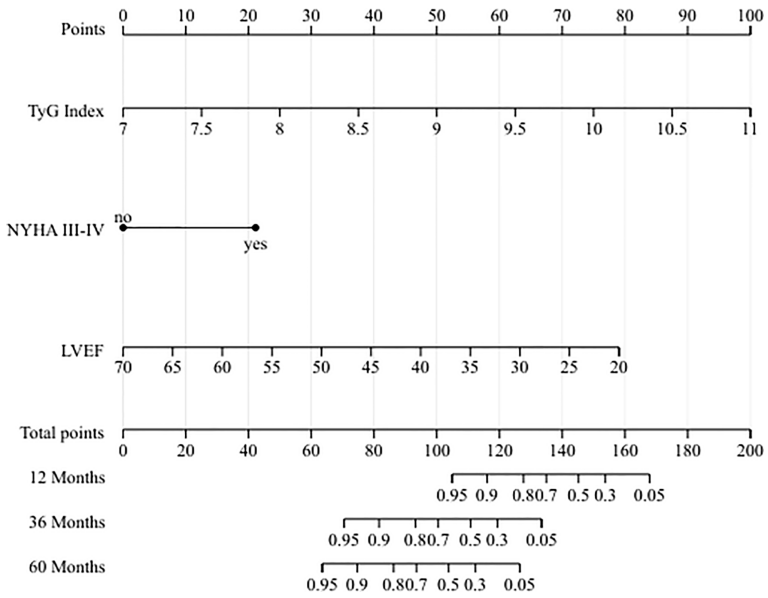


FIGURE 4
Nomogram for predicting 12-/36-/60-months incidence of MACEs.

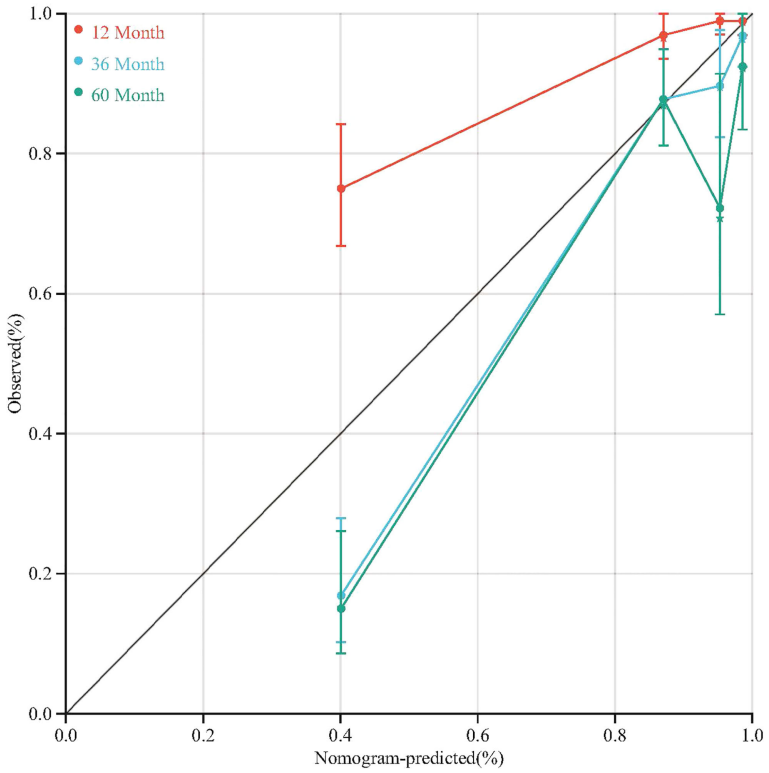


FIGURE 5
Calibration plots of the nomogram for 12-/36-/60-month incidence of MACEs.

MACEs at 12 and 36 months. Our results demonstrated that multivariate Cox regression analysis identified LVEF and NYHA functional class III/IV as independent risk factors for the development of MACEs. In addition, throughout early and middle-term follow-up, LVEF and NYHA categorization had a higher diagnostic value for the occurrence of MACEs, with AUCs of 0.84 for both. Despite no significant difference between high and low TyG in-hospital mortality, we found statistical significance in the absence of MACEs based on variations in the TyG index. The most substantial impact of diabetes mellitus is definitely on long-term outcomes. Also, LVEF and NYHA functional class III/IV significantly impact early- and mid-term outcomes (33, 34). A unique risk assessment approach for postoperative MACEs compatible with early-, mid-, and long-term follow-up is required. Therefore, we combined the TyG index, LVEF, and NYHA functional class III/IV, three independent risk variables, to find a novel assessment tool for predicting the risk of MACEs in post-CABG diabetic patients.

In addition, no clinical scores can predict the long-term risk of MACEs in T2DM patients after CABG surgery. The nomogram constructed from numerous independent predictors has been recognized as a practical and effective illness prediction tool. As a result, we developed a nomogram based on the multivariate analysis to predict MACEs at 12,36 and 60 months, which showed good accuracy and consistency. However, due to the limited number of cases and single-center investigation, this nomogram has not been externally validated, and the accuracy of its predictions in diverse populations requires further study. Further research is required to evaluate if diabetic persons with a high TyG index should be included in risk stratification algorithms for the occurrence of MACEs in post-CABG patients. In future clinical practice, a high TyG index could serve as an early warning signal for individuals to initiate lifestyle modifications that can minimize the progression or incidence of MACEs.

## Conclusions

In conclusion, the morbidity of MACEs is substantial in T2DM patients with a high TyG index having CABG. TyG index, LVEF, and NYHA functional class III/IV were identified as independent risk variables for the occurrence of MACEs in T2DM patients after CABG, according to multivariate analysis. In addition, a unique nomogram for predicting the incidence of MACEs comprised of the three independent risk variables was demonstrated to be a possible indicator for early intervention.

## Data availability statement

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

## Ethics statement

This study was approved by the Ethics Committee of Nanjing Drum Tower Hospital (NO.2020-281-01). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## Author contributions

Conception and design: HZ and DW. Administrative support: YX and DW. Provision of study materials or patients: BZ and DW. Collection and assembly of data: HZ and ZL. Data analysis and interpretation: HZ, HC, ZL, and KL. Manuscript writing: all authors. Final approval of manuscript: all authors.

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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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# High levels of oxidized fatty acids in HDL impair the antioxidant function of HDL in patients with diabetes

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**Aims:** Previous studies demonstrate that the antioxidant functions of high-density lipoprotein (HDL) are impaired in diabetic patients. The composition of HDL plays an important role in maintaining the normal functionality of HDL. In this study, we compared the levels of oxidized fatty acids in HDL from diabetic subjects and non-diabetic healthy controls, aiming to investigate the role of oxidized fatty acids in the antioxidant property of HDL.

**Methods:** HDL was isolated from healthy subjects (n=6) and patients with diabetes (n=6, hemoglobin A1c ≥ 9%, fasting glucose ≥ 7 mmol/L) using a dextran sulfate precipitation method. Cholesterol efflux capacity mediated by HDL was measured on THP-1 derived macrophages. The antioxidant capacity of HDL was evaluated with dichlorofluorescein-based cellular assay in human aortic endothelial cells. Oxidized fatty acids in HDL were determined by liquid chromatography-tandem mass spectrometry. The correlations between the levels of oxidized fatty acids in HDL and the endothelial oxidant index in cells treated with HDLs were analyzed through Pearson's correlation analyses, and the effects of oxidized fatty acids on the antioxidant function of HDL were verified *in vitro*.

**Results:** The cholesterol efflux capacity of HDL and the circulating HDL-cholesterol were similar in diabetic patients and healthy controls, whereas the antioxidant capacity of HDL was significantly decreased in diabetic patients. There were higher levels of oxidized fatty acids in HDL isolated from diabetic patients, which were strongly positively correlated with the oxidant index of cells treated with HDLs. The addition of a mixture of oxidized fatty acids significantly disturbed the antioxidant activity of HDL from healthy controls, while the apolipoprotein A-I mimetic peptide D-4F could restore the antioxidant function of HDL from diabetic patients.

**Conclusion:** HDL from diabetic patients displayed substantially impaired antioxidant activity compared to HDL from healthy subjects, which is highly correlated with the increased oxidized fatty acids levels in HDL.

KEYWORDS

high-density lipoprotein, diabetes, antioxidant activity, oxidized fatty acids, cardiovascular disease

# Introduction

Epidemiological studies report that patients with diabetes suffer significantly increased cardiovascular disease (CVD) morbidity and mortality. High-density lipoprotein (HDL) in plasma normally functions with antioxidant (1), endothelial-protective (2) anti-inflammatory (3), and anti-atherogenic (4) properties by preventing oxidation of low-density lipoprotein (LDL) and promoting cholesterol efflux from the arterial wall. The antioxidant and anti-inflammatory activities of HDL are impaired in type 2 diabetes compared to healthy control subjects (5, 6). The mechanisms underlying the loss of the beneficial effects of HDL in diabetic patients are likely multifactorial, and compositional changes in the HDL proteome and lipidome may be key players.

HDL is an assembly of a neutral lipid core and an outer shell consisting of polar lipids and proteins. The antioxidant function of HDL is partially mediated by the surface proteins such as apolipoprotein A-I (apoA-I), paroxonase-1 (PON1), and platelet-activating factor-acetyl hydrolase (PAF-AH) (4). In addition, lipidome composition also plays an important role in maintaining HDL function. Lipid peroxidation products of HDL are derived from the degradation of polyunsaturated omega-6 fatty acids, arachidonic acid (AA), and linoleic acid (LA), which are further converted to bioactive eicosanoids through the cyclooxygenase and lipoxygenase pathway, which leads to the formation of hydroxyeicosatetraenoic acids (HETEs) and hydroxyoctadecadienoic acids (HODEs) (7). Previous work shows increases in HODEs and HETEs in the HDL of patients with diabetes and atherosclerotic vascular disease (8), in the plasma of humans and rodents with pulmonary hypertension (9, 10), and in the HDL and LDL from patients with rheumatoid arthritis (11). However, whether the levels of oxidative lipids in HDL from diabetic patients are directly associated with the impaired function of HDL in diabetes still remains elusive. In the present study, we examined the level of oxidized fatty acids in HDL from healthy and diabetic subjects, and as well as whether these changes play a role in the antioxidant function of HDL.

# Materials and methods

## Study population

For Study 1, six consecutive patients with diabetes without known CVDs and other complication syndromes between 18-80 years, as well as six age- and sex-matched healthy control subjects without diabetes were enrolled in this study from the health examination center of Shenzhen Sami International Medical Center within a two-month period. The diagnostic criteria for diabetes in the present study was as follows: a fasting plasma glucose ≥ 7.0 mmol/L and a 2-hour plasma glucose value in a 75 g oral glucose tolerance test ≥ 11.1 mmol/L or a glycated hemoglobin A1c (HbA1c) ≥ 6.5%, as defined by the American Diabetes Association (ADA) (12). The inclusion criteria for diabetes participants was HbA1c ≥ 9% and fasting glucose ≥ 7 mmol/L, and the exclusion criteria was as follows: cardiovascular disease, previous diagnosis of cancer, and moderate-severe chronic kidney or liver disease. Age- and sex- matching in this study refers to that a pair of diabetic subject and healthy control is of the same sex and the age variation is no more than 2 years. Characteristics of participants in Study 1 are shown in Table 1. To verify the result of Study 1, another six pairs of diabetic and healthy control subjects with the same inclusion criteria and exclusion criteria were enrolled in Study 2 from the Outpatient Department of Shenzhen Sami International Medical Center within one month. Characteristics of participants in Study 2 are shown in Table 2. Venous blood was collected from all study subjects after fasting. Serum was separated by the local clinical laboratory and stored at 4°C for up to 1 week before HDL isolation. The study protocol was approved by the ethics committee of Shenzhen Sami International Medical Center (Ethical Approval No.: SMCC-2022-15).

## Isolation of HDL

HDL was isolated from the fasting serum of non-diabetic healthy subjects and diabetic patients by dextran sulfate

TABLE 1 Clinical and laboratory characteristics of diabetic patients and non-diabetic healthy controls in Study 1.

| | Diabetic (n = 6) | Healthy (n = 6) | *p* value |
|---|---|---|---|
| Age, y | 49 ± 10 | 50 ± 9 | 0.17 |
| Sex, male/female | 4/2 | 4/2 | 1.0 |
| BMI, kg/m$^2$ | 24.9 ± 2.4 | 25.9 ± 4.4 | 0.73 |
| HbA1c, % | 10.9 ± 1.3 | 5.6 ± 0.4 | <0.001 |
| Fasting glucose, mmol/L | 12.9 ± 2.2 | 5.5 ± 0.2 | <0.001 |
| TC, mmol/L | 5.0 ± 1.2 | 4.6 ± 1.2 | 0.23 |
| LDL-c, mmol/L | 3.6 ± 1.2 | 3.2 ± 1.0 | 0.44 |
| HDL-c, mmol/L | 1.0 ± 0.3 | 1.2 ± 0.2 | 0.32 |
| TG, mmol/L | 2.2 ± 0.8 | 1.2 ± 0.5 | <0.05 |
| ALT, U/L | 28.8 ± 15.7 | 27.0 ± 6.9 | 0.83 |
| AST, U/L | 21.8 ± 8.7 | 25.5 ± 4.8 | 0.51 |

Values are expressed as the mean ± SD or number of subjects. *p* values were obtained from analysis of paired Student' s t tests. HbA1c, hemoglobin A1c; BMI: body mass index; TC: total cholesterol; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; TG: triglycerides; ALT: alanine transaminase; AST: aspartate transaminase.

precipitation using a commercially available kit from Cell Biolabs (STA-607, CA), as described previously (13). The purified HDL was subsequently dialyzed in PBS using Slide-A-Lyzer MINI Dialysis devices (10K MWCO, Thermo Fisher, MA). The protein levels of HDL were measured using a bicinchoninic acid (BCA) kit (Thermo Fisher, Waltham, MA) and further identified through sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The concentrations of HDL used in the present study were based on protein content of HDL.

## Cell culture

Human aortic endothelial cells (HAECs) were purchased from LMAI Bio (Shanghai, China), and were cultured in endothelial cell medium (ScienCell Research Laboratories, Carlsbad, CA) consisting of basal medium, endothelial cell supplement, 2% fetal bovine serum (FBS) and 1% penicillin/streptomycin antibiotic solution (Hyclone, Logan, UT). THP-1 monocytes were obtained from the National Infrastructure of Cell Line Resource (Beijing, China), and cultured in RPMI 1640 medium (Hyclone) supplemented with 10% FBS and 1% penicillin-streptomycin (Hyclone). All cells were maintained at 37°C in a humidified atmosphere of 5% $CO_2$ and were used within 10 passages.

## Measurement of cellular cholesterol efflux capacity

The capacity of HDL to support cholesterol efflux was analyzed in the human monocyte cell line THP-1, a widely-used *in vitro* model for cholesterol efflux (14). N–(7–nitrobenz–2–oxa–1,3 –diazol–4–yl)amino)–23,24–bisnor–5–xholen–3β–ol (NBD) –cholesterol was used as an substitute for [$^3$H]-cholesterol for the measurement of cholesterol efflux in THP-1 derived macrophages (14). THP-1 cells were seeded in black 96-well plates (Corning Inc., New York, NY) at a density of 1.5^10$^4$ cells per well in triplicates and incubated with 100 ng/mL of phorbol-12-myristate-13-acetate (PMA, Sigma-Aldrich, MO) for 72 h. The

TABLE 2 Clinical and laboratory characteristics of diabetic patients and non-diabetic healthy controls in Study 2.

| | Diabetic (n = 6) | Healthy (n = 6) | *p* value |
|---|---|---|---|
| Age, y | 51 ± 9 | 51 ± 9 | 1.0 |
| Sex, male/female | 3/3 | 3/3 | 1.0 |
| HbA1c, % | 9.3 ± 0.3 | 5.5 ± 0.3 | <0.001 |
| Fasting glucose, mmol/L | 10.5 ± 3.0 | 5.2 ± 0.3 | <0.01 |
| TC, mmol/L | 5.0 ± 1.6 | 4.5 ± 1.5 | 0.64 |
| LDL-c, mmol/L | 3.2 ± 0.9 | 2.8 ± 1.4 | 0.62 |
| HDL-c, mmol/L | 1.1 ± 0.4 | 1.2 ± 0.3 | 0.68 |
| TG, mmol/L | 2.4 ± 2.5 | 1.6 ± 0.8 | 0.58 |
| ALT, U/L | 22.2 ± 10.4 | 19.5 ± 6.3 | 0.65 |
| AST, U/L | 16.3 ± 5.90 | 16.8 ± 3.2 | 0.98 |

Values are expressed as the mean ± SD or number of subjects. *p* values were obtained from analysis of paired Student' s t tests. HbA1c, hemoglobin A1c; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; TG, triglycerides; ALT, alanine transaminase; AST, aspartate transaminase.

PMA-differentiated macrophages then were incubated in phenol red-free RPMI1640 medium containing 5 μM NBD–cholesterol (N1148, Thermo fisher) for 4 h at 37°C. Following incubation, the cells were rinsed with PBS three times and were then incubated with HDL (100 μg/mL), as lipid acceptors. The cells were harvested after 4 h, and the medium and cell lysate were collected for the detection of fluorescence intensity. The percentage of NBD-cholesterol efflux was calculated by dividing the fluorescence intensity in the medium by the sum of the fluorescence intensity in the medium and inside the cells.

## Measurement of intracellular reactive oxygen species

The production of ROS was evaluated with an oxidation-sensitive fluorescent probe, 2',7'-Dichlorodihydrofluorescein-diacetate (DCFH-DA). DCFH-DA is a non-fluorescent lipophilic ester that easily penetrates the cells and could be oxidized into 2',7'-dichlorofluorescein (DCF) in the presence of oxidants, resulting in a green fluorescence. Therefore, the fluorescence intensity of DCF indicates the levels of ROS in the cells. In brief, HAEC cells were grown in 96-well culture plates with a density of 2^$10^4$ cells per well overnight and then pretreated with 10 μM DCFH-DA (Beyotime Biotech Inc., Shanghai, China) in serum-free medium at 37°C for 20 min. Then cells were washed with serum-free medium for three times and further exposed to different treatments for 30 min, and the fluorescence of each well was measured using a multi-plate reader at an excitation wavelength of 485 nm and an emission wavelength of 525 nm. The fluorescence intensity produced by intracellular DCF was used to indicate the oxidant stress in cells.

## Lipidomic analysis of oxidized fatty acids in HDL

A reversed-phase liquid chromatography/tandem mass spectrometry (LC-MS/MS) method was developed to simultaneously analyze oxidized fatty acids including AA and its metabolites such as prostaglandins (PGs), thromboxane ($TXB_2$), epoxyeicosatrienoic acids (EETs), dihydroxyeicosatrienoic acids (DHETs), HETEs, and HODEs. To extract the oxidized fatty acids in HDL, 100 μg HDL (indicated by protein content) was mixed with cold methanol (with deuterium labeled 15-HETE-d8 and AA-d8 as internal standards) and formic acid. Then, ethyl acetate extraction was performed for sample preparation after sonication in an ice bath (3 sec on/2 sec off for 60 cycles at 400w), and the organic phase was dried under vacuum and dissolved in methanol for LC/MS-MS analysis. LC/MS-MS was performed using a quadrupole mass spectrometer (Applied Biosystems Division, Life Technologies, Carlsbad, CA) equipped with an electrospray ionization (ESI) source. Chromatography was performed on an Agilent 1290 system (Agilent Technologies Inc., MA, USA) using a Waters symmetry C18 column (250 mm×4.6 mm, 5 μm) with a security guard cartridge maintained at 35°C. The mobile phase was composed of a gradient elution of (A) acetonitrile and (B) water containing 0.05% formic acid (v/v) at a flow rate of 1 mL/min with an injection volume of 10 μL. Metabolites were separated within 50 min with a gradient elution procedure as follows: 0-10 min, 40% A; 10-20 min, 40%-65% A; 20-35 min, 65% A; 35-40 min, 65%-90% A; 40-50 min, 90% A. Mass spectrometric detection was accomplished using multiple reaction monitoring (MRM) mode with negative electrospray ionization.

## Bioinformatics of lipidomic analysis

The analysis workflow of differential metabolites was performed on the ONE-MAP analytical platform (Dashuo Biotech Co., Dalian, China). In brief, the processed peak intensity data acquired from mass spectrometric detection was subjected to multivariate statistical analysis. Linear transformation was used to preserve the variance of the original data in the lower dimensionality of the output data using principal component analysis (PCA) score plots. Significantly differential metabolites were identified using partial least squares-discriminant analysis (PLS-DA). The heatmap was produced in ONE-MAP. In brief, peak intensities of metabolites with a fold change ≥1.5 and $p$ value <0.05 were normalized and a heatmap was generated using the pheatmap function. Colors in the heatmap correspond to normalized abundance of each metabolite by a gradient of color from blue (low abundance) to red (high abundance).

## Statistical analysis

The results of all experiments are expressed as the means ± standard deviation (S.D.). All distributions of data were assumed to be normal. Statistical analysis was performed using paired Student's $t$ test with Graphpad Prism Software version 7. The Pearson's correlation coefficient assumes that X and Y are jointly distributed as bivariate normal, i.e., X and Y each are normally distributed, and that they are linearly related (15). Pearson's correlation analysis between variables were performed in an all-group comparison method when applicable (16). A $p$ value < 0.05 was considered statistically significant.

# Results

## Demographic, laboratory, and clinical characteristics of the Study 1 population

The characteristics of the two groups in Study 1 are shown in Table 1. The gender and age distribution, as well as the body

mass index (BMI) were similar between the two groups. Glycemic control was poor in diabetic patients (HbA1c: 10.9 ± 1.3%). The serum levels of fasting glucose and HbA1c in the diabetic group were markedly higher than that in the healthy control group (HbA1c: 10.9 ± 1.3% vs 5.6 ± 0.4%, $p<0.001$; fasting glucose: 12.9 ± 2.2 mmol/L vs 5.5 ± 0.2 mmol/L, $p<0.001$). There was no significant difference in the serum levels of total cholesterol (TC), LDL-cholesterol (LDL-c), HDL-cholesterol (HDL-c), alanine transaminase (ALT), or aspartate transaminase (AST) between the diabetic and healthy group, except that serum triglycerides (TG) was slightly higher in diabetic patients compared with healthy controls (2.2 ± 0.8 mmol/L vs 1.2 ± 0.5mmol/L, $p<0.05$) (Table 1).

## HDL from diabetic patients is pro-oxidative compared to HDL from healthy control subjects

HDLs were isolated from the serum of the subjects in the two groups in Study 1. SDS-PAGE analysis of HDLs is shown in Figure 1A, displaying a main band comparable to apoA-I (the major apolipoprotein in HDL, molecular weight: 28 kD). The cholesterol efflux capacity and the effect on ROS production of HDLs were examined on THP-1 and HAEC cells, respectively. THP-1 derived macrophages were loaded with NBD-cholesterol firstly, and then HDL was added to induce the efflux of NBD-cholesterol. It was found that there was no difference in the ability of HDL to induce cholesterol efflux between diabetic patients and controls (Figure 1B). To compare the effect of HDL from the two groups on the oxidation status of cells, HAEC cells were treated with HDLs from diabetic patients ($HDL_{diabetic}$) and healthy controls ($HDL_{healthy}$), respectively, and the levels of intracellular ROS, an oxidant index was analyzed as indicated with the fluorescence intensity of DCF. As shown in Figure 1C, the ROS levels in cells treated with $HDL_{diabetic}$ was significantly higher than that in cells treated with $HDL_{healthy}$, indicating that $HDL_{diabetic}$ was pro-oxidant compared to $HDL_{healthy}$.

## Oxidized fatty acids were increased in HDL from patients with diabetes compared to healthy controls

The levels of oxidized fatty acids in HDLs from subjects in Study 1 were measured by LC/MS-MS. To compare the overall features between the two groups, PCA was performed to visualize the global variations between the two groups. PCA of all detected features unveiled two distinct cluster marked by HDLs from diabetic and healthy subjects, respectively (Figure 2A). In particular, the diabetic group clustered in the negative sector of PC1, while the healthy group clustered in the positive sector of PC1. The PCA score plot revealed a good separation especially along the first principal component PC1 (PC1 and PC2 accounted for 89.6% and 5.6% of the total variance, respectively). This result indicates that differences were present between the two groups. Then, supervised classification using PLS-DA model was also performed to search for discriminating features for the separation between the two groups. A clear separation between the two groups was also observed in the PLS-DA score plot (Figure 2B), confirming the distinct features between the two groups. Moreover, a heat

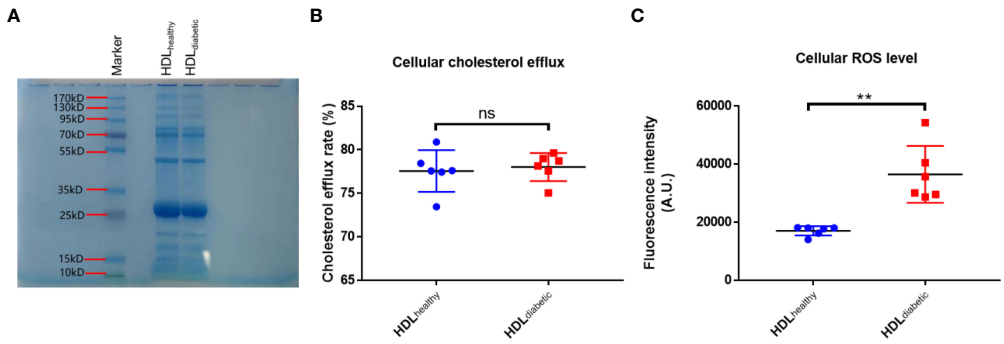


FIGURE 1
Analysis of the pro-oxidative activity of HDLs from diabetic patients. **(A)** SDS-PAGE analysis of HDLs purified by the sulfate dextran precipitation method. Ten micrograms of HDL purified using a commercial kit was loaded on a 4-20% Bis-Tris gel and stained with Coomassie Brilliant Blue dye. **(B)** Comparison of cholesterol efflux capacity of HDL from diabetic patients and healthy controls. Differentiated THP-1 cells were loaded with NBD-cholesterol for 4 h, then treated with 100 ug/mL HDL from healthy controls (n=6) or diabetic subjects (n=6) for another 4 h. The fluorescence intensity in cells and in the medium was determined. Cholesterol efflux rate was calculated by dividing the fluorescence intensity in the medium by the sum of fluorescence intensity in the medium and cells. **(C)** Comparison of the level of ROS in HAECs treated with HDL from healthy subjects and diabetic patients. HAECs were pre-incubated with 10 uM DCFH-DA for 20 min, then treated with 500 ug/mL HDL from healthy controls (n=6) or diabetic subjects (n=6) for 30 min. The intracellular fluorescence intensity produced by DCF was determined. ns: not significant. $^{**}p < 0.01$, ns: not significant. *p* value was obtained from analysis of paired Student' s *t*-tests.

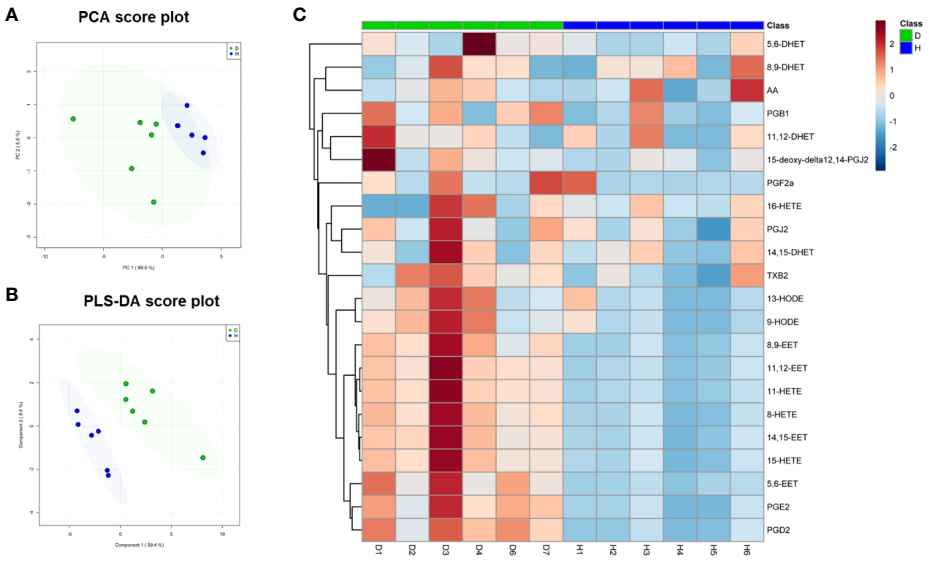


FIGURE 2
Multivariate analysis of the oxidized fatty acid lipidome of HDL from diabetic subjects and healthy controls in Study 1. **(A)** Principal Component Analysis (PCA) and **(B)** Partial Least Squares Discriminant Analysis (PLS-DA) of HDL from diabetic patients (D, n=6) and healthy subjects (H, n=6). The shaded areas indicate 95% confidence ellipse regions based on the data points for individual groups. **(C)** Heatmap visualization based on the significantly changed oxidized fatty acids (fold change ≥1.5, $p$<0.05). Colors in the heatmap correspond to normalized abundance of each metabolite by a gradient of color from blue (low abundance) to red (high abundance).

map of all of the differential metabolites was produced to visualize the relative concentration of oxidized fatty acids in each sample. The relative concentration of each metabolite in each sample is expressed with different colors. It is clear that there were higher levels of oxidized fatty acids in HDL from diabetic patients compared to the healthy control group (Figure 2C). Among the 19 studied metabolites, 10 oxidized fatty acids with a false discovery rate (FDR) <0.05, fold-change >2 were further picked out. As can be seen in Figure 3, the levels of the ten oxidized metabolites of AA and LA, including PGs, HODE, HETEs, and EETs, were significantly elevated in HDL from patients with diabetes.

## The levels of oxidized fatty acids in HDL were positively correlated with the oxidant index of HDL-treated cells

To investigate the correlation between elevated oxidized fatty acids and impaired HDL function, Pearson's correlation analyses were performed between the oxidant index of HAEC cells treated with each HDL sample as shown in Figure 1C and the intensity of the 10 significantly changed oxidized fatty acids as shown in Figure 3 after confirming that the assumptions of Pearson's correlation were satisfied. Despite the small numbers in the subset of subjects, Pearson's correlation analyses demonstrated strong positive correlations between the levels of 9 significantly changed oxidized fatty acids except 9-HODE (data not shown) and the cellular oxidant index with $r$ values > 0.6 and $p$ values < 0.05 (Figure 4).

## Elevated levels of oxidized fatty acids lead to impaired antioxidant capacity of HDL

Oxidative stress results in excessive accumulation of ROS, which directly damages cell membranes, protein and DNA, compromising cell function and threatening cell survival (17, 18). Other researchers have shown that the effects of experimentally induced ROS induced in the vascular wall were completely abolished by a daily infusion of HDL (1). HDL also possess the ability to protect endothelial cells from primary apoptosis and to reduce intracellular ROS induced by oxidized LDL (19). In addition, HDL has also been shown to protect mesenchymal stem cells from hydrogen peroxide ($H_2O_2$)-induced oxidative stress and apoptosis (20). The pro-oxidative property of diabetic HDL has been revealed in Figure 1C. To further investigate whether the impaired antioxidant function of HDL from diabetic patients is caused by elevated levels of oxidized fatty acids, *in vitro* DCF-based cell assays were performed with individual HDLs from subjects of Study 2 (characteristics of the study population see Table 2). $H_2O_2$, which is widely used as an oxidant, was applied to induce an

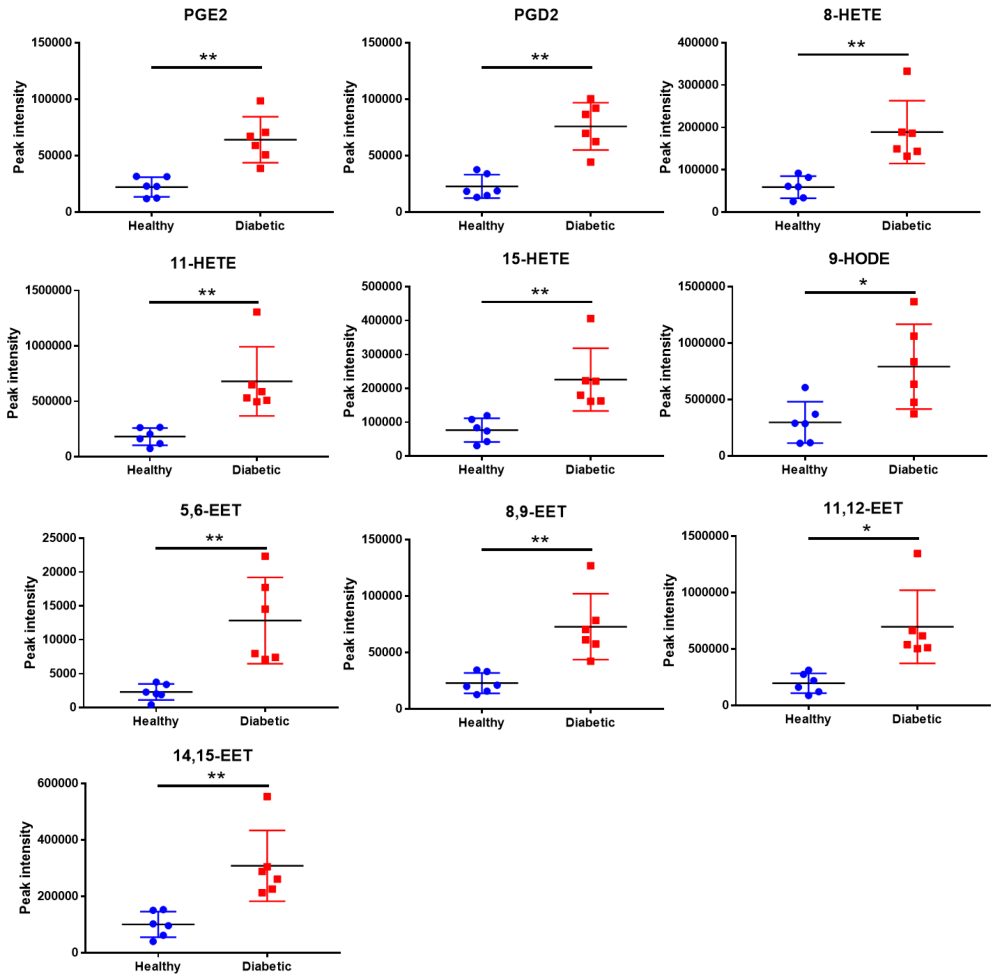


FIGURE 3
Quantitative comparison of oxidized fatty acids that significantly changed between the diabetic and healthy groups. $^{**}p < 0.01$, $^{*}p < 0.05$. *p* values were obtained from analysis of paired Student' s *t*-tests.

oxidative stress model in HAEC cells. As shown in Figure 5, $H_2O_2$ caused an evident increase in cellular ROS, while HDLs isolated from healthy subjects substantially reduced $H_2O_2$-stimulated oxidative stress, indicating a potent antioxidant property of HDL from healthy subjects on the endothelium. However, the addition of 20 ng/mL a mixture of oxidized fatty acids ($PGD_2$, 9-HODE, 8-HETE, and 5(6)-EET) significantly disrupted the antioxidant function of $HDL_{healthy}$ (Figure 5). Compared to HDL from healthy controls, HDL from diabetic patients lost the capacity to protect endothelial cells from oxidative stress induced by $H_2O_2$ (Figure 5). The apoA-I mimetic peptide 4F, which forms a class A amphipathic helix similar to those found in apoA-I, is reported to have anti-inflammatory and antioxidant effects due to its binding to oxidized fatty acids such as HETEs and HODEs (21), and improve the function of HDL (22). To determine whether 4F could restore the impaired antioxidant function of HDL, HAEC cells were co-incubated with D-4F and dysfunctional $HDL_{diabetic}$. Incubation of HDL with 50 μg/mL D-4F rescued the antioxidant capacity of $HDL_{diabetic}$ (Figure 5). These results strongly indicate that the reduced antioxidant capacity of HDL is, at least partially, caused by increased oxidized fatty acids.

## Discussion

CVD remains the leading cause of death in the developed and developing countries, and is prevalent in diabetes mellitus with 68% CVD-related mortality (1, 17). Circulating HDL plays cardiovascular protective roles by promoting cholesterol efflux and antioxidant effects, *etc* (2–5). In the present study, we disclosed that there was a dramatic descend in the antioxidant capacity of HDL from diabetic patients while the cholesterol efflux capacity was not altered. There was also an abnormal

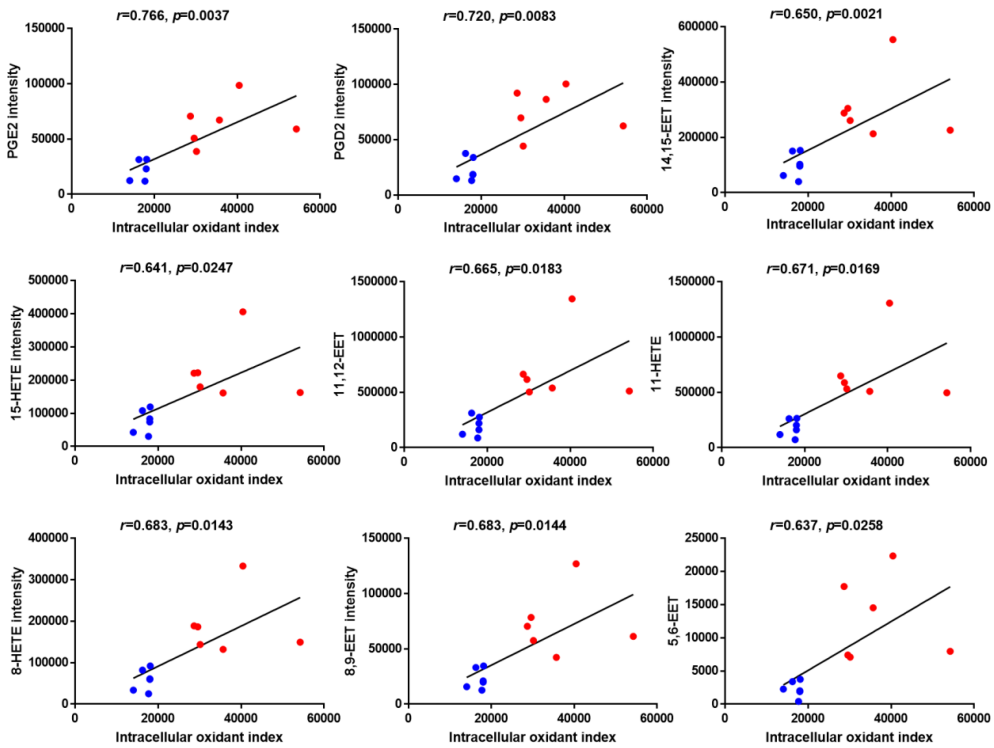


FIGURE 4
Correlation analysis between the levels of significantly changed oxidized fatty acids and the intracellular oxidant index of HAECs treated with HDLs determined by Pearson's analysis. Blue dots indicate HDL from healthy control group and red dots indicate HDL from diabetic group. N=6 for each group.

increase of oxidized fatty acids inside HDL, which was associated with the impaired antioxidant activity of HDL in diabetes.

Previous epidemiological studies demonstrate strong, inverse associations between HDL-c and CVD (23). However, efforts to raise HDL-c level *via* CETP inhibitors turned out be disappointing in outcome studies despite substantial increases in HDL-c (24, 25), questioning the causal role of HDL-c in CVD risk development (26). Furthermore, researchers proposed that the functional properties, rather than the cholesterol in HDL, are more important indices for HDL-targeted therapies (27). It is in fact the capacity to transport cholesterol from the periphery to the liver, anti-inflammatory properties, antioxidant effects and the endothelial-vasoprotective effects of HDL that renders it atheroprotective (27). Dysfunctional HDL has shown to have strong and independent associations with CVD risk (3, 4, 28) and impaired HDL function in diabetes has been proposed as a mechanism underlying the increased CVD risk in diabetes (2).

Cholesterol efflux capacity is a key metric of the anti-atherosclerotic functionality of HDL. Thus far, variable changes in the cholesterol efflux capacities of HDL from diabetes patients have been reported. While some found decreased (29–33), others reported unchanged (34–36), or even increased HDL cholesterol efflux in diabetic patients (37). The differences in protocols, for example, the cell type (macrophages or fibroblast), the stimulation or not of cholesterol transporters, the tracers (radioactive or fluorescent), the type of acceptor (HDL, total plasma, or apolipoprotein B (apoB)-depleted plasma) are likely to explain the divergent results. It is important to point out that no gold standard is currently agreed upon for cholesterol efflux assays. ATP-binding cassette transporter A1 (ABCA1)-mediated cholesterol efflux is the most important determinant of total efflux from THP-1 macrophages (38). The use of HDL isolated by ultracentrifugation or dextran sulfate precipitation instead of apoB-depleted plasma may also be a source of discrepancy because of the elimination of some pre-β-HDL, which plays an important role in ABCA1-dependent cholesterol efflux (39). In our study, there was no difference in the cholesterol efflux from THP-1 monocytes-derived macrophages either from normal or from diabetic patients. One of the possible reasons might be the concentration of HDL used in the cholesterol efflux assay was relatively low (100 μg/mL), which was lower than that we used in ROS assay (500 μg/mL) and the commonly used concentration of HDL described in other studies (150~800 μg/mL) (34, 40). Another reason might be the lack of pre-β-HDL in the HDLs isolated by the dextran sulfate precipitation which is dominated

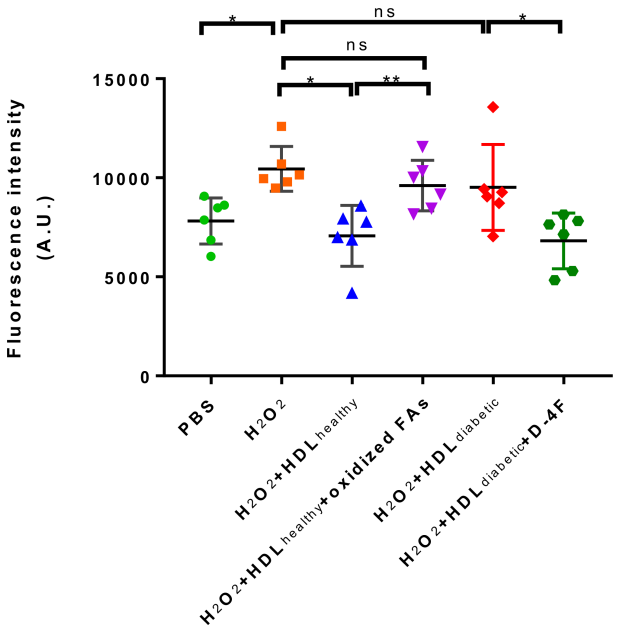


FIGURE 5
The effects of oxidized fatty acids and D-4F on the antioxidant functions of HDLs. HAECs were pre-incubated with 10 uM DCFH-DA for 20 min, then treated with PBS, 500 uM $H_2O_2$, $H_2O_2$ plus 500 ug/mL HDL from healthy controls (n=6), $H_2O_2$ plus 500 ug/mL HDL from healthy controls (n=6), and a mixture of oxidized fatty acids (FAs) composed of 20 ng/mL PGD2, 9-HODE, 8-HETE, and 5(6)-EET, $H_2O_2$ plus 500 ug/mL HDL from diabetic subjects (n=6) and 50 ug/mL D-4F for 30 min, respectively. The intracellular fluorescence intensity produced by DCF was determined. $^{**}p < 0.01$, $^{*}p < 0.05$, ns: not significant. *p* values were obtained from analysis of paired Student' s *t*-tests.

by mature HDLs (41). Wijtske et al., working with THP-1 macrophages and apoB-depleted plasma, also reported that HDL cholesterol efflux function was not impaired in diabetes but was lower in metabolic syndrome, partly dependent on plasma HDL-c levels (35). Damien et al. also reported that the HDL cholesterol efflux was not altered in type 2 diabetes despite lipidomic abnormalities (34). The surprisingly increased HDL cholesterol efflux in diabetes reported by Low et al. was linked to the higher levels of CETP activity observed in diabetic patients (37), Overall, there is no consensus on how diabetes affects the cholesterol efflux capacity of HDL, and we can conclude that not all of HDL functions are modified with HDL oxidation. As studied here, only the antioxidant function of HDL was impaired by oxidation and can be restored by D-4F, while the capacity of cholesterol efflux remained unchanged.

Although there was no significant difference in HDL-c and the ability to support cholesterol efflux between the two groups, the antioxidant capacity of HDL was significantly dampened in diabetes, confirming that HDL particles can become dysfunctional independent of HDL-c levels. The antioxidative property of HDL is associated with compositional changes in the HDL proteome and lipidome. Proteins compose 35-65% of the molecular weight of HDL particles (42). Thus far, there have been more than 90 protein species identified in HDL. The most commonly observed major protein groups are apolipoproteins, enzymes, lipid transfer proteins, and acute-phase-response proteins. The levels of apolipoproteins and enzymes (43) the posttranslational modification of apolipoproteins (44), and the protein-protein interactions in HDL (45) could affect the functions of HDL.

Beyond the proteome, oxidized lipids including HETEs and HODEs in HDL are also reported to be associated with the higher risk of CVD in populations with diabetes (8). As the oxidized lipid metabolites of AA and LA, HETEs and HODEs have been implicated as important mediators of the immune response (46) and directly implicated in the pathogenesis of atherosclerosis (47, 48). The presence of oxidized fatty acids such as HETEs and HODEs in HDL indicates the occurrence of lipid peroxidation in response to inflammation and oxidative stress. Also, it is possible that oxidation of lipoproteins during the HDL isolation may affect HDL lipid composition. However, it should be noted that HDL from all samples in the present study was isolated under identical conditions using the same methods. Therefore, differences in oxidized fatty acids among groups are less likely due to oxidation of lipoproteins during isolation. Previous studies suggest that hyperglycemia may promote pathological effects through glycation of lipoproteins (49), which leads to increased susceptibility to oxidation (50). This increased oxidative stress contributes to the formation of certain oxidized lipids (47). Therefore, it is plausible that the increased

levels of oxidized lipid metabolites in HDL from patients with diabetes is caused by hyperglycemia.

In the current study, the levels of oxidized fatty acids were significantly elevated in the HDL from diabetic patients compared to matched healthy controls. Moreover, high levels of oxidized fatty acids in HDL were significantly associated with worse antioxidant function of HDL. Our study suggests a potential mechanism in which hyperglycemia in diabetic patients results in increased oxidized fatty acids in HDL, thereby leading to impairment in HDL functionality, and increased CVD risk. The main findings of our study are consistent with those by Morgantini et al. (51) that the function of HDL is highly correlated with oxidized fatty acids inside the lipoprotein. There are some variations in the species of oxidized fatty acids detected between two studies, possibly due to differences in the methods for lipoprotein isolation and lipodomic analyses by LC-MS/MS. Besides common HETEs and HODEs, our study also reported the elevations of $PGD_2$, $PGE_2$, and EETs that are produced in different ways from HODEs and HETEs in the HDLs of diabetic patients and their correlations with HDL dysfunction, providing additional evidence for the mechanism underlying oxidized fatty acid-leading HDL dysfunction.

ApoA-I, the major protein in HDL is a selective target for oxidation by myeloperoxidase, which results in impaired HDL function. ApoA-1 mimetic peptides may have the ability to modify the lipid and protein content of HDL and convert dysfunctional HDL to functional. The apoA-I mimetic peptide 4F was designed to contain a class A amphipathic helix with a polar and a nonpolar face that allows it to bind lipids (52). Actually, the mimetic peptide 4F bound oxidized fatty acids derived from AA and LA with an astoundingly higher affinity than apoA-1 (53). Oral administration or injection of 4F restore the anti-inflammatory and antioxidant activities of HDL as well as the capacity to promote cholesterol efflux in various models. For example, D-4F could restore the anti-inflammatory properties of HDL after influenza infection in mice by preventing macrophage infiltration into the aortic arch (54). Administration of 13(*S*)-HPODE, a kind of oxidized products of polyunsaturated fatty acids, increased plasma 13-HODE and 9-HODE levels and decreased HDL anti-inflammatory properties in mice, while injection of 4F decreased the levels of 13-HODE and 9-HODE, and remarkably rescued the anti-inflammatory property of HDL impaired by 13(*S*)-HPODE (22). Watanabe et al. reported that administration of 4F rescued the anti-inflammatory activity of HDL by disturbing the association of hemoglobin with HDL in hyperlipidemic mice (55). Meanwhile, oral administration or injection of 4F can also restore the antioxidant activities of HDL as well as the capacity to promote cholesterol efflux in various models. A study by van Lenten et al. found that treatment of mice with D-4F resulted in an increase in HDL-c and PON1 activity, and the ability to inhibit LDL-induced monocyte chemotactic activity (54). Novab et al. reported that oral administration of D-4F led to a substantial increase in the protective capacity of HDL to protect LDL against oxidation and dramatically reduced atherosclerosis in LDL receptor-null mice but independent of changes in total plasma or HDL-c (56). Further mechanism study by the same group revealed that D-4F caused the formation of pre-β-HDL, reduced lipid hydroperoxides in HDL and increased PON1 activity of HDL in mice (57) and monkeys (58). Pre-β-HDL is generally considered to be the most active HDL fraction in promoting reverse cholesterol transport, and the cycling of cholesterol through pre-β HDL is generally considered to be protective against atherosclerosis (59).

How could D-4F decrease HDL lipid hydroperoxide content and increase PON1 activity? PON1 is a HDL-associated enzyme and has the ability to prevent the formation of proinflammatory oxidized phospholipids (60), thereby mainly responsible for the antioxidative properties of HDL. Forte et al. have shown that the activity of PON1 are reversibly inhibited by lipid hydroperoxides (61). It can be concluded that effective sequestration of lipid hydroperoxides by peptides such as D-4F may active PON1, while the active PON1 further reduces the levels of oxidized lipids, finally forming a positive feedback loop (62). Consistent with the fact that administration of the 4F peptide improved HDL function in various *in vivo*, *in vitro* or *ex-vivo* models, herein our results revealed that addition of D-4F reverted pro-oxidant $HDL_{diabetic}$ into antioxidant, supporting the notion that oxidized lipids in HDL are strongly related to HDL function since the effect of the apoA-I mimetic is attributable to its ability to bind oxidized lipids.

Although interesting, there are some limitations to the present study. One limitation is that the findings are kind of hypothesis-driven based on existing literature, and limited by the small sample size, which makes the Pearson's correlation between oxidized fatty acids and HDL antioxidant functionality based on the two separate clusters should be interpreted prudently. The correlations may not exist when calculated separately in diabetic subjects and in healthy controls, since the range of observed values in the predictor variable was too small. Another limit is the unknown size distribution of the HDL particles due to limited amount of serum samples. HDL particle sizes are differentially associated with and may mediate atherosclerotic CVDs (63). Future studies will evaluate levels of oxidized fatty acids in HDL with different sizes from larger diabetes cohorts in order to better understand how HDL is altered in diabetes and to determine the effect of specific treatments. In addition, further work is warranted to determine whether these assessments of HDL composition beyond HDL-c levels are more useful markers for cardiovascular risk in diabetes patients.

## Conclusions

In summary, the present study indicated the relevance between increased oxidized fatty acids in HDL and the

impaired antioxidant function of HDL from diabetic patients, supporting the link between higher CVD risk in patients with diabetes and HDL composition beyond HDL-c concentration.

## Data availability statement

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

## Ethics statement

The studies involving human participants were reviewed and approved by the ethics committee of Shenzhen Sami International Medical Center (Ethical Approval No.: SMCC-2022-15). The patients/participants provided their written informed consent to participate in this study.

## Author contributions

RL and PY conceived and designed the study. JF contributed to conduction of study, data analysis and writing the manuscript. YW, YZ and YL contributed to subject recruitment and data collection. WL contributed to conduction of study. XY and SL contributed the review of the manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

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

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# Lipoprotein profiles of fat distribution and its association with insulin sensitivity

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**Background:** Fat deposition is associated with adverse outcomes. Waist-to-hip (WHR) ratio is a simple feasible index to assess fat distribution. Lipoprotein particle composition in relation to WHR and to what extent their association is mediated by insulin sensitivity are less investigated.

**Methods:** In 504 randomly recruited Flemish (mean age: 48.9 years; women: 51.6%), we analyzed the lipoprotein particle constitutions using nuclear magnetic resonance spectroscopy. WHR obesity described a WHR of ≥ 0.85 for women or 0.9 for men. Insulin sensitivity was evaluated by the homeostasis model assessment-estimated insulin resistance (HOMA-IR). SCORE-2 risk algorithm was applied to estimate 10-year cardiovascular risk. Statistical methods included multivariable-adjusted linear regression analysis, logistic regression analysis, and mediation analysis.

**Results:** The prevalence of WHR obesity was 54.6%, approximately 3 times of BMI-determined obesity (19.1%). Individuals with WHR obesity had significantly higher metabolic complications, such as hypertension (57.1%), dyslipidemia (61.8%), and insulin resistance (14.2%). WHR and WHR obesity were positively associated with total very-low-density lipoprotein (VLDL) particle concentration, remnant cholesterol, and triglycerides, but were negatively associated with VLDL particle size ($P \leq 0.027$), independent of body mass index and other covariates. WHR was inversely associated with total high-density lipoprotein (HDL) particle concentration, whereas WHR obesity was inversely associated with HDL cholesterol ($P \leq 0.039$). Neither WHR nor WHR obesity was associated with the concentration of total low-density lipoprotein (LDL) particles, LDL particle size, and LDL cholesterol ($P \geq 0.089$). In the mediation analysis, insulin sensitivity significantly mediated the effect of WHR on total VLDL particle concentration (mediation percentage: 37.0%), remnant cholesterol (47.7%), and HDL cholesterol (41.1%). Individuals with WHR obesity were at increased cardiovascular risk, regardless of LDL cholesterol ($P \leq 0.028$).

In WHR obesity, higher total VLDL particle concent36ration and remnant cholesterol, and lower HDL cholesterol were associated with an increased cardiovascular risk (P≤ 0.002).

**Conclusions:** Upper-body fat deposition was independently associated with an unfavorable lipoprotein profile, and insulin sensitivity significantly mediated this association. LDL cholesterol might underestimate lipid abnormality for people with upper-body obesity and lowering VLDL particles and remnant cholesterol might potentially reduce the residual cardiovascular risk.

KEYWORDS

Obesity, waist-to-hip ratio, lipoprotein, insulin resistance, cardiovascular risk

# Introduction

The prevalence of obesity, one of the top threats to global public health, has nearly tripled from 1975 to 2016 (1). Obesity strikingly increases the risk of various noncommunicable diseases, including type 2 diabetes mellitus, cardiovascular disease, and mortality (2). It is extensively acknowledged that obesity is generally accompanied by metabolic comorbidities, including insulin resistance and dyslipidemia. Apart from excessive overall fat accumulation, adipose tissue distribution, especially abdominal fat deposition, is strongly associated with an increased risk of all-cause mortality, cardiovascular events, and insulin resistance (3–7). Since body mass index (BMI) is prevailingly used to assess overall fat accumulation and to associate with cardiovascular disease, most studies on lipid associations investigated BMI as a surrogate of obesity (8–12). Waist-to-hip ratio (WHR) is a simple and cheap indicator of fat distribution, and is relatively less correlated with BMI, compared to waist circumference (13). The positive association between WHR and cardiovascular risk has been suggested by large prospective studies (13, 14). WHR has been repetitively associated with conventional lipid parameters, such as low-density lipoprotein (LDL) cholesterol, whereas the association between WHR and more detailed lipoprotein particle compositions is less investigated (15–18). Linking WHR to complex lipoprotein composition might promote the utilization of WHR, provide more information for lipid-lowering options, and recognize the residual cardiovascular risk for individuals with abdominal obesity.

Insulin resistance, a prevalent metabolic complication of obesity, has a profound impact on lipid metabolism. Numerous epidemiological studies have investigated the association of insulin resistance with the anthropometric indices of obesity and the cholesterol contents or particles constitutions of different lipoproteins (19, 20). However, it remains unclear to what extent insulin sensitivity intermediates the effect of fat distribution on lipoproteins. Determining the effect mediated by insulin sensitivity could further uncover the mechanism of lipid abnormality in individuals with upper-body obesity and potentially facilitate the development of effective intervention strategies. Thus, a simultaneous investigation of insulin sensitivity, WHR, and lipoprotein particle constitutions is necessary to quantify the mediated effect of insulin sensitivity using mediation analysis.

Therefore, this study evaluated the association between lipoprotein particle composition and WHR, and further investigated the mediator role of insulin sensitivity in the general population. We additionally associated the lipoprotein profile of WHR with the estimated cardiovascular risk score to sharpen the understanding of the lipoprotein profile.

# Materials and methods

## Participants

All participants were from the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO), a large prospective study that included 3343 individuals from the Flemish region from 1985 until 2004 with a participation rate of 78% at enrollment (21). The present study was approved by the University of Leuven Ethics Committee and written informed consent was obtained from all participants prior to study participation. All procedures were in accordance with the ethical principles of the Declaration of Helsinki. Participants who underwent lipoprotein profiling using nuclear magnetic resonance (NMR) spectroscopy were eligible for this study. Of 593 eligible participants, 89 participants who received lipid-lowering drugs were excluded due to the potential influence of lipid-lowering drugs on lipoprotein constitutions. Thus, this study eventually included 504 participants.

## Anthropometric measurements

The measurement of weight and height was performed with standardized equipment and procedures. BMI was calculated by body weight (kg) divided by the square of height ($m^2$). Waist circumference was measured at the midway between the lower ribs and the top of the iliac crest to the nearest 0.1 cm. Hip circumference was measured at the widest portion of the buttocks. Waist-to-hip ratio (WHR) indicated the ratio of waist circumference and hip circumference. The measurement was completed when participants were in the upright position, and waist circumference was measured at the end of expiration. BMI obesity was defined as a BMI of ≥ 30 $kg/m^2$. WHR obesity described a WHR of ≥ 0.85 for women or 0.9 for men (22).

## Nuclear magnetic resonance (NMR) spectroscopy measured lipoproteins

The fasting venous blood samples were centrifuged after collection, and the obtained plasma samples were preserved under -80°C until further analysis. The lipoprotein profiling was measured by 2D diffusion-ordered $^1$HNMR spectroscopy (DOSY) at INCLIVA Molecular and Metabolomics Image Lab, Valencia, Spain (23). The sample preparation and the protocol of analysis were detailed elsewhere (24). After being transferred into 5 mm NMR tubes, the prepared samples were randomized, and kept at 4°C until measurement (mean time until measurement: 6 hours). Samples were then inserted in the magnet, warmed to 37°C and $^1$H NMR spectra were acquired in a Bruker Avance III 600 spectrometer with an operating frequency at 600.20 MHz. The double stimulated echo pulse program with bipolar gradient pulses and a longitudinal eddy current delay was used. The obtained lipoproteins signals were deconvoluted and analyzed separately based on the diffusion properties and its associated NMR size into main fractions: very-low-density lipoprotein (VLDL) (38.6-81.9 nm), low-density lipoprotein (LDL) (18.9-26.5 nm), high-density lipoprotein (HDL) (7.8-11.5 nm) as detailed elsewhere (24). Particle size was then calculated based on its diffusion properties and each fraction was further divided into large, medium, and small particle subclass according to their particle diameters. The average lipoprotein particle size was calculated by averaging the NMR area of each fraction by its associated size.

## Other measurements

The venous blood samples were collected from August 2005 to March 2015 and obtained after at least 8 hours of fasting for the conventional lipid profile measurements, plasma glucose, and insulin. The conventional lipid measures included total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides, which were measured by using automated methods in certified laboratories. Specifically, since remnant cholesterol, indicative of the cholesterol content of the triglyceride-rich lipoproteins, has been associated with cardiovascular risk recently, it was also included as a conventional lipid parameter in this study. Remnant cholesterol was estimated by total cholesterol minus LDL cholesterol minus HDL cholesterol. The homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated by multiplying plasma glucose (mmol/L) by insulin (uIU/mL), divided by 22.5. Diabetes mellitus was defined as fasting blood glucose of ≥126 mg/dL or receiving antidiabetic drugs. Hypertension was an office blood pressure of ≥140 mmHg systolic or ≥90 mmHg diastolic, or the use of antihypertensive drugs. Dyslipidemia was defined as LDL-cholesterol ≥ 3.36 mmol/L (130 mg/dL) or total cholesterol ≥ 5.17 mmol/L (200 mg/dL) or HDL-cholesterol ≤ 1.29 mmol/l (50 mg/dL) in women and 1.03 mmol/l (40 mg/dL) in men or fasting triglycerides ≥ 1.70 mmol/L (150 mg/dL) according to the criteria of Adult Treatment Panel III (25). Insulin resistance was defined as HOMA-IR of ≥ 2.5. Glomerular filtration rate was estimated using the chronic kidney disease epidemiology collaboration creatinine equation (26). The 10-year fatal and non-fatal cardiovascular risk (%) was estimated with SCORE2 risk prediction algorithms based on sex, age, smoking status, total and HDL cholesterol, and systolic blood pressure (27).

## Statistical analyses

Data analyses were performed with SAS software, version 9.4 (SAS Institute, Cary, NC, USA). Means and proportions were compared by t-test and Wilcoxon test as appropriate. Statistical significance was a two-sided P value of 0.05. The concentration of lipoprotein particles was normalized by the transformation of the logarithm to base 2. The correlation between the NMR-measured lipoprotein particles and conventional lipid variables was assessed by Spearman's rank correlation. Multivariable-adjusted linear regression models were applied to assess the association of continuous WHR with lipid parameters. The following variables were considered as covariates: sex, age, current smoking, current alcohol assumption, and blood glucose. These covariates were considered based on their clinical relevance, the association with obesity, and literature (8, 28, 29). The collinearity of linear models was examined. In categorical analysis, the association of WHR obesity with lipid parameters was assessed using multivariable-adjusted logistic regression models with the adjustment of the same covariates.

The mediation analysis was performed by the following steps: 1) To examine whether insulin sensitivity was a potential mediator, the associations between insulin sensitivity, WHR, and lipid parameters were examined. The association of WHR with HOMA-IR indicated the effect (a) of WHR on the

mediator. The association of HOMA-IR with a lipid parameter denoted the effect (b) of mediator on lipid parameter. 2) The association of WHR with a lipid parameter was the total effect (c). 3) Whether insulin sensitivity intermediated the association between WHR and lipid parameters was analyzed by the mediation model using HOMA-IR as a mediator. The total effect (c) of WHR on a parameter comprised direct effect and indirect effect. The indirect effect (a*b) represented the mediated effect of WHR on a lipid parameter through insulin sensitivity (WHR → HOMA-IR → a lipid parameter). The direct effect (c') referred to the remaining effect of WHR on lipid parameters, not intermediating *via* insulin sensitivity (WHR → other paths → a lipid parameter). The proportion of indirect effect to total effect was the mediation percentage of HOMA-IR. The mediation analysis was performed in SAS with the PROC CAUSALMED Procedure. All associations in mediation models were expressed as β coefficients and were adjusted for sex and age.

# Results

## Participant characteristics

Table 1 shows the characteristics of 504 participants. The age (SD) averaged 48.9 ( ± 15.4) years, and 260 (51.6%) were female. Of 504 participants, 275 (54.6%) had WHR obesity, approximately 3 times of BMI obesity (96, 19.1%). Individuals with WHR obesity had significantly higher cardiometabolic complications: higher prevalence of hypertension (57.1% vs. 27.1%), dyslipidemia (61.8% vs. 44.1%), insulin resistance

TABLE 1 Participant characteristics.

| Characteristics | All (n = 504) | Normal WHR (n = 229) | WHR obesity (n = 275) | P |
|---|---|---|---|---|
| Number with characteristic (%) | | | | |
| Female | 260 (51.6) | 144 (62.9) | 116 (42.2) | <0.0001 |
| Current Smoking | 76 (15.1) | 38 (16.6) | 38 (13.8) | 0.45 |
| Current alcohol | 369 (73.2) | 174 (76.0) | 195 (70.9) | 0.23 |
| BMI ≥ 30 kg/m$^2$ | 96 (19.1) | 13 (5.7) | 83 (30.2) | <0.0001 |
| Diabetes mellitus | 8 (1.6) | 1 (0.4) | 7 (2.6) | 0.077 |
| Cardiovascular diseases | 29 (5.8) | 11 (4.8) | 18 (6.6) | 0.45 |
| Hypertension | 219 (43.5) | 62 (27.1) | 157 (57.1) | <0.0001 |
| Treatment of hypertension | 87 (17.3) | 27 (11.8) | 60 (21.8) | 0.003 |
| Dyslipidemia | 271 (53.8) | 101 (44.1) | 170 (61.8) | <0.0001 |
| Insulin resistance | 50 (9.9) | 11 (4.8) | 39 (14.2) | 0.0005 |
| Mean ± SD or median (IQR) of characteristic | | | | |
| Age, years | 48.9 ± 15.4 | 43.2 ± 15.7 | 53.6 ± 13.5 | <0.0001 |
| BMI, kg/m$^2$ | 26.1 ± 4.6 | 23.7 ± 3.4 | 28.0 ± 4.5 | <0.0001 |
| WHR | 0.88 ± 0.08 | 0.81 ± 0.05 | 0.94 ± 0.06 | <0.0001 |
| Waist circumference, cm | 90.7 ± 13.0 | 81.5 ± 8.8 | 98.4 ± 10.7 | <0.0001 |
| Hip circumference, cm | 102.9 ± 8.8 | 100.6 ± 8.1 | 104.9 ± 8.9 | <0.0001 |
| Systolic blood pressure, mmHg | 129.9 ± 17.2 | 125.7 ± 17.4 | 133.5 ± 16.1 | <0.0001 |
| Diastolic blood pressure, mmHg | 81.9 ± 9.8 | 78.7 ± 9.3 | 84.5 ± 9.4 | <0.0001 |
| Total cholesterol, mmol/L | 4.94 ± 0.90 | 4.79 ± 0.85 | 5.07 ± 0.92 | 0.0003 |
| LDL cholesterol, mmol/L | 2.92 ± 0.78 | 2.73 ± 0.72 | 3.07 ± 0.79 | <0.0001 |
| HDL cholesterol, mmol/L | 1.52 ± 0.42 | 1.65 ± 0.41 | 1.41 ± 0.39 | <0.0001 |
| Remnant cholesterol, mmol/L | 0.50 ± 0.27 | 0.41 ± 0.18 | 0.59 ± 0.40 | <0.0001 |
| Triglycerides, mmol/L | 1.09 ± 0.61 | 0.89 ± 0.39 | 1.26 ± 0.71 | <0.0001 |
| Non-HDL, mmol/L | 3.42 ± 0.90 | 3.14 ± 0.79 | 3.67 ± 0.92 | <0.0001 |
| Blood glucose, mmol/L | 5.10 (3.40-8.10) | 4.00 (2.80-6.00) | 6.10 (4.00-9.00) | 0.21 |
| Insulin, uIU/mL | 1.03 (0.67-1.69) | 0.82 (0.56-1.20) | 1.28 (0.82-1.95) | <0.0001 |
| HOMA-IR | 2.22 (0.88-4.79) | 1.19 (0.44-2.95) | 3.57 (1.60-5.97) | <0.0001 |
| 10-years SCORE2, % | 0.81 (0.71-0.95) | 0.77 (0.68-0.89) | 0.85 (0.73-0.98) | <0.0001 |
| eGFR, ml/min/1.73m$^2$ | 95.3 ± 16.7 | 100.8 ± 16.0 | 90.8 ± 16.0 | <0.0001 |

WHR obesity was defined as WHR ≥ 0.85 for women, 0.9 for men. BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; IQR, interquartile range; LDL, low-density lipoprotein; SD, standard deviation; WHR, waist-to-hip ratio.

(14.2% vs. 4.8%), compared to those without WHR obesity. The clinical risk factors in individuals with WHR obesity presented an unfavorable trend as well: older, higher blood pressure, blood glucose, insulin, HOMA-IR, and 10-year cardiovascular risk score (P <0.0001).

## Lipid parameters in individuals with WHR obesity

For conventional lipid parameters, individuals with WHR obesity had elevated concentrations of total cholesterol, LDL cholesterol, remnant cholesterol, and triglycerides, but lower HDL cholesterol (Table 1). Table 2 shows NMR-measured lipoprotein constitutions in individuals with and without WHR obesity. Compared to the normal WHR group, individuals with WHR obesity had significantly higher total VLDL particle concentrations (P <0.0001), and lower total HDL particle concentrations (P=0.0003). However, neither total LDL particle concentration nor any LDL particle subclass concentration (large, medium, and small fraction) showed a particular trend across the BMI categories (P ≥ 0.054). The correlation between the NMR spectrometry measured lipoprotein particle parameters and conventional lipid parameters is shown in Table S1 in the supplementary information. Remnant cholesterol and triglycerides from the conventional lipid measurement were highly correlated with VLDL particle concentration (r: 0.914 and 0.913, respectively). LDL particle concentration was proportionally correlated with LDL cholesterol and total cholesterol (r: 0.723 and 0.776, respectively). HDL cholesterol was positively correlated with HDL particle concentration (r: 0.608), whereas it was negatively correlated with VLDL particle concentration (r: -0.504).

TABLE 2 NMR spectrometry-measured lipoprotein particle concentration and size.

| | Normal WHR (n = 229) | WHR obesity (n = 275) | P |
|---|---|---|---|
| Lipoprotein particle concentration, nmol/L | | | |
| VLDL particles | | | |
| Total | 24.63 (17.32-33.61) | 36.91 (25.38-55.44) | <0.0001 |
| Large | 0.74 (0.53-1.01) | 0.93 (0.73-1.39) | <0.0001 |
| Medium | 3.16 (2.20-4.36) | 4.76 (3.18-7.50) | <0.0001 |
| Small | 20.75 (14.69-27.64) | 30.99 (21.51-46.78) | <0.0001 |
| Cholesterol | 3.62 (0.25-8.34) | 9.09 (3.83-16.96) | <0.0001 |
| TG | 39.28 (29.22-50.85) | 54.08 (40.01-79.08) | <0.0001 |
| IDL particles | | | |
| Cholesterol | 2.90 (0.68-5.36) | 3.96 (1.76-6.90) | 0.0003 |
| TG | 3.54 (1.37-5.66) | 4.43 (2.52-7.17) | 0.0004 |
| LDL particles | | | |
| Total | 507.14 (358.50-664.78) | 544.30 (402.66-705.84) | 0.088 |
| Large | 77.34 (54.96-103.99) | 83.63 (57.74-109.31) | 0.070 |
| Medium | 173.40 (123.13-225.07) | 175.44 (135.51-234.29) | 0.240 |
| Small | 257.05 (190.16-340.95) | 279.78 (206.74-365.00) | 0.054 |
| Cholesterol | 68.88 (50.13-89.63) | 75.14 (52.68-98.81) | 0.061 |
| TG | 9.49 (4.30-14.58) | 9.68 (5.29-14.49) | 0.50 |
| HDL particles | | | |
| Total | 22.76 (19.01-27.99) | 19.59 (15.17-26.03) | 0.0003 |
| Large | 0.23 (0.15-0.39) | 0.26 (0.15-0.42) | 0.48 |
| Medium | 7.24 (5.45-8.79) | 5.56 (4.11-7.63) | <0.0001 |
| Small | 15.36 (12.47-18.78) | 14.11 (10.52-18.07) | 0.009 |
| Cholesterol | 51.63 (43.05-59.95) | 42.82 (34.40-53.30) | <0.0001 |
| TG | 4.28 (0.54-8.18) | 6.19 (2.27-10.80) | <0.0001 |
| Average particle size, nm | | | <0.0001 |
| VLDL particles | 42.01 (41.70-42.38) | 41.81 (41.57-42.15) | 0.0001 |
| LDL particles | 21.08 (21.02-21.14) | 21.09 (21.01-21.17) | 0.70 |
| HDL particles | 8.24 (8.22-8.25) | 8.23 (8.20-8.25) | <0.0001 |

WHR obesity was defined as WHR ≥ 0.85 for women, 0.9 for men. The lipid particle concentrations and average sizes are expressed as median (interquartile range). HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; VLDL, very low-density lipoprotein, WHR, waist-to-hip ratio.

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## The association of WHR with lipids parameters

Table 3 displays the adjusted linear association of WHR with the lipoprotein constitutions with adjustment of sex, age, current smoking, current alcohol assumption, and blood glucose (model 1). Total VLDL particle concentration and the concentration of VLDL particle subclasses, cholesterol, and triglycerides contents were proportionally increased with WHR (P ≤ 0.0001, Table 3), independent of covariables. With the additional adjustment of BMI (model 2), WHR was still positively associated with the concentration of total VLDL particle and VLDL particle subclasses (P ≤ 0.047). Total HDL particle concentration, medium and small HDL particle concentration, and cholesterol and triglycerides contents were inversely associated with WHR (P ≤ 0.011). The association of WHR with the concentration of total and medium HDL particle remained significant after further adjusting for BMI. High WHR was

TABLE 3 Linear association of WHR with lipid parameters.

| | WHR | | | |
|---|---|---|---|---|
| | Model 1: coefficient (95% CI) | P | Model 2: coefficient (95% CI) | P |
| Lipoprotein particle concentration | | | | |
| VLDL particles | | | | |
| Total | 0.024 (0.018 to 0.030) | <0.0001 | 0.010 (0.004 to 0.016) | 0.001 |
| Large | 0.021 (0.014 to 0.029) | <0.0001 | 0.0071 (0.0001 to 0.0141) | 0.047 |
| Medium | 0.022 (0.016 to 0.028) | <0.0001 | 0.008 (0.003 to 0.014) | 0.005 |
| Small | 0.024 (0.018 to 0.031) | <0.0001 | 0.010 (0.004 to 0.016) | 0.001 |
| Cholesterol | 0.005 (0.003 to 0.007) | <0.0001 | 0.0012 (-0.0005 to 0.0029) | 0.16 |
| Triglyceride | 0.028 (0.021 to 0.036) | <0.0001 | 0.012 (0.005 to 0.019) | 0.0009 |
| IDL particles | | | | |
| Cholesterol | 0.002 (-0.0003 to 0.003) | 0.10 | -0.0001 (-0.0017 to 0.0016) | 0.94 |
| Triglyceride | 0.004 (0.001 to 0.007) | 0.012 | 0.001 (-0.002 to 0.004) | 0.45 |
| LDL particles | | | | |
| Total | 0.001 (-0.007 to 0.009) | 0.78 | 0.001 (-0.006 to 0.008) | 0.80 |
| Large | 0.002 (-0.006 to 0.010) | 0.56 | 0.003 (-0.004 to 0.010) | 0.41 |
| Medium | -0.002 (-0.010 to 0.006) | 0.60 | -0.0004 (-0.0072 to 0.0064) | 0.91 |
| Small | 0.002 (-0.006 to 0.010) | 0.58 | 0.001 (-0.006 to 0.008) | 0.76 |
| Cholesterol | -0.001 (-0.008 to 0.006) | 0.83 | 0.0004 (-0.0055 to 0.0063) | 0.90 |
| Triglyceride | 0.001 (-0.002 to 0.003) | 0.57 | 0.0005 (-0.0015 to 0.0024) | 0.64 |
| HDL particles | | | | |
| Total | -0.011 (-0.018 to -0.004) | 0.003 | -0.006 (-0.012 to 0.000) | 0.039 |
| Large | -0.001 (-0.006 to 0.004) | 0.72 | -0.0004 (-0.0047 to 0.0039) | 0.85 |
| Medium | -0.013 (-0.020 to -0.007) | <0.0001 | -0.007 (-0.013 to -0.002) | 0.013 |
| Small | -0.009 (-0.016 to -0.002) | 0.011 | -0.0057 (-0.0116 to 0.0003) | 0.063 |
| Cholesterol | -0.034 (-0.045 to -0.023) | <0.0001 | -0.016 (-0.026 to -0.006) | 0.002 |
| Triglyceride | 0.004 (0.002 to 0.006) | 0.001 | 0.0016 (-0.0002 to 0.0033) | 0.081 |
| Particle size | | | | |
| VLDL | -0.026 (-0.036 to -0.015) | <0.0001 | -0.016 (-0.026 to -0.007) | 0.0005 |
| LDL | -0.017 (-0.049 to 0.015) | 0.30 | 0.010 (-0.017 to 0.037) | 0.47 |
| HDL | -0.330 (-0.482 to -0.178) | <0.0001 | -0.107 (-0.241 to 0.028) | 0.12 |
| Conventional lipid measures | | | | |
| Total cholesterol | 0.021 (-0.002 to 0.043) | 0.068 | 0.015 (-0.004 to 0.034) | 0.13 |
| LDL cholesterol | 0.015 (0.000 to 0.030) | 0.049 | 0.007 (-0.005 to 0.020) | 0.25 |
| HDL cholesterol | -0.043 (-0.059 to -0.027) | <0.0001 | -0.011 (-0.026 to 0.004) | 0.14 |
| Remnant cholesterol | 0.027 (0.018 to 0.035) | <0.0001 | 0.009 (0.001 to 0.017) | 0.024 |
| Non-HDL cholesterol | 0.030 (0.015 to 0.045) | 0.0001 | 0.0128 (-0.0005 to 0.0260) | 0.059 |
| Triglyceride | 0.027 (0.018 to 0.036) | <0.0001 | 0.009 (0.001 to 0.017) | 0.027 |

For model 1, coefficients were adjusted for sex, age, current smoking, current alcohol assumption, blood glucose, while for model 2 coefficients were additionally adjusted for BMI. Coefficients were calculated for a doubling of the lipid concentration or 1 nm increment of the averaged lipoprotein particle size. Abbreviation: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; WHR, waist-to-hip ratio.

associated with smaller VLDL particle size, independent of BMI (P = 0.0005). Notably, higher WHR was not associated with LDL particle concentration, cholesterol and triglycerides contents, and LDL particle size (P ≥ 0.56). Along the same line, the conventional lipid parameters presented similar associations with WHR, as indicated by elevated remnant cholesterol and triglycerides, mainly derived from VLDL particles (P ≤ 0.027). The associations of WHR with HDL-cholesterol and LDL cholesterol were disappeared when additionally adjusting for BMI (P ≥ 0.14).

The association of WHR obesity with lipid parameters was similar, as shown in Table 4. In the multivariable-adjusted logistic regression models, WHR obesity was significantly associated with higher concentration of total VLDL particle, VLDL particle subclasses, triglycerides contents, and smaller VLDL particle size, independent of BMI and other covariables (P

TABLE 4 Association of WHR obesity with lipid parameters.

| | WHR obesity vs. Normal WHR | | | |
|---|---|---|---|---|
| | **Model 1: OR (95% CI)** | **P** | **Model 2: OR (95% CI)** | **P** |
| Lipoprotein particle concentration | | | | |
| VLDL particles | | | | |
| Total | 2.48 (1.89-3.26) | <0.0001 | 1.71 (1.28-2.29) | 0.0003 |
| Large | 2.14 (1.59-2.88) | <0.0001 | 1.46 (1.06-2.01) | 0.021 |
| Medium | 2.28 (1.77-2.93) | <0.0001 | 1.60 (1.22-2.10) | 0.0007 |
| Small | 2.50 (1.91-3.28) | <0.0001 | 1.72 (1.29-2.31) | 0.0002 |
| Cholesterol | 1.17 (1.09-1.25) | <0.0001 | 1.07 (0.99-1.15) | 0.090 |
| Triglyceride | 2.99 (2.16-4.12) | <0.0001 | 1.94 (1.37-2.74) | 0.0002 |
| IDL particles | | | | |
| Cholesterol | 1.06 (1.00-1.14) | 0.06 | 1.01 (0.93-1.08) | 0.87 |
| Triglyceride | 1.17 (1.05-1.30) | 0.00 | 1.09 (0.96-1.23) | 0.18 |
| LDL particles | | | | |
| Total | 0.99 (0.74-1.31) | 0.93 | 0.93 (0.67-1.30) | 0.68 |
| Large | 1.04 (0.79-1.36) | 0.80 | 1.03 (0.74-1.42) | 0.88 |
| Medium | 0.88 (0.66-1.16) | 0.36 | 0.87 (0.62-1.21) | 0.40 |
| Small | 1.02 (0.78-1.33) | 0.90 | 0.94 (0.68-1.29) | 0.71 |
| Cholesterol | 0.97 (0.76-1.24) | 0.81 | 0.97 (0.73-1.30) | 0.85 |
| Triglyceride | 1.00 (0.92-1.08) | 0.89 | 0.97 (0.89-1.06) | 0.49 |
| HDL particles | | | | |
| Total | 0.79 (0.61-1.02) | 0.07 | 0.88 (0.68-1.15) | 0.35 |
| Large | 1.05 (0.89-1.25) | 0.55 | 1.09 (0.90-1.31) | 0.37 |
| Medium | 0.70 (0.55-0.91) | 0.01 | 0.83 (0.65-1.07) | 0.15 |
| Small | 0.83 (0.65-1.07) | 0.15 | 0.91 (0.70-1.18) | 0.47 |
| Cholesterol | 0.38 (0.24-0.58) | <0.0001 | 0.62 (0.39-0.98) | 0.042 |
| Triglyceride | 1.14 (1.06-1.22) | 0.00 | 1.09 (1.01-1.17) | 0.038 |
| Particle size | | | | |
| VLDL | 0.92 (0.88-0.96) | <0.0001 | 0.94 (0.90-0.98) | 0.002 |
| LDL | 0.97 (0.86-1.09) | 0.57 | 1.09 (0.93-1.28) | 0.28 |
| HDL | 0.34 (0.19-0.60) | 0.0002 | 0.61 (0.33-1.13) | 0.12 |
| Conventional lipid measures | | | | |
| Total cholesterol | 2.19 (1.02-4.70) | 0.044 | 2.07 (0.86-4.96) | 0.10 |
| LDL cholesterol | 1.90 (1.14-3.17) | 0.014 | 1.67 (0.93-3.00) | 0.089 |
| HDL cholesterol | 0.18 (0.10-0.33) | <0.0001 | 0.41 (0.21-0.79) | 0.008 |
| Remnant cholesterol | 2.69 (1.91-3.78) | <0.0001 | 1.68 (1.16-2.43) | 0.007 |
| Non-HDL cholesterol | 3.23 (1.86-5.59) | <0.0001 | 2.21 (1.18-4.14) | 0.013 |
| Triglyceride | 2.70 (1.92-3.79) | <0.0001 | 1.67 (1.15-2.42) | 0.007 |

For model 1, odds ratios (ORs) were adjusted for sex, age, current smoking, current alcohol assumption, blood glucose, while for model 2 ORs were additionally adjusted for BMI. ORs and 95% confidence intervals were calculated for a doubling of the lipid concentration or for a 0.1 nm increment of the averaged lipoprotein particle size. WHR obesity was defined as WHR ≥ 0.85 for women, 0.9 for men.

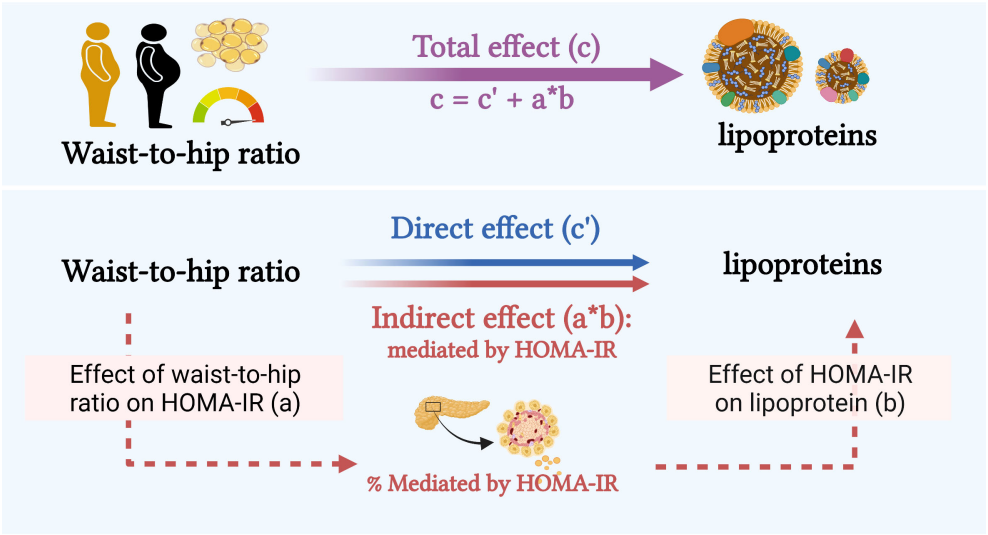
≤ 0.021). The cholesterol and triglycerides contents, but not the concentration of HDL particles, were associated with WHR obesity (P ≤ 0.042). The associations between WHR and LDL particles concentrations, cholesterol and triglycerides contents, LDL particle size were null (P ≥ 0.28). Likewise, for these lipid parameters from the conventional lipid measurement, WHR obesity was associated with higher remnant cholesterol and triglycerides, but lower HDL cholesterol (P ≤ 0.013). The unadjusted associations of WHR and WHR obesity with lipid parameters are presented in supplementary Tables S2, S3.

## Insulin sensitivity as a mediator in the association of obesity with lipoproteins

WHR was positively associated with HOMA-IR (β coefficient: 0.573, 95% CI: 0.443-0.702 for 0.1 increment in WHR), while HOMA-IR was positively associated with total VLDL particle concentration (β: 0.336, 95% CI: 0.270-0.403), remnant cholesterol (β: 0.264, 95% CI: 0.214-0.315), but was inversely associated with HDL cholesterol (β: -0.113, 95% CI: -0.142- -0.085) after adjustment of sex and age. Subsequently, the effect of WHR on these lipid parameters intermediated by HOMA-IR was assessed using mediation analysis, as illustrated in Figure 1. In mediation models, 37% of the effect of WHR on VLDL particle concentration was mediated by HOMA-IR (indirect effect: β=0.158 [95% CI: 0.104-0.211], P <0.0001). For remnant cholesterol and HDL cholesterol, the mediation percentage was 47.7% and 41.1% (P ≤ 0.0003), respectively.

## The relationship between 10-year cardiovascular risk score, WHR obesity, and lipid parameters

Figure 2 shows the 10-year cardiovascular risk score across the LDL cholesterol categories and WHR obesity categories. Individuals with higher LDL cholesterol were at an increased




| | Total effect, β | Direct effect, β | Indirect effect, β | Mediation, % |
|---|---|---|---|---|
| VLDL particle numbers | 0.426 | 0.268 | 0.158 | 37.0 |
| Remnant cholesterol | 0.278 | 0.145 | 0.132 | 47.7 |
| HDL cholesterol | -0.133 | -0.078 | -0.055 | 41.1 |

FIGURE 1
Insulin sensitivity mediated around 40-50% of the association of waist-to-hip ratio with VLDL particle concentration, remnant cholesterol, and HDL cholesterol. In the mediation model, HOMA-IR determined insulin sensitivity was considered a mediator between waist-to-hip ratio and VLDL particle concentration, remnant cholesterol, and HDL cholesterol. The effect of waist-to-hip ratio on HOMA-IR was defined as effect (a). The effect of HOMA-IR on a lipid parameter was defined as effect (b). The total effect of waist-to-hip ratio on a lipid parameter effect (c) comprised direct effect (c') and indirect effect (a*b) mediated by HOMA-IR. These effects were assessed by β coefficients. The mediation percentages were the proportions explained by insulin sensitivity. All effects were adjusted for sex and age and P < 0.05. The figure was created with BioRender.com. HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; VLDL, very-low-density lipoprotein.

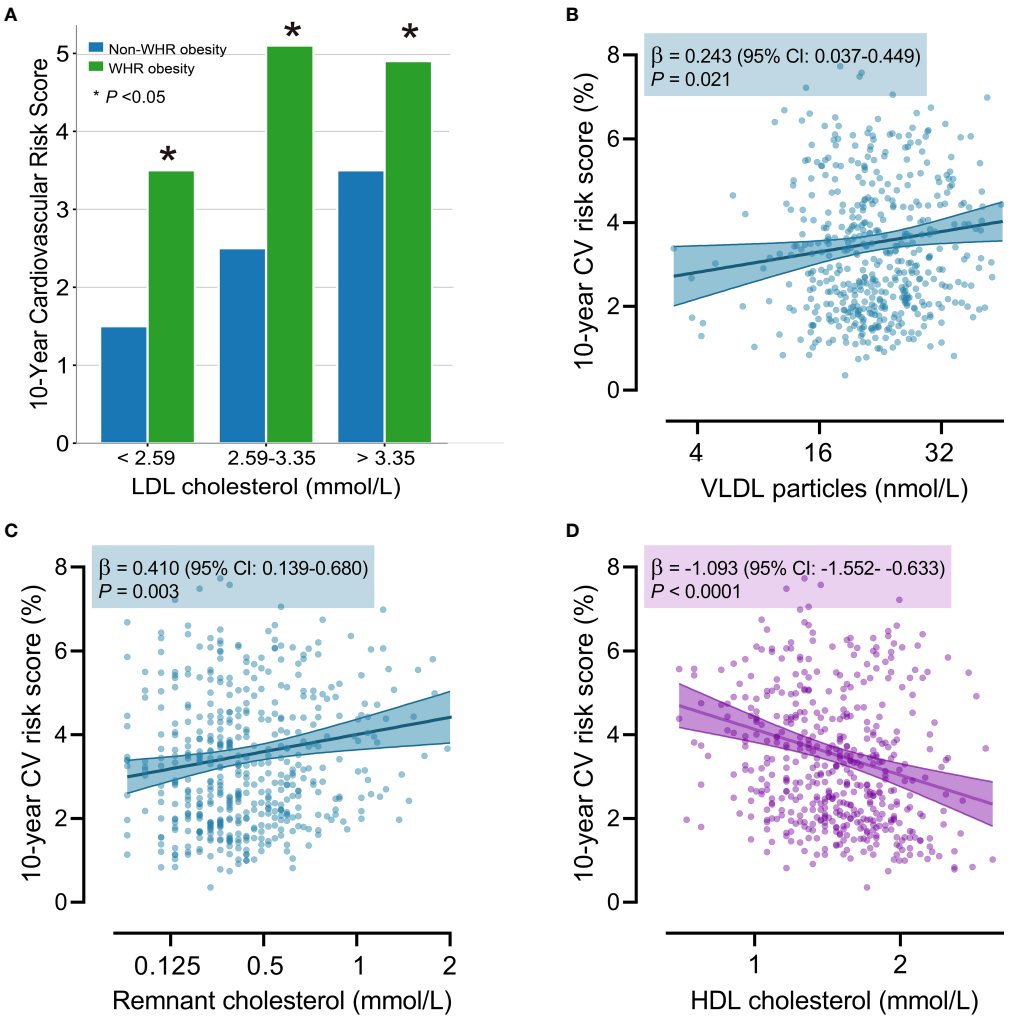



FIGURE 2
The relationship between 10-years cardiovascular risk score, WHR obesity, and lipid parameters. **(A)** The 10-years cardiovascular risk score across the LDL cholesterol categories and WHR obesity categories. The star (*) indicated a P-value < 0.05 between two groups in the same LDL cholesterol category. The linear association of cardiovascular risk with total VLDL particle concentration **(B)**, remnant cholesterol **(C)**, and HDL cholesterol **(D)**. The linear association was adjusted for sex and age. The solid line represents the regression line. The band with two solid lines indicates the 95% confidence limits of the regression line, and the transparent band refers to the 95% prediction limits of the regression model. β coefficients were calculated for a doubling of the lipid concentration. The 10-years cardiovascular risk score was estimated by the SCORE2 algorithm. HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; WHR, waist-to-hip ratio.

cardiovascular risk, independent of WHR obesity. However, regardless of the LDL cholesterol categories, individuals with WHR obesity had a consistently higher cardiovascular risk score compared with those without WHR obesity (P ≤0.028). This might indicate a residual cardiovascular risk for individuals with WHR obesity. Besides, WHR-associated lipid parameters showed a significant association with the estimated cardiovascular risk. As shown by Figure 2, VLDL particle concentrations and remnant cholesterol, positively associated with WHR, were significantly associated with an increased cardiovascular risk (β=0.261, 95% CI: 0.047-0.475 and β=0.434, 95% CI: 0.156-0.713, P≤ 0.002), whereas HDL particle concentration, inversely associated with WHR, presented a negative association with cardiovascular risk score (β=-1.330, 95% CI: -1.836- -0.825, P <0.0001).

## Discussion

The main findings of the current study can be summarized as 1) WHR-defined obesity was far more prevalent than BMI-defined obesity, and individuals with WHR obesity had higher metabolic complications compared to those with normal WHR; 2) the prominent lipid alterations associated with WHR included

increased VLDL particle concentration and remnant cholesterol, and decreased HDL particle concentration, rather than LDL particle concentration or LDL cholesterol; 3) insulin sensitivity mediated roughly 40-50% of the association between WHR and the major altered lipid parameters; 4) individuals with WHR obesity were at higher cardiovascular risk and the WHR obesity-associated lipoprotein alteration was associated an increased cardiovascular risk.

The lipoprotein profile of WHR obesity is consistent with previous findings that abdominal obesity is associated with atherogenic lipid alteration (8). Visceral abdominal fat determined by computerized tomography scan was associated with higher VLDL particle concentration and LDL particle concentration, and smaller LDL particles and HDL particles (8). Our study further suggested the similarity of the association of lipoproteins with upper-body fat distribution and overall fat deposition. Even if BMI is prevailingly used to define obesity, BMI remarkably underestimates the prevalence of abdominal obesity. Moreover, a previous study suggested that WHR provided the highest sensitivity (94.1% for males and 86.7% for females) for the detection of metabolic syndrome in 1104 participants, outperforming both BMI and waist circumference (30). Therefore, WHR is a promising alternative to identify abdominal obesity and metabolic syndrome carriers, delivered by a feasible, simple anthropometric measurement. In line with the solid evidence that WHR is independently associated with mortality and cardiovascular risk (4–6, 31, 32), our findings also supported that WHR obesity seemed to capture the residual cardiovascular risk on top of LDL cholesterol. We found that the estimated cardiovascular risk was consistently higher in individuals with WHR obesity, even with optimal LDL cholesterol levels. The application of WHR may pave the way for early intervention of abdominal obesity and properly assess residual cardiovascular risk for individuals with central body fat distribution.

The present finding also confirmed that VLDL particle concentration was more strongly related to fat distribution as compared to LDL particle concentration. The underlying clinical relevance is that LDL cholesterol might underrate the lipid abnormality in people with upper-body obesity, and VLDL-derived lipid parameters would be more informative. For instance, we found that remnant cholesterol, an estimate for the cholesterol of VLDL particles and VLDL particle remnants, was consistently associated with upper-body obesity. High remnant cholesterol has been demonstrated to be associated with an increased cardiovascular risk (33, 34). In this study, we also observed the positive association between remnant cholesterol and the estimated cardiovascular risk score. High remnant cholesterol may partly explain the residual cardiovascular risk in people with abdominal obesity, even when LDL cholesterol levels are optimal. Noteworthy, remnant cholesterol could be estimated by the existing conventional lipid measures without extra cost. Another lipid parameter derived from VLDL particles is triglycerides. Although elevated triglycerides are the hallmark of the lipid abnormality of obesity, the association of triglycerides per se with cardiovascular risk has been debated for decades (35–37). The altered lipid metabolism characterization in people with obesity might be responsible for the insignificant association between LDL-cholesterol and abdominal obesity. Another possible explanation may relate to the influence of genetic factors on LDL-cholesterol. Among genetic lipid disorders, LDL-cholesterol is more commonly affected (38, 39). Large-scale genome-wide studies integrated the genetic variants associated with LDL-cholesterol with polygenic risk scores responsible for 11-21% of the variance of LDL-cholesterol (40, 41). The correlation between the polygenic risk score and HDL was 0.11 (41).

Apart from VLDL particles, smaller LDL particles and HDL particles are prominent factors in relation to obesity. With the persistent status of high triglyceride-enriched VLDL particle concentration in people with obesity, cholesteryl ester transfer protein (CETP) promotes the exchange of cholesteryl esters from LDL particles and HDL particles for triglycerides from VLDL particles (42). The growing triglycerides contents in LDL and HDL particles tend to be hydrolyzed by hepatic lipase, which generates small, dense LDL and HDL particles. Smaller LDL particles are prone to deposit within arteries; thus, they are atherogenic and associated with the risk of cardiovascular disease (42–45). By contrast, smaller HDL particles contain fewer cholesterol contents, and the smaller particle size is susceptible to degradation by lipases, leading to decreased HDL cholesterol (46).

Our study found that insulin sensitivity is an essential mediator in the association between fat distribution and dyslipidemia, which is in the agreement with previous findings (47, 48). On the one hand, abdominal obesity has been strongly associated with insulin sensitivity and the mechanistic links are considered multifactorial (49). The hypertrophic adipocytes and adipose tissues induce pre-inflammatory cytokines, such as tumor necrosis factor α (TNF-α), that prevent insulin signaling and lead to insulin resistance (46). The impaired free fatty acids storage of enlarged adipose tissues increases free fatty acids in circulation and leads to ectopic fat deposition in the pancreas that dysregulates β-cells and contributes to insulin resistance as well (50, 51). On the other hand, insulin resistance plays a pivotal role in the pathogenesis of obesity-induced lipid disorders, especially in the overproduction of VLDL particles and the reduction of HDL particles (47, 48). The role of insulin resistance can be found in diverse pathways. For instance, an interesting study recently reported that the link between HOMA-IR and the level of PCSK9 is evident in people with obesity (52). Moreover, the effect of depression on HOMA-IR was partially mediated by PCSK9 level, providing a potential treatment strategy for the improvement of insulin sensitivity (52). Based on the existed evidence, our findings emphasized the significance of insulin sensitivity in the development of lipid

abnormality in people with upper-body obesity. Given the mediation percentage (40-50%) of insulin sensitivity, the lifestyle modifications, such as weight loss and carbohydrate-restricted diets, might effectively intervene against dyslipidemia in individuals with obesity on multiple levels, as it has been associated with the improvement in both obesity and insulin sensitivity (53, 54).

Along with adopting healthy lifestyles, restrictive dietary strategies have been reported to influence cardiovascular risk. Long-term fasting (14 days, daily calorie intake: 200–250 kcal) can limit the generation of chylomicrons, stimulate the mobilization of fatty acids from the adipose tissue, and subsequently relate to decreased triglyceride-enriched VLDL particles and LDL cholesterol, as well as higher large HDL particles (55). The lipoprotein profile improved by fasting is less atherogenic and associated with lower cardiovascular risk (55). Pharmacological therapy is another option for lipid-lowering therapy in people with obesity. Statins are the first-line lipid-lowering option to reduce LDL cholesterol and cardiovascular risk (36). However, the efficiency of statins on triglycerides is marginal (36). Given the association of fat distribution with lipoprotein constitutions, specific treatments targeting the elevated VLDL particle concentration and remnant cholesterol hold the potentiality to reduce the remaining cardiovascular risks in individuals with abdominal obesity. It was supported by a large clinical trial of 9423 participants. In this clinical trial, the reduction of VLDL particles with statin was found to associate with reduced risk of atherosclerotic cardiovascular disease, independent of the existence of low LDL cholesterol (56). The proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors provide an alternative approach to lower LDL cholesterol. However, its effect on VLDL particle concentration or remnant cholesterol remains inconclusive (36). A large randomized, controlled trial (PROSPER) recently compared the effect of pravastatin (40 mg/d) and a loss-of-mutation in PCSK9 gene on NMR spectrometry-measured lipoprotein profile, and it demonstrated that PCSK9 inhibition has a weaker effect on VLDL particle concentration (57). In parallel, alirocumab, a monoclonal antibody of PCSK9 was shown to have no impact on the metabolism of VLDL particles (58). These findings contradicted with other reports on monoclonal antibodies of PCSK9 (59, 60). A real-world study in 350 patients receiving PCSK9 antibodies (alirocumab or evolocumab) suggested that PCSK9 inhibitors reduce small VLDL particle concentration (60). Moreover, targeting angiopoietin-like protein 3 (ANGPTL3) shows the potentiality to effectively reduce VLDL particle concentration (reduction of 27.9 to 60.0%), remnant cholesterol (reduction of 38%), and cardiovascular risk (61–63).

A strength of our study is the inclusion of well-characterized participants from the general population since the observed early association might be involved in the development and prevention of cardiometabolic complications. Other major strengths included the incorporation of NMR spectrometry-measured lipoproteins and conventional lipid measures, the application of multivariate analysis with the adjustment of potential confounders, and mediation analysis to identify potential mechanisms for the association of obesity and dyslipidemia. This study also has several limitations. As a cross-sectional study, the observed association between waist-to-hip ratio and atherogenic lipid profile cannot prove causality. Furthermore, given the atherogenic property of lipoprotein (a), accumulating evidence has revealed the causality between long-term exposure to higher lipoprotein (a) and increased risk of cardiovascular events (36). However, the lipoprotein profile of our study did not measure lipoprotein (a) because it was not included in the measurements protocol when the participants were recruited. Besides, cardiovascular risk was not calculated by cardiovascular events in the follow-up but was estimated by the SCORE2 algorithm which includes clinical and biochemical risk factors. SCORE2 is not validated for patients with diabetes or with already known cardiovascular disease, where it underestimates risk. However, the prevalence of diabetes and previous cardiovascular diseases in the studied population was 1.6% and 5.8%, respectively. The findings from sensitivity analyses excluding these participants were confirmative.

## Conclusions

WHR obesity, more prevalent than BMI-determined obesity, was independently associated with an unfavorable lipoprotein profile. Insulin sensitivity was a pivotal mediator that links upper-body fat deposition to lipid disorders. VLDL particle concentration and remnant cholesterol are more strongly associated with obesity than LDL cholesterol. Lowering VLDL particle concentration and remnant cholesterol might further reduce the residual cardiovascular risk for individuals with obesity.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material.

## Ethics statement

The studies involving human participants were reviewed and approved by The University of Leuven Ethics Committee. Written informed consent to participate in this study was provided by the participants and the participants' legal guardian/next of kin.

## Author contributions

DW and Z-YZ conceptualized and designed the study. VM, DM, JR, and Z-YZ contributed to data acquisition. DW and

Z-YZ performed analysis. All authors interpreted the data. DW initially drafted the manuscript. DW, LV, TV, and Z-YZ critically revised the manuscript. All authors reviewed 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



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.978745/full#supplementary-material

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# The effect of total cholesterol/high-density lipoprotein cholesterol ratio on mortality risk in the general population

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**Background:** The relationship between the total cholesterol/high-density lipoprotein cholesterol (TC/HDL-C) ratio and all–cause and cardiovascular mortality has not been elucidated. Herein, we intend to probe the effect of the TC/HDL-C ratio on all-cause and cardiovascular mortality in the general population.

**Methods:** From the 1999–2014 National Health and Nutrition Examination Surveys (NHANES), a total of 32,405 health participants aged ≥18 years were included. The TC/HDL-C levels were divided into five groups: Q1: <2.86, Q2: 2.86–3.46, Q3: 3.46–4.12, Q4: 4.12–5.07, Q5: >5.07. Multivariate Cox regression models were used to explore the relationship between the TC/HDL-C ratio and cardiovascular and all-cause mortality. Two–piecewise linear regression models and restricted cubic spline regression were used to explore nonlinear and irregularly shaped relationships. Kaplan–Meier survival curve and subgroup analyses were conducted.

**Results:** The population comprised 15,675 men and 16,730 women with a mean age of 43 years. During a median follow-up of 98 months (8.1 years), 2,859 mortality cases were recorded. The TC/HDL-C ratio and all-cause mortality showed a nonlinear association after adjusting for confounding variables in the restricted cubic spline analysis. Hazard ratios (HRs) of all-cause mortality were particularly positively related to the level of TC/HDL-C ratio in the higher range >5.07 and in the lower range <2.86 (HR 1.26; 95% CI 1.10, 1.45; HR 1.18; 95% CI 1.00, 1.38, respectively), although the HRs of cardiovascular disease mortality showed no difference among the five groups. In the two-piecewise linear regression model, a TC/HDL-C ratio range of ≥4.22 was positively correlated with cardiovascular mortality (HR 1.13; 95% CI 1.02, 1.25). In the subgroup analysis, a nonlinear association between TC/HDL-C and all-cause mortality was found in those aged <65 years, men, and the no lipid drug treatment population

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**Conclusion:** A nonlinear association between the TC/HDL-C ratio and all-cause mortality was found, indicating that a too-low or too-high TC/HDL-C ratio might increase all-cause mortality. However, for cardiovascular mortality, it does not seem so. The cutoff value was 4.22. The individuals had higher cardiovascular mortality with a TC/HDL-C ratio >4.22.

KEYWORDS

total cholesterol/high-density lipoprotein cholesterol ratio, all-cause mortality, cardiovascular mortality, nonlinear association, prognostic capacity

# Introduction

Cardiovascular disease and cancer are the primary causes of mortality worldwide. A report from America shows the United States see 1 million deaths from cardiovascular disease per year (1). Cholesterol contributes significantly to cardiovascular disease and cancer (2, 3). Impaired intracellular cholesterol metabolism is related to the procedure of many diseases (4).

Although low-density lipoprotein cholesterol (LDL-C) level is used as the primary target of therapy, the risk of cardiovascular disease among statin-treated individuals remains high and not fully explained. Other lipid profiles may interpret some causes of the risk of cardiovascular disease and mortality. Guidelines recommend consideration of lipoprotein ratios [total cholesterol (TC)/high-density lipoprotein cholesterol (HDL-C)] in the management of cardiovascular risk (5). Previous studies have indicated a link between the TC/HDL-C ratio and cardiovascular events; however, findings from these studies have been controversial due to partially inconsistent results (6, 7). The TC/HDL-C ratio remains to be related to cardiovascular mortality among statin-treated individuals; patients in the highest range >2.83 have an increased risk of cardiovascular mortality that is 63% higher than those in the lowest range <2.23 (8, 9). The TC/HDL-C ratio is associated with cardiovascular morbidity and mortality in the general population, independently of triglycerides (TGs), albuminuria, and high-sensitivity C-reactive protein (10). It also has been proven to be an effective predictor of future cardiac events among healthy US women aged 45 years or older (11). Calculating the ratio TC/HDL-C can help us better judge cardiovascular risk when TC levels and HDL-C levels are difficult to determine. Exploring the relationship between TC/HDL-C and death can focus on the residual risk of death after LDL-C treatment.

However, few studies have examined the association of the TC/HDL-C ratio with all-cause mortality. The optimal range of the TC/HDL-C ratio for avoiding mortality is still unknown. Evidence from a large cohort among the general population is needed to address the knowledge gap.

# Materials and methods

## Population

The National Health and Nutrition Examination Survey (NHANES) was a program of studies designed to assess the health and nutritional status of adults and children in the United States. Data from the NHANES for the years 1999–2014 were used for analysis, and a total of 32,405 participants aged ≥18 years with lipid data were included (Figure 1). From 1999 to 2014, people with cardiovascular disease (7,021 people) or cancer (2,719 people) were excluded during the baseline. The study protocol was agreed upon by the Centers for Disease Control and Prevention of the United States. All participants signed an informed consent form.

## Data collection

Demographic information was collected through questionnaires by trained personnel, including age, gender, race

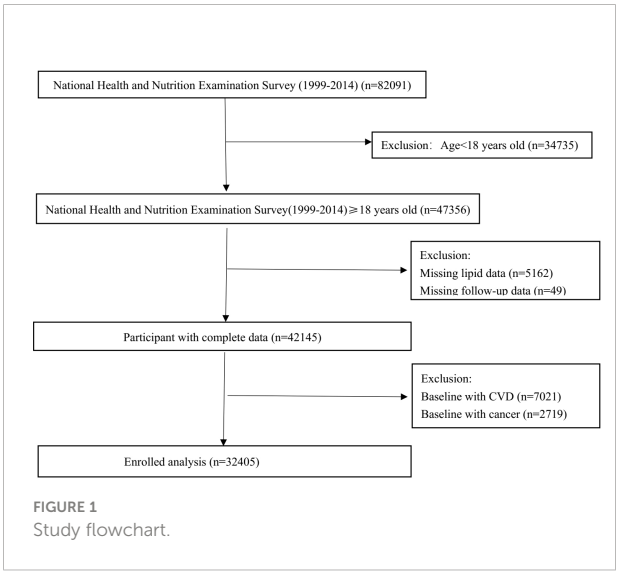


FIGURE 1
Study flowchart.

(white or non-white), medical history (hypertension or diabetes), and smoking status. Height, weight, and blood pressure were measured by trained personnel in a standard operating procedure. Body mass index (BMI) was calculated using weight (kg) divided by the square of height ($m^2$). The estimated glomerular filtration rate (eGFR) was computed using the Modification of Diet in Renal Disease (MDRD) formula (12). Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg or self-reported history of hypertension (13). Type 2 diabetes was defined as fasting blood glucose ≥126 mg/dl (7.0 mmol/L), self-reported history of diabetes, hemoglobin A1c (HbA1C) ≥6.5%, or using hypoglycemic drugs (14).

## Lipid measurement

Lipid blood sample collection and measurement were conducted according to a standardized protocol from the Centers for Disease Control and Prevention. HDL-C was measured by direct immunoassay or by precipitation (15). Serum TC and HDL-C levels were measured enzymatically with a Hitachi 704 Analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN, USA) (16). LDL-C was measured by the Friedewald formula [LDL-C = TC – HDL-C – (TG/5)] if the TG level was ≤400 mg/dl (17).

## Clinical outcomes

Primary outcomes were all-cause mortality and cardiovascular mortality (heart disease and stroke mortality). These participants were followed up until 31 December 2015. Mortality data were extracted from the 1999–2014 NHANES public-use linked mortality files. The International Classification of Diseases, Tenth Revision, codes (I00-I09, I11, I13, I20-I51, I60-69) were used to define cardiovascular mortality.

## Statistical analysis

We applied population-weighted parametric and nonparametric tests when appropriate for exploring the associations of baseline characteristics (18). In the analysis, continuous variables were expressed as means [standard deviation (SD)] for normally distributed variables. Categorical variables were expressed as percentages (number of individuals). Baseline characteristics of participants were grouped by the TC/HDL-C ratio (Q1: <2.86, Q2: 2.86–3.46, Q3: 3.46–4.12, Q4: 4.12–5.07, Q5: >5.07). The chi-square, one-way ANOVA, Kruskal–Wallis H-test were carried out to examine the differences among these groups. Multivariate-adjusted Cox-restricted cubic spline regression was used to explore the relationship. Survival analysis was explored by using standardized Kaplan–Meier curves and log-rank tests.

Multivariate Cox regression models were conducted to examine independent factors for all-cause and cardiovascular mortality. Multivariate-adjusted Cox-restricted cubic spline regression models and a generalized additive model were used to explore the nonlinear relationship between the TC/HDL-C ratio and mortality. If nonlinear relationships were identified, a two-piecewise Cox proportional hazards model on both sides of the inflection point and log likelihood ratio test were performed. TC/HDL-C was included in the model as a continuous variable and fit a coefficient above/below cutoff value separately. We used a two-piecewise linear regression model to evaluate the nonlinear relationships between the TC/HDL-C ratio and mortality, and the optimal cutoff points were set by testing all possible values and selecting the cutoff values with the highest likelihood. Subgroup analysis was performed. All analyses were performed with R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria), with statistical significance being identified at the level of $P < 0.05$.

# Results

## Baseline characteristics

The baseline characteristics according to the TC/HDL-C ratio groups were presented in Table 1. In total, 32,405 participants were included in this analysis with mean age of 43 years old. Among them, 51.63% were women, 67.69% were white, and 45.23% smoked. In addition, the proportion of participants with hypertension and diabetes was 33.22% and 10.04%, respectively. The proportion of antihypertensive drugs, hypoglycemic agents, and lipid-lowering drugs was 17.67%, 5.09%, and 8.81%, respectively. There were significant differences in age, gender, smoking, BMI, SBP, DBP, TC, eGFR, baseline proportion of diabetes, hypertension, and the use of lipid-lowering, antihypertensive, and hypoglycemic drugs among groups according to the TC/HDL-C concentrations (all $P < 0.05$), except race.

## Incidence of cardiovascular and all-cause mortality

During a median follow-up of 98 months (8.1 years), 2,859 mortality cases occurred; 551 mortality cases were due to cardiovascular disease. The incidence rate of all-cause and cardiovascular mortality among the TC/HDL-C groups was shown in Table 1.

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TABLE 1 Demographic and clinical characteristics according to the TC/HDL-C ratio quintiles.

| | TC/HDL-C | | | | | | |
|---|---|---|---|---|---|---|---|
| | Total | Q1 | Q2 | Q3 | Q4 | Q5 | P for trend |
| Number | 32,405 | 6,481 | 6,483 | 6,479 | 6,484 | 6,478 | |
| Age, years | 43.9 (0.18) | 42.4 (0.31) | 43.8 (0.30) | 44.1 (0.24) | 44.9 (0.26) | 44.5 (0.24) | <0.001 |
| Gender-female, % | 51.6 (0.27) | 70.6 (0.74) | 62.1 (0.88) | 53.1 (0.75) | 42.2 (0.86) | 30.4 (0.69) | <0.001 |
| Race-white, % | 67.6 (1.19) | 66.6 (1.28) | 67.5 (1.21) | 68.0 (1.38) | 66.5 (1.42) | 69.5 (1.43) | 0.086 |
| Smoking, % | 45.2 (0.57) | 41.5 (0.82) | 41.2 (0.85) | 44.8 (0.87) | 45.0(0.88) | 53.2 (1.00) | <0.001 |
| Body mass index, kg/m$^2$ | 28.4 (0.07) | 25.1 (0.10) | 27.3 (0.11) | 28.8 (0.10) | 30.0 (0.11) | 30.7 (0.12) | <0.001 |
| Systolic blood pressure, mmHg | 121.1 (0.18) | 118.2 (0.30) | 119.4 (0.31) | 120.9(0.26) | 122.3(0.30) | 124.5 (0.33) | <0.001 |
| Diastolic blood pressure, mmHg | 71.1 (0.16) | 68.5 (0.26) | 69.2 (0.22) | 71.0 (0.21) | 72.4 (0.23) | 74.3 (0.28) | <0.001 |
| eGFR, mg/min/1.73 m$^2$ | 88.7 (0.32) | 89.9 (0.53) | 88.7(0.52) | 89.6 (0.56) | 87.4 (0.46) | 87.8 (0.44) | <0.001 |
| Total cholesterol, mg/dl | 198.3 (0.39) | 173.8 (0.56) | 185.7 (0.58) | 194.6 (0.70) | 206.4 (0.61) | 230.5 (0.74) | <0.001 |
| HDL cholesterol, mg/dl | 52.8 (0.16) | 71.4 (0.27) | 58.8 (0.19) | 51.5 (0.19) | 45.3 (0.14) | 37.4 (0.13) | <0.001 |
| TC/HDL-C ratio | 4.1 (0.01) | 2.4 (0.00) | 3.1 (0.00) | 3.7 (0.00) | 4.5 (0.00) | 6.2 (0.02) | <0.001 |
| Comorbidities, % | | | | | | | |
| Hypertension | 33.2 (0.44) | 26.9 (0.70) | 30.2 (0.75) | 34.4 (0.80) | 36.1 (0.83) | 38.2 (0.87) | <0.001 |
| Diabetes | 10.0 (0.23) | 6.8 (0.41) | 8.0 (0.43) | 9.9 (0.42) | 11.5 (0.50) | 13.8 (0.55) | <0.001 |
| Treatment, % | | | | | | | |
| Antihypertensive drugs | 17.6 (0.35) | 14.8 (0.59) | 17.7 (0.65) | 19.5 (0.66) | 19.2 (0.71) | 17.0 (0.59) | 0.002 |
| Hypoglycemic agents, | 5.1 (0.16) | 4.1 (0.34) | 4.5 (0.31) | 5.3 (0.32) | 5.3 (0.34) | 5.9 (0.40) | 0.001 |
| Lipid-lowering drugs | 8.8 (0.25) | 9.1 (0.43) | 10.5 (0.56) | 10.2 (0.51) | 8.3 (0.42) | 5.8 (0.37) | <0.001 |
| Outcomes, % | | | | | | | |
| Cardiovascular disease mortality | 1.0(0.06) | 0.88(0.11) | 0.8 (0.12) | 1.1(0.13) | 1.0 (0.12) | 1.3(0.14) | 0.008 |
| All-cause mortality | 6.1 (0.19) | 5.6 (0.31) | 5.5 (0.29) | 5.7 (0.33) | 6.2 (0.33) | 7.3 (0.35) | <0.001 |

Results are mean (SD) or percentage (number of individuals).
Abbreviations: Q, quintiles; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; Q1, <2.86; Q2, 2.86–3.46; Q3, 3.46–4.12; Q4, 4.12–5.07; Q5,>5.07.

## TC/HDL-C ratio and all-cause or cardiovascular mortality

Table 2 shows the estimated hazard ratio (HR) and confidence intervals (CIs) of all-cause and cardiovascular mortality according to the different TC/HDL-C ratio groups. When compared to the reference group (TC/HDL-C ratio: 3.16–3.78) in model III, the multivariable-adjusted HRs for all-cause mortality was 1.26 (1.10, 1.45) for group 5 ($P < 0.05$). HRs for all-cause mortality in the first group was 1.18 (1.00, 1.38). Among the large sample size of 32,405 participants, only 15,106 participants have the data for LDL-C. LDL-C has a strong relationship with cardiovascular risk. We tried to include LDL-C in the model of the 15,106 participants. The results showed severe collinearity between LDL-C and TC (Supplementary Table S1). LDL-C and TC showed a strong correlation (Supplementary Table S2). We adjusted TC in model 3, and LDL-C was excluded from the analysis. Multivariate-adjusted Cox-restricted cubic spline regression models and a generalized additive model were used to explore the nonlinear relationship between the TC/HDL-C ratio and mortality. Multivariate-adjusted Cox-restricted cubic spline regression was shown in Figure 2. After adjusting for some potential confounders, the relationship between TC/HDL-C and all-cause mortality was revealed to be U-shaped ($P < 0.001$) (Figure 2A), as both low and high concentrations were associated with high all-cause mortality risk. However, the nonlinear association between TC/HDL-C and cardiovascular mortality appeared to be not significant ($P = 0.07$) (Figure 2B). The results of the two-piecewise linear regression model between TC/HDL-C and mortality were demonstrated in Table 3. After adjusting for potential confounders, the cutoff value of all-cause and cardiovascular mortality was 3.66 and 4.22, respectively. More or less than 3.66 was related to a higher risk of all-cause mortality (all $P < 0.05$). When the TC/HDL-C ratio was >4.22, the association was significantly positive for cardiovascular mortality ($P < 0.05$). A TC/HDL-C ratio increase of 1 SD leads to a 13% risk increase for cardiovascular mortality. The cumulative survival probability of all-cause (Figure 3A) and cardiovascular mortality (Figure 3B) among the participants as stratified by TC/HDL-C levels was demonstrated in Figure 3. There were no significant differences among the five groups (all log-rank $P > 0.05$).

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TABLE 2 Multivariate Cox regression analysis of the TC/HDL-C ratio with all-cause mortality and cardiovascular mortality.

| | Event rate/1,000 person-years | Model I HR (95% CI), P-value | Model II HR (95% CI), P-value | Model III HR (95% CI), P-value |
|---|---|---|---|---|
| All-cause mortality | | | | |
| TC/HDL-C ratio quintiles | | | | |
| Q1 | 10.57 | 1.07 (0.93, 1.22), 0.3449 | 1.28 (1.12, 1.47), 0.0002 | 1.18 (1.00, 1.38), 0.0534 |
| Q2 | 10.21 | 1.01 (0.88, 1.16), 0.9124 | 1.08 (0.95, 1.23), 0.2417 | 1.06 (0.92, 1.22), 0.3976 |
| Q3 | 10.43 | Ref | Ref | Ref |
| Q4 | 11.17 | 1.08 (0.93, 1.25), 0.3325 | 1.04 (0.89, 1.20), 0.6286 | 1.05 (0.90, 1.23), 0.5207 |
| Q5 | 11.37 | 1.19 (1.04, 1.37), 0.0120 | 1.24 (1.09, 1.40), 0.0007 | 1.26 (1.10, 1.45), 0.0010 |
| P for trend | | 0.0216 | 0.5215 | 0.1408 |
| Cardiovascular mortality | | | | |
| TC/HDL-C ratio quintiles | | | | |
| Q1 | 2.07 | 0.85 (0.60, 1.21), 0.3676 | 1.04 (0.72, 1.51), 0.8255 | 1.06 (0.71, 1.57), 0.7853 |
| Q2 | 1.60 | 0.78 (0.54, 1.13), 0.1959 | 0.84 (0.58, 1.23), 0.3744 | 0.94 (0.64, 1.38), 0.7507 |
| Q3 | 2.09 | Ref | Ref | Ref |
| Q4 | 2.20 | 0.92 (0.66, 1.29), 0.6386 | 0.89 (0.64, 1.24), 0.4807 | 0.86 (0.62, 1.19), 0.3603 |
| Q5 | 2.38 | 1.15 (0.85, 1.56), 0.3645 | 1.23 (0.92, 1.66), 0.1673 | 1.09 (0.78, 1.53), 0.6111 |
| P for trend | | 0.0324 | 0.1332 | 0.7329 |

HR, hazard ratio; CI, confidence interval; Q, quintiles; Q1, <2.86; Q2, 2.86–3.46; Q3, 3.46–4.12; Q4, 4.12–5.07; Q5, >5.07.
Model I adjusted for none.
Model II adjusted for age, gender, and race.
Model III adjusted for age, gender, race, smoking, body mass index, systolic blood pressure, estimated glomerular filtration rate, total cholesterol, comorbidities (diabetes and hypertension), and medicine use (antihypertensive drugs, hypoglycemic agents, and lipid-lowering drugs).

We analyzed the association of the TC/HDL-C ratio and cancer mortality. Supplementary Table S3 showed HR and 95% CI of the multivariable Cox regression model. The patients with the highest TC/HDL-C in the Q5 group had HRs ranging from 0.99 to 2.04 compared with patients in the Q3 group. It seems that a higher TC/HDL-C was related to cancer mortality, consistent with cardiovascular mortality. However, due to the very few outcomes of cancer mortality, the P-value was not significant.

## Subgroup analyses

Subgroup analysis was presented in Figure 4. After adjusting for some confounders, the results showed differences in the subgroups. The two-piecewise linear relationship between TC/HDL-C and all-cause mortality was significant in the populations that were aged <65 years, men, not taking lipid-lowering drugs, and no matter white or not. For those treated

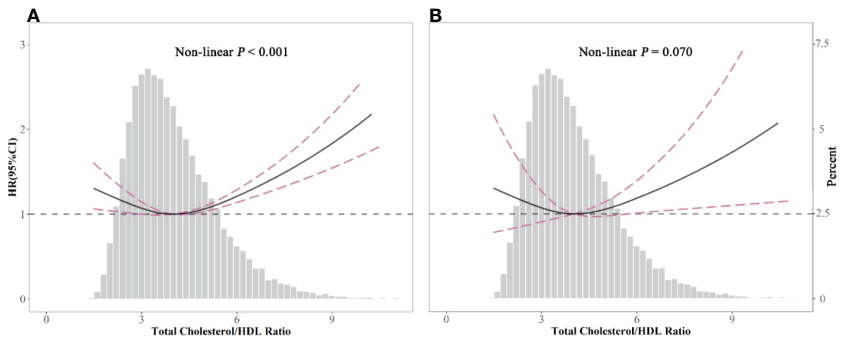


FIGURE 2
Spline analyses of all-cause **(A)** and cardiovascular **(B)** mortality by the total cholesterol/high-density lipoprotein cholesterol (TC/HDL-C) ratio in the overall cohort, and the probability distribution histogram is represented in the background. (Spline analyses were adjusted for age, gender, race, smoking, body mass index, systolic blood pressure, estimated glomerular filtration rate, diabetes and hypertension, antihypertensive drugs, hypoglycemic agents, and lipid-lowering drugs.).

TABLE 3 The results of two-piecewise linear regression model between the TC/HDL-C ratio and all-cause mortality and cardiovascular mortality.

| | All-cause mortalityHR (95% CI) P-value | Cardiovascular mortalityHR (95% CI) P-value |
|---|---|---|
| Cutoff value | 3.66 | 4.22 |
| <Cutoff value | 0.84 (0.75, 0.93) 0.001 | 0.91 (0.75, 1.10) 0.331 |
| ≥Cutoff value | 1.13 (1.09, 1.17) <0.001 | 1.13 (1.02, 1.25) 0.015 |
| P for log likelihood ratio test | <0.001 | 0.076 |

HR, hazard ratio; CI, confidence interval.
The two-piecewise linear regression model was adjusted for age, gender, race, smoking, body mass index, systolic blood pressure, estimated glomerular filtration rate, total cholesterol, comorbidities (diabetes and hypertension), and medicine use (antihypertensive drugs, hypoglycemic agents, and lipid-lowering drugs).

with lipid-lowering drugs, above the cutoff value had shown a significant association with all-cause mortality. Although a single lipid variable controlled well, the TC/HDL-C ratio contributed to additional lipid evaluation value. The two-piecewise linear relationship between TC/HDL-C and cardiovascular mortality was not significant in the subgroup.

## Discussion

The principal finding of this study was that the TC/HDL-C ratio had a nonlinear connection with all-cause mortality but not cardiovascular mortality. For cardiovascular mortality, the TC/HDL-C ratio >4.22 had higher cardiovascular mortality.

Abnormal blood lipid metabolism is a necessary condition for the occurrence of atherosclerosis. TC, LDL-C, and TG are the most often evaluated in clinical work. However, a single lipid index has been shown to be poorly predictive of cardiovascular disease. Among the patients treated with a statin, lipoprotein ratios provided additional value. In a cohort study (10), a high TC/HDL-C ratio was associated with a higher risk of cardiovascular and malignancy mortality in participants without previous cardiovascular disease and who did not use lipid-lowering drugs initially. Another analysis from the Atherosclerosis Risk in Communities (ARIC) study (19), a large cohort of participants free from atherosclerotic cardiovascular disease (ASCVD) at baseline and followed up for more than 20 years with five visits, indicated that those with a TC/HDL-C ratio ≥4.2 had a higher risk of ASCVD, independent of other clinical risk factors and the use of lipid-lowering medications. These two studies are extensive cohort studies with an extended follow-up time; our finding was consistent with these results. The ARIC study recruited participants from 1987 to 1989 in the United States from four communities, almost the same as ours; the risk of cardiovascular mortality increased for a TC/HDL-C ratio ≥4.22. However, NHANES was a program of studies conducted in the whole United States. Data from 1999 to 2014 were used. The results of our study would be more suitable nowadays. A 1 SD increase in the TC/HDL-C ratio resulted in a 13% increased risk of cardiovascular mortality. The proportion of participants with hypertension and diabetes was only 33.22% and 10.04%, respectively. The mean age was 43 years old, and the mean SBP and DBP were normal. Therefore, the population was less at risk for cardiovascular mortality. This may be the reason that those with a TC/HDL-C ratio <4.22 showed no significance. In addition, we did not distinguish coronary heart disease mortality and cerebrovascular disease mortality from cardiovascular mortality. The adjustment of different confounding factors may also have a certain effect on the results.

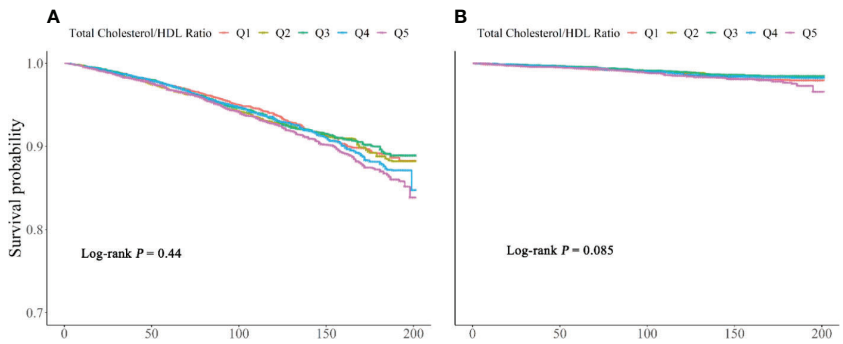


FIGURE 3
Kaplan–Meier survival curves for all-cause **(A)** and cardiovascular **(B)** mortality between Q1, <2.86, Q2, 2.86–3.46, Q3, 3.46–4.12, Q4, 4.12–5.07, and Q5, >5.07.

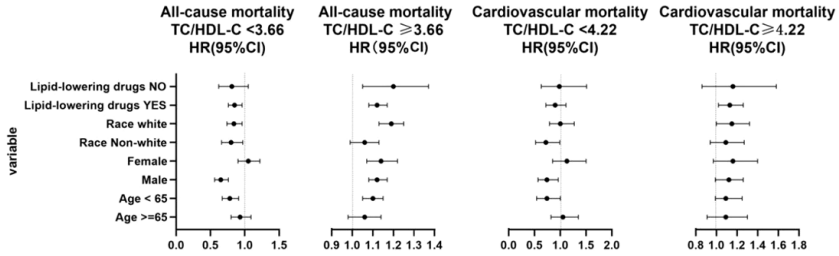


FIGURE 4
Subgroup analysis of the total cholesterol/high-density lipoprotein cholesterol (TC/HDL-C) ratio based on cutoff value. When analyzing a subgroup variable, age, gender, race, smoking, body mass index, systolic blood pressure, estimated glomerular filtration rate, total cholesterol, comorbidities (diabetes and hypertension), and medicine use (antihypertensive drugs, hypoglycemic agents, and lipid-lowering drugs) were adjusted except the variable itself.

Our study found that the TC/HDL-C ratio had a nonlinear association with all-cause mortality. Both extremely high and low ratios indicated a high risk of all-cause mortality in populations that were aged <65 years, men, white, and not taking lipid-lowing drugs. A retrospective study (20) from China has shown that TC/HDL-C ≥3.37 had a predictive value for mortality. Moreover, a U-shaped relationship was found between TC and all-cause mortality in the general Korean population regardless of sex and age (21) and patients with type 2 diabetes mellitus (22). The association between HDL-C concentrations and all-cause mortality was U-shaped for both men and women, with both extremely high and low concentrations being associated with high all-cause mortality risk in the Copenhagen City Heart Study and the Copenhagen General Population Study (23). Our study first showed a U-shaped association between the TC/HDL-C ratio and all-cause mortality in the general population. In the follow-up, 2,859 individuals died in our research. However, 694,423 individuals died in the general Korean population study during follow-up. In addition, 5,619 men died and 5,059 women died in the Copenhagen City Heart Study and the Copenhagen General Population Study. Fewer outcomes and fewer gaps among groups meant the cumulative survival probability analysis showed no difference in all-cause mortality and cardiovascular mortality.

TC and TC/HDL-C showed a moderate correlation in our report. Although the treatment for lowering TC and LDL-C was ongoing for reducing cardiovascular events, the incidents of cardiac events are still high. A single lipid index is poorly predictive of cardiovascular disease. Among the patients treated with a statin, lipoprotein ratios provide additional value. In the report by Beale et al. (24), TC was U-shaped associated with mortality in the no lipid drug population; in the lipid drug-treated population, TC showed no difference between groups. However, our results showed after adjusting TC that the TC/HDL-C ratio had additional clinical value in the population treated with lipid-lowering drugs. A higher TC/HDL-C ratio in treated patients still had a higher risk of all-cause mortality, not cardiovascular mortality. Maybe the decreased TC reduced the artery atherosclerosis of the heart and brain; however, the TC/HDL-C ratio influenced all-cause mortality with other mechanisms. The results make us pay attention to lipoprotein ratios in all-cause mortality.

In the subgroup, a two-piecewise linear connection with all-cause mortality only had significance in the populations that were aged <65 years, men, not taking lipid-lowering drugs, and white or not white. It seems that atherosclerosis caused by cholesterol was not the leading cause of mortality in these populations, with extreme control of the TC/HDL ratio for avoiding all-cause mortality and remote control of the TC/HDL ratio for avoiding cardiovascular mortality. The population aged ≥65 years may have many comorbidities, such as hypertension, diabetes, or hyperuricemia. Recently, older women with a higher risk of heart failure with preserved ejection fraction (HFpEF) had been identified by several studies (24). Then, more risk factor management is needed to focus on these populations, not only the TC/HDL-C ratio.

There are several limitations to the study. First, the population-based sampling of NHANES permitted our analyses to represent men and women living in the United States. Second, we did not show time-fixed and time-varying follow-up lipid data. Third, despite adjusting for known or hypothesized variables to influence or confound the TC/HDL-C ratio and mortality relationship, we cannot exclude the possibility of residual confounding by unmeasured factors, such as inflammation markers, physical markers, physical activity, and uric acid.

## Conclusions

In summary, the TC/HDL-C ratio had a nonlinear connection with all-cause mortality but not with cardiovascular mortality. The cutoff value was 4.22. Individuals had higher cardiovascular mortality with a TC/HDL-C ratio >4.22. The prognostic capacity of the TC/HDL-C ratio provides complementary tools

to assess the deleterious health effects of dysfunctional lipid composition. For statin-treated patients, the TC/LDL-C ratio contributes more value than TC does.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

## Ethics statement

The studies involving human participants were reviewed and approved by Protocol #2018-01. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

DZ, YF contributed to the conception and design of the study. DZ drafted the manuscript. XL contributed to the acquisition of data, interpretation of data, and analysis of data. KL, YH contributed to the interpretation of data and critical revision of the article for important intellectual content. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

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

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1012383/full#supplementary-material

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# The elevated visceral adiposity index increases the risk of hyperuricemia in Chinese hypertensive patients: A cross-sectional study

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**Background:** Uncertainty still remained about the relationship between visceral adiposity index (VAI) and hyperuricemia. The aim of this study was to investigate whether VAI was an independent risk factor for hyperuricemia in hypertensive Chinese patients.

**Methods:** A cross-sectional study including 13176 hypertensive participants (6478 males) recruited from Wuyuan County, Jiangxi province, was conducted. All patients received anthropometric measurements, completed questionnaires and provided blood samples for biochemical testing. VAI was calculated by waist circumference, BMI, triglyceride and high-density lipoprotein cholesterol. Hyperuricemia was defined as serum uric acid ≥ 7 mg/dL in men and ≥ 6 mg/dL in women.

**Results:** Overall, the average level of uric acid was 7.8 ± 2.0 mg/dL in males and 6.34 ± 1.78 in females and prevalence of hyperuricemia was 61.4% and 51.30%, respectively. In multivariate logistic regression analysis, the risk of hyperuricemia increased 1.77 times and 1.88 times with the increase of ln VAI in males (OR:1.77, 95% CI: 1.62, 1.94) and females (OR:1.88, 95% CI: 1.73, 2.04). For males, compared to quartile 1, the risk of hyperuricemia in the second, third and the forth quartile of visceral adiposity index were 1.34 (95% CI: 1.14, 1.57),1.82(95% CI: 1.54, 2.14) and 2.97 (95% CI: 2.48, 3.57). For females, compared to quartile 1, the risk of hyperuricemia in the second, third and the forth quartile of visceral adiposity index were 1.48 (95% CI: 1.28, 1.72), 1.99 (95% CI: 1.71, 2.32) and 2.92 (95% CI: 2.50, 3.42).

**Conclusions:** This study found that VAI was an independent risk factor for hyperuricemia among hypertensive patients, which may provide some strategies for reducing the level of uric acid.

KEYWORDS

visceral adiposity index, uric acid, hyperuricemia, hypertension, obesity

# Introduction

The improvement of living standards, changes in diet and living habits have gradually increased the incidence of hyperuricemia (1). Hyperuricemia, a critical public health issue, is not only a direct cause of gout, but also a risk factor for many cardiovascular diseases and their risk factors. Accumulated studies have demonstrate that high uric acid was associated with hypertension (2), heart failure (3, 4), diabetes (5), kidney disease (6), stroke (7) and coronary heart disease (8, 9). Moreover, increased uric acid was also an independent predictor of all-cause (10) and cardiovascular death (11). Consequently, it is critical to identify hyperuricemia timely and take measures to prevent disease in clinical practice.

Obesity is regarded as a common risk factor for hyperuricemia. Previous studies reported that some traditional obesity index, such as body mass index (BMI) (12), waist circumference (13), waist height ratio (WHtR) (14) and neck circumference (15) had a impact on serum uric acid metabolism. However, they does not have enough ability to distinguish between muscle and fat accumulation (16), as well as visceral fat and subcutaneous fat (17). In contrast to some traditional adiposity indices, such as body mass index (BMI), waist circumference(WC), in which VAI can take into account the effects of sex, triglycerides, and HDL cholesterol levels on body fat distribution and function, VAI has greatly improved the accuracy of visceral fat and its functional assessment, and is currently a valid index for evaluating metabolic diseases (18). Xiaolin Huang et al. demonstrated that VAI increased the risk of hyperuricemia among middle-aged and elderly Chinese adults (19). Another study consisting of 7632 adult subjects from the China Health and Nutrition Survey 2009 suggested that VAI was closely related to hyperuricemia (20). It has also been confirmed that VAI can predict the risk of hypertension (21). However, there are limited reports to explore the relationship between VAI and hyperuricemia in a population prone to adverse events such as hypertension, and we think it is necessary to clarify the exact association between VAI and hyperuricemia in hypertensive patients.

**Abbreviations:** VAI, visceral adiposity index; BMI, body mass index; WC, circumference; SUA, serum uric acid; 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; HCY, homocysteine; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease.

# Methods

## Study design and participants

This study was a sub-study of the China H-type Hypertension Registry Study (registration number: CHiCTR1800017274) carried out in Wuyuan County, Jiangxi Province. It was a real world and observational study with the aim of setting up a national H-type hypertension cohort study, investigating the incidence and treatment rate of H-type hypertension in China and evaluating the related risk factors. Those who met the following criteria were considered as qualified: 1) Aged 18 years or older; 2) Diagnosed with hypertension which defined as Systolic blood pressure (SBP)≥140mmHg and/or diastolic blood pressure (DBP) ≥90mmHg; or self-reported history of hypertension; or taking anti-hypertensive drugs); 3) Signing informed consent. From March 2018 to August 2018, our team recruited 14,268 participants altogether in Wuyuan County, Shangrao City, Jiangxi Province, China. Since VAI was calculated on triglycerides and HDL, we excluded patients taking blood lipid-lowering drugs (n=506) in order to avoid the influence of blood lipids itself. In addition, individuals with data missing (n=12) were excluded. 13, 176 participants included in final data analysis (Supplementary Figure 1).

## Data collection and measurement

At baseline, participants described the the information about sociodemographic characteristics (marital status, education, occupation), their living habits (smoking, drinking, physical exercise), medical history (hypertension, diabetes, chronic kidney disease, etc.) and medication through questionnaires. Weight, Height, circumference (WC) and hip circumference (HC) were measured by trained staff. Body mass index (BMI) was calculated by dividing body weight (kg) by the square of height (m). Blood pressure measurements for all patients would be

performed three times with an electronic sphygmomanometer (Omron; Dalian, China) after 10 minutes of rest. The blood pressure in this study was the average of three blood pressure values. All individuals were informed to keep an empty stomach for more than 10 hours in order to collect their blood samples in the next morning and store them in the Biaojia Biotechnology Laboratory (Shenzhen). Biochemical indicators, including fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), serum uric acid (SUA), homocysteine (Hcy) and creatinine were detected in the core laboratory of national kidney disease clinical research center (Guangzhou, China). The newly developed Chronic Kidney Disease-Epidemiology Collaboration equation was used to estimate glomerular filtration rate (22).

## Definitions

Hyperuricemia was defined as uric acid ≥ 7 mg/dL in men and ≥ 6 mg/ml in women (23). VAI was calculated according to the following formula (24):

$$\text{VAI (males)} = [\text{WC}/(39.68 + 1.89 \times \text{BMI})] \times (\text{TG}/0.81) \times (1.52/\text{HDL} - \text{C}$$

$$\text{VAI (females)} = [\text{WC}/(36.58 + 1.89 \times \text{BMI})] \times (\text{TG}/1.03) \times (1.31/\text{HDL} - \text{C}$$

## Statistical analysis

All data analysis used R software version 4.0.3 (https://www.R-project.org) and Empower (R) version 3.0 (www.empowerstats.com). A two-sided P < 0.05 was defined as significant differences. In the current study, continuous variables were showed in mean ± standard deviation (SD), while categorical variables were presented as percentage. T-test was used to compare continuous variables and Chi-square for categorical variables. The association between visceral adiposity index and hyperuricemia was assessed by logistic regression model. In detail, the current study constructed three models: unadjusted, partially adjusted (age), and fully adjusted (age, smoking, drinking, SBP, DBP, FBG, TC, LDL-C, eGFR, Hcy, anti-hypertensive drugs, glucose-lowering drugs). Generalized additive model (GAM) and smooth curve fitting (penalized spline method) were preformed to characterize the shape of the association between VAI and hyperuricemia clearly. Due to gender differences in serum uric acid levels, multiple regression and subgroup analysis were performed separately in men and women.

# Results

## Characteristics of participants

Table 1 listed the baseline characteristics of males according to quartile of VAI. In general, the mean age was 63.79 ± 9.84 years old, and and the prevalence of hyperuricemia was 61.49%. The prevalence of hyperuricemia in first, second, third and fourth quartile was 49.88%, 57.75%, 64.48% and 73.83%, respectively. Patients with higher VAI values seemed to be younger, have higher level of WC, BMI, DBP, FBG, TC, TG, LDL, Hcy, and eGFR, but lower level of SBP, HDL-C. No difference was detected in history of CKD and number of people taking antihypertensive drugs. Table 2 listed the baseline characteristics of females according to quartile of VAI. The mean age was 63.78 ± 8.99 years old, and and the prevalence of hyperuricemia was 51.30%. The prevalence of hyperuricemia in first, second, third and fourth quartile was 36.91%, 48.59%%, 55.56% and 64.14%, respectively. Significant differences were observed in age, drinking, WC, BMI, DBP, FPG, TC, TG, HDL-C, LDL-C and eGFR (all P values < 0.05). No difference was detected in the status of smoking, SBP, history of CKD and stroke.

## The association between VAI and hyperuricemia

Because of the non normal distribution of VAI, it was transformed by logarithmic function. Table 3 displayed the association between ln VAI and hyperuricemia. For males, the risk of hyperuricemia increased by 70% (95% CI: 1.59, 1.83) for each unit of ln VAI in the unadjusted model. In partially and fully adjusted models, ln VAI was independently associated with hyperuricemia, with adjusted ORs of 1.77 (95% CI: 1.65, 1.91) and 1.77 (95% CI: 1.62, 1.94), respectively (*P* for trend <0.001). When ln VAI was converted from a continuous variable to a categorical variable, the relative risk of hyperuricemia in the second, the third, the fourth quartiles were 1.34 (95% CI:1.14, 1.57), 1.82 (95% CI: 1.54, 2.14) and 2.97 (95% CI: 2.48, 3.57) compared to first quartile. For females, the risk of hyperuricemia in the unadjusted model, partially adjusted model and fully adjusted model increased by 1.89times, 1.98 times and 1.88 times, respectively. The relative risk of hyperuricemia in the second, the third, the fourth quartiles were 1.48 (95% CI:1.28, 1.72), 1.99 (95% CI: 1.71, 2.32) and 2.92 (95%CI: 2.50, 3.42) compared to those in first quartile (*P* for trend <0.001). Figures 1, 2 showed the association between ln VAI and hyperuricemia clearly using smooth curves in males and females. In addition, we also found that WC and BMI were positively associated with hyperuricemia in hypertensive patients regardless of men and women in the logistic

TABLE 1 Baseline characteristics of study participants across visceral adiposity index (VAI) quartiles in males.

| Characteristics | Total participants (n = 6478) | Quartile 1 (< 0.7) n = 1620 | Quartile 2 (0.7-1.1) n = 1619 | Quartile 3 (1.1-2.0) n = 1619 | Quartile 4 (≥ 2.0) n = 1620 | *P*-value |
|---|---|---|---|---|---|---|
| Age (years) | 63.79 ± 9.84 | 67.20 ± 8.67 | 65.84 ± 9.32 | 62.93 ± 9.59 | 59.22 ± 9.82 | <0.001 |
| Smoking (n, %) | 3165 (48.87%) | 828 (51.11%) | 807 (49.88%) | 754 (46.57%) | 776 (47.90%) | 0.047 |
| Drinking (n, %) | 2622 (40.49%) | 801 (49.44%) | 606 (37.48%) | 618 (38.17%) | 597 (36.85%) | <0.001 |
| WC (cm) | 84.23 ± 9.90 | 76.87 ± 8.11 | 82.42 ± 8.90 | 86.84 ± 8.59 | 90.79 ± 8.16 | <0.001 |
| BMI (kg/m2) | 23.35 ± 3.91 | 21.22 ± 4.65 | 22.65 ± 3.18 | 24.08 ± 3.22 | 25.44 ± 3.02 | <0.001 |
| VAI | 1.64 ± 1.67 | 0.50 ± 0.13 | 0.91 ± 0.12 | 1.49 ± 0.23 | 3.64 ± 2.29 | <0.001 |
| SBP (mmHg) | 146.34 ± 17.90 | 147.10 ± 18.07 | 146.87 ± 18.56 | 146.12 ± 17.43 | 145.25 ± 17.45 | 0.014 |
| DBP (mmHg) | 90.25 ± 11.04 | 87.95 ± 10.86 | 89.17 ± 11.07 | 90.63 ± 10.84 | 93.26 ± 10.69 | <0.001 |
| FPG (mmol/L) | 90.25 ± 11.04 | 5.77 ± 1.11 | 5.88 ± 1.09 | 6.05 ± 1.29 | 6.55 ± 2.10 | <0.001 |
| TC (mmol/L) | 4.96 ± 1.04 | 4.86 ± 0.97 | 4.88 ± 1.01 | 5.04 ± 1.06 | 5.07 ± 1.12 | <0.001 |
| TG (mmol/L) | 1.66 ± 1.25 | 0.78 ± 0.20 | 1.13 ± 0.24 | 1.61 ± 0.37 | 3.14 ± 1.67 | <0.001 |
| HDL-C (mmol/L) | 1.53 ± 0.44 | 1.96 ± 0.44 | 1.59 ± 0.32 | 1.41 ± 0.28 | 1.19 ± 0.26 | <0.001 |
| LDL-C (mmol/L) | 2.86 ± 0.77 | 2.53 ± 0.68 | 2.79 ± 0.70 | 3.04 ± 0.77 | 3.09 ± 0.78 | <0.001 |
| HCY (μmol/L) | 20.51 ± 13.65 | 19.47 ± 11.43 | 21.02 ± 13.56 | 20.88 ± 14.71 | 20.67 ± 14.61 | 0.004 |
| eGFR (ml/min/1.73m2) | 85.91 ± 20.42 | 86.27 ± 19.42 | 83.52 ± 21.23 | 85.93 ± 20.23 | 87.93 ± 20.52 | <0.001 |
| SUA (mmol/L) | 7.82 ± 2.00 | 7.31 ± 1.89 | 7.61 ± 1.88 | 7.91 ± 1.91 | 8.47 ± 2.12 | <0.001 |
| Hyperuricemia (n, %) | 3983 (61.49%) | 808 (49.88%) | 935 (57.75%) | 1044 (64.48%) | 1196 (73.83%) | <0.001 |
| Diabetes (n, %) | 1007 (15.54%) | 142 (8.77%) | 199 (12.29%) | 262 (16.18%) | 404 (24.94%) | <0.001 |
| CKD (n, %) | 358 (5.53%) | 77 (4.75%) | 90 (5.56%) | 102 (6.30%) | 89 (5.49%) | 0.293 |
| Stroke (n, %) | 477 (7.36%) | 88 (5.43%) | 138 (8.52%) | 137 (8.46%) | 114 (7.04%) | 0.002 |
| Antihypertensive drugs (n, %) | 4131 (63.78%) | 1017 (62.78%) | 1018 (62.92%) | 1055 (65.16%) | 1041 (64.26%) | 0.435 |
| Glucose-lowering drugs (n, %) | 256 (3.95%) | 31 (1.91%) | 51 (3.15%) | 69 (4.26%) | 105 (6.48%) | <0.001 |

VAIm visceral adiposity index; BMIm body mass index; WCm circumference; SUAm serum uric acid; SBPm systolic blood pressure; DBPm diastolic blood pressure; FBGm fasting blood glucose; TCm total cholesterol; TGm triglyceride; LDL-Cm low density lipoprotein cholesterol; HDL-Cm high density lipoprotein cholesterol; HCYm homocysteine; eGFRm estimated glomerular filtration rate; CKDm chronic kidney disease.

regression model between WC and BMI with hyperuricemia, and the prevalence of hyperuricemia increased with increasing WC and BMI (Supplementary Tables 1, 2). Subsequently, we found that the area under curve(AUC) of these three obesity measures for the presence of hyperuricemia were 0.615, 0.588, 0.576 in men (Supplementary Figure 2) and 0.620, 0.606, 0.588 in female (Supplementary Figure 3), it shows that the correlation between VAI and hyperuricemia is strongest in both female and male.

## Subgroup analysis

For the stability of the results, subgroup analysis Figures 3, 4 were conducted. Selected subgroups were as follows: age (<65, ≥ 65 years), smoking (no, yes), drinking (no, yes), SBP (<140, ≥ 140mmHg), DBP (<90, ≥ 90 mmHg), Hcy (low, high), eGFR (<60, ≥ 60 ml/min/1.73m2) and antihypertensive drugs (no, yes) (all *P* for interaction > 0.05).

# Discussion

In this large-scale cross-sectional study, we found that VAI was positively associated with hyperuricemia in hypertensive patients. The prevalence of hyperuricemia increased with the increase of VAI. This result might provide a strategy for individuals to reduce uric acid.

According to an epidemiological study in the coastal areas of southeast China, the prevalence of hyperuricemia was 32.4% (25), meanwhile the prevalence of hyperuricemia in the current study was more than 50% in both men and women, which indicating the increasing trend of hyperuricemia.

Previous studies had reported that some obesity traditional indices, such as BMI, WC and waist to height ratio, were associated with hyperuricemia (12, 14, 26). Even earlier, studies had reported the effect of visceral fat accumulation on hyperuricemia and demonstrated that visceral fat was a stronger factor than BMI (27). In recent years, study began to focus on the association between adiposity indices and hyperuricemia. A cross-

TABLE 2 Baseline characteristics of study participants across visceral adiposity index (VAI) quartiles in females.

| Characteristics | Total participants (n = 7238) | Quartile 1 (< 1.3) n = 1810 | Quartile 2 (1.3-2.0) n = 1809 | Quartile 3 (2.0-3.1) n = 1809 | Quartile 4 (≥ 3.1) n = 1810 | *P*-value |
|---|---|---|---|---|---|---|
| Age (years) | 63.78 ± 8.99 | 65.08 ± 9.35 | 64.04 ± 9.07 | 63.54 ± 8.77 | 62.48 ± 8.58 | <0.001 |
| Smoking (%) | 399 (5.51%) | 94 (5.19%) | 105 (5.81%) | 93 (5.14%) | 107 (5.91%) | 0.638 |
| Drinking (%) | 383 (5.29%) | 124 (6.85%) | 104 (5.75%) | 83 (4.59%) | 72 (3.98%) | <0.001 |
| WC (cm) | 83.30 ± 9.79 | 77.65 ± 9.35 | 82.60 ± 9.42 | 85.56 ± 8.95 | 87.38 ± 8.56 | <0.001 |
| BMI (kg/m$^2$) | 23.77 ± 3.59 | 22.20 ± 3.63 | 23.58 ± 3.57 | 24.40 ± 3.38 | 24.90 ± 3.15 | <0.001 |
| VAI | 2.63 ± 2.47 | 0.93 ± 0.23 | 1.61 ± 0.20 | 2.46 ± 0.32 | 5.53 ± 3.45 | <0.001 |
| SBP (mmHg) | 150.49 ± 17.52 | 151.19 ± 17.78 | 150.42 ± 17.29 | 150.27 ± 17.33 | 150.08 ± 17.68 | 0.235 |
| DBP (mmHg) | 87.95 ± 10.38 | 87.41 ± 10.78 | 87.63 ± 10.44 | 87.92 ± 10.06 | 88.86 ± 10.17 | <0.001 |
| FPG (mmol/L) | 6.26 ± 1.67 | 5.97 ± 1.26 | 6.18 ± 1.66 | 6.28 ± 1.58 | 6.62 ± 2.02 | <0.001 |
| TC (mmol/L) | 5.37 ± 1.11 | 5.33 ± 1.03 | 5.41 ± 1.10 | 5.43 ± 1.13 | 5.32 ± 1.18 | 0.003 |
| TG (mmol/L) | 1.92 ± 1.23 | 0.98 ± 0.25 | 1.41 ± 0.29 | 1.89 ± 0.40 | 3.40 ± 1.57 | <0.001 |
| HDL-C (mmol/L) | 1.61 ± 0.41 | 1.99 ± 0.40 | 1.67 ± 0.31 | 1.50 ± 0.28 | 1.27 ± 0.27 | <0.001 |
| LDL-C (mmol/L) | 3.12 ± 0.81 | 2.89 ± 0.77 | 3.17 ± 0.80 | 3.25 ± 0.81 | 3.17 ± 0.81 | <0.001 |
| HCY (umol/L) | 15.68 ± 7.28 | 15.50 ± 7.00 | 7.75 ± 1.90 | 15.88 ± 7.96 | 15.68 ± 7.37 | 0.483 |
| eGFR (ml/min/1.73m$^2$) | 90.49 ± 19.74 | 92.04 ± 18.90 | 90.35 ± 19.81 | 90.17 ± 19.55 | 89.42 ± 20.59 | <0.001 |
| SUA (mg/dL) | 6.34 ± 1.78 | 5.79 ± 1.62 | 6.17 ± 1.66 | 6.50 ± 1.80 | 6.90 ± 1.85 | <0.001 |
| Hyperuricemia | 3713 (51.30%) | 668 (36.91%) | 879 (48.59%) | 1005 (55.56%) | 1161 (64.14%) | <0.001 |
| Diabetes | 1429 (19.74%) | 229 (12.65%) | 304 (16.80%) | 375 (20.73%) | 521 (28.78%) | <0.001 |
| CKD | 310 (4.28%) | 70 (3.87%) | 75 (4.15%) | 77 (4.26%) | 88 (4.86%) | 0.507 |
| Stroke | 342 (4.73%) | 77 (4.25%) | 81 (4.48%) | 93 (5.14%) | 91 (5.03%) | 0.532 |
| Antihypertensive drugs | 4650 (64.26%) | 1104 (60.99%) | 1166 (64.49%) | 1166 (64.46%) | 1214 (67.11%) | 0.002 |
| Glucose-lowering drugs | 405 (5.60%) | 60 (3.31%) | 89 (4.92%) | 101 (5.58%) | 155 (8.56%) | <0.001 |

WC, waist circumference; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglycerides; LDL- C, low-density cholesterol lipoprotein; HDL-C, high-density lipoprotein cholesterol; HCY, homocysteine; SUA, serum uric acid; VAI, visceral adiposity index.

TABLE 3 Odds ratios and 95% CIs for hyperuricemia according to VAI as continuous variables and quartiles.

| | Model 1 OR (95% CI) | *P* | Model 2 OR (95% CI) | *P* | Model 3 OR (95% CI) | *P* |
|---|---|---|---|---|---|---|
| **Males** | | | | | | |
| Continuous of ln VAI | 1.70 (1.59, 1.83) | <0.001 | 1.77 (1.65, 1.91) | <0.001 | 1.77 (1.62, 1.94) | <0.001 |
| Quartile of VAI | | | | | | |
| Q1(< 0.7) | Ref. | | Ref. | | Ref. | |
| Q2(0.7-1.1) | 1.37 (1.20, 1.58) | <0.001 | 1.39 (1.21, 1.60) | <0.001 | 1.34 (1.14, 1.57) | <0.001 |
| Q3(1.1-2.0) | 1.82 (1.58, 2.10) | <0.001 | 1.90 (1.64, 2.19) | <0.001 | 1.82 (1.54, 2.14) | <0.001 |
| Q4(≥ 2.0) | 2.83 (2.45, 3.29) | <0.001 | 3.05 (2.61, 3.56) | <0.001 | 2.97 (2.48, 3.57) | 0.001 |
| *P* for trend | <0.001 | | <0.001 | | <0.001 | |
| **Females** | | | | | | |
| Continuous of ln VAI | 1.89 (1.76, 2.04) | <0.001 | 1.98 (1.84, 2.13) | <0.001 | 1.88 (1.73, 2.04) | <0.001 |
| Quartile of VAI | | | | | | |
| Q1(< 01.3) | Ref. | | Ref. | | Ref. | |
| Q2(1.3-2.0) | 1.62 (1.41, 1.85) | <0.001 | 1.67 (1.46, 1.91) | <0.001 | 1.48 (1.28, 1.72) | <0.001 |
| Q3(2.0-3.1) | 2.14 (1.87, 2.44) | <0.001 | 2.25 (1.96, 2.57) | <0.001 | 1.99 (1.71, 2.32) | <0.001 |
| Q4(≥ 3.1) | 3.06 (2.67, 3.50) | <0.001 | 3.32 (2.89, 3.81) | <0.001 | 2.92 (2.50, 3.42) | <0.001 |
| *P* for trend | <0.001 | | <0.001 | | <0.001 | |

Model 1:Adjusted for none.
Model 2:Adjusted for age.
Model 3:Adjusted for age, smoking, drinking, SBP, DBP, FPG, TC, LDL-C, HCY, eGFR, diabetes, stroke, antihypertensive drugs, glucose-lowering drugs.

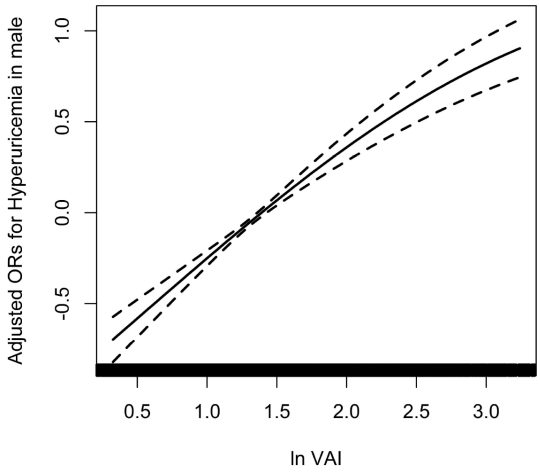


FIGURE 1
Association between ln VAI and hyperuricemia in males adjusted for age, smoking, drinking, BMI, SBP, DBP, TC, LDL-C, Hcy, eGFR, anti-hypertensive drugs, glucose-lowering drugs.

sectional research involving 633 individuals of health check-up demonstrated that VAI was a better indicator of hyperuricemia than waist circumference. The area under receiver operating characteristic curve of VAI and WC were 0.618 and 0.556, respectively (28, 29). Huimin Dong et al. recruited 7632 adult subjects from the China Health and Nutrition Survey 2009 to investigate whether VAI was independent of hyperuricemia. It turned out that VAI was significantly associated with hyperuricemia, independent of metabolic health and obesity phenotypes (20). In addition, VAI was reported to modify the relationship between urinary albumin/creatinine ratio and serum uric acid. In this study of patients with hypertensive but normal renal function, the risk of hyperuricemia increased with the increase of VAI. However, not all research results were consistent. A study of 174698 adults analyzed the relationship between multiple nontraditional adiposity indices and hyperuricemia, and found a negative correlation between VAI and hyperuricemia (30). The reasons for the discrepancy in results

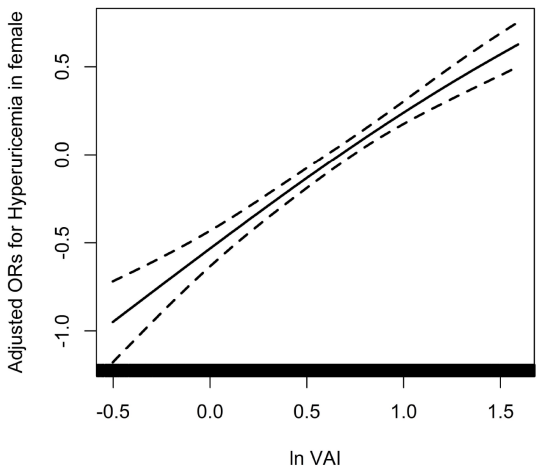


FIGURE 2
Association between ln VAI and hyperuricemia in females adjusted for age, smoking, drinking, BMI, SBP, DBP, TC, LDL-C, Hcy, eGFR, anti-hypertensive drugs, glucose-lowering drugs.

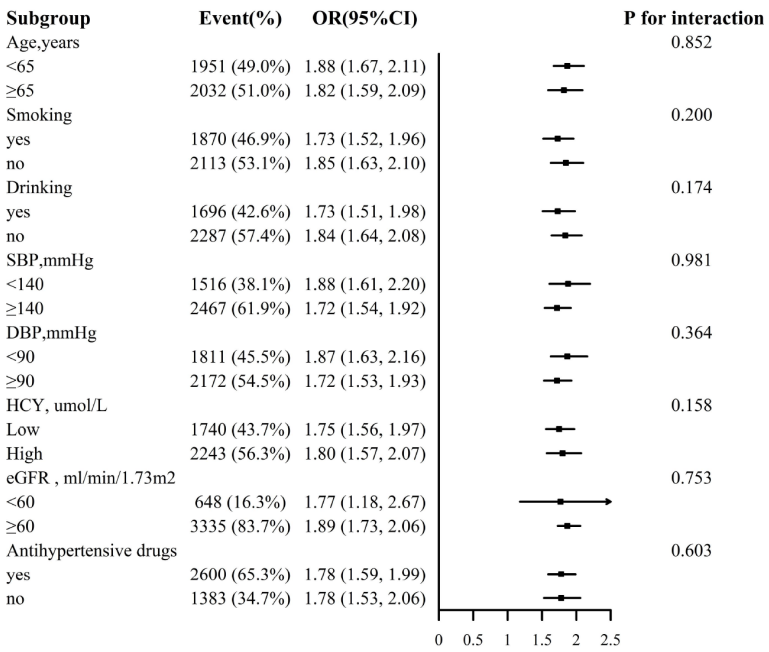


FIGURE 3
Association between ln VAI and hyperuricemia in males in various subgroups. The models adjusted for age, smoking, drinking, BMI, SBP, DBP, TC, LDL-C, HDL, HCY, eGFR, anti-hypertensive drugs, glucose-lowering drugs except for the stratify.

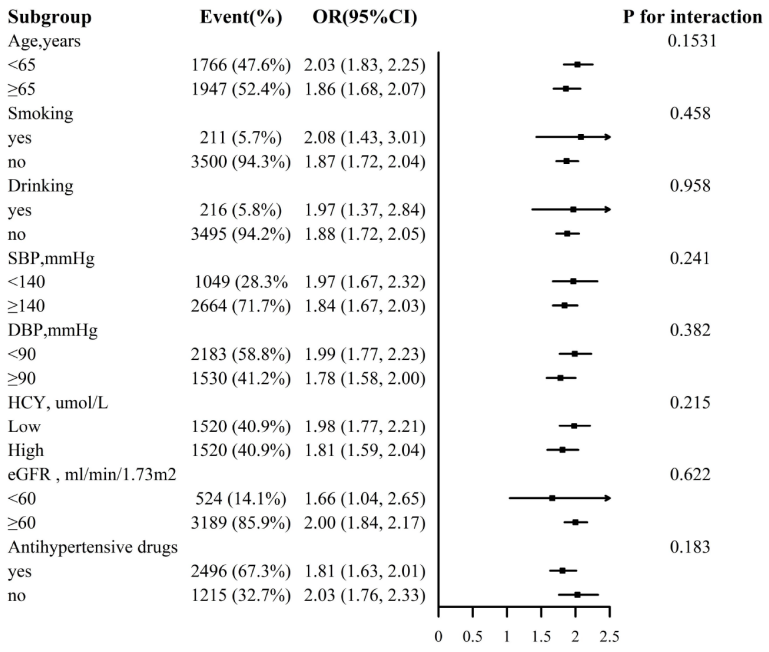


FIGURE 4
Association between ln VAI and hyperuricemia in females in various subgroups. The models adjusted for age, smoking, drinking, BMI, SBP, DBP, TC, LDL-C, HDL, HCY, eGFR, anti-hypertensive drugs, glucose-lowering drugs except for the stratify.

may be attributed to the study population, sample size, statistical analysis, and adjustment factors.

In the current study of hypertensive patients, we found that VAI, an obesity index, was associated with hyperuricemia. The possible mechanisms of hyperuricemia were as follows. First of all, as a hypertensive population, renal microvascular damage and renal ischemia caused by hypertension itself can lead to decreased uric acid excretion and increased production (9). Then, the possible mechanisms of increased risk of hyperuricemia caused by VAI include the following aspects. (1) poor dietary habits and changes in dietary components can lead to obesity. As shown in Table 1, patients with high VAI had higher level of BMI and fasting glucose, and higher prevalence of dyslipidemia and hyperuricemia. This suggested that the increase of visceral fat was often accompanied by the metabolic disorder of blood glucose, blood lipid and uric acid. Second, obesity patients tended to be excessive of energy intake, resulting in increased purine synthesis and uric acid production. (2) Fatty acid metabolites could inhibit the excretion of uric acid and promote the increase of serum uric acid level indirectly. (3) Obesity was often associated with insulin resistance, which acted on the kidney and increased uric acid reabsorption and decrease excretion of uric acid, leading to hyperuricemia finally (31, 32). (4) Some obesity-related adipocytokines, such as adiponectin and leptin, have been reported to be associated with hyperuricemia (33, 34). However, the specific physiological mechanism still needs to be confirmed.

Our highlight was the first study to explore the relationship between VAI and hyperuricemia in Chinese hypertensive patients. In addition, large sample size, accurate statistical methods and strict adjustment of confusion factors ensured the reliability of the results. Certainly, some limitations of this study could not be ignored. First, it was not cautious to established causal inference because of the cross-sectional nature of our study and further prospective studies are needed to explain the exact role of visceral fat accumulation in metabolism of serum uric acid and progression of hyperuricemia. Secondly, present study only evaluated the relationship between VAI and hyperuricemia in Chinese hypertensive patients, whose results might not be extrapolated to other populations. Finally, we did not consider the effect of diet and drugs on serum uric acid level due to the limitation of data.

To sum up, this study demonstrated that VAI was closely related to hyperuricemia in hypertensive patients, which might provide a strategy for reducing uric acid in for obesity patients in clinical practices.

## Data availability statement

The datasets presented in this article are not readily available because data access is obtained according to individual contributions to the study. Requests to access the datasets should be directed to the corresponding authors. Requests to access these datasets should be directed to XC, xiaoshumenfan126@163.com.

## Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Anhui Medical University Biomedical Institute. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

All authors contributed to: (1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, and, (3) final approval of the version to be published. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

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

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1038971/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Flow charts of participants.

SUPPLEMENTARY FIGURE 2
ROC curves for incident hyperuricemia comparing VAI, BMI, and WC in female.

SUPPLEMENTARY FIGURE 3
ROC curves for incident hyperuricemia comparing VAI, BMI, and WC in male.

SUPPLEMENTARY TABLE 1
Odds ratios and 95% CIs for hyperuricemia according to WC as continuous variables and quartiles

SUPPLEMENTARY TABLE 2
Odds ratios and 95% CIs for hyperuricemia according to BMI as continuous variables and quartiles.

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# Association between serum carcinoembryonic antigen and cardiometabolic risks: Implication for cardiometabolic prevention

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**Background:** Serum carcinoembryonic antigen (CEA) is a biomarker commonly used to detect colorectal cancer. CEA levels are affected by many factors, including cardiometabolic diseases, such as cardiovascular diseases (CVDs) and diabetes. Cardiometabolic diseases and cancer share a similar pathological inflammatory pathway, which correlates with an unhealthy lifestyle. Hence, establishing an adequate CEA cut-off value might be a valuable reference for developing precision healthcare programs for cardiometabolic disease prevention. This study aimed to investigate the association between cardiometabolic risks and serum CEA and the underlying factors.

**Methods:** A community-based, cross-sectional study was conducted between March and December 2021 on the western coast of Taiwan. Lifestyle data were assessed using a structured questionnaire. The cardiometabolic biomarkers, serum CEA, urine malondialdehyde, and 1-hydroxypyrene were quantified by the central laboratory of the collaborating hospital. Chi-square and binary multivariable logistic regression implemented in R version 4.0.2 were used to identify factors defining the risk of high serum CEA levels.

**Results:** A total of 6,295 adult residents without cancer-related diseases completed the study. The mean age was 48.6 (SD = 16.4) years, 56% were female, 32% had metabolic syndrome, and 23% and 10% had CVDs and diabetes, respectively. Multivariate logistic regression showed that age ≥ 65 years, male sex, alcohol consumption, smoking, infrequent use of dental floss, fewer remaining teeth, CVDs, diabetes, and oxidative stress were significantly associated with serum CEA ≥ 3 ng/mL. The discriminatory performance of the area under the receiver operating characteristic curve was 0.75 (0.73–0.76), showing that this model was suitable for distinguishing high CEA levels.

**Conclusion:** Our findings highlight the importance of understanding cardiometabolic diseases, unhealthy lifestyles, and oxidative stress, which contribute to high serum CEA. This study demonstrates that CEA, a well-known tumor marker, can help the early detection and prevention of cardiometabolic diseases *via* personalized lifestyle modification.

KEYWORDS

tumor marker, carcinoembryonic antigen (CEA), oxidative stress, 1hydroxypyrene (1-OHP), malondialdehyde (MDA), cardiometabolic diseases (CMDs), unhealthy lifestyle

# 1 Introduction

Recently, researchers studying the interplay between carcinogenesis and cardiometabolic diseases (CMDs) have focused on reactive oxygen species (ROS) and redox imbalance (1–3). ROS, such as oxygen, nitrogen, and sulfur, are highly reactive derivatives of oxygen metabolism and are considered normal cellular metabolites. ROS can be used as a biomarker for oxidative stress. ROS plays a double-edged role in cellular damage and protection (4, 5). Levels of oxidative stress biomarkers, such as urinary 1-hydroxypyrene (1-OHP) and malondialdehyde (MDA), were significantly higher in patients with colorectal cancer (CRC) and correlated with aging, smoking, liver diseases, and CMDs (2, 6). ROS activate the pro-inflammatory signaling pathway and cytokines that induce endothelial cell dysfunction and cause vascular smooth muscle migration and hyperplasia. Serial reactions result in atheroma formation and further CMDs such as hypertension, heart disease, stroke, and type 2 diabetes (2, 7, 8). Serum carcinoembryonic antigen (CEA) is an inflammatory biomarker commonly used to detect colorectal cancer. CEA levels are affected by many factors, including CMDs.

CMDs are recognized as systemic diseases induced by dysregulation of systemic inflammation, immunity, and metabolism and have been shown to have direct effects on atherosclerotic plaques, insulin resistance, and diabetes (9, 10). Furthermore, CMDs and cancer are the leading causes of morbidity and mortality worldwide (11, 12) and in Taiwan (13, 14), with similar biological mechanisms related to the inflammation process, as well as many modifiable risk factors, such as smoking, low vegetable, and fruit intake, obesity, physical inactivity, hypertension, dyslipidemia, and non-modifiable aging, as well as genetic factors (9, 15, 16). According to the literature, most CMDs and cancers can be prevented through modifiable risk factors, such as reduced tobacco and alcohol consumption, changes in an unhealthy diet, and physical activity (9, 11, 15). Additionally, cardiometabolic risks can be detected early, before progression to CMDs, *via* primary health examination. Cardiometabolic risks are a cluster of risk factors such as abdominal obesity, impaired glucose tolerance, elevated blood pressure, triglycerides, and low high-density lipoprotein cholesterol, which increases the risk of CMDs. Furthermore, the presence of three or more of these risk factors is known as metabolic syndrome (MetS) (9, 14, 17).

Except for fecal occult blood tests, serum carcinoembryonic antigen (CEA) is commonly used for the early detection of CRC in many annual health examination settings. CEA, a surface glycoprotein mainly found in epithelial and mucus-secreting cells of the colon, participates in cancer invasion and metastasis (18, 19). CEA is a malignant transformation and chronic inflammation marker and was first identified as a colon cancer antigen; it was previously used as a prognostic marker in CRC and monitoring response to therapy (20, 21). Previous studies showed increased serum CEA levels in CRC and chronic diseases, such as hyperglycemia, CVDs, and type 2 diabetes (17, 19, 22). European cardiologists recently reported that CEA was associated with the severity of heart failure outcomes, including cardiovascular morbidity and mortality (3, 18, 23). The underlying mechanism might be due to imbalanced oxidative damage and endoplasmic reticulum stress production, triggering redox imbalance and increasing oxidative damage to proteins, lipids, and DNA (24)

However, no cut-off reference value is available to distinguish high serum CEA levels in clinical practice. Traditionally, clinicians used serum CEA for tumor detection in CRC and to monitor the response to further treatment. Few studies have linked CEA levels and cardiometabolic risks in primary prevention to mitigate CMDs pathogenesis and progression. Hence, we aimed to investigate the possible modifiable factors associated with high serum CEA levels and establish a cut-off value of serum CEA levels for the prevention of CMDs among adults in rural communities.

# 2 Materials and methods

## 2.1 Design and population

This study was part of a series of health promotion programs designed to explore health needs and provide tailored health care for adults in rural areas. Community-based annual health screening was conducted in collaboration with a local hospital between March and December 2021 in western coastal Yunlin County, Taiwan. Participants were selected using convenience sampling. The inclusion criteria were as follows: (1) age ≥ 20 years, (2) the ability to complete questionnaires in a Mandarin or Taiwanese dialect *via* a face-to-face interview, and (3) agreement to participate in the study after providing informed consent. The exclusion

criteria were as follows: (1) inability to complete the questionnaires, (2) inability to perform self-care or walk independently, (3) diagnosis of cancer-related diseases, and (4) incomplete health surveys or laboratory data.

## 2.2 Procedure and ethical considerations

This study conformed to the principles outlined in the Declaration of Helsinki and was approved by the Institutional Review Board of the Research Ethics Committee (IRB no: 202000109B0C101). All participants were informed about the study's purpose, procedures, benefits, and potential risks agreed to participate, and signed an informed consent form. Five registered nurses were recruited as research assistants and trained by the investigators. The one-on-one questionnaire interview included health-related lifestyle behaviors and was established from a previous study (25). The questionnaire designed was based on the relationships between a healthy lifestyle and anti-inflammatory reactions, such as adequate diets, regular exercise, and oral hygiene are benefits for cardiometabolic health (15, 25). Blood and urine samples were drawn and stored according to the standard procedure by the central laboratory of the collaborating hospital.

## 2.3 Measurements

### 2.3.1 Demographic and health history

Demographic and health history included age, sex, level of education (years of education received), and self-reported comorbidities diagnosed by a physician (diabetes, hypertension, heart disease, and stroke).

### 2.3.2 Substance use was assessed

Substance use was assessed: (a) regular alcohol consumption at least three times per week and (b) cigarette smoking, with responses categorized as "never" vs. "yes: former or current user."

### 2.3.3 Healthy diet

Healthy diet was assessed using the frequency of at least three portions of vegetables and two portions of fruit per day, with responses categorized as "less: never or seldom" and "often: usually or always."

### 2.3.4 Regular exercise

Regular exercise was based on whether the participants usually or always (often) exercised for > 30 min, at least three times per week, or seldom or never (less) engaged in exercise.

### 2.3.5 Oral health

Oral health was measured as follows: (a) the number of natural teeth and fixed dentures were self-reported, and (b) frequency of using dental floss before bed with responses of "less: never or seldom" or "often: usually or always."

### 2.3.6 Cardiometabolic risk factors

Cardiometabolic risk factors were based on the national standard (14), including the presence of five physiological biomarkers: (a) elevated central obesity (waist circumference) in males and females > 90 and 80 cm, respectively, (b) elevated systolic/diastolic blood pressure > 130/85 mmHg, (c) low serum high-density lipoprotein-cholesterol (HDL-C) in males and females < 40 and 50 mg/dL, respectively, (d) elevated serum fasting blood glucose (FBG) > 100 mg/dL, and (e) elevated serum triglyceride (TG) levels > 150 mg/dL. MetS were defined by the presence of three or more risk factors.

### 2.3.7 Carcinoembryonic antigen

Carcinoembryonic antigen (CEA, ng/mL) was measured by electrochemiluminescence immunoassay (ECLIA) on Roche Cobas e801 analyzer. Instead of using reference intervals published in manufacturers' package inserts, we used CEA ≥ 3.0 ng/mL as the cut-off value for the high serum level group based on previous studies, considering age, sex, and smoking habits (18, 22, 26).

### 2.3.8 Urine 1-hydroxypyrene and malondialdehyde

Urine 1-hydroxypyrene (1-OHP) and malondialdehyde (MDA) (μg/g CRE): Spot urine samples were collected and sent to the central laboratory of the collaborating hospital for analysis. Urinary 1-OHP was analyzed using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and urinary MDA was quantified using standard thiobarbituric acid reactive substances (TBARS) assay. The urinary creatinine concentration was used for urinary 1-OHP and MDA adjustments (5, 6).

## 2.4 Statistical analysis

This study used the R version 4.0.2 software (The R Foundation for Statistical Computing, Vienna, Austria) for data analysis, including (1) Chi-square and t-tests performed to confirm the differences according to the CEA category (CEA < 3 or CEA ≥ 3 ng/mL); (2) binary multivariable logistic regression used to identify the factors affecting the risk of CEA ≥ 3; (3) to measured effects of data discrepancies. The dataset was randomly divided into two subsets using the Caret R package, with 80% of the data (n = 5036) in the training subset and the remaining 20% (n = 1259) in the validation set. In the training cohort, significant variables ($p < 0.05$) were selected for binary multivariable logistic regression analysis in the univariate analysis. The model of the training cohort used backward elimination processes to predict the risk of CEA levels ≥ 3 ng/mL. The fitted model was applied to the training and validation subsets. The probability of CEA levels ≥ 3 was calculated based on the beta coefficients of the training subset. The area under the receiver operating characteristic (ROC) curve (AUC) values of the training and validation datasets were calculated using the pROC R package, and (4) To evaluate overfitting, the logistic regression model was fitted to the 1000 bootstrap samples, and the

corresponding values for the AUC were calculated. The results were averaged to provide a final bootstrap estimate for AUC optimism. The differences in the values for the averaged AUC and training subset AUC provided an estimate of optimism.

# 4 Results

## 4.1 Demographic characteristics

A total of 6,295 participants aged ≥ 20 years who completed the community annual health examination were included, of whom 3507 (56%) were female and 1204 (19.1%) were classified as having a CEA ≥ 3 ng/mL (Table 1). The mean age of the participants was 48.6 years (SD = 16.4, range 20–90 years), with more than three-quarters of those aged < 65 years.

## 4.2 Factors associated with high serum CEA level

Univariate analysis showed that male sex ($p < 0.001$), age ≥ 65 years ($p < 0.001$), alcohol consumption ($p < 0.001$), cigarette smoking ($p < 0.001$), less consumption of vegetables and fruits ($p < 0.01$), reduced use of dental floss ($p < 0.001$), and fewer than 20 natural teeth ($p < 0.001$), were significantly associated with high serum CEA levels (Table 1). To compare participants with or without cardiometabolic risks, those with increased abdominal obesity ($p < 0.01$), elevated systolic/diastolic blood pressure ($p < 0.001$), elevated serum FBG ($p < 0.001$), low HDL-C ($p < 0.01$), elevated TG ($p < 0.001$), and MetS ($p < 0.001$) were significantly associated with higher serum CEA level. Participants who reported having been diagnosed with CVDs (hypertension or heart disease, $p < 0.001$) and diabetes ($p < 0.001$) by a physician

TABLE 1 Univariate analysis of factors associated with higher serum level of CEA (N=6295).

| Variables | | Total | CEA[12] <3 | CEA≥3 | $\chi^2$/t | p-value |
|---|---|---|---|---|---|---|
| | | n (%) | n (%) / M±SD | n (%) / M±SD | | |
| Age (years) | <65 | 4952 (79) | 4197 (82) | 755 (63) | 225.9 | <0.001 |
| | ≥65 | 1343 (21) | 894 (18) | 449 (37) | | |
| Gender | Female | 3507 (56) | 3033 (60) | 474 (39) | 161.1 | <0.001 |
| | Male | 2788 (44) | 2058 (40) | 730 (61) | | |
| Healthy diet | Often[1] | 3361 (53) | 2765 (54) | 596 (50) | 9.1 | <0.01 |
| | Less | 2934 (47) | 2326 (46) | 608 (50) | | |
| Alcohol | Never | 5277 (84) | 4398 (86) | 879 (73) | 128.6 | <0.001 |
| | Yes[2] | 1018 (16) | 693 (14) | 325 (27) | | |
| Smoking | Never | 5462 (87) | 4597 (90) | 865 (72) | 288.8 | <0.001 |
| | Yes | 833 (13) | 494 (10) | 339 (28) | | |
| Exercise | Often[1] | 3414 (54) | 2775 (55) | 639 (53) | 0.8 | 0.37 |
| | Less | 2881 (46) | 2316 (45) | 565 (47) | | |
| Dental floss | Often[1] | 3203 (51) | 2746 (54) | 457 (38) | 99.5 | <0.001 |
| | Less | 3092 (49) | 2345 (46) | 747 (62) | | |
| Remaining teeth | ≥20 | 5143 (82) | 4300 (84) | 843 (70) | 135.9 | <0.001 |
| | <20 | 1152 (18) | 791 (16) | 361 (30) | | |
| WC (cm)[3] | < 80/90 | 3735 (59) | 3060 (60) | 675 (56) | 6.6 | 0.01 |
| | ≥ 80/90 | 2560 (41) | 2031 (40) | 529 (44) | | |
| BP (mmHg)[4] | < 130/85 | 3505(56) | 2966(58) | 539(45) | 71.8 | <0.001 |
| | ≥ 130/85 | 2790(44) | 2125(42) | 665(55) | | |
| FBG (mg/dL)[5] | < 100 | 3886 (62) | 3312 (65) | 574 (48) | 124.5 | <0.001 |
| | ≥ 100 | 2409 (38) | 1779 (35) | 630 (52) | | |
| HDL-C (mg/dL)[6] | > 40/50 | 5111 (81) | 4164 (82) | 947 (79) | 6.3 | 0.01 |
| | ≤ 40/50 | 1184 (19) | 927 (18) | 257 (21) | | |

*(Continued)*

TABLE 1 Continued

| Variables | | Total | CEA[12] <3 | CEA≥3 | $\chi^2$/t | p-value |
|---|---|---|---|---|---|---|
| | | n (%) | n (%) / M±SD | n (%) / M±SD | | |
| TG (mg/dL)[7] | < 150 | 5083 (81) | 4187 (82) | 896 (74) | 38.4 | <0.001 |
| | ≥ 150 | 1212 (19) | 904 (18) | 308 (26) | | |
| MetS[8] | < 3 risk factors | 4309 (68) | 3611 (71) | 698 (58) | 75.7 | <0.001 |
| | ≥ 3 risk factors | 1986 (32) | 1480 (29) | 506 (42) | | |
| CVD[9] | No | 4838 (77) | 4084 (80) | 754 (63) | 169.5 | <0.001 |
| | Yes | 1457 (23) | 1007 (20) | 450 (37) | | |
| Diabetes | No | 5677 (90) | 4709 (92) | 968 (80) | 161.0 | <0.001 |
| | Yes | 618 (10) | 382 (8) | 236 (20) | | |
| MDA (μg/g CRE)[10] | | 0.47 (0.71) | 0.45 (0.70) | 0.57 (0.72) | 4.9 | <0.001 |
| 1-OHP (μg/g CRE)[11] | | 0.09 (0.17) | 0.08(0.16) | 0.12 (0.18) | 7.2 | <0.001 |

[1] Often: usually/always; Less: never/seldom;
[2] Yes: former or current user.
[3] Waist circumferences: male/female;
[4] Blood pressure: systolic/diastolic;
[5] Fasting blood glucose;
[6] High-density lipoprotein-cholesterol: male/female;
[7] Triglyceride;
[8]Metabolic syndrome;
[9] cardiovascular diseases: hypertension or heart disease;
[10] malondialdehyde;
[11] 1-hydroxypyrene;
[12] carcinoembryonic antigens.

were classified as having high serum CEA levels. Owing to the lack of reference values for urine 1-OHP and MDA concentration levels, we further compared the mean differences and found that higher levels of urine MDA ($p < 0.001$) and 1-OHP ($p < 0.001$) were significantly associated with high serum CEA (Table 1).

The multivariable logistic regression model shows that the estimated odds of participants with ages ≥ 65 [odds ratio (OR) = 2.25, 95% confidence interval (CI) 1.88–2.70], male sex (OR = 1.71, 95% CI 1.47–1.98), alcohol consumption (OR = 1.25, 95% CI 1.04–1.5), cigarette smoking (OR = 3.11, 95% CI 2.56–3.77), less using dental floss (OR = 1.32, 95% CI 1.14–1.53), fewer remaining teeth (OR = 1.32, 95% CI 1.11–1.57), CVDs (OR = 1.41, 95% CI 1.20–1.67), diabetes (OR = 1.81, 95% CI 1.48–2.21), urine MDA (OR = 1.14, 95% CI 1.04–1.24), and 1-OHP (OR = 1.90, 95% CI 1.33–2.73) were significantly associated with higher serum CEA levels (Table 2). Overall, the discriminatory performance of the full model revealed an AUC of 0.747 (0.733–0.762) (Figure 1A), indicating the suitability of this model in identifying participants with high serum CEA levels.

## 4.3 Training and validation

From the dataset of the 6295 participants, we used 80% of the entries as training data (n = 5036) and 20% for testing (n = 1259). To clarify the potential confounding variables in the training set, backward elimination by binary multivariable logistic regression was used to assess the association between CEA ≥ 3 and various factors. A comparison of the ROC curves for the training and validation data indicated an area difference of 0.003 (0.749–0.746, $p = 0.87$), reflecting a small disparity between the two curves and suggesting a small decay in the model performance in prospective testing (Figure 1B).

The partial area under the ROC curve (pAUC) allows us to focus on the area of interest on the left/right side of the ROC plot (Figures 1C, D), i.e., average sensitivity, between 80–100% specificity values, and average specificity, between 80–100% sensitivity values. Figure 1C shows a slight disparity ($p = 0.4$) in the model's performance in prospective testing under a high valid negative rate. However, Figure 1D shows a slight disparity ($p = 0.3$) in the model performance in prospective testing under a high true positive rate. To validate this difference, bootstrap processes were repeated 1000 times, and the results were averaged to provide an optimum correction for an AUC of 0.003 (AUC range = 0.758–0.79), indicating the lack of overfitting.

# 5 Discussion

To the best of our knowledge, this study is the first to investigate the relationship between serum CEA ≥ 3 (ng/mL), oxidative stress biomarkers, unhealthy lifestyle factors (such as poor oral hygiene, smoking, and fewer remaining teeth), and cardiometabolic risks. The present study provides valuable findings for further interventional studies and evidence-based lifestyle modifications for the early detection and prevention of

TABLE 2 Logistic regression analysis of factors associated higher serum level of CEA[6].

| Variables | | Full Model (All=6295) | | Training Model(n=5036) | |
|---|---|---|---|---|---|
| | | OR (95% CI) | p-value | OR (95% CI) | p-value |
| Age (years) | <65 | Ref | <0.001 | Ref | <0.001 |
| | ≥65 | 2.25 (1.88-2.70) | | 2.34 (1.91-2.86) | |
| Gender | Female | Ref | <0.001 | Ref | <0.001 |
| | Male | 1.71 (1.47-1.98) | | 1.67 (1.42-1.99) | |
| Healthy diet | Often [1] | Ref | 0.08 | Ref | <0.01 |
| | Less | 1.16 (0.98-1.36) | | 1.28 (1.07-1.53) | |
| Alcohol | Never | Ref | 0.02 | Ref | 0.03 |
| | Yes[2] | 1.25 (1.04-1.5) | | 1.25 (1.02-1.54) | |
| Smoking | Never | Ref | <0.001 | Ref | <0.001 |
| | Yes | 3.11 (2.56-3.77) | | 3.14 (2.54-3.90) | |
| Exercise | Often [1] | Ref | 0.89 | – | – |
| | Less | 1.01 (0.88-1.16) | | – | |
| Dental floss | Often [1] | Ref | <0.001 | Ref | <0.001 |
| | Less | 1.32 (1.14-1.53) | | 1.35 (1.15-1.59) | |
| Remaining teeth | ≥20 | Ref | <0.01 | Ref | 0.03 |
| | <20 | 1.32 (1.11-1.57) | | 1.24 (1.02-1.50) | |
| CVD[3] | No | Ref | <0.001 | Ref | <0.001 |
| | Yes | 1.41 (1.20-1.67) | | 1.47 (1.23-1.78) | |
| Diabetes | No | Ref | <0.001 | Ref | <0.001 |
| | Yes | 1.81 (1.48-2.21) | | 1.76 (1.40-2.20) | |
| MDA (μg/g CRE)[4] | | 1.14 (1.04-1.24) | <0.01 | 1.10 (1-1.21) | 0.05 |
| 1-OHP (μg/g CRE)[5] | | 1.90 (1.33-2.73) | <0.001 | 1.87 (1.26-2.76) | <0.01 |

[1] Often: usually/always; Less: never/seldom; [2] Yes: former or current user.
[3] cardiovascular diseases; hypertension or heart disease; [4] malondialdehyde; [5]1-hydroxypyrene; [6] carcinoembryonic antigens.

cardiometabolic risks. Three crucial findings were obtained from this study. First, a high prevalence of cardiometabolic risks was observed, which was significantly associated with high serum CEA levels. Second, an unhealthy lifestyle was significantly associated with a high serum CEA level. Third, the oxidant stress biomarkers 1-OHP and MDA were also positively associated with high serum CEA levels.

## 5.1 Serum CEA can be used for the early detection of cardiometabolic risks

The present study demonstrated that a high prevalence of cardiometabolic risk is significantly associated with high serum CEA levels. For instance, 44%, 41%, 38%, 32%, 23%, and 10% of participants had elevated blood pressure, central obesity, elevated FBG level, MetS, CVDs, and diabetes, respectively. In addition, almost all cardiometabolic risk factors were significantly associated with high serum CEA levels. Similar to previous studies, CEA levels did not only increase in CRC but were also higher in some chronic diseases, especially CVDs, MetS, and diabetes (17, 19, 22). This finding implies that clinicians can use serum CEA as a useful biomarker for the early detection of cardiometabolic risks and unhealthy lifestyles rather than solely as a tumor marker for CRC. Furthermore, if participants had smoked and suffered from CRC and cardiometabolic risks, it is important to clarify which factor primarily contributed to their high serum CEA levels. Huang et al. (19) demonstrated that postoperative serum CEA levels could not predict survival in CRC patients with type 2 diabetes. Type 2 diabetes and cigarette smoking influence serum CEA levels, which may cause a prognostic bias.

The commonly used clinical threshold value for tumor detection is serum CEA ≥ 5 (ng/mL) (19, 26, 27). However, based on previous studies [18,22] and considering smoking, age, and sex, we used CEA ≥ 3 (ng/mL) as a cut-off value and used this model to distinguish high serum CEA levels based on the area under the ROC curve (AUC). Considering that nearly one-third of the total death rate is caused by CMDs, the increase was higher than ever of cancer in Taiwan (13, 14). The findings presented herein could guide further studies for the early detection and

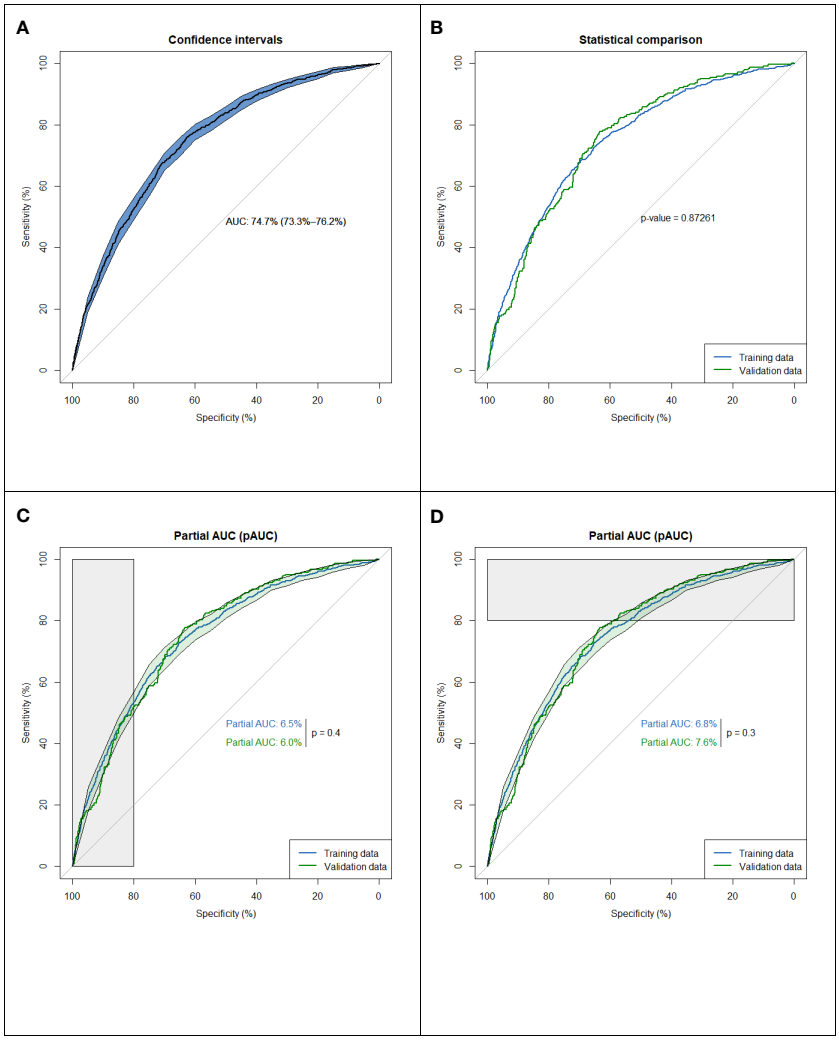


FIGURE 1
Assessing the discrimination of a fitted logistic model, via the ROC curve. **(A)** Discriminatory performance of the full model (n=6295). **(B)** Comparison of ROC curves for the training data (n=5036) and the validation data (n=1259). **(C)** Average sensitivity, between 80%-100% specificity values. A little disparity (p=0.4) in the model's performance in prospective testing under high true negative rate. **(D)** Average specificity, between 80%-100% sensitivity values a little disparity (p=0.3) in the model's performance in prospective testing under ospective high true positive rate.

prevention of cardiometabolic risks using serum CEA levels as a useful biomarker.

## 5.2 Serum CEA levels can be reduced by adopting a healthier lifestyle

Despite male sex and aging factors, the present study revealed that an unhealthy diet (e.g., inadequate amounts of vegetables and fruits), alcohol consumption, and smoking significantly increased serum CEA levels. Furthermore, the present study indicated that urinary 1-OHP and MDA levels correlated with higher serum CEA levels. A possible mechanism might be due to ROS and redox imbalance. Evidence supports that ROS activates the inflammation process and induces endothelial cell dysfunction, causing vascular smooth muscle migration and hyperplasia (10, 15, 16). These findings agreed with previous studies showing that aging, substance use, and an unhealthy diet correlated with elevated CEA levels (24, 28, 29). On the other hand, some dietary compounds and metabolites, such as components of the Mediterranean diet pattern (rich in whole grains, fish, fruits, and vegetables), directly affect HDL-C composition and enhance anti-inflammatory and vasoprotective properties (9, 15, 30).

Increased oxidative stress plays a significant role in cardiometabolic risk as well as the initiation and progression of atherosclerosis. However, adopting a healthy diet and engaging in regular exercise are associated with the prevention of CMDs by reducing the inflammatory process (10, 31, 32). Therefore, the American Heart Association (AHA) guidelines suggest that all adults consume a healthy diet that emphasizes the intake of vegetables and fruits, in addition to exercising for at least 150 min per week (9). However, the present study did not accurately account for the effect of regular exercise on serum CEA levels, which could have been insufficiently characterized, as our questionnaire only

asked whether the participants exercised for > 30 min, at least three times per week. This criterion does not meet the AHA recommendation of 150 mins per week. Further studies should use more precise tools to gauge exercise behavior.

Moreover, the present study showed that infrequent use of dental floss before bed and tooth loss with < 20 remaining teeth were associated with high serum CEA levels. Several studies have shown that lifestyle modifications, including oral hygiene, regular exercise, a healthy diet, and weight control, are important in managing cardiometabolic risks (9, 33). This finding echoed those of previous studies reporting that poor oral hygiene facilitates infections by *Helicobacter pylori* and other bacteria that increase the inflammatory reaction *via* dental plaque, which in turn increases the possibility of periodontal disease, type 2 diabetes, and CVDs (33–35). Hence, it is worth initiating further interventional studies for adults with high serum CEA through personalized healthcare, including smoking and alcohol cessation, maintaining an adequate number of natural teeth through good oral hygiene, and following a Mediterranean diet.

### 4.3 Strengths and limitations

This is the first study involving large-scale reporting of the relationship between the traditional use of serum CEA as a tumor marker and cardiometabolic diseases, identifying determinant factors associated with higher serum CEA levels. Moreover, we used R version statistical analysis to identify CEA levels ≥ 3 as a reasonable cut-off value to distinguish factors associated with high serum CEA, which can be applied to clinical and community settings for early detection of unhealthy lifestyles and providing personalized health promotion programs. However, this study had some limitations. First, it was conducted in only one county, which may limit the generalizability of the findings. Second, the health-related behavior questions were mostly self-reported, which might generate measurement bias and affect the study findings. For instance, the frequencies relative to vegetable consumption and exercise might be inaccurate. In addition, owing to the coronavirus disease pandemic, the number of remaining teeth was self-reported and not counted by the research assistants. Furthermore, our study lacks deep probing into the history of cardiometabolic diseases, such as prescribed medications for hypertension, heart disease, and diabetes. Hence, the prevalence of cardiometabolic risks may be underestimated.

## 6 Conclusion

A high prevalence of cardiometabolic risk factors was associated with high serum CEA levels. Furthermore, unhealthy lifestyles and oxidative stress biomarkers contributed to high serum CEA levels. CEA ≥ 3 ng/mL was a meaningful threshold value for classifying significant risk factors. Therefore, in addition to being a tumor marker for CRC, CEA could be used in clinical and community settings for the early detection and prevention of CMDs through individualized lifestyle modifications.

## 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 institutional review board of the Chang Gung Memorial Hospital Foundation (IRB no: 202000109B0C101). The patients/participants provided their written informed consent to participate in this study.

## Author contributions

C-HC and H-HW contributed to statistical analysis. M-YC and C-HC conceived and designed the study and interpreted the data. C-NL, H-HW, Y-CL, and T-JH collected the data and contributed to the study direction. 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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# Triglycerides and leptin soluble receptor: Which one is the target to protect β-cells in patients with type 2 diabetes?

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**Objectives:** to study the relationships of leptin and leptin SR with adiposity indices, and glycemic indices in patients with type 2 diabetes mellitus (T2DM) compared to healthy subjects.

**Methods:** This cross-sectional study involved 65 patients with T2DM and 63 healthy controls. Fasting plasma levels of leptin, leptin SR, insulin and lipid profile were measured by enzyme linked immunosorbent essay, basal insulin resistance and beta-cell function were assessed using the homeostasis model assessment.

**Results:** leptin SR level was significantly higher in T2DM patients than in controls (5.8 ± 1.6 and 4.8 ± 1.3 respectively; p= 0.001). In patients with T2DM, leptin SR was negatively correlated with homeostasis model of β-cell function and body fat mass while it has a significant positive correlation with glycosylated hemoglobin (HbA1c). The independent predictors for leptin SR in patients with T2DM were triglycerides (TG) and HbA1c.

**Conclusions:** elevated serum leptin SR level in patients with T2DM was positively correlated with TG and abnormal glucose metabolism which indicate that it plays a role in pathophysiology of T2DM. The association of elevated leptin SR level with high TG and deterioration of β-cell function indicate that in some individuals, particularly non-obese, dyslipidemia might be a cause rather than a complication of diabetes.

KEYWORDS

**leptin soluble receptor, body composition, diabetes mellitus, HbA1c, triglycereides, insulin resistance**

## Introduction

The hormone leptin is secreted mainly by adipose tissues and plays many important roles in the regulation of energy balance by suppressing the intake of food and stimulating thermogenesis, thus leading to loss of weight (1). Serum leptin levels are positively correlated with the percentage of body fat and body mass index (2). It has been suggested that obese

people are resistant to the effects of endogenous leptin and even after administration of exogenous leptin there is no significant effect on weight loss (3). When leptin binds to its receptor in the hypothalamus, it stimulates many anorexigenic peptides and inhibit several orexigenic neuropeptides (4).

In humans' plasma, leptin is found in bound, inactive form and free active forms. There is an equilibrium between the circulating binding protein and the free leptin. In lean subjects, most of serum leptin is bound to circulating binding proteins while in obese individuals the majority of circulating leptin remains free (5). Leptin acts by binding to leptin receptor (OB-R), which has a single transmembrane domain and belongs to class 1 cytokine receptor family. The shedding of OB-R extracellular domain produces the main binding protein for leptin in the blood, leptin SR. Animal studies and tissue culture experiments showed that an increased serum leptin SR was associated with inhibition of leptin signal transduction (6). Additionally, a study conducted in young populations found a relationship between serum leptin and its soluble binding protein levels on one hand with measures of adiposity and metabolic syndrome score on the other hand (7). Morioka and coworkers suggested that leptin SR is a factor that can affect pancreatic beta cells secretory functions in patients with T2DM (8). Additionally, Kang et al. found that triglycerides/glucose index can predict insulin resistance (9). Recently, a group of researchers reported that TG level can significantly predict the risk of developing prediabetes and diabetes (10). The present study aimed to investigate the levels of leptin, leptin SR and their correlation with lipid profile, obesity and glycemic control in patients with T2DM. We hypothesize that both leptin SR and TG have a profound effect on beta cell function and a complex interrelationship that needs further investigation.

## Methodology

This cross-sectional study enrolled 128 participants, 65 subjects were diabetic patients (34 males and 31 females) and 63 subjects were healthy controls (36 males and 27 females). The control subjects were healthy employees recruited by local advertisement and the diabetic patients were recruited from primary care clinics at King Khalid University Hospital, Riyadh, Saudi Arabia. This study was conducted at the Department of Physiology, College of medicine at King Saud University. The control group were evaluated using detailed history, clinical examination and investigations. Recruited patient were known to have T2DM for at least six months and the duration ranges between six months and 11 years. All patients with T2DM were receiving oral hypoglycemic drugs and only 18 (24%) patients were on lipid lowering agents. We excluded any patient with type 1 diabetes, acute infection, cardiovascular complications, nephropathy, neuropathy, amputation or those who needed admission. Patients and controls with pregnancy, using oral contraceptive pills or glucocorticoids were excluded.

The study protocol was approved by the ethical committee of institutional review board of college of medicine, King Saud University with approval number: 03/1342/R. All participants signed an informed consent and confidentiality was assured.

Body composition measurement obtained using the body composition analyzer (Biospace-InBody 3.0. SNBS 300504E 2003/04.272-Iyongieong-vi, yipjang-myeon, chanan-si, chungcheongnam-do, South Korea). The measurements included: body mass index (BMI), % body fat, lean body mass and waist-hip ratio (WHR). Before those measurements were taken, the subjects palms and soles were cleaned with electrolytes tissue, and information about their height, sex and age were fed to the machine. The subject was asked to stand with barefoot on the platform of the machine

Fasting venous blood samples were analyzed for blood glucose, HbA1c, basal insulin, lipid profile, leptin and leptin SR. Measurement of HbA1c was performed by Helena Glyco-Tek Affinity Column method, (Helena Biosciences, Europe, Colima Avenue, Sunderland Enterprise Park, Sunderland, Tyne & Wear, SR53 x B, UK). Lipid profile was estimated using Knonelab Itelligent Diagnostics Systems (Konelab Corporation, Ruukintie 18, FIN-02320 Espoo, Finland).

Insulin, leptin and leptin SR immunoassays were performed by quantitative standard sandwich ELISA technique using monoclonal antibody specific for these parameters with kits supplied by R&D Systems, (Abingdon, United Kingdom). The indices of basal insulin resistance and beta-cell function were assessed using the homeostasis model assessment (HOMA/IR and HOMA/B) in which HOMA/IR (mmol/L x μIU/mL) = fasting glucose (mmol/L) x fasting insulin (μIU/mL)/22.5 and HOMA/B = fasting insulin (μIU/mL) x 20/[fasting glucose (mmol/L) – 3.5].

For further analysis of the effect of obesity on leptin and leptin SR we subdivided the study group into four groups, non-obese control (n=30), non-obese diabetic (n=30), obese control (n=33) and obese diabetic (n=35). Obesity was defined as BMI more than 30 $kg/m^2$ (according to WHO criteria). To test for the impact of glycemic control on leptin and leptin SR we subdivided patients into two groups, one with good control (HbA1c ≤ 7.5) which included 35 patients and the other with uncontrolled diabetes (HbA1c >7.5) represented by 30 patients.

## Statistical analysis

The data were analyzed by the computer software program Statistical Package for Social Sciences (SPSS version 20, Chicago). Descriptive characteristics and the lipid profile of the subjects were expressed as Mean± Standard Deviation (SD). Kolmogorov-Smirnov[a] and Shapiro-Wilk tests were used to see that data is following normal distribution or not. Those parameters which were not following normal distribution were analyzed by non-parametric Mann Whitney test. For continuous data with normal distribution Student's t-test was used. Correlations between leptin, leptin SR, HbA1c, BFM and markers of insulin resistance were determined by simple regression analysis. A stepwise linear regression model was constructed for leptin and leptin SR as dependent variables to find the independent predictors for these variables in patients with

T2DM. Anova test was used to compare the level of leptin and leptin SL between four groups (control non-obese, diabetic non-obese, control obese and diabetic obese) then *post-hoc* test was performed.

## Results

Demographic characteristics, clinical features, insulin resistance indices and body composition for controls and T2DM patients were presented in Table 1. Age for control subjects ranges between 25 and 62 years (Mean: 47.22 ± 7.73) and for patients was 30-66 years (49.45 ± 10.2).

Leptin SR level was significantly higher in T2DM patients than in controls (5.8 ± 1.6 and 4.8 ± 1.3 respectively, P=0.001) while the difference was not significant for leptin (32.2 ± 19.5 and 30.6 ± 19.8 respectively, P=0.331).

Using simple regression analysis we determined the correlations between levels of leptin and leptin SR with BFM and HOMA-IR, HOMA-B and HbA1c in patients with T2DM. Leptin SR correlated negatively with HOMA-B (r= -0.416, p=0.001), while the correlation of leptin with HOMA-B was non-significant (r=0.222, p=0.075) (Figures 1A, B). Leptin correlated significantly with HOMA-IR (r=0.248, p=0.048) while the correlation of leptin SR with HOMA-IR was not significant (r=0.037, p=0.771) (Figures 1C, D).

Leptin SR had a significant negative correlation (r= -0.297, p=0.016) with fat mass which was positively correlated with leptin level (r=0.652, p=0.001) (Figures 1E, F). Significant positive correlation between HbA1c and leptin SR was observed (r=0.440, p=<0.001), while the correlation was not significant with leptin levels (r=0.005, p=0.971) (Figures 1G, H).

A multivariate regressions analysis model was constructed to find significant predictors of both serum total leptin and leptin SR among adiposity measures, insulin resistance indices and lipid markers for patients with T2DM. We adjusted for age, sex, BMI, systolic blood pressure, HbA1c, TG, HDL and LDL (Table 2). The independent predictors for leptin in diabetic patients were BFM (B=0.705, p<0.001) and HbA1c (B= -0.311, p=0.003) and for leptin SR were TG (B=0.325, p=0.003) and HbA1c (B=0.262, p=0.015) Table 2.

The serum level of leptin was significantly higher in obese control vs non-obese control (p=0.001) and in obese control vs non-obese diabetic patients (p<0.0001). Leptin was significantly higher

TABLE 1 Comparison of clinical characteristics, body composition and insulin resistance indices between healthy subjects and patients with T2DM.

| Variables | Controls (n= 63) | Patients (n= 65) | P value |
|---|---|---|---|
| M/F | 36/27 | 34/31 | |
| Age (years) | 47.22 ± 7.73 | 49.45 ± 10.2 | 0.790 |
| BMI | 28.9 ± 4.2 | 31.4 ± 5.7 | 0.005* |
| WHR | 0.97 ± 0.07 | 1.03 ± 0.08 | 0.001* |
| SBP (mmHg) | 117.3 ± 14.6 | 128.1 ± 16.8 | 0.001* |
| DBP (mmHg) | 78.8 ± 9.8 | 80.6 ± 9.0 | 0.256 |
| TC (mmol/L) | 5.1 ± 0.8 | 5.0 ± 0.9 | 0.390 |
| HDL (mmol/L) | 1.2 ± 0.3 | 0.9 ± 0.2 | 0.001* |
| TG (mmol/L) | 1.3 ± 0.7 | 1.9 ± 0.9 | 0.001* |
| FBG (mmol/L) | 5.0 ± 0.5 | 7.9 ± 2.6 | 0.001* |
| LDL (mmol/L) | 3.3 ± 0.7 | 3.2 ± 0.8 | 0.346 |
| HbA1c (%) | | 7.3 ± 1.8 | – |
| Fat Mass (kg) | 26.6 ± 8.5 | 30.2 ± 10.6 | 0.040* |
| Body Fat % | 34.9 ± 8.2 | 37.6 ± 7.4 | 0.032* |
| Basal Insulin uIU/ml | 6.5 ± 3.3 | 10.5 ± 14.4 | 0.028* |
| Homa IR | 1.5 ± 0.8 | 2.9 ± 2.4 | 0.010* |
| Homa B(%) | 95.5 ± 61.3 | 48.6 ± 34.5 | 0.001* |
| Leptin ng/ml | 30.6 ± 19.8 | 32.2 ± 19.5 | 0.331 |
| Leptin SR ng/ml | 4.8 ± 1.3 | 5.8 ± 1.6 | 0.001* |

Values are expressed as mean ± SD, Insulin and leptin levels were compared by Mann. Whitney test. All other parameters were compared by t test.
*P value significant when p≤ 0.05.

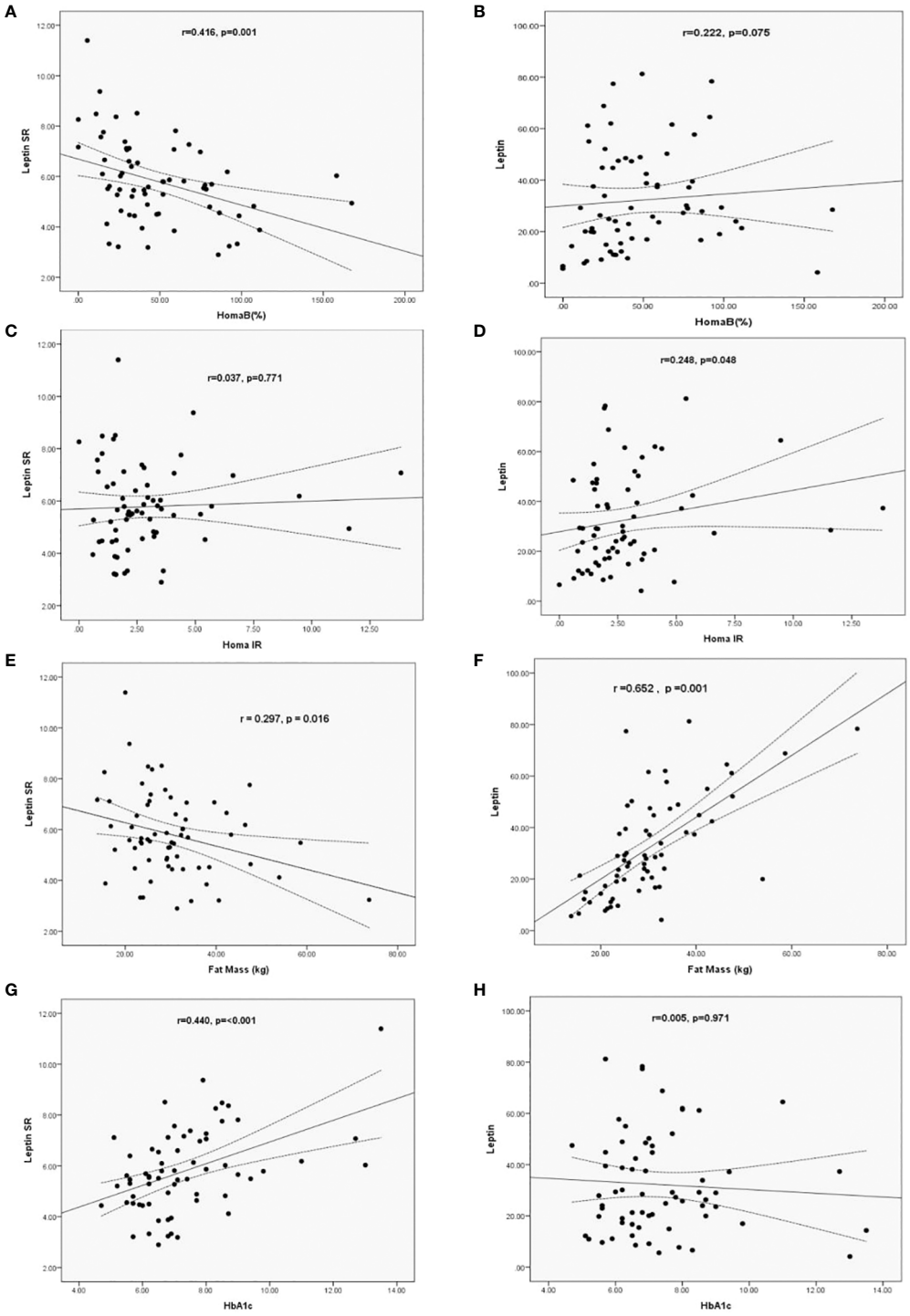


FIGURE 1
Correlation of Leptin SR **(A, C, E, G)** and leptin **(B, D, F, H)** levels with HOMA B, HOMA-IR, HbA1c and Fat Mass in patients with T2DM.

TABLE 2 Multivariate regression analysis for significant factors associated with serum leptin and leptin SR levels in patients with T2DM.

| | B | S.E (E) | *P* |
|---|---|---|---|
| Leptin | | | |
| Body Fat Mass | 0.705 | 0.250 | 0.000 |
| HbA1c | -0.311 | 0.210 | 0.003 |
| Leptin SR | | | |
| TG | 0.325 | 0.207 | 0.003 |
| HbA1c | 0.262 | 0.026 | 0.015 |

In this table we showed the significant predictors only.

in obese diabetic patients vs non-obese control ($p<0.001$) and in Obese diabetic patients vs non-obese diabetic patients, ($p<0.0001$) as shown in Figure 2. Leptin SR level was significantly higher in non-obese diabetic vs Non obese control group ($p=0.006$) and in non-obese diabetic vs obese control group ($p=0.002$) as shown in Figure 3.

The level of leptin and leptin SR were associated with the glycemic control, we observed that diabetic patients with poor control of blood glucose level (HbA1c >7.5) tend to have a higher serum level of leptin SR and a lower leptin level compared to patients with good control (Figure 4).

## Discussion

Our study demonstrated that regardless of obesity, serum leptin SR level was significantly higher in patients with T2DM compared to healthy subjects. The elevated level of leptin SR was linked to dyslipidemia specifically TG level. On the other hand, leptin level was elevated in obese subjects whether they have diabetes or not and it was correlated with BFM. A group of researchers observed that leptin level increased suddenly at BMI of 24.6 while the level of leptin SR decreased rapidly at BMI of 30. However, further increase in BMI was not associated with a further decrease in synthesis of leptin SR (11).

Interestingly leptin SR level showed a strong positive correlation with HbA1c and a significant negative association with beta cell function. Moreover, leptin SR level increases while leptin level decreases with poor glycemic control. These associations of leptin SR with glycemic indices in our patients indicate that elevated leptin SR might play a significant role in disease manifestations and severity. Data is scarce with regards to the association of leptin SR with body composition, glycemic indices and HOMA-IR in patients with T2DM (7, 12 –13). Sun and colleagues reported that independent of obesity and leptin levels, there was a strong inverse association between high levels of circulating leptin SR and the risk for development of T2DM in American women (12). In contrast, our findings suggested that increased plasma level of leptin SR in a milieu of low leptin level might play a role in pathophysiology of T2DM by causing impairment of β-cell function. One reason for low leptin SR level in Sun's et al. study among patients with diabetes might be the higher leptin level associated with obesity in these subjects. In a previous study we reported that increased leptin level was related to obesity regardless of associated diabetes while elevated level of tumor necrosis factor-α was linked to obesity that is associated with diabetes (14).

In the current study we observed that although leptin SR was not correlated with BFM it was significantly associated with high level of TG and uncontrolled diabetes. Ogawa and coworkers' study showed a positive correlation between leptin SR level and high density lipoprotein level (13). Another study reported that serum leptin SR contributed to carotid intima media thickness in patients with T2DM (15). Recently, Horii et al. concluded that accumulation of lipid droplets in β-cells was associated with insulin resistance which can lead to high levels of free fatty acids (FFAs) derived from degradation of triglycerides, the accumulated FFAs can flow into β-cell. In patients with T2DM due to insulin resistance, hyperglycemia combined with excess FFAs are linked to accumulation of lipid droplets in β-cell (16). Additionally, a longitudinal study which lasted for 15 years found that patients with

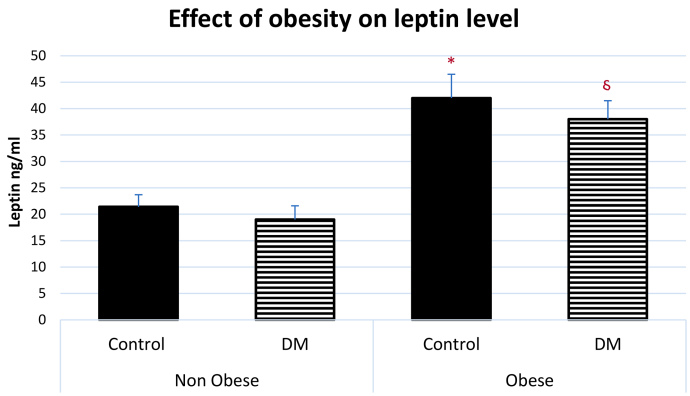


FIGURE 2
Effect of obesity on serum leptin level (mean ± SEM). Non-obese control (n=30), Non-obese diabetic (n=30), obese control (n=33), obese diabetic (n=35) * Obese control vs non-obese control, p=0.001, Obese control vs non-obese diabetic patients, p<0.0001. § Obese diabetic patients vs non-obese control, p<0.001, Obese diabetic patients vs non-obese diabetic patients, p<0.0001.

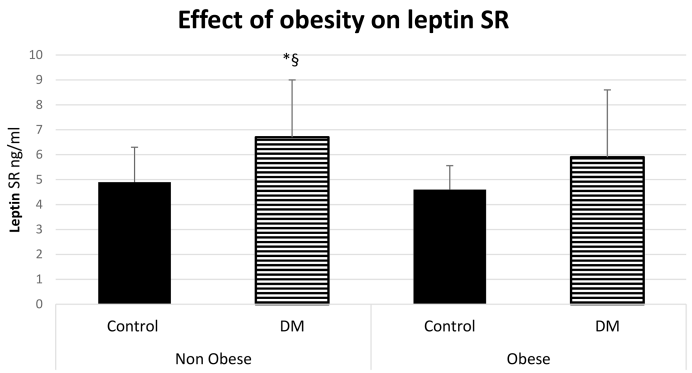


FIGURE 3
Effect of obesity on leptin soluble receptor (Leptin SR) level (mean ± SD), DM: Diabetes mellitus. Non-obese control (n=30), Non-obese diabetic (n=30), obese control (n=33), obese diabetic (n=35). § Non obese DM vs Non obese contol group (p=0.006).*Non-obese DM vs obese control group (p=0.002).

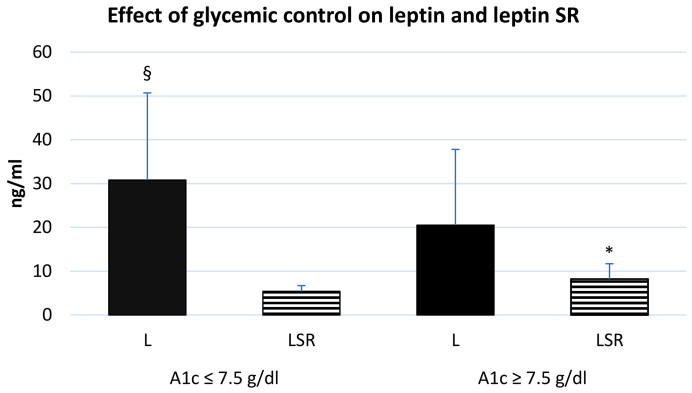


FIGURE 4
effect of glycemic control on the serum level of leptin and leptin SR. A1c: glycosylated hemoglobin A1c, L: leptin, LSR: leptin soluble receptor. Well controlled (n=35), poorly controlled (n=30). § Significant difference in L with poor glycemic control (p<0.03), * Significant difference in LSR with poor glycemic control (p<0.001).

familial combined hyperlipidemia were at high risk to develop T2DM (17). Substantially all steps in the pathway of lipotoxicity, starting with high food intake to the point of synthesis of ceramide, is protectively influenced by leptin (18). The transport of leptin was found to be inhibited by TG through direct binding with leptin or its transporter while pharmacological intervention that reduces the level of TG reversed this inhibition of leptin transport (19).Also, we observed that leptin SR level have a strong positive correlation with HbA1c value and a negative correlation with HOMA-β. Similarly, previous studies demonstrated that leptin SR had positive correlation with HbA1c in patients with type1 diabetes (20) and negative correlation with HOMA-β in patients withT2DM (8).

We postulate that the elevated leptin SR in patients with T2DM in our study played a role in leptin resistance, in addition to its role in the impairment of β-cell function. Leptin was reported to decrease β cell apoptosis and lower α cell insulin resistance which usually leads to inhibition of the pathways leading to T2DM. Lowering TG level should be an early important step in management of diabetes to protect β cells and endothelial cells from apoptosis and atherosclerosis.

In conclusion, our study showed that leptin SR is higher in patients with T2DM and is associated with abnormal β-cell function and thus it might be considered as an important marker in the pathogenesis of diabetes. Furthermore, leptin SR level is linked to HbA1c value and TG level so it can be used in monitoring the response to treatment in patients with diabetes. More studies are needed to explore the complex pathophysiological mechanisms in diabetes mellitus, and specifically focusing on investigating whether lowering TG levels would decrease leptin SR level and protect β-cell from its deleterious effect. Finding new pathways to manage T2DM will help in implementing precision medicine.

## 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 Institutional Review Board, College of Medicine, King Saud University, Riyadh, Saudi Arabia. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

HA, literature review, data analysis, and writing manuscript. LA, idea, data collection, and manuscript revision. 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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# Association between weight-adjusted waist index and arterial stiffness in hypertensive patients: The China H-type hypertension registry study

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**Objective:** Exploring the relationship between (weight-adjusted waist index) WWI and arterial stiffness (AS) in the total and different BMI populations among patients with hypertension.

**Methods:** This study enrolled 5232 hypertensive subjects, a subset of the China H-type Hypertension Registry Study. WWI was calculated as WC (cm) divided by the square root of weight (kg). Brachial-ankle pulse wave velocity (baPWV) was measured to determine AS.

**Results:** The mean WWI was 10.97 (0.78)cm/$\sqrt{}$kg. In multiple logistic analyses showed that there were significant dose-dependent association between WWI with baPWV in a dose-dependent manner in total population (β 57.98, 95% CI 44.06-71.90), and in different BMI group: group 1 (BMI<18.5kg/m$^2$) (β 94.30, 95% CI 39.36-149.23), group 2 (18.5-23.9kg/m$^2$) (β 74.21, 95% CI 54.57-93.85), group 3 (≥24kg/m$^2$) (β 26.11, 95% CI 5.22-47.01). In stratified analysis, stronger associations between WWI and baPWV were observed in patients with higher BP or lower BMI. Sensitivity analysis by excluding patients treated with lipid-lowering agents did not change the association between WWI and baPWV.

**Conclusion:** For hypertensive patients, we found that WWI was positively associated with baPWV in different BMI groups. WWI might be considered as an intervening factor in preventing and treatment of AS, besides BP management.

KEYWORDS

**weight-adjusted waist index, arterial stiffness, BMI, hypertension, obesity**

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# Introduction

Arterial stiffness (AS) is an independent predictor of cardiovascular diseases (CVD) and mortality (1, 2). Brachia-ankle pulse wave velocity (baPWV) measurement is commonly used to assess arterial wall stiffness in epidemiological studies, as it is accurate, simple and non-invasive. Recently, increasing numbers of studies have revealed that baPWV is positively related to future risk of CVD events (3), diabetes (4), and all-cause mortality (5).

As generally known, obesity is closely associated with AS and can independently predict the future risk of AS (6–8). Several studies have shown that weight loss achieved through lifestyle measures can improve AS (9, 10). Moreover, existing studies show that muscle indices are negatively related to the risk of AS (11). Body mass index (BMI) is the major measure of obesity and is widely used in many epidemiological studies because of its low cost, simplicity, and availability. Nevertheless, current researches are still controversial about the relationship between BMI and AS among different populations. In the past decade, some studies have found a positive association between BMI and AS (12–14), while others have found a negative association (15, 16), and others have found no association (17, 18). These discrepancies between the different studies may be attributed to different sample sizes, ethnic and regional variations. Moreover, BMI is not able to discriminate between muscle and fat. Weight-adjusted waist index (WWI) was first proposed in 2018 as a new anthropometric index for predicting the risk of CVD events and mortality (19). Moreover, a study from Korea shows that elevated WWI is closely related to high body fat and low muscle mass (20). Draw a question, whether WWI would better identify the risk of AS compared to BMI.

Hypertension is a significant risk factor for the development of AS (21). According to China Hypertension Survey (2012-2015), the prevalence of hypertension among Chinese adults is 23.2% (22). Obesity is a proven risk factor for hypertension (23). The co-existence of obesity and hypertension significantly increases the risk of developing AS. According to a cohort study of 10338 subjects, WWI is closed associated with future risk of hypertension (24). However, the ability of WWI for assessing the risk of AS among the hypertensive population is poorly understood. Therefore, this study aimed to explore the association between WWI and AS among hypertensive patients in the total population and different BMI populations. In addition, we further assess the mediating roles of blood pressure (BP) satisfaction on the association between WWI and AS.

# Methods

## Study participants

Our study participants came from China H-type Hypertension Registry Study that previously reported (25, 26) (Registration number: ChiCTR1800017274). In brief, this is a real-world, multicenter, observational registry study carried out in southern China from March 2018 to August 2018. Inclusion was carried out in participants with hypertension aged 18 years and older. Hypertension was defined as seated, resting BP≥140/90mmHg at the screening, self-report, or undergoing anti-hypertensive treatment. The exclusion criteria included neurological abnormalities, inability to follow up according to the study protocol, or plans to relocate shortly, and the patients, who were not suitable for inclusion or for long-term follow-up assessed by study physicians. All participants provided written informed consent. The protocol was approved by the Ethics Committee of Institute of Bio-medicine, Anhui Medical University (NO. CH1059) and the Second Affiliated Hospital of Nanchang University (NO. 2018019).

Our study was conducted on a subset of 5233 subjects with complete baPWV data from China H-type Hypertension Registry Study. After excluding participants with loss of WWI data (n =1), 5232 participants were included in the final analysis, as shown in Figure S1.

## Assessment of covariates

According to a standard operating procedure, all subjects were interviewed by a trained study coordinator. The information related to age, sex (male or female), smoking, drinking, physical activity (mild, moderate or vigorous), and medical history (diabetes mellitus, hyperlipidemia, duration of hypertension and current medication usage) were abstracted from a standard questionnaire. The heights, weights, and waist circumference (WC) of all patients were collected by trained researchers according to the standard process. BMI was calculated as weight (kg) divided by the square of height ($m^2$). BMI was defined as underweight (<18.5kg/$m^2$), normal (18.5-23.9kg/$m^2$), overweight or obesity (≥24kg/$m^2$) according to the cut-off point for Chinese adults (27). WWI (cm/√kg) was calculated as WC (cm) divided by the square root of weight (kg). Resting blood pressure (BP) was measured by the automated electronic device (Omron; Dalian, China) in a standardized manner.

After a 10 h fasting period, blood samples were obtained from all subjects. The blood samples would be measured in Biaojia Biotechnology Laboratory, Shenzhen, China. Fasting plasma glucose (FPG), fasting lipids (total cholesterol, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides) and homocysteine (Hcy) were determined using automatic clinical analyzers (Beckman Coulter).

## BaPWV measurements

The baPWV was assessed with Omron Colin BP-203RPE III device (Omron Health Care, Kyoto, Japan) following the recommended standard procedures. The specific details of baPWV measurement were described previously (26).

## Statistical analysis

Baseline characteristics are shown as mean ± standard deviation (SD) for continuous variables, and as n (%) for categorical variables. Descriptive analyses were conducted according to WWI quartiles using t-test or Chi-square tests to compare between-group differences. Dose-response association of WWI with baPWV was assessed using a generalized additive model (GAM) and a fitted smoothing curve (penalized spline method). We used a multivariate linear regression model (beta coefficient [β] and 95% confidence interval [CI]) to assess the relationship between WWI and baPWV in the total and different BMI populations by controlling the confounders in three models. Model 1: adjusted for age; Model 2: Model 1 plus sex, current smoking, current drinking, physical activity, BMI; Model 3: Model 2 plus systolic blood pressure (SBP), diastolic blood pressure (DBP), duration of hypertension, diabetes mellitus, hyperlipidemia, antihypertensive agents, antidiabetes agents, lipid-lowering agents, FPG, Hcy, triglyceride, HDL-C, and LDL-C. The general linear model was determined to compare the difference of baPWV in total and different BMI populations. Subgroup and stratified analyses were also done. A sensitivity analysis was also conducted. In sensitivity analyses, the same analyses were performed after excluding patients treated with lipid-lowering agents. In addition, To determine whether the association between WWI and baPWV was mediated by BP (SBP or DBP), a simple mediation analysis was completed (Figures S2A, B).

All data analyzed were using the statistical package R (http://www.r-project.org) and Stata software, version 14.0 (StataCorp). A 2-tailed P < 0.05 was considered to be statistically significant.

# Results

## Characteristics of the subjects

Overall, 5232 hypertensive subjects were enrolled in the final analysis (2603 men and 2629 women, aged 64.7 ± 9.5 years). The mean WWI was 10.97 (0.78) cm/√kg. Mean baPWV was 1858.4 (420.7) mm/s. Table 1 shows the characteristics of the patients according to the WWI quartiles. The participants with higher WWI had higher values of age, weight, WC, BMI, SBP, duration of hypertension, diabetes mellitus, antihypertensive agents, antidiabetes agents, FBG, and LDL-C. In addition, higher WWI level was negatively associated with lower values in male, smoking, drinking, physical activity, Hcy, and HDL-C.

## Association between WWI and baPWV in total population

As shown in Figure 1, there was a significantly positive association between WWI and baPWV. Moreover, the significant relationship between WWI with baPWV was observed in Table 2. After correction for different confounding factors, the positive associations between WWI and baPWV were found in three models (*P* for trend <0.001). For a 1-unit increase in WWI, the baPWV is changed in 57.98mm/s (95% CI 44.06-71.90) in the fully adjusted model. The subjects were stratified into four groups by the quartile value of WWI, comparing the Q1 (<10.5 cm/√kg), the adjusted β of Q2 (≥10.5, <10.9), Q3 (≥10.9, <11.5), and Q4 (≥11.5) were 30.07 (95% CI 3.53-56.62), 67.49 (95% CI 39.59-95.38), 113.65 (95% CI 83.46-143.84) in the model 3.

## Association between WWI and baPWV in different BMI groups

As shown in Table 3, the positive associations between WWI and baPWV were maintained in different categories of BMI, even in the normal BMI group (18.5-23.9kg/m$^2$). In the normal BMI group, per 1-unit increase in WWI, the baPWV increases by 74.21mm/s (95% CI 54.57-93.85) in model 3. Moreover, the baPWV values increased as the quartiles of WWI (*P* for trend<0.05). Consistently, the same associations between WWI and baPWV were observed among underweight (BMI <18.5) and overweight (BMI ≥24) subjects. Figure 2 shows the levels of baPWV by quartiles of WWI in total and different BMI populations. The levels of baPWV showed an increasing trend across quartiles of WWI in total and different BMI populations, but this trend was not evident in the underweight group.

## Stratification analysis

We conducted a list of stratified analyses by sex, age (<65 and ≥65 years), BMI (<18.5, 18.5-23.9 and ≥24kg/m$^2$), smoking (yes/no), drinking (yes/no), physical activity (mild, moderate and vigorous), SBP (<140 and ≥140mmHg), DBP (<90 and ≥90mmHg), diabetes mellitus (yes/no), and hyperlipidemia (yes/no) as shown in Figure 3. We observed that the relationship between WWI and baPWV was consistent in all subgroups except for BMI (*P* for interaction =0.001), SBP (*P* for interaction <0.001), and DBP (*P* for interaction =0.021).

We further divided the population into four groups based on SBP and DBP (group 1: SBP<140mmHg and DBP<90mmHg, group 2: SBP≥140mmHg or DBP≥90mmHg, group 3: SBP≥160mmHg or DBP≥100mmHg, and group 4: SBP≥180mmHg or DBP≥110mmHg), and to explore the association of WWI and baPWV (Table S1). Per 1-unit increase in WWI, the baPWV increase by 23.39mm/s (95% CI 2.36-44.42), 72.80mm/s (95% CI 53.84-91.75), 79.03mm/s (95% CI 50.21-107.85), 189.74mm/s (95% CI 96.37-283.11) respectively. A more accentuated increase was observed in the higher BP group.

WWI (cm/√kg) was calculated as WC (cm) divided by the square root of weight (kg). As shown in Table S2, significant differences in weight and WC between BMI subgroups. Therefore, there was an interaction between WWI and BMI.

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TABLE 1 Clinical characteristics of the study population.

| | Weight-adjusted waist index, cm/√kg | | | | *P* value |
|---|---|---|---|---|---|
| | Q1 (<10.5) | Q2 (≥10.5, <10.9) | Q3 (≥10.9, <11.5) | Q4 (≥11.5) | |
| N | 1308 | 1308 | 1308 | 1308 | |
| Age, y | 63.2 ± 9.8 | 63.3 ± 9.8 | 64.6 ± 9.1 | 67.5 ± 8.8 | <0.001 |
| Male, n (%) | 887 (67.8) | 791 (60.4) | 615 (47.0) | 311 (23.8) | <0.001 |
| Weight, kg | 73.7 (7.7) | 81.6 (7.8) | 85.2 (7.3) | 88.5 (8.3) | <0.001 |
| WC, cm | 54.6 (10.3) | 58.5 (11.0) | 58.4 (9.9) | 55.4 (10.6) | <0.001 |
| BMI, kg/m$^2$ | 21.4 ± 3.2 | 23.3 ± 3.3 | 24.0 ± 3.1 | 24.4 ± 3.6 | <0.001 |
| Smoking, n (%) | 753 (57.6) | 690 (52.8) | 587 (44.9) | 417 (31.9) | <0.001 |
| Drinking, n (%) | 592 (45.3) | 541 (41.4) | 454 (34.7) | 320 (24.5) | <0.001 |
| Physical activity, n (%) | | | | | <0.001 |
| Mild | 643 (49.2) | 717 (54.8) | 733 (56.0) | 796 (60.9) | |
| Moderate | 319 (24.4) | 310 (23.7) | 307 (23.5) | 241 (18.4) | |
| Vigorous | 346 (26.5) | 281 (21.5) | 268 (20.5) | 271 (20.7) | |
| SBP, mmHg | 145.2 ± 17.9 | 146.5 ± 17.9 | 147.4 ± 17.2 | 149.2 ± 17.3 | <0.001 |
| DBP, mmHg | 88.7 ± 10.9 | 89.5 ± 11.4 | 89.0 ± 10.6 | 87.2 ± 11.0 | <0.001 |
| Duration of hypertension, y | 4.0 (2.0-9.0) | 5.0 (2.0-10.0) | 5.0 (2.0-10.0) | 6.0 (2.0-10.0) | <0.001 |
| Diabetes mellitus, n (%) | 178 (13.6) | 215 (16.4) | 287 (21.9) | 305 (23.3) | <0.001 |
| Hyperlipidemia, n (%) | 185 (14.1) | 265 (20.3) | 260 (19.9) | 230 (17.6) | <0.001 |
| Antihypertensive agents, n (%) | 760 (58.1) | 799 (61.1) | 815 (62.3) | 833 (63.7) | 0.025 |
| Antidiabetes agents, n (%) | 34 (2.6) | 53 (4.1) | 83 (6.3) | 69 (5.3) | <0.001 |
| Lipid-lowering agents, n (%) | 39 (3.0) | 42 (3.2) | 51 (3.9) | 49 (3.7) | 0.530 |
| Homocysteine, umol/L | 19.5 ± 12.6 | 18.9 ± 12.8 | 18.3 ± 11.8 | 17.7 ± 9.6 | <0.001 |
| FBG, mmol/L | 5.9 ± 1.3 | 6.1 ± 1.4 | 6.3 ± 1.9 | 6.3 ± 1.8 | <0.001 |
| Triglyceride, mmol/L | 1.4 ± 1.2 | 1.8 ± 1.2 | 1.9 ± 1.3 | 1.9 ± 1.3 | <0.001 |
| HDL-C, mmol/L | 1.6 ± 0.4 | 1.5 ± 0.4 | 1.4 ± 0.4 | 1.4 ± 0.4 | <0.001 |
| LDL-C, mmol/L | 2.7 ± 0.8 | 2.9 ± 0.8 | 3.0 ± 0.8 | 3.1 ± 0.8 | <0.001 |

Data are the mean ± SD or median (interquartile range), or number (percentage).
WC, waist circumference; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Hcy, Homocysteine; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

## Sensitivity analyses

As shown in Table S3A, the sensitivity analysis was conducted by the exclusion of subjects treated with lipid-lowering agents, and the result was stable. Moreover, we performed a sensitivity analysis restricting the patients treated with lipid-lowering agents, the results remained significant and consistent (Table S3B).

## Mediation effect of BP

The unstandardized regression coefficients for the effect of WWI on baPWV without and with SBP and DBP as mediators are shown in Table 4. As in Table 4, the results indicated that SBP and DBP mediated 28.3% and 8.6% of the relationship of increasing WWI and baPWV, respectively.

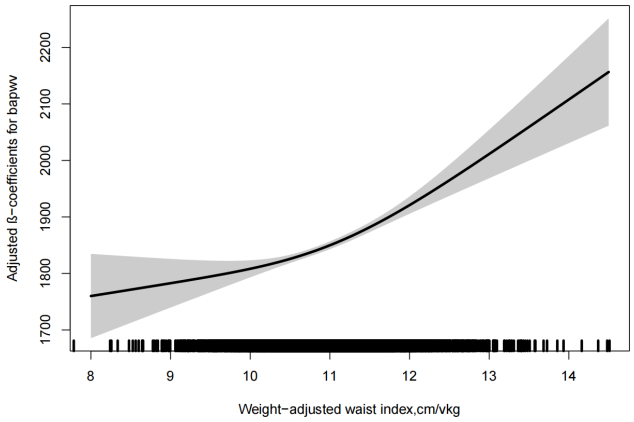


FIGURE 1
Dose-response relationship between weight-adjusted waist index and baPWV. Models were adjusted for age, sex, current smoking, current drinking, physical activity, BMI, SBP, DBP, duration of hypertension, diabetes mellitus, hyperlipidemia, antihypertensive agents, antidiabetes agents, lipidlowering agents, FPG, homocysteine, triglyceride, HDL-C and LDL-C.

## Discussion

This cross-sectional study evaluated the association between WWI and baPWV in a group of middle-aged and older hypertensive patients. The main findings encompassed the following. We observed that WWI was positively associated with baPWV among patients with hypertension, even though these patients had normal BMI. The association was more significant in patients with higher BP or lower BMI levels. Moreover, this association was stable after excluding the patients treated with lipid-lowering agents. In this relationship, SBP mediated around 28.3% of the total effects. These findings suggest that WWI could be considered as an intervening factor in preventing and treatment of AS, besides BP management.

Numbers of epidemiological studies have demonstrated that obesity was significantly associated with high arterial stiffness (7, 8). A previous meta-analysis of 20 studies showed that modest weight loss was associated with reduced PWV (9). Obesity is usually defined by BMI. Many clinical studies indicated that BMI is positively associated with increased PWV (12, 13). However, several studies suggested that BMI is negatively associated with high arterial stiffness (15, 16). Moreover, Liao et al. conducted a longitudinal study of 1553 subjects found that there was no relationship between the BMI and AS after correction for SBP and DBP (18). There were several reasons for these apparent discrepancies including different study cohorts, ethnic and regional disparity. Furthermore, BMI does not differentiate between lean mass and body fat mass. WWI is an anthropometric index that can be calculated easily. A cross-sectional study suggested that WWI could reflect both body fat and muscle mass (20). In addition, several clinical studies showed that WWI is closely related to an increased risk of diabetes (28), hypertension (24), metabolic syndrome (29), CVD (19), and mortality (30). However, to our knowledge, this is the first study to explore the association between WWI and baPWV in a large

TABLE 2 Association between weight-adjusted waist index and baPWV in different models.

| Weight-adjusted waist index, cm/√kg | | Model 1 | Model 2 | Model 3 |
|---|---|---|---|---|
| | | β (95% CI) | β (95% CI) | β (95% CI) |
| Per 1 unit increase | 5232 | 59.69 (46.26, 73.13) | 72.76 (57.43, 88.09) | 57.98 (44.06, 71.90) |
| Q1 (<10.5) | 1308 | Reference | Reference | Reference |
| Q2 (≥10.5, <10.9) | 1308 | 24.16 (-4.85, 53.17) | 43.36 (13.76, 72.97) | 30.07 (3.53, 56.62) |
| Q3 (≥10.9, <11.5) | 1308 | 65.85 (36.80, 94.90) | 91.69 (60.85, 122.53) | 67.49 (39.59, 95.38) |
| Q4 (≥11.5) | 1308 | 111.47 (82.08, 140.86) | 137.83 (104.51, 171.14) | 113.65 (83.46, 143.84) |
| *P* for trend | | <0.001 | <0.001 | <0.001 |

Model 1: adjusted for age.
Model 2: adjusted for age, sex, current smoking, current drinking, physical activity, BMI.
Model 3: adjusted for sex, age, current smoking, current drinking, physical activity, BMI, SBP, DBP, duration of hypertension, diabetes mellitus, hyperlipidemia, antihypertensive agents, antidiabetes agents, lipid-lowering agents, FPG, Hcy, triglyceride, HDL-C and LDL-C.

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TABLE 3 Association between weight-adjusted waist index and baPWV in different BMI subgroups.

| Weight-adjusted waist index, cm/√kg | | Model 1 | Model 2 | Model 3 |
|---|---|---|---|---|
| | | β (95% CI) | β (95% CI) | β (95% CI) |
| BMI (<18.5kg/m2) | | | | |
| Per 1 unit increase | 409 | 108.88 (53.26, 164.50) | 112.78 (53.93, 171.63) | 94.30 (39.36, 149.23) |
| Q1 (<10.5) | 102 | Reference | Reference | Reference |
| Q2 (≥10.5, <10.9) | 102 | -70.62 (-207.69, 66.44) | -76.35 (-213.37, 60.68) | -79.26 (-203.86, 45.33) |
| Q3 (≥10.9, <11.5) | 102 | 14.93 (-122.81, 152.67) | 30.07 (-110.13, 170.27) | 44.13 (-83.53, 171.78) |
| Q4 (≥11.5) | 103 | 201.23 (61.97, 340.50) | 205.81 (59.25, 352.37) | 163.38 (25.32, 301.44) |
| *P* for trend | | 0.003 | 0.003 | 0.009 |
| BMI (18.5-23.9kg/m2) | | | | |
| Per 1 unit increase | 2221 | 90.74 (71.26, 110.22) | 85.27 (63.78, 106.75) | 74.21 (54.57, 93.85) |
| Q1 (<10.5) | 673 | Reference | Reference | Reference |
| Q2 (≥10.5, <10.9) | 671 | 53.51 (12.20, 94.83) | 62.22 (20.34, 104.10) | 30.03 (-7.43, 67.50) |
| Q3 (≥10.9, <11.5) | 674 | 108.16 (66.70, 149.61) | 110.46 (67.65, 153.27) | 89.22 (50.39, 128.04) |
| Q4 (≥11.5) | 673 | 169.19 (126.87, 211.51) | 156.90 (110.64, 203.16) | 126.90 (84.88, 168.92) |
| *P* for trend | | <0.001 | <0.001 | <0.001 |
| BMI (≥24kg/m2) | | | | |
| Per 1 unit increase | 2101 | 41.17 (19.06, 63.28) | 45.59 (21.87, 69.30) | 26.11 (5.22, 47.01) |
| Q1 (<10.5) | 533 | Reference | Reference | Reference |
| Q2 (≥10.5, <10.9) | 532 | 18.21 (-22.49, 58.91) | 22.79 (-18.16, 63.75) | 16.94 (-18.83, 52.70) |
| Q3 (≥10.9, <11.5) | 533 | 40.58 (-0.53, 81.70) | 47.31 (5.28, 89.33) | 20.50 (-16.48, 57.48) |
| Q4 (≥11.5) | 533 | 73.20 (30.80, 115.61) | 82.99 (37.40, 128.58) | 58.22 (18.11, 98.32) |
| *P* for trend | | <0.001 | <0.001 | 0.007 |

Model 1: adjusted for age.
Model 2: adjusted for age, sex, current smoking, current drinking, physical activity.
Model 3: adjusted for age, sex, current smoking, current drinking, physical activity, SBP, DBP, duration of hypertension, diabetes mellitus, hyperlipidemia, antihypertensive agents, antidiabetes agents, lipid-lowering agents, FPG, Hcy, triglyceride, HDL-C and LDL-C.

population. In this study, it seems that WWI is positively related to baPWV in total and different BMI populations. Moreover, this relationship between 2 was independent of other variables in the multivariate linear regression and mediation analysis.

Notably, the association between WWI and baPWV appeared to be more pronounced in patients with SBP ≥140mmHg or DBP ≥90mmHg. As is well known, hypertension is a major risk for AS (21). Therefore, it is reasonable to postulate that higher BP status may amplify the adverse impact of WWI on baPWV. Our results support this assumption. In stratified analysis by BP, a larger association between WWI and baPWV is observed in patients with higher BP status (Table S1).

Several underlying mechanisms might explain the association between WWI and baPWV. WWI was positively associated with fat mass and negatively associated with lean body mass (20). Numbers experimental and human studies suggested that adipocyte hyperplasia and hypertrophy might induce adipokine dysregulation (31). And adipokine dysregulation may lead to vascular inflammation, endothelial dysfunction, and vascular remodeling, resulting in AS (32, 33). Moreover, lower lean mass induces less glucose intake into the muscle and physical activity increases lean mass and improves endothelial function, oxidative stress, insulin resistance, and inflammation, and subsequently improves AS (9). In addition, WWI was an independent risk factor for diabetes (28) and metabolic syndrome (29), all of these diseases can worsen arteriosclerosis.

The strength of our study is that we assess the association between WWI and baPWV in different BMI groups. Moreover, the present study included a large hypertension cohort, subgroup analysis, and

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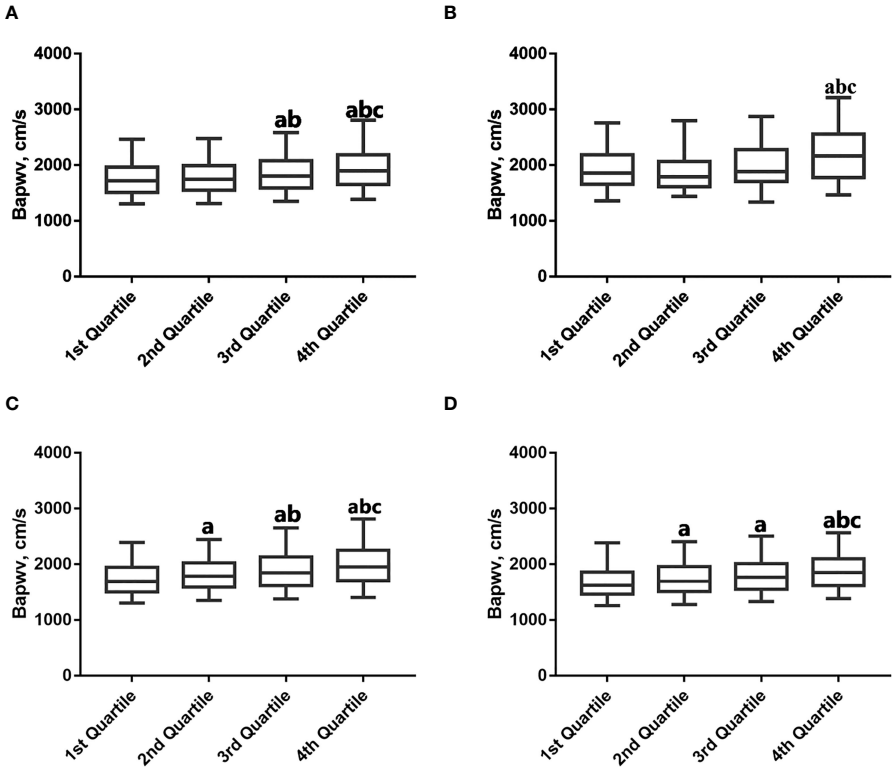


FIGURE 2
Mean baPWV by weight-adjusted waist index quartiles according to BMI category. **(A)** Total; **(B)** BMI (<18.5kg/m2); **(C)** BMI (18.5-23.9kg/m2); **(D)** BMI (≥24kg/m2). a $p < 0.05$ compared with the 1st Quartile. b $p < 0.05$ compared with the 2nd Quartile. c $p < 0.05$ compared with the 3rd Quartile.

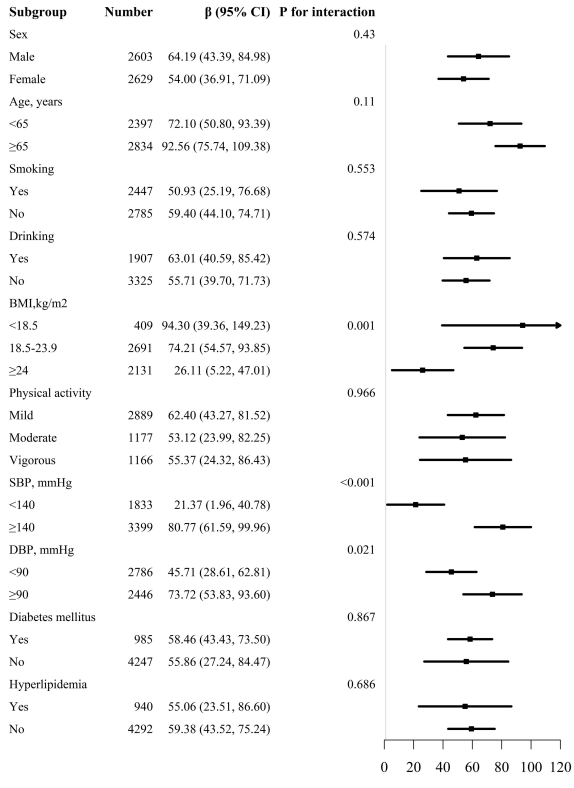


FIGURE 3
Subgroup analyses of the effect of WWI on baPWV.

TABLE 4 Direct and indirect effects of WWI on markers of Bapwv with blood pressure as mediators in hypertensive patients.

| Variables | Total Effect (c) | | Direct Effect (c') | | Indirect Effect (ab) | | Proportion of Mediation (%) |
|---|---|---|---|---|---|---|---|
| Mediators and Outcomes | β (95% CI) | *p*-Value | β (95% CI) | *p*-Value | β (95% CI) | *p*-Value | |
| WWI (cm/√kg) | 59.69 (46.26, 73.13) | <0.001 | | | | | |
| *via* SBP (mmHg) | | | 42.81 (30.52, 55.10) | <0.001 | 16.89 (11.19, 22.50) | <0.001 | 28.3 |
| *via* DBP (mmHg) | | | 55.16 (42.60, 67.71) | <0.001 | 4.54 (-0.26, 9.34) | 0.064 | 7.6 |

Regression coefficients c, a, b and c' are shown in Figure S1. All estimates were adjusted for the potential effects of age.

mediation analysis. However, this study contains several limitations. First, this study was a cross-sectional study. Hence, the association cannot prove causality. Second, although multivariate correction, it was difficult to exclude any potential confounding effect. Third, the conclusion of the current study applies to the Chinese hypertensive population that may not be directly extrapolated.

## Conclusion

For hypertensive patients, we found that WWI was positively associated with baPWV in different BMI groups. Further longitudinal studies are required to corroborate our findings. Moreover, WWI might be considered as an intervening factor in preventing and treatment of AS, besides BP management.

## Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. Requests to access these datasets should be directed to XC, xiaoshumenfan126@163.com.

## Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Institute of Bio-medicine, Anhui Medical University and the Second Affiliated Hospital of Nanchang University. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

YX, XH, HB and XC conceived and designed the study. YX, XH, WZ and were involved in the acquisition of data. YX and CY analyzed the data. YX wrote this paper. HB and XC contributed to the revision of manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

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

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1134065/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Study flow diagram.

SUPPLEMENTARY FIGURE 2
Represent a single mediator model used to test the association between WWI and Bapwv, SBP and DBP as mediators. **(A)** Path c, represents the simple total effect of WWI on baPWV, without adjusting mediators; **(B)** Path c, represents the direct (Path c') and indirect effect (product of path a and b, ab) of WWI on Bapwv after controlling for the effect of the mediator.

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# Nonlinear correlation between fatty liver index and carotid intima media thickness among individuals undergoing health examination

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**Background:** Fatty liver index (FLI) is a predictor of non-alcohol fatty liver disease (NAFLD). This study aimed to assess the association between FLI and carotid intima media thickness (CIMT).

**Methods:** In this cross-sectional study, we enrolled 277 individuals for health examination from the China-Japan Friendship Hospital. Blood sampling and ultrasound examinations were conducted. Multivariate logistic regression and restricted cubic spline analyses were performed to evaluate the association between FLI and CIMT.

**Results:** Overall, 175 (63.2%) and 105 (37.9%) individuals had NAFLD and CIMT, respectively. The multivariate logistic regression analyses results showed that high FLI was independently associated with a high risk of increased CIMT, T2 vs. T1 (odds ratio [OR], 95% confidence interval [CI]): 2.41, 1.10–5.25, p = 0.027; T3 vs. T1 (OR, 95% CI): 1.58, 0.68–3.64, p = 0.285. The association between FLI and increased CIMT exhibited a J-shaped curve (nonlinear, p = 0.019). In the threshold analysis, the OR for developing increased CIMT was 1.031 (95% CI: 1.011–1.051, p = 0.0023) in participants with FLI < 64.247.

**Conclusion:** The relationship between FLI and increased CIMT in the health examination population is J-shaped, with an inflection point of 64.247.

KEYWORDS

fatty liver index (FLI), carotid intima-media thickness, J curve, health examination, athrosclerosis

**Abbreviations:** CVD, Cardiovascular disease; CIMT, Carotid intima media thickness; CT, Computed tomography; FLI, Fatty liver index; GGT, Gamma glutamyl transferase; hba1c, Glycosylated haemoglobin; HDL-CHigh-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; NAFLD, Non-alcohol fatty liver disease.

# 1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by fatty liver that is related to over-nutrition in the absence of excessive alcohol consumption (1, 2). NAFLD is now the most common liver disease with a prevalence of approximately 20–30% among adults worldwide, and a very high disease burden in Asia (3). NAFLD is growing at a rate that is parallel to that of the obesity epidemic, and people with NAFLD have increased risk of cardiovascular disease (CVD) according to meta-analyses of large observational studies (4). Subclinical atherosclerosis, an early stage of CVD, is of vital clinical significance. The onset of clinical CVD events can be delayed or prevented if screening and precautionary measures are available at this stage (5). Accordingly, metrics that indicate poor cardiovascular prognosis, such as increased carotid intima media thickness (CIMT) for arterial wall thickness (6) and increased brachial-ankle pulse wave velocity for stiffness (7), are particularly important in patients with NAFLD.

NAFLD is diagnosed using modalities, such as ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), and liver biopsy. Non-invasive serum-based methods of diagnosing hepatic steatosis, such as fatty liver index (2, 8) (FLI, an index based on body mass index [BMI]), waist circumference, triglyceride level, and gamma glutamyl transferase (GGT) level, were mentioned in previous studies, although their diagnostic cut-off values were not consistent. FLI might serve as a prognostic indicator of death and morbidity including CVD, cancer, respiratory disease, and liver disease (9). Hepatic steatosis index and FLI are independently correlated with carotid atherosclerosis in patients with type 2 diabetes mellitus (10). However, whether FLI influences the risk of increased carotid intima-media thickness (CIMT) during health examination remains unknown.

# 2 Methods and materials

## 2.1 Data sources and study population

This cross-sectional study was conducted at the Health Examination Center of China-Japan Friendship Hospital in Beijing, China. We continuously recruited 943 individuals who underwent health examinations, including anthropometry, laboratory tests, and liver and carotid ultrasound examination, between September 2018 and October 2021. Standardized questionnaires (to collect data, such as demographic information, lifestyle, and history of disease) were completed under the guidance of the researchers. We determined whether a participant had increased CIMT based on the reports of carotid ultrasound examination, and data on FLI was collated. Six hundred and sixty-six participants without data on FLI and carotid ultrasound examination were excluded. Pregnant and lactating women; participants with a history of severe brain disease, coronary heart disease, lung disease, kidney disease, blood disease, psychiatric disease, infectious disease, and malignancy; and those with missing information were excluded. Finally, 277 participants who underwent carotid ultrasound were recruited (Figure 1). This study

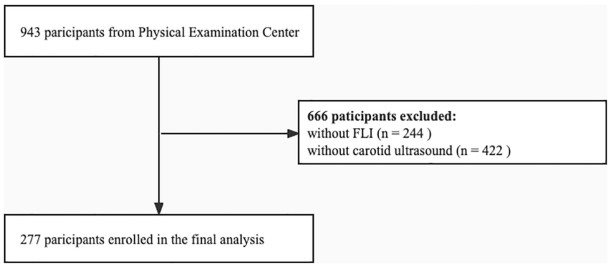


FIGURE 1
Flowchart of patient selection. FLI, fatty liver index.

was conducted in accordance with the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of China-Japan Friendship Hospital (2018-110-K79-1). All participants voluntarily agreed to participate in this study and provided written informed consent.

## 2.2 Anthropometric and biochemical measurements

The health examinations were performed in the morning. Anthropometric indicators were measured by professionally trained physicians. Height, weight, and waist circumference were measured while the participants were in the standing position without shoes and heavy clothing. After 10 min of rest, the blood pressure was measured using an upper arm electronic sphygmomanometer. Fasting blood samples were stored and measured in the laboratory of China-Japan Friendship Hospital. Laboratory investigation data were obtained from the electronic medical record system, including data on total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), alanine aminotransferase, aspartate aminotransferase, GGT, and alkaline phosphatase.

The determination of previous disease (hypertension, NAFLD, and diabetes) was based on a question in the questionnaire that asked whether they had been previously informed by a doctor that they had the condition. Body mass index (BMI) was calculated as the weight (in kilograms) divided by the square of the height (in meters). Waist circumference was the circumference at the level of the flat navel. FLI was calculated using a previously published formula (8).

## 2.3 Determination of CIMT and carotid plaque

Carotid atherosclerosis was evaluated using high-resolution ultrasonography to determine the atherosclerotic indices of intima media thickness in the common carotid arteries, carotid artery bulbs, and internal and external carotid arteries. CIMT was measured from the upper edge of the clavicle to the lower edge of

the mandible. The mean-intima media thickness was defined as the mean intima media thickness of the proximal and distal walls of both common carotid arteries on a longitudinal scan at a point 10 mm proximal to the beginning of the dilation of each carotid artery bulb. A single trained medical technologist performed all examinations using high-resolution ultrasonography with a 7.5-MHz transducer that produced an axial resolution of 0.1 mm (Siemens, Erlangen, Germany). In this study, the average value was considered the final CIMT value. Normal CIMT was defined as CIMT < 1.0 mm, whereas carotid intima-media thickening was defined as CIMT ≥ 1.0 mm as previously described. Based on the results of carotid ultrasonography, participants with carotid plaques were classified as the plaque group and those without carotid plaques were classified as the non-plaque group.

## 2.4 Statistical analyses

Baseline characteristics are described as the mean (standard deviation) and median (interquartile range) for continuous variables, and numbers (percentage) for categorical variables. One-way Analysis of Variance (ANOVA) or Kruskal–Wallis H test was used for continuous variables, and Chi-square or Fisher's exact test was used for categorical variables to determine differences between the groups. Prior to regression analysis, missing values for covariates were imputed using multiple imputations of the fully conditional normative (FCS-MI) method. This method enables the specification of multivariate imputation models on a variable-by-variable basis and uses a principled but malleable approach to address missing data. Five datasets were established and modeled individually, with missing data imputed.

Univariate and multivariate logistic regression models were used to determine the odds ratios (OR) and 95% confidence intervals (CIs) for the relationship between FLI and CIMT. We selected these confounders based on their association with the outcomes of interest or a change in effect estimate of more than 10% (11). Model 1: adjusted for age and sex; Model 2: adjusted for variables included in Model 1, smoking, drinking, systolic blood pressure, diastolic blood pressure, HDL-C, and HbA1c; and Model 3: adjusted for variables included in Model 2, hypertension history, and NAFLD history. Additionally, restricted cubic spline regression was performed with four knots at the 5th, 35th, 65th, and 95th percentiles of FLI to assess linearity and examine the curve between FLI and CIMT. We used a two-piece-wise logistic regression model with smoothing to analyze the association threshold between FLI and CIMT after adjusting the variables in Model 3. The likelihood-ratio test and the bootstrap resampling method were used to determine the inflection points.

In sensitivity analyses, the analysis was repeated for data without multiple imputation. Furthermore, potential modifications of the relationship between FLI and CIMT were assessed, including the following variables: sex, age (< 45 vs. >50 years), hypertension history (yes or no), and NAFLD history (yes or no). Heterogeneity among subgroups was assessed using multivariate logistic regression analysis, and interactions between subgroups and FLI were examined using likelihood ratio testing. All analyses were performed using the statistical software packages R 3.3.2 (http://www.R-project.org, The R Foundation, Shanghai, China) (accessed on March 10, 2022) and Free Statistics software version 1.7 (12). A descriptive study was conducted on all participants. A two-tailed p-value <0.05 was considered significant.

# 3 Results

## 3.1 Demographics and baseline information

In total, 277 individuals had sufficiently reliable data to meet the inclusion criteria of our study. Table 1 illustrates the baseline characteristics of all subjects according to their FLI. There were 105 (37.9%) individuals with carotid intima media thickness. The average age of the study participants was 47.1 (10.9) years, and 212 (76.5%) were male. Higher FLI was associated with increased smoking and higher systolic blood pressure, diastolic blood pressure, FBG, HbA1c, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase levels (P < 0.001 for all).

## 3.2 Nonlinear correlation between FLI and CIMT

The multivariable logistic regression analyses results of the associations between FLI and CIMT is presented in Table 2. The analysis was performed after adjusting for confounding factors, including age, sex, smoking, drinking, systolic blood pressure, diastolic blood pressure, HDL-C, HbA1c, hypertension history, and NAFLD history. Compared with individuals with lower FLI T1 (≤ 38.70), the adjusted OR values for FLI and CIMT in T2 (38.70–67.45) and T3 (≥ 67.45) were 2.41 (95% CI: 1.10–5.25, $p$ = 0.027), and 1. 58 (95% CI: 0.68–3.64, $p$ = 0. 285), respectively. Accordingly, the association between FLI and CIMT exhibited a J-shaped curve (nonlinear, $p$ = 0. 027) in the restricted cubic spline (Figure 2). In the threshold analysis, the OR of developing CIMT was 1.031 (95% CI: 1.011–1.051, $p$ = 0.002) in participants with FLI < 64.247 (Table 3). This means that the risk of increased CIMT increases by 3.1% with every 1 unit increase in FLI. The association between FLI and CIMT was stable when the FLI was ≥ 64.247 (Table 3).

## 3.3 Stratified and sensitivity analysis

In several subgroups, stratified analysis was performed to assess the potential effect of modifications on the relationship between FLI and CIMT. No significant interactions were found in any subgroups after stratifying by age, sex, NAFLD history, and hypertension history (Supplementary Figure 1). In sensitivity analyses, results obtained without using multiple imputations remained consistent (Supplementary Tables 1, 2). Compared with individuals with lower FLI in T1 (≤38.70), the adjusted OR values for FLI and CIMT in T2 (38.70–67.45) and T3 (≥67.45) were 3.32 (95% CI: 1.00–11.04, $p$ = 0.05), and 1.67 (95% CI: 0.39–7.24, $p$ = 0. 491), respectively.

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TABLE 1 Baseline characteristics of the study participants stratified by the tertiles of the FLI.

| Characteristic | FLI | | | | |
|---|---|---|---|---|---|
| | Total | T1 (≤38.70) | T2 (38.70-67.45) | T3 (≥67.45) | *P*-value |
| No. | 277 | 92 | 92 | 93 | |
| Age, years | 47.1 ± 10.9 | 45.3 ± 11.5 | 47.9 ± 11.1 | 48.2 ± 10.0 | 0.145 |
| Sex, Male (%) | 212 (76.5) | 59 (64.1) | 73 (79.3) | 80 (86) | 0.002 |
| Smoking, n (%) | 99 (35.7) | 17 (18.5) | 37 (40.2) | 45 (48.4) | < 0.001 |
| Drinking, n (%) | 93 (33.6) | 26 (28.3) | 30 (32.6) | 37 (39.8) | 0.245 |
| Hypertension History, n (%) | 177 (63.9) | 44 (47.8) | 57 (62) | 76 (81.7) | < 0.001 |
| NAFLD History, n (%) | 161 (58.1) | 43 (46.7) | 58 (63) | 60 (64.5) | 0.025 |
| DM, n (%) | 9 (3.2) | 4 (4.3) | 2 (2.2) | 3 (3.2) | 0.764 |
| NAFLD, n (%) | 175 (63.2) | 30 (32.6) | 67 (72.8) | 78 (83.9) | < 0.001 |
| Pulse (bpm) | 75.5 ± 10.5 | 72.7 ± 10.2 | 77.4 ± 10.5 | 76.5 ± 10.3 | 0.006 |
| SBP (mmHg) | 132.9 ± 16.8 | 126.7 ± 16.5 | 132.9 ± 15.3 | 138.9 ± 16.4 | < 0.001 |
| DBP (mmHg) | 81.7 ± 12.4 | 75.9 ± 12.7 | 81.6 ± 9.8 | 87.6 ± 11.8 | < 0.001 |
| WC (cm) | 93.7 ± 9.5 | 85.2 ± 7.2 | 93.7 ± 5.9 | 102.0 ± 6.6 | < 0.001 |
| HC (cm) | 103.2 ± 6.7 | 98.6 ± 5.7 | 103.9 ± 5.0 | 107.0 ± 6.4 | < 0.001 |
| BMI ($Kg/m^2$) | 27.0 ± 3.2 | 24.3 ± 2.3 | 26.9 ± 1.7 | 29.7 ± 3.0 | < 0.001 |
| TC (mmol/L) | 4.8 (4.2, 5.5) | 4.8 (4.3, 5.5) | 4.6 (4.2, 5.5) | 4.8 (4.2, 5.4) | 0.812 |
| TG (mmol/L) | 1.6 (1.1, 2.2) | 1.0 (0.7, 1.3) | 1.6 (1.3, 2.0) | 2.4 (1.8, 3.3) | < 0.001 |
| HDL-C (mmol/L) | 1.2 (1.1, 1.4) | 1.4 (1.2, 1.6) | 1.2 (1.0, 1.4) | 1.1 (1.0, 1.3) | < 0.001 |
| LDL-C (mmol/L) | 2.8 (2.4, 3.4) | 2.8 (2.4, 3.4) | 2.8 (2.4, 3.6) | 2.8 (2.4, 3.4) | 0.433 |
| FBG (mmol/L) | 5.8 ± 1.5 | 5.4 ± 1.0 | 5.7 ± 1.3 | 6.4 ± 1.9 | < 0.001 |
| HbA1c (%) | 5.7 ± 0.8 | 5.6 ± 0.6 | 5.6 ± 0.6 | 5.9 ± 1.0 | < 0.001 |
| Antihypertension Drugs, n (%) | 17 (6.1) | 5 (5.4) | 4 (4.3) | 8 (8.6) | 0.456 |
| ALT (IU/L) | 29.0 (21.0, 40.0) | 22.0 (16.8, 28.2) | 30.0 (21.0, 39.2) | 37.0 (26.0, 52.0) | < 0.001 |
| AST, (IU/L) | 22.0 (18.0, 27.0) | 20.0 (17.8, 23.0) | 21.0 (19.0, 26.0) | 25.0 (21.0, 30.0) | < 0.001 |
| TBil, (μmol/L) | 12.4 (9.4, 16.3) | 12.6 (9.5, 16.6) | 12.3 (8.8, 16.1) | 12.6 (9.7, 16.3) | 0.724 |
| DBil, (μmol/L) | 1.9 (1.6, 2.4) | 1.8 (1.5, 2.3) | 2.0 (1.5, 2.5) | 2.0 (1.6, 2.4) | 0.268 |
| ALP, (IU/L) | 70.0 (61.0, 82.0) | 65.0 (57.0, 79.0) | 68.5 (61.8, 81.5) | 77.0 (64.0, 86.0) | 0.001 |
| GGT, (IU/L) | 28.0 (20.0, 42.0) | 19.0 (14.0, 25.2) | 28.0 (23.0, 34.2) | 47.0 (29.0, 73.0) | < 0.001 |
| CIMT, n (%) | 105 (37.9) | 24 (26.1) | 42 (45.7) | 39 (41.9) | 0.015 |
| Carotid Plaques, n (%) | 111 (40.1) | 31 (33.7) | 42 (45.7) | 38 (40.9) | 0.25 |

Data are presented as means ± SD or medians (inter-quantile range (IQR)) for continuous variables and number (percentages) for categorical variables. FLI, fatty liver index; BMI, body mass index; T, tertiles; DM, diabetes mellitus; NAFLD, non-alcoholic fatty liver disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; HC, hip circumference; BMI, body mass index; TC, Total Cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBil, total bilirubin; DBil, direct bilirubin; GGT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; CIMT, carotid intima media thickness.

# 4 Discussion

This cross-sectional study of participants who underwent health examination demonstrated a J-shaped relationship between FLI and CIMT, with an inflection point of approximately 64.247. Both the stratified and sensitivity analyses showed that the relationship between FLI and CIMT remained robust.

The leading cause of death in individuals with NAFLD is CVD (13). A recent meta-analysis reported that NAFLD was associated with a moderately increased risk of fatal or non-fatal CVD events

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TABLE 2 Multivariate logistic regression analyses of associations between FLI and CIMT.

| | No. | Unadjusted | | Model 1 | | Model2 | | Model3 | |
|---|---|---|---|---|---|---|---|---|---|
| | | OR (95%CI) | *P*-value | OR (95%CI) | *P*-value | OR (95%CI) | *P*-value | OR (95%CI) | *P*-value |
| FLI | 277 | 1.01 (1.00~1.02) | 0.0190 | 1.01 (0.997~1.02) | 0.120 | 1.01 (0.99~1.02) | 0.220 | 1.01 (0.995~1.02) | 0.210 |
| T1 | 92 | Ref | | Ref | | Ref | | Ref | |
| T2 | 92 | 2.38 (1.28~4.43) | 0.006 | 2.23 (1.09~4.55) | 0.028 | 2.27 (1.06~4.85) | 0.034 | 2.41 (1.10~5.25) | 0.027 |
| T3 | 93 | 2.05 (1.1~3.81) | 0.024 | 1.79 (0.87~3.67) | 0.111 | 1.56 (0.68~3.56) | 0.296 | 1.58 (0.68~3.64) | 0.285 |
| P for trend | | | 0.027 | | 0.138 | | 0.351 | | 0.357 |

Model 1: Adjust for age and sex.
Model 2: Adjust for Smoking, Drinking, SBP, DBP, HDL-C, HbA1c in addition to model 1.
Model 3: Adjust for Hypertension history, NAFLD history in addition to model 2.
FLI, fatty liver index; CIMT, carotid intima media thickness; T, tertiles; OR, odds ratio; CI, confidence interval; Ref, reference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; HbA1c, glycosylated hemoglobin; NAFLD, non-alcoholic fatty liver disease.

(pooled HR: 1.45, 95% CI: 1.31–1.61). This risk markedly increases across the severity of NAFLD, especially in the fibrosis stage (pooled HR: 2.50, 95% CI: 1.68–3.72) (4). The mechanism by which steatosis increases the risk of CVD is unclear. Cardiometabolic disease, characterized by metabolic disorder triggered cardiovascular events, is a leading cause of death and disability. However, CVD events are a late step in the process of atherogenesis which makes it difficult to ascertain the contribution of steatosis itself.

We postulated that if steatosis played an independent role in the development of atherosclerosis, it should promote the occurrence and progression of early, pre-atherosclerotic lesions. In Familial Combined Hyperlipidemia patients with more severe steatosis, the risk of atherosclerotic plaque development was significantly increased in patients with liver fibrosis, suggesting that dyslipidemia and insulin resistance may be processes between liver disease and atherosclerotic damage (14). Metabolic syndrome could worsen CIMT in patients with NAFLD (15). In rats with NASH, CIMT correlated with hepatic inflammation score (16). Moreover, mechanism studies have found that the accumulation of fat in the liver could increase free fatty acid levels, which are involved in inflammatory responses and endothelial injury during the development of atherosclerosis (17). NAFLD might accelerate the progress of increased CIMT by a common metabolic dysfunction, such as insulin resistance, glucose and lipid metabolism disorders (18, 19), and low-grade inflammation (20).

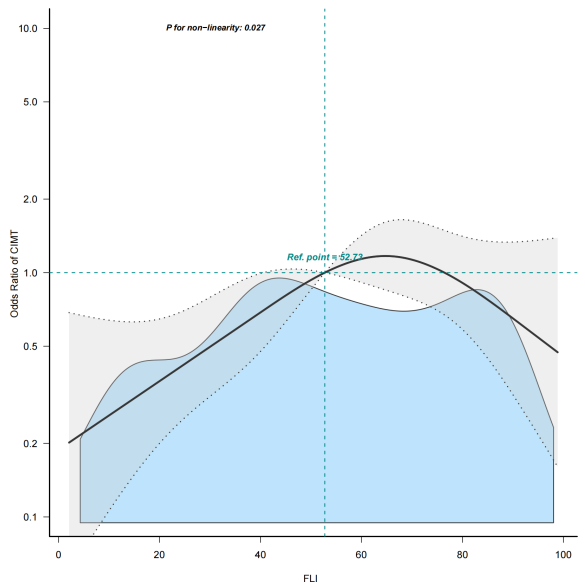


FIGURE 2
Restricted cubic spline plot of the association between FLI and CIMT. Solid and dashed lines represent the predicted and 95% confidence interval. FLI, fatty liver index; CIMT, carotid intima-media thickness.

FLI is a predictor of NAFLD and is calculated using serum triglyceride and GGT levels, BMI, and waist circumference. FLI integrates obesity, central obesity, lipid metabolism disorders and GGT, which might serve as a marker of metabolic dysfunction (21) and low-grade inflammation (22–24). Furthermore, non-invasive hepatic steatosis indices are positively correlated with fasting blood insulin, C peptide, triglyceride, total cholesterol, and LDL-C levels, but negatively correlated with HDL-C levels, which are also associated with atherosclerosis. In this study, we demonstrated that a threshold effect was presented by restricted cubic spline, which might be an indicator of CIMT, a pre-atherosclerotic lesion that predicts cardiovascular events. Therefore, we believe that NAFLD may contribute to the occurrence and development of carotid atherosclerosis by aggravating metabolic dysfunction and inflammation.

Predicting early atherosclerosis plays an important role in decreasing CVD events. A J-shaped relationship was demonstrated between FLI and CIMT in this study. The CIMT increased proportionally with FLI, and this association was independent of traditional cardiometabolic risk factors.

Our study has several limitations. First, this was a cross-sectional study and the results of this study should not be used to draw causal conclusions. Second, the overall sample size of our study was small due to missing data from laboratory results. Third, even though regression models were constructed, and stratified analyses and sensitivity analysis were performed, residual confounding effects from unmeasured or unknown factors could not be entirely excluded. Therefore, further studies between FLI and CIMT are required in the future.

TABLE 3 Threshold effect analysis of the relationship of FLI with CIMT.

| FLI | Model | |
|---|---|---|
| | OR (95% CI) | *P*-value |
| <64.247 | 1.031(1.011 – 1.051) | 0.0023 |
| ≥64.247 | 0.966(0.924 – 1.009) | 0.1229 |
| log likelihood ratio test | | 0.008 |

FLI, fatty liver index; CIMT, carotid intima media thickness; OR, odds ratio; CI, confidence interval.

In conclusion, the results of this study indicate that FLI, which is readily available in clinical practice, may be more useful as a risk assessment index for CIMT among people undergoing health examination. This may have clinical utility and guide treatment choices.

## 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 clinical research ethics committee of China-Japan Friendship Hospital. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

YZ, data curation, software, writing - review and editing. SD and RW, data curation, writing - review and editing. JC and SY, conceptualization, methodology, investigation, resources, data curation, visualization, supervision, funding acquisition, writing - review and editing.

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## Conflict of interest

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

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1120581/full#supplementary-material

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# Association of long-term triglyceride-glucose index level and change with the risk of cardiometabolic diseases

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**Objective:** The triglyceride-glucose (TyG) index is considered as a pivotal factor for various metabolic, cardiovascular, and cerebrovascular diseases. However, there is currently a paucity of relevant studies on the association between long-term level and change of TyG-index and cardiometabolic diseases (CMDs) risk. We aimed to explore the risk of CMDs in relation to the long-term level and change of TyG-index.

**Methods:** Based on the prospective cohort study, a total of 36359 subjects who were free of CMDs, had complete data of triglyceride (TG) and fasting blood glucose (FBG) and underwent four health check-ups from 2006 to 2012 consecutively were followed up for CMDs until 2021. The associations between long-term level and change of TyG-index and CMDs risk were assessed by Cox proportional hazards regression models to compute hazard ratios (HRs) and 95% confidence intervals (CIs). The TyG-index was calculated as ln [TG, mg/dL) × FBG, mg/dL)/2].

**Results:** During the median observation period of 8 years, 4685 subjects were newly diagnosed with CMDs. In multivariable-adjusted models, a graded positive association was observed between CMDs and long-term TyG-index. Compared with the Q1 group, subjects with the Q2-Q4 group had increased progressively risk of CMDs, with corresponding HRs of 1.64(1.47-1.83), 2.36(2.13-2.62), 3.15 (2.84-3.49), respectively. The association was marginally attenuated, after further adjustment for the baseline TyG level. In addition, compared with stable TyG level, both loss and gain in TyG level were associated with increased CMDs risk.

**Conclusions:** Long-term elevated level and change of TyG-index are risk factors for the incident CMDs. Elevated TyG-index in the early stage remains to exert cumulative effects on the occurrence of CMDs even after accounting for the baseline TyG-index.

KEYWORDS

**triglyceride-glucose index, triglyceride-glucose index change, cardiometabolic diseases, cumulative effect, cohort study**

# Introduction

Cardiometabolic diseases (CMDs), encompassing type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVDs), remain the most predominant cause of permanent disability and mortality as a cluster of metabolic disorders on a global scale (1–3).

CMDs still impose a substantial burden on the medical and health industry, accounting for approximately 31% of total mortality worldwide, as identified by the latest World Health Organization (WHO) report (1). Early detection of groups at high risk and the recognition and control of risk factors, in particular modifiable factors, are therefore pivotal to preventing CMDs. The traditional risk factors, such as unhealthy living practices characterized by a lack of physical activity and poor diet (including smoking, alcohol consumption, and obesity), contribute to but cannot fully explain the increased risk of CMDs (4–6).

The existing robust epidemiological evidence implies that insulin resistance (IR), which has been recognized as an independent risk factor, contributes to the initiation and perpetuation of CMDs (7, 8). Nevertheless, the TyG-index, known as an alternative method for measuring IR (9–11), could be applied for clinical purposes and has shown a significant relation with IR (12, 13). Previous studies have demonstrated a positive correlation between the risk of developing CVD and the TyG-index (14). Moreover, a Korean cohort study with a 12-year follow-up reported that the TyG-index is regarded as a strong predictor for the development of T2DM among middle-aged and aged populations (15). Notably, however, measurements of the TyG-index in these related studies at a single time point might not be sufficient to indicate the long-term longitudinal effect of the TyG level on CMDs accurately. Moreover, both at home and abroad, the association between CMDs and the long-term dynamic changes in the TyG-index are scarce. Only one study, Wang et al. (16), has explored the association between CVD and change in the TyG-index thus far. However, the abovementioned research is inherently limited to a single event in CMDs and does not take into account the direction of change in the TyG-index and the time-varying effect of CMDs.

Therefore, based on the Kailuan study, this finding was aimed at examining the cumulative effects of the TyG-index, including its long-term level and change, on the risk of developing CMDs using up to 15 years of biennial longitudinal data.

**Abbreviations:** TyG, Triglyceride-glucose; CMDs, cardiometabolic diseases; TG, Triglyceride; FBG, Fasting blood glucose; IR, Insulin resistance; MI, myocardial infarction; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; hs-CRP, High-sensitivity C-reactive protein; TC, total cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate. CI, Confidence interval.

# Subjects and methods

## Reasearch subjects

The Kailuan Study, which launched in July 2006 and remained ongoing, was an epidemiological survey that investigated and intervened in the risk factors for cardiovascular and cerebrovascular diseases among current and retired individuals at the Kailuan Group. The Kailuan Group is a massive-scale energy and chemical enterprise primarily dominated by coal mining products, where male employees make up a majority of the proportion. All the participants were followed up biennially by responding to validated questionnaires on demographics, health-related behaviors, and medical conditions, undergoing clinical examinations and laboratory tests. Detailed descriptions of the study design have been published (17, 18).

Given the long-term level and change in the TyG-index, all 49435 subjects ≥18 years old or above underwent four health-checks consecutively from 2006–2007, 2008–2009, 2010–2011, and 2012-2013 (index year). For these remaining subjects, we excluded those who experienced a history of stroke (n=2449), myocardial infarction (n=1392), or diabetes (n=7748) and with missing data on triglyceride (TG) or fasting blood glucose (FBG) (n=1787) in or prior to 2012–2013. Ultimately, 36359 subjects were included to analyze the association between long-term level and change in the TyG-index with incident CMDs (Figures 1, S1). The study was approved by the ethics committee of Kailuan General Hospital, and all the subjects gave written informed consent according to the guidelines of the Declaration of Helsinki.

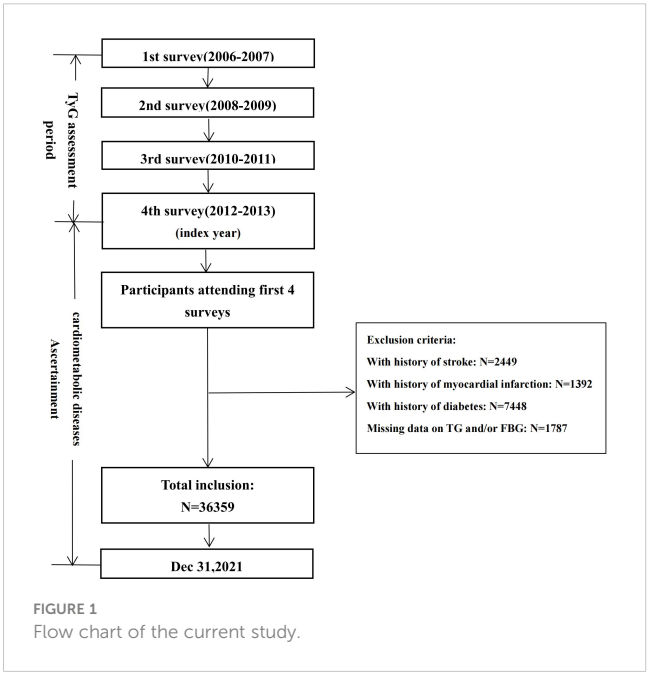


FIGURE 1
Flow chart of the current study.

# Data collection and definitions

## TyG-index calculation

The TyG-index was calculated as Ln [TG (mg/dl) ×FBG (mg/dl)/2] (19). The FBG and TG data were assayed using standard laboratory procedures in the central laboratory of the Kailuan General Hospital. Approximately 5 ml samples were extracted from the cephalic vein into an anticoagulant (EDTA) tube from 7:00–9:00 a.m. on the day of the health examination after overnight (>8 h) fasting. The FBG and serum TG were assessed using the hexokinase method (the coefficient of variation was <2%, and the upper limit of linearity was 33.3 mmol/L) and enzymatic colorimetric method, respectively. The hematological parameters of the blood were analyzed by an autoanalyzer (Hitachi, Tokyo, Japan). The corresponding kits were purchased from Zhongsheng North Control Biotechnology Co., Ltd. The professional quality controller regularly monitored these parameters.

## Average TyG-index and change of TyG-index were performed using the following formula:

① Average TyG index$_{06-12}$= (TyG$_{06}$ × Time$_{1-2}$) + (TyG$_{08}$ × Time$_{2-3}$) + (TyG$_{10}$ × Time$_{3-4}$)]/Time$_{1}$.4

② Average TyG index= [(TyG$_{06}$ × Time$_{1-2}$) + (TyG$_{08}$ × Time$_{2-3}$)+(TyG$_{10}$ × Time$_{3-4}$) + (TyG$_{12}$ × Time$_{4-}$)]/Total Time

③ Total TyG index change= [(TyG$_{08}$−TyG$_{06}$) × Time$_{1-2}$) + (TyG$_{10}$−TyG$_{08}$) × Time$_{2-3}$) + (TyG$_{12}$−TyG$_{10}$) × Time$_{3-4}$)] / Time$_{1-4}$

Time$_{1-2}$= 1$^{st}$measurement time (2006 − 2007) to 2$^{nd}$measurement time (2008 − 2009);

Time$_{2-3}$ =2$^{nd}$measurement time (2008 − 2009) to 3$^{rd}$measurement time (2010 − 2011);

Time$_{3-4}$ =3$^{rd}$measurement time (2010 − 2011) to 4$^{th}$measurement time (2011 − 2012);

Time$_{1-4}$ =1$^{st}$measurement time (2006 − 2007) to 4$^{th}$measurement time (2011 − 2012);

Time$_{4-}$ = 4$^{th}$measurement time (2011 − 2012) to end of follow − up;

Total Time = 1$^{st}$measurement time (2006 − 2007) to end of follow − up.

According to the grouping method described in the reference (20), the subjects were stratified by the average TyG-index into quartiles: Q1 group, <8.22 (as reference group), Q2 group, 8.22–8.53, Q3 group, 8.53–8.90, and Q4 group, ≥8.90. We further analyzed the impacts of the TyG-index change on CMDs, including the total TyG change (TyG$_{06}$-TyG$_{12}$), early TyG change (TyG$_{06}$-TyG$_{08}$), middle TyG change (TyG$_{08}$-TyG$_{10}$), and late TyG change (TyG$_{10}$-TyG$_{12}$), separately. We classified the total, early, middle, and late TyG changes into quintiles according to the quintiles of the TyG-index change: Q1 group<-0.14, -0.14≤Q2 group<-0.025, -0.025≤Q3 group<0.07 (as reference group), 0.07≤ Q4 group<0.19, and Q5 group≥0.19.

Additionally, we conducted latent variable modeling, which was implemented by a group-based approach with SAS Proc Traj (21), to identify four distinctive TyG trajectories: low-stable (7990, 22.0%), moderate-low stable (18802, 51.7%), moderate-high stable (8018, 22.1%), and high stable (1549, 4.3%). The TyG-index variability was calculated using the standard deviation (SD) and coefficient of variation (CV) across the four TyG measurements (in 2006–2007, 2008–2009, 2010–2011, and 2011-2012).

## Assessment of variability using the following formula:

$$SD = \sqrt{\frac{1}{n-1}\sum_{i-1}^{1}(xi-x)^2}$$

$$CV=(SD/MEAN) \times 100\,\%$$

## Assessment of outcomes

The first occurrence of CMDs event was the primary endpoint outcome during follow-up, including MI, stroke, or T2DM, as described previously (1). ICD codes from the Tenth Revisions (ICD-10) were used to identify diagnoses of CMD (either MI: I21, stroke: I60-I63, or T2DM: E11). The database of CMDs diagnoses was ascertained by searching the Hospital Discharge Register of the 11 hospitals and the Municipal Social Insurance Institution, which was updated annually throughout the follow-up period. All suspected new-onset CMDs events were reviewed by a panel of 3 physicians who scrutinized all pertinent medical records and further identified the diagnosis. Diagnostic evidence of MI included a history of clinical symptoms, changes in electrocardiography and elevated concentrations of cardiac enzymes (22), according to the WHO criteria, while that of stroke included focal neurofunctional deficit signs and symptoms, and neuroimaging examination (23). T2DM was diagnosed either by receiving hypoglycemic drugs, a self-reported history of diagnosed diabetes, or FBG ≥7.0 mmol/L, based on the 2020 American Diabetes Association Standards (24).

## Covariates

The demographics collected during this study included age, sex (male, female), lifestyle factors including alcohol consumption (never/ever, current), smoking (never/ever, current), education level (middle school and below, high school and above), physical activity, and salt intake level; medical history (hypertension, CMD); medication history (antihypertensive, hypoglycemic, or lipid-

lowering drugs), and other information *via* face-to-face interview questionnaires. Standard exercise was defined as performing aerobic exercise ≥3 times/week for >30 minutes each time continuously. The standard salt diet was defined as 10 g/day of salt intake. Hypertension was defined based on either receiving medications for hypertension, a self-reported history of hypertension, or blood pressure ≥140/90 mmHg (25).

All laboratory tests, including high-density lipoprotein cholesterol (HDL-C), high sensitivity C-reactive protein (hs-CRP), low-density lipoprotein cholesterol (LDL-C), and total cholesterol (TC), were performed using an automatic biochemical analyzer (7600-020).

## Statistical analysis

Statistical analysis was performed with R 4.1.1 and SAS 9.4 (SAS Institute Inc., Cary, NC). The baseline data are displayed as the means ± standard deviation (X ± SD), numbers (percentage), or medians (P25, P75), where appropriate. The incidence rate of new-onset CMDs was calculated by dividing the number of events by the total follow-up period (per 1000 person-years).

Cox proportional regression models were applied to assess the relationships for CMDs within a given category as follows: (i) average TyG-index (quartile 1<8.22 as the reference group), (ii) TyG-index change (-0.025≤quintile 3<0.07 as the reference group), (iii) TyG-index trajectory (low-stable trajectory as the reference group), and (iv) TyG-index variability (TyG-CV: quartile 1<3.76, TyG-SD: quartile 1<0.22 as the reference group). All the Cox proportional hazards models were adjusted for sex, age, educational level, smoking, salt intake, physical activity, alcohol consumption, LDL-C, hs-CRP, eGFR, HDL-C, BMI, lipid-lowering drugs, and antihypertensive drugs as potentially relevant confounders. The missing covariates were imputed using a fully conditional specification method.

To account for the association between CMDs and the level of TyG-index change over time, we ran time-dependent Cox regression models to repeat the above (i) and (ii) categories of analysis in which the time-varying covariates were incorporated into the models. In addition, we also assessed the impacts on CMDs risk at the four time points ($TyG_{06}$, $TyG_{08}$, $TyG_{10}$, and $TyG_{12}$).

We performed analyses stratified by gender (male, female), BMI (BMI ≤ 28, BMI>28), and age (≤60, >60 years) to assess whether the effect was modified. Furthermore, a subgroup analysis was conducted on the participants after stratification by the direction of change in TyG index (increase, decrease). To minimize the influence of potential bias from reverse causality, we also performed sensitivity analyses that excluded the first 1 year of follow-up. Considering the potential survival bias, we assessed the cross-sectional correlation of the TyG-index at baseline, the average TyG-index, and the TyG-index change with the risk of CMDs at baseline by running logistic regression models. $P<0.05$ was considered statistically significant for the bilateral tests.

# Results

## Baseline characteristics

Table 1 presents descriptive characteristics of the baseline demographic and biochemical profiles. Overall, 36359 subjects (mean age 53.11 ± 11.79 years old, male 73.87%) comprised the study population. We grouped the subjects according to the quartiles of the baseline TyG-index. When compared to the Q1 group, subjects in the other groups were more inclined to be more current smokers, current drinkers, and male, had a higher SBP, DBP, BMI, FBG, hs-CRP, TG, LDL-C, TC level, and a higher prevalence of hypertension ($P<0.01$).

## Average TyG-index and CMDs risk

During the mean observation period of 8.03 years, 4685 subjects were diagnosed with new-onset CMDs (including 1708 CVD events, 2772 diabetes events and 205 CVD and diabetes events). In addition, the cumulative incidence and the incidence rate for CMDs were 12.89% and 16.05 per 1000 person-years, respectively.

Table 2 shows the association between incident CMDs and the average TyG-index. The multivariable-adjusted HRs for subjects in the Q2-Q4 groups were 1.64(1.47-1.83), 2.36(2.13-2.62), and 3.15 (2.84-3.49), compared with those in the Q1 group. The associations of the average TyG-index with the CMDs risk became more significant compared with the primary analysis, after introducing confounders as time-varying covariates based on the time-dependent Cox regression models.

One goal of the analysis was to explore the relative importance of the average TyG-index and that of the most recent TyG-index values. Even after adjustment for the baseline TyG-index, the average TyG-index was correlated with CMDs risk in the Q2 (HR 1.34, 95% CI 1.20-1.49), Q3 (HR 1.83, 95% CI 1.65-2.03), and Q4 (HR 2.39, 95% CI 2.14-2.67) groups compared with the Q1 group (Table 3). In contrast, there was no strong association between the risk of CMDs and the current baseline TyG-index following adjustment for the average TyG-index (Figure 2).

When we assessed the HRs and 95% CIs for $TyG_{06}$, $TyG_{08}$, $TyG_{10}$, and $TyG_{12}$ separately, these results indicated that a high TyG level was positively related to an increased risk of subsequent incident CMDs at each time point (Supplementary Table S1).

## TyG-index change and CMDs risk

A Cox proportional hazards regression analysis, which was applied to explore the relationship between CMDs and the TyG-index change, is shown in Table 4. When compared to the stable group (0.025≤Quintile 3<0.07), we found a correlation between both loss and gain in TyG level and an increased CMDs risk. In the case of the total TyG-index change, the HRs (95% CI) for subjects in

TABLE 1 Baseline characteristics according to quartiles of baseline TyG index.

| | Total | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | *P* |
|---|---|---|---|---|---|---|
| Participants | 36359 | 9093 | 9084 | 9093 | 9089 | |
| Age, year | 53.11 ± 11.79 | 52.47 ± 12.34 | 53.58 ± 11.88 | 54.19 ± 11.56 | 52.22 ± 11.23 | <0.01 |
| Male, N (%) | 26857 (73.87) | 6228 (68.49) | 6699 (73.75) | 6787 (74.64) | 7143 (78.59) | <0.01 |
| SBP, mmHg | 128.55 ± 18.28 | 124 ± 18.22 | 127.76 ± 18.14 | 130.60 ± 18.32 | 131.82 ± 17.46 | <0.01 |
| DBP, mmHg | 82.97 ± 10.25 | 80.18 ± 10.03 | 82.42 ± 9.99 | 83.91 ± 10.20 | 85.37 ± 10.07 | 0.32 |
| BMI, Kg/m$^2$ | 24.99 ± 3.39 | 23.84 ± 3.17 | 24.62 ± 3.29 | 25.39 ± 3.33 | 26.11 ± 3.32 | <0.01 |
| FBG, mmol/L | 5.25 ± 0.63 | 4.99 ± 0.58 | 5.17 ± 0.58 | 5.36 ± 0.60 | 5.50 ± 0.65 | <0.01 |
| TC, mmol/L | 5.04 ± 0.98 | 4.62 ± 0.84 | 4.92 ± 0.88 | 5.20 ± 0.94 | 5.43 ± 1.03 | <0.01 |
| LDL-C, mmol/L | 2.49 ± 0.83 | 2.24 ± 0.71 | 2.51 ± 0.76 | 2.66 ± 0.84 | 2.55 ± 0.94 | <0.01 |
| HDL-C, mmol/L | 1.39 ± 0.39 | 1.54 ± 0.39 | 1.44 ± 0.35 | 1.36 ± 0.37 | 1.23 ± 0.39 | <0.01 |
| TG, mmol/L | 1.21 (0.87-1.83) | 0.68 (0.57-0.79) | 1.04 (0.94-1.15) | 1.45 (1.30-1.63) | 2.56 (2.10-3.48) | <0.01 |
| hs-CRP, mg/L | 1.20 (0.59-2.30) | 0.97 (0.49-1.90) | 1.12 (0.50-2.35) | 1.24 (0.61-2.38) | 1.42 (0.75-2.60) | 0.12 |
| eGFR, [mL/(min·1.73$^2$)] | 92.53 (75.59-105.32) | 95.17 (78.51-107.69) | 90.52 (74.24-103.86) | 91.62 (74.81-104.20) | 92.86 (75.27-105.80) | <0.01 |
| Current smoking, N (%) | 11806 (32.47) | 2553 (28.08) | 2720 (29.94) | 3011 (33.11) | 3522 (38.75) | <0.01 |
| Current drinking, N (%) | 11010 (30.28) | 2403 (26.43) | 2482 (27.32) | 2716 (29.87) | 3409 (37.51) | <0.01 |
| Physical exercisers, N (%) | 25885 (71.19) | 6246 (68.69) | 6694 (73.69) | 6546 (71.99) | 6399 (70.40) | <0.01 |
| Education level, N (%) | | | | | | <0.01 |
| High school diploma or below | 26871 (73.90) | 6472 (71.18) | 6878 (75.72) | 6862 (75.46) | 6659 (73.26) | <0.01 |
| High school diploma or above | 9488 (26.10) | 2621 (28.82) | 2206 (24.28) | 2231 (24.54) | 2430 (26.74) | <0.01 |
| Salt level, g/d | | | | | | <0.01 |
| <10 | 4389 (12.07) | 1147 (12.61) | 1016 (11.18) | 1134 (12.47) | 1092 (12.01) | <0.01 |
| >10 | 31970 (87.93) | 7946 (87.39) | 8068 (88.82) | 7959 (87.53) | 7997 (87.99) | <0.01 |
| Hypertension, N (%) | 14766 (40.61) | 2693 (29.62) | 34373 (38.23) | 4088 (44.96) | 4512 (49.64) | <0.01 |
| Antihypertensive treatment, N (%) | 4906 (13.49) | 775 (8.52) | 1012 (11.14) | 1425 (15.67) | 1694 (18.64) | <0.01 |
| Lipid-lowering treatment, N (%) | 395 (1.09) | 44 (0.48) | 56 (0.62) | 102 (1.12) | 193 (2.12) | <0.01 |

P, comparison of baseline characteristics between different TyG index groups
SBP systolic blood pressure, DBP diastolic blood pressure, BMI body mass index, FBG fasting blood glucose, TC total cholesterol, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol, hs-CRP high-sensitivity C reactive protein, TG triglyceride, eGFR estimated glomerular filtration rate.
TyG index: triglyceride glucose index.

the Q1, Q2, Q4 and Q5 groups were 1.14 (1.02-1.27), 1.09 (0.99-1.20), 1.08 (0.98-1.19), and 1.14 (1.02-1.27), respectively, compared with the stable TyG-index group. Furthermore, for early, middle, and late changes in the TyG-index, the multivariable-adjusted HRs (95% CI) within the Q5 group compared with the reference group were 1.13 (1.00-1.27), 1.10 (0.98-1.23), and 1.12 (1.00-1.26), respectively. Essentially similar results were revealed according to the time-dependent Cox regression models.

Figure 3 displays the association between the TyG-index change and CMDs, as fitted by restricted cubic splines ($P_{linearity}$<0.001, $P_{non\text{-}linearity}$=0.001), after adjustment for potential confounders. Furthermore, when considered as a continuous variable, the relationship between TyG-index change and CMDs risk followed a U-shaped curve.

## TyG-index trajectory, TyG-index variability and CMDs risk

As shown in Supplementary Table S2, for either the trajectory or variability in the TyG-index, the association between these variables and the risk of CMDs was assessed using the Cox

TABLE 2 Hazard ratios (95% CI) for risk of outcomes according to quartiles of Average TyG-index (1st measurement to end of follow-up).

| | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | *P* for trend |
|---|---|---|---|---|---|
| Multivariable adjusted HR (95% CI) | | | | | |
| Cases, N (%) | 531(5.84) | 942(10.36) | 1399(15.39) | 1813(19.94) | |
| Incidence, per1000 person-y | 7.03 | 12.76 | 19.42 | 25.67 | |
| Model.1 | Reference | 1.77(1.59-1.97) | 2.65(2.40-2.93) | 3.65(3.32-4.03) | 0.01 |
| Model.2 | Reference | 1.70(1.53-1.90) | 2.50(2.26-2.77) | 3.39(3.07-3.74) | 0.01 |
| Model.3a | Reference | 1.64(1.47-1.83) | 2.36(2.13-2.62) | 3.15(2.84-3.49) | 0.01 |
| Time-dependent variables adjusted HR (95% CI) | | | | | |
| Cases, N (%) | 531(5.84) | 942(10.36) | 1399(15.39) | 1813(19.94) | |
| Incidence, per1000 person-y | 7.03 | 12.76 | 19.42 | 25.67 | |
| Model.1 | Reference | 1.80(1.69-1.92) | 2.77(2.60-2.95) | 3.71(3.50-3.94) | 0.01 |
| Model.2 | Reference | 1.78(1.67-1.91) | 2.75(2.59-2.93) | 3.72(3.50-3.95) | 0.01 |
| Model.3b | Reference | 1.66(1.56-1.78) | 2.44(2.29-2.60) | 3.16(2.95-3.35) | 0.01 |

Model 1:adjusted for age and sex;
Model 2:adjusted for age, sex, Smoking, Drinking, Education level, Salt status and Physical activity, BMI;
Model 3:adjusted for all the variables in model 2 and LDL-C, HDL-C, hs-CRP, eGFR, Antihypertensive treatment, Lipid-lowering treatment.

TABLE 3 Hazard ratios (95% CI) for risk of outcomes according to quartiles of Average TyG-index (1st measurement to 4th measurement).

| | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | P for trend |
|---|---|---|---|---|---|
| Cases, N (%) | 576(6.34) | 892(9.81) | 1324(14.57) | 1893(20.83) | |
| Incidence, per1000 person-y | 7.62 | 12.06 | 18.31 | 26.99 | |
| Model.1 | Reference | 1.53(1.38-1.70) | 2.32(2.10-2.56) | 3.49(3.17-3.83) | 0.01 |
| Model.2 | Reference | 1.48(1.34-1.65) | 2.20(1.99-2.42) | 3.24(2.95-3.57) | 0.01 |
| Model.3c | Reference | 1.43(1.29-1.59) | 2.06(1.86-2.28) | 3.00(2.72-3.31) | 0.01 |
| Model.3d | Reference | 1.36(1.22-1.51) | 1.87(1.67-2.09) | 2.51(2.20-2.87) | 0.01 |
| Model.3e | Reference | 1.34(1.20-1.49) | 1.83(1.65-2.03) | 2.39(2.14-2.67) | 0.01 |

Model 1: adjusted for age and sex;
Model 2: adjusted for age, sex, Smoking, Drinking, Education level, Salt status and Physical activity, BMI;
Model 3c:adjusted for all the variables in model 2 and LDL-C, HDL-C, hs-CRP, eGFR, Antihypertensive treatment, Lipid-lowering treatment.
Model 3d:adjusted for all the variables in model 2 and LDL-C, HDL-C, hs-CRP, eGFR, Antihypertensive treatment, Lipid-lowering treatment, $tyg_{06}$.
Model 3e:adjusted for all the variables in model 2 and LDL-C, HDL-C, hs-CRP, eGFR, Antihypertensive treatment, Lipid-lowering treatment, $tyg_{12}$.

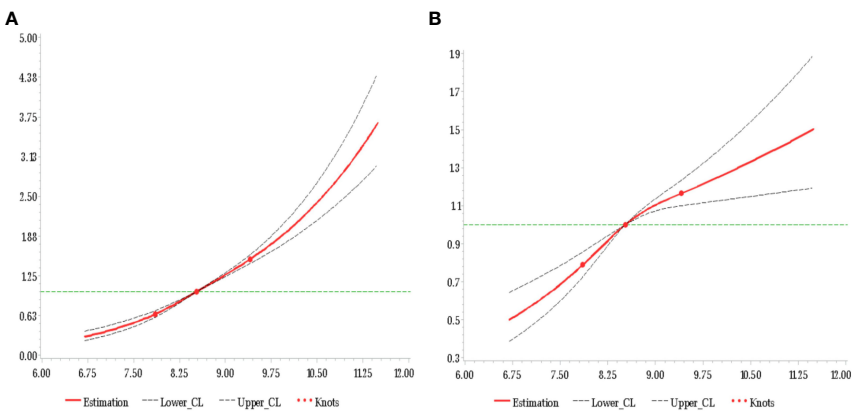


FIGURE 2
HR of CMDs and TyG-index at baseline adjusted for all covariates **(A)** and additional adjustment for average TyG-index up to baseline **(B)**.

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TABLE 4 Hazard ratios (95% CI) for risk of outcomes according to quintiles of TyG index change.

| | Cases, N (%) | Incidence, per 1000 person-y | Whole period (06-12) | Early period (06-08) | Middle period (08-10) | Late period (10-12) |
|---|---|---|---|---|---|---|
| Multivariable adjusted HR (95% CI) | | | | | | |
| Quintile 1 | 867 (11.92) | 14.81 | 1.14 (1.02-1.27) | 1.15 (1.02-1.29) | 1.09 (0.97-1.22) | 1.09 (0.97-1.22) |
| Quintile 2 | 995 (13.68) | 17.22 | 1.09 (0.99-1.20) | 1.06 (0.92-1.23) | 1.00 (0.87-1.15) | 1.06 (0.92-1.22) |
| Quintile 3 | 953 (13.11) | 16.42 | Reference | Reference | Reference | Reference |
| Quintile 4 | 919 (12.64) | 15.65 | 1.08 (0.98-1.19) | 1.04 (0.90-1.21) | 1.01 (0.87-1.16) | 1.10 (0.96-1.27) |
| Quintile 5 | 951 (13.08) | 16.16 | 1.14 (1.02-1.27) | 1.13 (1.00-1.27) | 1.10 (0.98-1.23) | 1.12 (1.00-1.26) |
| Time-dependent variables adjusted HR (95% CI) | | | | | | |
| Quintile 1 | 867 (11.92) | 14.81 | 1.06 (0.99-1.14) | 1.23 (1.14-1.32) | 1.06 (0.99-1.13) | 1.04 (0.97-1.11) |
| Quintile 2 | 995 (13.68) | 17.22 | 1.11 (1.05-1.18) | 1.16 (1.06-1.27) | 1.03 (0.95-1.12) | 1.01 (0.93-1.10) |
| Quintile 3 | 953 (13.11) | 16.42 | Reference | Reference | Reference | Reference |
| Quintile 4 | 919 (12.64) | 15.65 | 1.11 (1.04-1.17) | 1.11 (1.02-1.21) | 0.99 (0.91-1.08) | 1.05 (0.97-1.14) |
| Quintile 5 | 951 (13.08) | 16.16 | 1.18 (1.10-1.26) | 1.17 (1.09-1.26) | 1.06 (0.99-1.13) | 1.10 (1.03-1.18) |

Model:adjusted for age, sex, Smoking, Drinking, Education level, Salt status, Physical activity, BMI, LDL-C, HDL-C, hs-CRP, eGFR, Antihypertensive treatment, Lipid-lowering treatment,

proportional hazard model. When compared with the low-stable trajectory group, the risk of CMDs incidence in the high-stable trajectory group was highest (HR 3.86, 95% CI 3.35-4.45), followed by the moderate-high stable trajectory group (HR 2.95, 95% CI 2.65-3.29) and the moderate-low stable trajectory group (HR 1.82, 95% CI 1.65-2.01). For TyG-index variability, compared to those of the reference group, subjects with the highest degree of TyG-index variability (either TyG-SD or TyG-CV) were related to a higher CMDs risk (HR 1.13, 95% CI 1.04-1.22, HR 1.22, 95% CI 1.12-1.32, correspondingly).

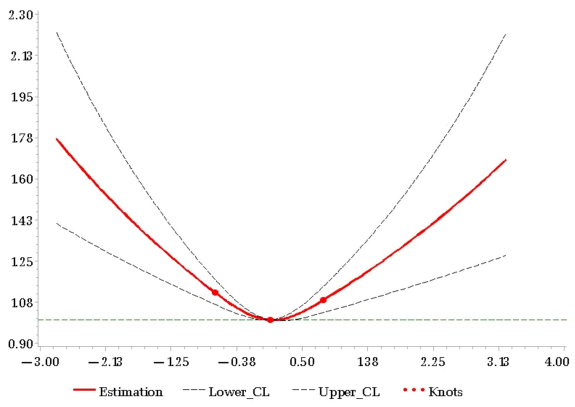


FIGURE 3
HR of CMDs and TyG-index change adjusted for all covariates.

## Hierarchical analyses and sensitivity analyses

After correction for the potential variables, the detailed results of the hierarchical analyses are presented in Supplemental Tables S3-S6. We found significant interactions between age (≤60, >60), gender (male, female), and BMI (BMI ≤ 28, BMI>28) in relation to the average TyG-index ($P_{Interaction}$<0.05). Moreover, the relative risks for CMDs were likely to be higher among ≤60 years old, female, and non-obese individuals by categories of the average TyG-index. For the change in the TyG-index, no significant interactions were detected between potential risk factors for the risk of CMDs, such as age (≤60, >60), sex, and BMI (BMI ≤ 28, BMI>28). Using the intermediate group (Q3) as a reference, the HRs (95% CI) within ≤60 years old, males, and non-obese groups were 1.25 (1.09-1.44), 1.14 (1.01-1.29), and 1.17 (1.03-1.33), respectively. However, no statistically significant differences were observed in elderly individuals, female, and obese groups. Supplementary Table S6 showed that whether increase or decrease in TyG index, the relationship between CMDs and change in TyG index is positively correlated with the magnitude of change.

In the sensitivity analyses, the results were consistent with the main analyses when a total of 330 CMDs cases were excluded after the first year of follow-up (Supplementary Table S7). The cross-sectional associations between the TyG-index and prevalent CMDs at baseline generally echoed the corresponding prospective associations (Supplementary Table S8). The results of the above sensitivity analyses demonstrated the good robustness of the association without reducing the estimate.

## Discussion

In the present research, the major discovery is that a long-term high TyG-index and a change in the TyG-index are independently important risk factors for CMDs and combine over time to increase the risk of having CMDs. Moreover, a high TyG-index in the early stage, even after accounting for the baseline TyG-index, exerted cumulative impacts on the development of CMDs. Moreover, an increased variability and longitudinal trajectory in the TyG-index were also correlated with an increased risk of CMD occurrence.

IR is widely known as a primary risk factor for T2DM and CVD, and associated with metabolic abnormalities (7, 8, 26). On account of the complex detection process and the clinical complexity, the hyperinsulinemic euglycemic glucose clamp (HEGC), which was considered as the gold standard for IR assessment (27). Additionally, homeostatic model assessment of insulin resistance (HOMA-IR), which is derived from fasting plasma glucose and fasting insulin, has been used as an alternative indictor for defining IR (28). Its measurement of insulin is consistently limited in clinical practice. Moreover, although Mounting evidence indicate that TyG index is correlate with the HOMA-IR, TyG index has high sensitivity for recognizing insulin resistance compared with the HOMA-IR (29). Thus, we focused on the TyG index due to its characteristics of simplicity and cost-efficient in this research.

Over the long-term follow-up (up to 8 years), we found that elevated TyG level are correlated with an increased risk of developing CMDs. Although the abovementioned idea has been demonstrated by previous studies (14, 15, 30–34), the measurement of the circulating TyG-index, which was measured only once, makes it impossible to reflect on the long-term influence between the TyG level and the development of CMDs. A meta-analysis combining 5 prospective studies showed a positive association between atherosclerotic cardiovascular disease (ASCVD) events and the TyG-index (33). A high TyG level has been reported to be closely correlated with T2DM in a prospective cohort study in Singapore (34). Our research further expands the pre-existing knowledge to explore the cumulative effect of the long-term TyG-index on the development of CMD occurrence in the Kailuan Study. The subjects in the fourth quartile had a 3.15-fold higher risk of developing CMDs than those in the first quartile. After correction for $TyG_{06}$ and $TyG_{12}$, when compared to the uncorrected data (HR: 3.00, 95% CI: 2.72-3.31), the HRs (95% CI) of the average $TyG_{06\text{-}12}$ index on CMDs were 2.51 (2.20-2.87) and 2.39 (2.14-2.67), respectively. This result suggests that the effect of the long-term TyG level on CMDs was independent and superior to the single point measurement of the TyG level. Moreover, when using updated confounders within the follow-up period, a significant short-term increase in the risk of CMDs with elevated TyG level was observed in this finding.

Given that TG and FBG, both of which are utilized to calculate the TyG-index, have been considered time-varying exposures. Of note, due to a single measurement of the TyG-index, this characteristic cannot be avoided for the potential regression dilution bias (35). It has been proposed that the above limitations and methodological insufficiencies can be addressed by assessing the influence of TyG-index change on the outcomes. Two cohort studies performed by Zhang et al (36)and Wang et al (16), demonstrated an association between change in the TyG-index and the risk of T2DM and CVD, respectively. However, those studies only considered the magnitude of the TyG-index change and not the direction. Compared with the stable TyG-index, our findings revealed that the effects of TyG level gain and loss were both directly correlated with a 14% increase in the risk of developing CMDs. This result suggests that the TyG-index change is positively related to the risk of CMDs occurrence, whether they are positive or negative. The results of this study were in accordance with previous studies on the effect of TyG-index gain on CMDs development; rather, the effect of TyG level loss has not been reported in the relevant literature, currently.

As a surrogate for identifying IR, the results from this research seem counterintuitive given that TyG-index loss was associated with CMDs. First, we found that the average TyG level was the highest in decrease group (Quintile 1) compared to other groups from the baseline table of change in TyG index (Table S9). Thus, it is reasonable to assume that, even if the current "ideal state" exists, long exposure to a high TyG-index during the early period of the observation window may increase the possibility of residual risk of CMDs before the TyG-index level decreases. Second, considering the fact that the determinants of TyG are the fasting glucose and triglycerides, similar conclusions have been reached in Table S9. For triglycerides, a clinical, randomized controlled trial (37) concluded that among patients with mild-to-moderate hypertriglyceridemia, the incidence of cardiovascular events was not lower among those who received pemafibrate than among those who received placebo, although pemafibrate lowered triglyceride level. Also, the"metabolic memory"effect may cause an increasing risk of CMDs when FBG is at a high level for a prolonged period of time in early life (38). Furthermore, owing to the possibility of reverse causality, participants in the TyG level loss category were more inclined to be at high risk for having a CMDs, such as drinkers and smokers (Table S9), who have greater odds of receiving health education about CMDs prevention, adherence to medication and consciously lowering TyG levels. Notably, although we have tried to adequately adjust for confounders, there are still other unconsidered or uncontrollable factors, such as some insidious diseases, that may cause unintentional TyG level loss during the protracted or chronic course of the disease. For the above reasons, it can be said that the true association between TyG level loss and CMDs risk may be distorted among subjects with relatively high TyG levels in the early period of life, which might cause the spurious association that the lower TyG-index increases CMDs risk.

Provocatively, we found that the TyG-index change in the early stage was more strongly associated with the occurrence of CMDs

after dividing the approximately 8-year observation window into three stages: early, intermediate and late. All these signs indicate that in a clinical setting, more attention should be given to individuals with significant TyG-index change. For high-risk individuals with an elevated TyG-index, early prevention and control added strong evidence-based support for the primary prevention of CMDs.

It was recently noted that the variability and longitudinal trajectory in the TyG-index, both regarded as novel and important indicators for outcome events, had strong predictive effects for cardiovascular diseases (39–42). With either variability or longitudinal trajectory in the TyG-index, the effects on CMDs risk in this research are consistent with a previous study that found those indicators (variability and trajectory) to be associated with CMDs risk. In the occurrence and development of CMDs, our findings reveal that long-term level and change in the TyG-index play an important role, further emphasizing the need to maintain TyG level homeostasis in CMDs prevention. From another long-term perspective, closely monitoring the TyG-index, could elicit the maximum potential health benefit. Simultaneously, our findings also provide novel ideas for a clinical solution to screen high-risk CMDs individuals.

Several potential mechanisms link the sustained TyG-index (insulin resistance) and CMDs. Endothelial dysfunction is considered a key pathophysiological process in atherosclerosis initiation and progression (43, 44). Basic experiments indicated that the impairment of IR can mediate the injury and functional alterations of smooth muscle cells, mononuclear macrophages, and vascular endothelial cells by interfering with the insulin signaling pathway, which in turn leads to endothelial dysfunction (44). Moreover, the prolonged hyperglycemia state induced by IR may result in oxidative stress, coagulation dysfunction and the chronic inflammatory reaction of the human body, which are considered to be the driving factors in the development of dyslipidemia, metabolic syndrome and CMDs (45). Lastly, individuals with a long-term high TyG-index tend to experience complications with other traditional risk factors and comorbidities, such as smoking, drinking, and hypertension, which significantly increases the risk of CMDs. Although adjusting for the above confounders, it might not completely eliminate effects that are biologically meaningful. To date, because addressing the power of all of the relevant questions is limited, further basic research is needed to elucidate the pathophysiological significance of this finding.

Our findings have salient public health and clinical implications regarding the prevention of CMDs event development. First, the importance of early well-controlled and prevention of the TyG-index among young and middle-age groups. Second, either long-term changes in TyG level or increased variability in the TyG-index increased the risk of developing CMDs events. Thus, health education, physical exercise and a balanced diet could be regarded as some measures to maintain the long-term TyG-index at the "ideal level". Third, along with the current TyG-index, we cannot ignore the importance of considering the history of TyG levels when assessing CMDs risks. Therefore, in terms of clinical application, the dynamic monitoring of the TyG-index and refined information on electronic medical records (including change and variability of the TyG-index) all shed new light on the prevention of CMDs.

This research has several strengths. The primary strength is that we conducted long-term follow-up of a large community-based population based on the Kailuan study to obtain more comprehensive biological information. Second, this is the first massive-scale prospective study to explore the relationships between long-term TyG-index levels and changes and incident CMDs. Moreover, several limitations of the current study must be acknowledged. First, the sex distribution of the study population was uneven (male 73.87%), while the characteristics and mechanisms of CMD occurrence may differ between males and females. Second, longitudinal data are naturally hampered by missing data at different time points according to an 8-year observation window. Third, due to the observational nature of the study, the association between long-term level and change in the TyG-index may not necessarily reflect any causal link. Fourth, although the diagnosis of diabetes in this study took into account the medical history and use of glucose-lowering drugs during the follow-up time, a proportion of the population in this study was still diagnosed based on a single measurement of FBG without the oral glucose tolerance test or detection of glycated hemoglobin levels, thus possibly underestimating the prevalence of T2DM as well as CMDs. However, this consideration is widely applied in many large-scale epidemiological studies at home and abroad, such as the Framingham study.

## Conclusion

To summarize, an increased CMDs risk was associated with long-term elevated level and change in the TyG-index, and even the related risk of CMDs from exposure during the early stage tended to be higher. This finding strongly underlined the importance of tracking the long-term maintenance of an appropriate TyG-index within the desirable range over time to prevent the development of CMDs effectively.

## 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 study was performed according to the guidelines of the Helsinki Declaration and was approved by the Ethics Committee of Kailuan General Hospital (approval number:2006-05). All participants agreed to take part in the study and provided informed written consent.

## Author contributions

WX wrote the main manuscript text and conceived and designed the study. LGao, HZ, LGuo, JL, HL, JS, and AX contributed to acquisition of data, analysis and interpretation of data and revision of the drafting of the manuscript. YW and SW

performed the manuscript review. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

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

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1148203/full#supplementary-material

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# A bibliometric analysis and visualization of literature on non-fasting lipid research from 2012 to 2022

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**Background:** Non-fasting lipid assessment can help predict cardiovascular disease risks and is linked to multiple diseases, particularly diabetes. The significance of non-fasting lipid levels in routine screening and postprandial lipid tests for potential dyslipidemia has not been conclusively determined. Various new lipid-lowering strategies have been developed to improve non-fasting dyslipidemia. Therefore, analysis of scientific outputs over the past decade is essential to reveal trends, hotspots, and frontier areas for future research in this field.

**Methods:** The Science Citation Index Expanded in the Web of Science Core Collection database was searched for publications related to non-fasting lipid research from 2012 to 2022. The regional distributions, authors, disciplines, journals, references, and keywords of the studies were analyzed using the bibliometric software VOSviewer and CiteSpace.

**Results:** A total of 4160 articles and reviews that met the inclusion criteria were included in this study. The output trend was established to be stable and the number of citation indices has been persistently increasing. A total of 104 countries/regions, 4668 organizations, and 20782 authors were involved in this research area. In terms of country, the United States had the largest number of publications (979). The University of Copenhagen was the most productive institution, publishing 148 papers. Professor Børge G Nordestgaard has made the most significant contribution to this field. *Nutrients* was the most productive journal while the *American Journal of Clinical Nutrition* was the highest co-cited journal. Analysis of co-cited references indicated that lipid-lowering strategies, statin therapy, high-fat meals, insulin resistance, physical exercise, and fructose were hotspots. Analysis of co-cited keywords revealed that apolipoprotein B, especially apolipoprotein B48, is becoming a key research focus. The keywords "gut microbiota" and "meal timing" were the most extensively studied.

**Conclusion:** The causal relationship between non-fasting dyslipidemia and diseases is currently being explored and the standards for non-fasting or postprandial lipid assessment are continuously being updated. Among the hotspots, lipid-lowering strategies are a potential research direction. Apolipoprotein B48, gut microbiota, and

chrononutrition are the research frontiers. This initial bibliometric analysis of non-fasting lipids will enable researchers to monitor swift transformations and recognize novel concepts for upcoming research.

KEYWORDS

non-fasting lipid, postprandial lipid, cardiovascular diseases, diabetes, oral fat tolerance test, bibliometric analysis, VOSviewer, citespace

# 1 Introduction

Lipid profile standard measurements include triglycerides (TG) and cholesterol. They also include lipoprotein (a), apolipoprotein B (Apo B), apolipoprotein A1 (Apo A1), lipoprotein subfractions, other apolipoproteins, and metabolomics phenotyping in expanded or additional lipid profiles (1). Guidelines and consensus statements from nearly ten years ago mandated that lipid profile samples be collected after a period of fasting. Study subjects were required to fast overnight for at least 8 h before blood samples were drawn. However, compared to fasting TG, non-fasting TG performed better in cardiovascular risk prediction (2). Evidence from prospective cohort studies based on large populations revealed that elevated non-fasting TG levels are independently associated with increased risks of myocardial infarctions, ischemic heart disease, death (3), total cardiovascular events (2), and ischemic stroke (4). Furthermore, blood sampling in the non-fasting state was convenient for both laboratory personnel and patients (5). In 2009, the Danish Society of Clinical Biochemistry was the first society to recommend the testing of lipid profiles in the non-fasting state to predict cardiovascular risk (6). Currently, the debate on fasting and non-fasting lipid measurements is ongoing. Societies, guidelines, and statements from the United States (7–9), Europe (10, 11), Canada (12), Brazil (13), and India (14) recommend routine screening via non-fasting lipid measurements.

The fasting state only occurs in a short period before breakfast, which does not reflect the real lipid concentration throughout a 24-hour day (5). Intestinal-derived lipoproteins, especially postprandial triglyceride-rich lipoproteins (TRL), can be assessed in blood samples after meals as supplementary to liver-origin lipoproteins in fasting samples. Among the lipid profiles, TG levels fluctuate with different meals and meal times. Immensely high TG levels interfere with estimation of LDL-C levels (15). Therefore, establishing optimal cut-offs for non-fasting lipid profiles is a major challenge in clinical practice. Postprandial TG concentration, which can be detected by an oral fat tolerance test (OFTT), is also a cardiometabolic risk factor (16). The OFTT standards have not been fully established (10).

Non-fasting lipid metabolism is not only associated with cardiovascular disease (CVD), but also with diabetes (17), metabolic syndromes (18), non-alcoholic fatty liver disease (19), and polycystic ovarian syndrome (20) among others. The pathophysiological processes associated with postprandial metabolic responses have a connection with insulin resistance (21), oxidative stress (22), endothelial dysfunctions (23), and gut microbiota (24) among others. Nutrition (25), physical exercise and medication (26) play an important role in prevention and treatment strategies. Given the high number of studies, it is important to summarize the worldwide status, trends, and major topics in non-fasting lipid research.

Bibliometrics is a cross-disciplinary technique applied to the quantitative and qualitative analysis of knowledge. It can reveal trends and hotspots by countries/regions, institutions, researchers, keywords, and references based on publications in a field. In this study, we used bibliometrics software to analyze the characteristics of emerging trends and directions for future research on non-fasting lipids.

# 2 Methods

## 2.1 Literature search and data collection

We searched the Science Citation Index Expanded 1900-present (SCI-E) of Web of Science Core Collection (WoSCC) from 1$^{st}$ January 2012 to 1$^{st}$ November 2022. This database contains impactful global academic publications, and the downloadable data information is suitable with the bibliometrics software. The search strategy was composed of the following topic words with MESH Unique ID and free words: ("Lipids (D008055)" OR "Hyperlipidemias (D006949)" OR "Triglycerides (D014280)" OR "Hypertriglyceridemia (D015228)" OR "Cholesterol (D002784)" OR "Hypercholesterolemia (D006937)") AND ("Postprandial period (D019518)" OR "non-fasting" OR "nonfasting"). The articles and reviews written in the English language were included in this study. Data were collected as plain text files with full records and cited references. The data acquisition flow chart is shown in Figure 1.

## 2.2 Data analysis and visualization

VOSviewer (1.6.18 version), CiteSpace (6.1.R4 basic version) and Microsoft Excel (2019 version) were used for bibliometric analyses and visualization. VOSviewer is a computer program

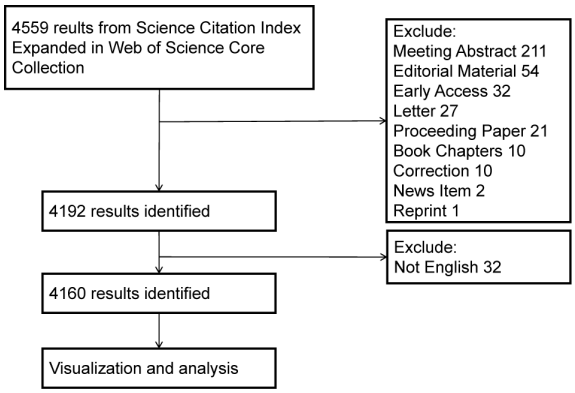


FIGURE 1
Retrieval and screening process flowchart. CiteSpace allows efficient evaluation of network visualization, while the VOSviewer provides clearer, user-friendly visualization.

supported by the Centre of Science at Leiden University in the Netherlands. VOSviewer was utilized to conduct co-authorship analysis, co-occurrence analysis, and bibliographic coupling, followed by visualizing intellectual structure (27). CiteSpace, created by Chaomei Chen at Drexel University in the United States, is a Java-based application (28). Evolution analysis was performed to investigate the development process of the research based on an understanding of its structure using CiteSpace. VOSviewer and CiteSpace use colorful nodes and links to display the characteristics and connections using information extracted from publications. Node sizes refer to the occurrences of a trait. The color of the nodes and connections depends on the cluster. Different clusters are diversely colorized.

First, we summarized the annual publications and citations and built a graph using Microsoft Excel. Second, we used VOSviewer to create a knowledge map based on co-authorship of countries/regions and organizations. The global geographical distribution of publications was established on www.mapchart.net. Third, the networks of authors and co-cited authors were built by VOSviewer. Author productivity and impact are measured by the H-Index in Web of Science. The H-index is defined by the number of publications *h*, where each publication has at least *h* citations (29). Fourth, the research areas summary was downloaded from the literature search website. The dual-map overlay of journals was created by CiteSpace. Fifth, bibliographic coupling journals and co-cited journals visualization were achieved using VOSviewer. Sixth, analysis of co-cited references by CiteSpace provided structural metrics, such as betweenness centrality (BC), citation burst, and cluster mapping (30). Nodes with BC (> 0.10) represented significant connections between clusters. To identify emerging hotspots, burst detection was conducted for a specific period. Burst strength can reflect the influence of an article or a keyword over time. Cluster view of the co-citation network automatically acquired the cluster labels by using "All in One" function of CiteSpace. The Modularity Q value and Sihouette S value were important in interpreting the cluster network. The Q value range was [0, 1]. A clustering modularization was considered significant when the Q value exceeded 0.3. The cluster network was considered better with elevated Q value. The S value range was [-1, 1]. When the S value is close to 1, the cluster is isolated from others. When the S value surmounted 0.3, 0.5, or 0.7, the cluster network was considered homogenous, reasonable, and highly credible. Finally, author keywords for each publication were extracted to create a timeline view of cluster networks and show the strongest citation bursts using CiteSpace. The number of publications can provide a general estimate of the amount of work produced by a team or facility, while citation rates are commonly considered as measures of a paper's quality, relevance, or interest, indicating the research's impact. Bibliometric maps provide a great deal of detail. Exploring fields and comparing maps to users' own expertise should enable users to access this underlying information. A cluster of research specialties is often identified. The map is used to visualize the relationship between countries and research fields (subfields). Co-citations provide a forward-looking assessment of document similarity. Citations can still vary over time due to changes in academic fields. A co-word analysis is used to identify a research field's structure based on keyword co-occurrences in publications.

# 3 Results

## 3.1 Annual trend of publications and citations

The annual distribution of publications and citations is shown in Figure 2. Based on our search strategy, we identified 4,160 papers (3,599 articles and 561 reviews), with no duplicates. In the past decade, the number of outputs in the non-fasting lipid field stably fluctuated between 300 and 450 per year. The peak of publications was in 2021, with 439 papers. The H index of this field, as reported by WoSCC (SCIE), was 110 and the average citation per item was 22.09. A regression model [$y = 87.958x^2 + 920.95x - 609.32$, $R^2 = 0.9973$] was adopted to fit the citation over time (2012–2021). 319 publications and 13576 citations from January to November 2022. The findings indicate that there is increasing attention being paid to the field of lipid research that is not limited to fasting conditions.

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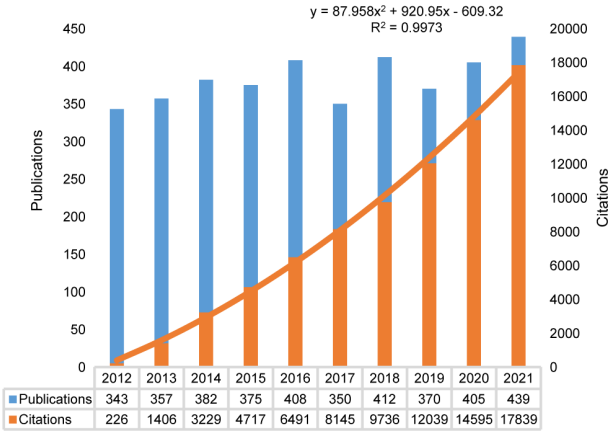


FIGURE 2
Annual trend of publications and citations. The orange line showed a regression model to illustrate citations per year (2012-2021). A total of 319 publications and 13576 citations were identified from January to November 2022.

## 3.2 Distribution of countries/regions and institutions

The obtained papers in the non-fasting lipid field were from 104 countries or regions. The top 10 countries/regions contributing to publications and citations were shown in Figure 3. The world map of publication distribution was shown in Supplementary Figure S1A. Almost half of the output was papers from the United States (979, 18.38%), China (608, 11.41%), the United Kingdom (432, 8.11%), Japan (301, 5.65%), and Canada (267, 5.01%). Publications from the United States (27957, 19.50%), the United Kingdom (12432, 8.67%), Denmark (9411, 6.56%), and China (8757, 6.11%) received the most citations. Regarding the average citation per item, the highest was Italy (30.49), followed by Canada (30.36), the United States (27.13), Netherlands (26.93), and Australia (26.8). Denmark, which had 193 outputs, ranked 11$^{th}$ among the productive countries and had 48.76 average citations per paper. Thirty countries published more than 30 papers. Co-authorship countries/regions network was as shown in Supplementary Figure S1B. Studies are active throughout countries in Western Europe, North America, Asia, Oceania, and South America. Cooperation among countries was found to be comprehensive (for instance, China collaborated actively with the United States, the United Kingdom, Canada, Denmark, and the Netherlands).

The analysis revealed 4668 organizations which were engaged in non-fasting lipid research. In Table 1, among the top 10 institutions, the University of Copenhagen ranked first in terms of the number of papers (148) and citations (8295). A total of 63 institutions published at least 20 papers in this field. The co-authorship institution network was as presented in Supplementary Figure S1C. European institutions, such as the University of Copenhagen, Maastricht University, and Instituto de Salud Carlos III had close cooperation with the University of Toronto and Harvard Medical School in North America, Shanghai Jiao Tong University and National University of Singapore in Asia,

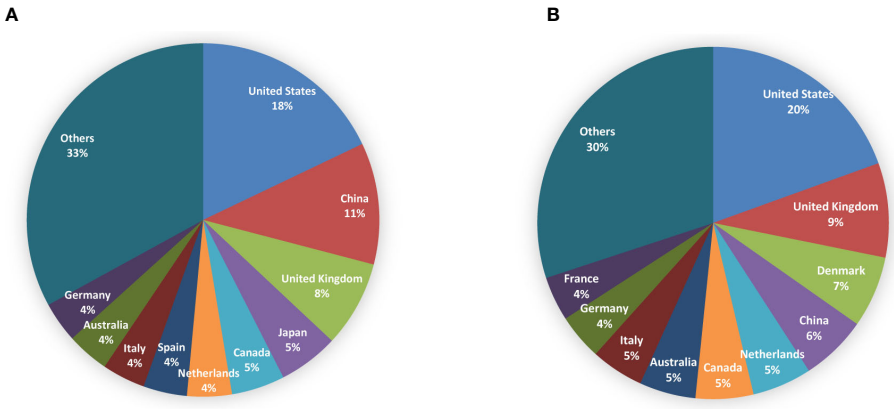


FIGURE 3
Pie charts for top 10 countries/regions contributing to publications **(A)** and citations **(B)** in the field of non-fasting lipid.

TABLE 1 Top 10 institutions engaged in research on non-fasting lipid.

| Rank | Institutions | Country | Publications | Citations | Citations per Document |
|---|---|---|---|---|---|
| 1 | University of Copenhagen | Denmark | 148 | 8295 | 56.05 |
| 2 | Maastricht University | Netherlands | 64 | 1647 | 25.73 |
| 3 | University of Toronto | Canada | 59 | 2331 | 39.51 |
| 4 | University of California, Davis | USA | 57 | 1696 | 29.75 |
| 5 | Instituto de Salud Carlos III | Spain | 52 | 1583 | 30.44 |
| 6 | Tufts University | USA | 42 | 1375 | 32.74 |
| 7 | French National Institute for Agricultural Research | France | 41 | 1508 | 36.78 |
| 8 | King's College London | United Kingdom | 40 | 1693 | 42.33 |
| 9 | University of São Paulo | Brazil | 40 | 866 | 21.65 |
| 10 | University of Helsinki | Finland | 39 | 1995 | 51.15 |

University of Western Australia in Oceania, as well as the University of São Paulo in South America.

## 3.3 Authors and co-cited authors

Globally, 20782 authors were involved in research on non-fasting lipid. The top 10 productive and co-cited authors, most of whom were from Europe, are shown in Table 2. Børge G Nordestgaard at the University of Copenhagen in Denmark ranked first with 43 publications. Out of the authors who published more than 10 papers in this particular field, a total of 89 authors were identified, and 58 of them had formed a collaboration network (Figure 4A). Børge G Nordestgaard closely cooperated with José López-Miranda, Pablo Perez-Martinez, and Marja-Riitta Taskinen among others. In Figure 4B, Børge G Nordestgaard was the highest co-cited author, with 1144 co-citations. Eighty-four authors were co-cited at least 100 times. Scott M Grundy, Jens Juul Holst, Børge G Nordestgaard, and Marja-Riitta Taskinen each had an H-index higher than 100, suggesting their contributions were significant.

## 3.4 Distribution of disciplines

The top 10 disciplines of non-fasting lipid research in WoSCC (SCI-E) are categorized in Figure 5A. Most of the papers referred to the disciplines of Nutrition Dietetics, followed by Endocrinology Metabolism, Biochemistry Molecular Biology, Food Science Technology, and Pharmacology Pharmacy among others.

In the dual-map overlay of journals (Figure 5B), major associations between disciplines of citing and cited journals are presented by the orange and green curves. The orange curve represents papers belonging to molecular/biology/genetics and health/nursing/medicine that are cited by molecular/biology/genetics. The green curve represents research output belonging to molecular/biology/genetics, health/nursing/medicine and environmental/toxicology/nutrition that are cited by medicine/medical/clinical researchers.

TABLE 2 Top 10 productive authors and co-citation authors in the research field of non-fasting lipid.

| Rank | Authors | Publications | H-index | Co-cited Authors | Co-citations | H-index |
|---|---|---|---|---|---|---|
| 1 | Børge G Nordestgaard | 43 | 144 | Børge G Nordestgaard | 1144 | 144 |
| 2 | José López-Miranda | 27 | 60 | Sandeep Bansal | 479 | 20 |
| 3 | Pablo Perez-Martinez | 26 | 48 | Anette Varbo | 395 | 36 |
| 4 | Jens Juul Holst | 25 | 159 | Scott M Grundy | 382 | 166 |
| 5 | Marja-Riitta Taskinen | 24 | 105 | Antonio Ceriello | 378 | 93 |
| 6 | Javier Delgado-Lista | 23 | 38 | Genovefa D Kolovou | 321 | 37 |
| 7 | Jan Borén | 22 | 66 | Jason M.R. Gill | 300 | 44 |
| 8 | Julie A Lovegrove | 22 | 51 | Kim G Jackson | 277 | 29 |
| 9 | Kim G Jackson | 21 | 29 | David R Matthews | 276 | 90 |
| 10 | Gerald F Watts | 21 | 89 | Anne Langsted | 272 | 32 |

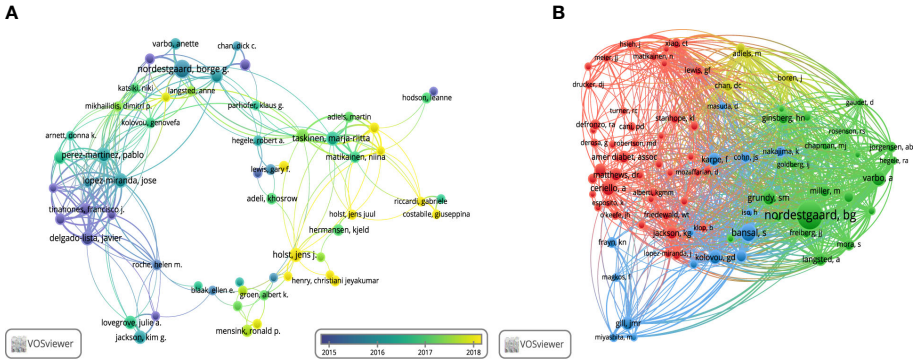


FIGURE 4
Visualization of authors. **(A)** Collaboration network of co-authors. **(B)** Network visualization of co-citation authors.

## 3.5 Journals and co-cited journals

Among the most productive journals, *Nutrients* (169, 4.1%) had the largest output, followed by *Plos One* (104, 2.5%), *British Journal of Nutrition* (84, 2%), *American Journal of Clinical Nutrition* (66, 1.6%), *Journal of Clinical Endocrinology & Metabolism* (59, 1.4%), and *Journal of Nutrition* (58, 1.4%) among others. Of note, 41 of the 940 journals published more than 20 papers in the non-fasting lipid field. The bibliographic coupling network of journals is shown in Figure 6A. The network of the co-cited journals is shown in Figure 6B. The *American Journal of Clinical Nutrition*, which was co-cited 6501 times, ranked first, followed by *Diabetes Care* (5272), *Diabetes* (4377), *Circulation* (4170), *Journal of Lipid Research* (3677), *Atherosclerosis* (3570), *Journal of Clinical Endocrinology & Metabolism* (3238), and *Journal of Nutrition* (3159). The *New England Journal of Medicine* and *JAMA-Journal of the American Medical Association* had the highest impact factors of 176.082 and 157.375, according to JCR 2021.

## 3.6 Co-cited references and references bursts

Co-cited references analysis was performed using CiteSpace, with setting parameters: timeline from 2012 to 2022, 1 year per slice, node type of references, and selection criteria g index (k = 5). We established a 245-node 1204-link network (Supplementary Figure S2A). The nodes surrounded by purple circles had relatively high BC, and were considered key turning points in the non-fasting lipid research field. Guidelines from European Atherosclerosis Society (EAS) about lipids (Chapman MJ, 2011; BC = 0.22) had the highest BC (31), followed by results from the Copenhagen City Heart Study

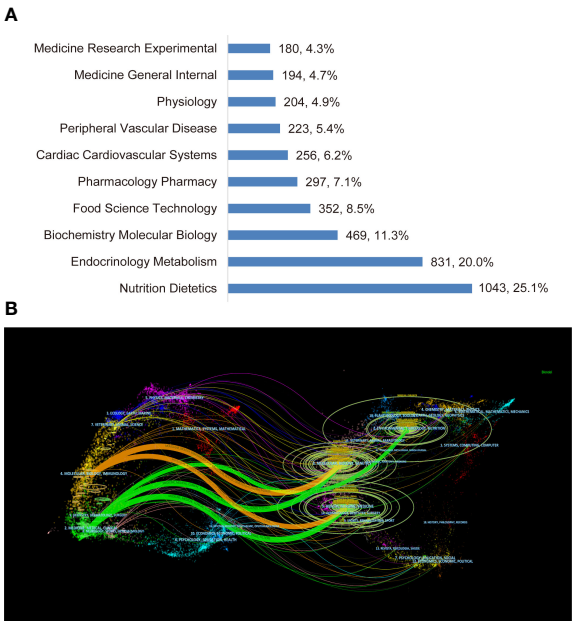


FIGURE 5
Visualization of disciplines. **(A)** The top 10 disciplines. **(B)** The dual-map overlay of journals.

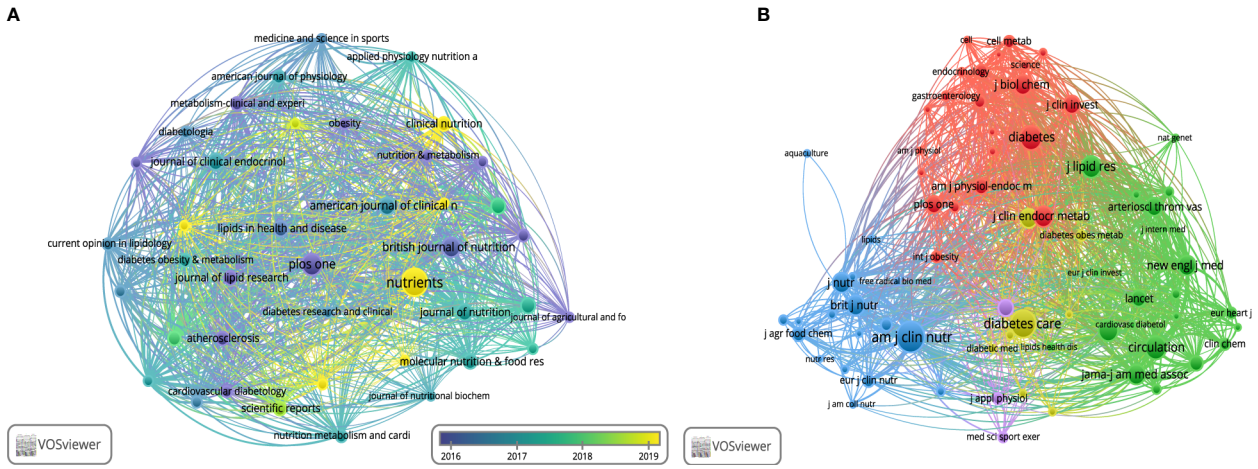


FIGURE 6
Visualization of journals. **(A)** Bibliographic coupling journals. **(B)** Co-citation journals.

(Nordestgaard BG, 2007; BC = 0.21) (3), a meta-analysis about diabetes and CVD (Sarwar N, 2010; BC = 0.12) (32), and a clinical trial about fructose and fat (Stanhope KL, 2009; BC = 0.11) (33).

We used the "All in One" button to automatically cluster and label the co-cited references. The network, consisting of 7 major clusters (Supplementary Figure S2B), was significant with a Q value of 0.472, and highly reliable with an S value of 0.832. From cluster #0 to #6, the number of articles in each cluster declined. The largest cluster #0 lipid-lowering strategies (n = 41, S = 0.814), followed by cluster #1 statin-treated patient (n = 40, S = 0.804), cluster #2 high-fat meal (n = 40, S = 0.67), cluster #3 metabolic response (n = 24, S = 0.901), cluster #4 insulin-resistant state (n = 23, S = 0.941), cluster #5 prior exercise (n = 19, S = 0.977), and cluster #6 syrup-sweetened beverage (n = 17, S = 0.914). The annual transformation of co-cited references is presented in Figure 7.

The burst detection function was run to screen citation bursts (Supplementary Figure S2C). The articles with the most powerful burst strengths were two prospective follow-up studies published in *JAMA-Journal of the American Medical Association* in 2007. One with a burst of 69.82 has been mentioned above (Nordestgaard BG, 2007) (3), the other with a burst of 60.04 reported on the association between non-fasting TG levels and cardiovascular events (Bansal S, 2007) (2). Review of TRL and a statement about determination of lipid profiles in non-fasting state (Nordestgaard BG, 2016) (34, 35) have received continuous attention from 2017 to 2022. Recent studies by Bhatt DL et al. (36), Ference BA et al. (37), Anderson TJ et al. (12), Mach F et al. (15), Ference BA et al. (38), Langsted A et al. (5), and Grundy SM et al. (7) have achieved significance.

## 3.7 Keyword evolution and bursts

Author keywords reflect the most crucial information in articles. These keywords were analyzed using CiteSpace with parameters; from 2012 to 2022, 1 year per slice, node types of keywords, with the selection criteria as the top 50 per slice. The "All in One" function was used to automatically recognize the clusters and labels. We established a 279-node 1283-link cluster network in Supplementary Figure S3A. The timeline view of author keywords revealed the evolution of research (Figure 8). Duplicates of keywords such as "triglycerides" and "triglyceride" were merged by thesaurus. Clusters were significant, with a Q value of 0.3263, and reasonable with an S value of 0.6561. The cluster label was the most important keyword for each cluster. The most outstanding cluster was cluster #0 apolipoprotein B, followed by cluster #1 type 2 diabetes, cluster #2 insulin resistance, cluster #3 cardiovascular disease, cluster #4 physical activity, cluster #5 fatty acids, and cluster #6 lipid metabolism. Among the nodes of clusters, postprandial lipemia (n = 176, BC = 0.17, #0), type 2 diabetes (n = 358, BC = 0.47, #1), insulin resistance (n = 188, BC = 0.13, #2), metabolic syndrome (n = 152, BC = 0.12, #2), cardiovascular disease (n = 248, BC = 0.37, #3), lipid metabolism (n = 94, BC = 0.12, #6) acted as joints between clusters with high frequencies of co-citation and BC. Oxidative stress (n = 98, #4) and fatty acid (n = 56, #5) have also been exhaustively investigated. Apo B48 (n = 27, #0), one of Apo B transcripts, has particularly become important in research.

The top 20 keywords with citation bursts are shown in Supplementary Figure S3B and are also highlighted with red nodes in Figure 8. The keyword with the strongest citation burst was "systematic review" (strength = 5.82), followed by "gut microbiota" (strength = 5.44), and "oral fat tolerance test" (strength = 5.34). These were potential topics that were associated with transformative discoveries. The keywords, "remnant cholesterol", "blood glucose", "gut microbiota", "systematic review", "lipid profile", "weight loss", and "meal timing", showed strong citation bursts until 2022.

# 4 Discussion

## 4.1 General information

A scientometric analysis and visualization were performed using studies on non-fasting lipid published from 2012 to 2022.

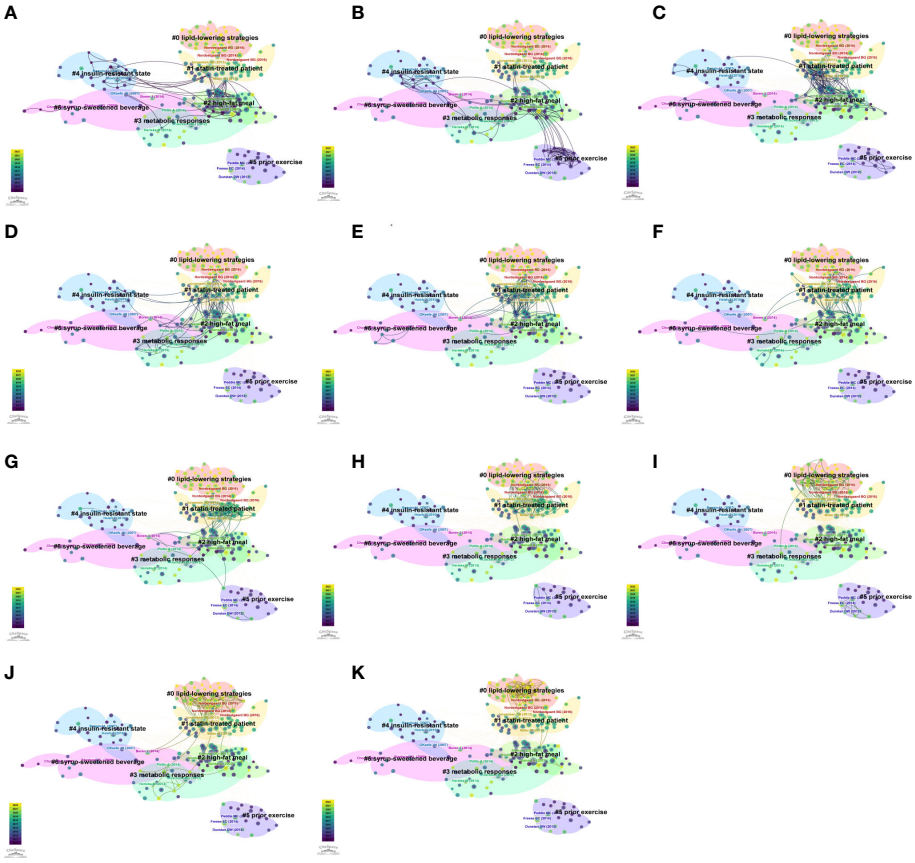


FIGURE 7
The annual trends of co-citation references network. **(A)** 2012. **(B)** 2013. **(C)** 2014. **(D)** 2015. **(E)** 2016. **(F)** 2017. **(G)** 2018. **(H)** 2019. **(I)** 2020. **(J)** 2021. **(K)** 2022.

The number of publications maintained a steady increase, with rapid citations. This implies that the non-fasting lipid research field has a bright future. Studies in this field were found to be relatively mature in the United States and the United Kingdom, with both productive publications and high citations per document. Although Denmark was the 11$^{th}$ most productive country, the average citation times for each document was the highest, indicating that Danish papers are of a higher quality. In terms of publications, the outputs are burgeoning and booming in China, however, the citations per document are relatively low, suggesting that there is still a long way for Chinese researchers. There were strong collaborations among countries/regions regarding research in this field. Globally, the

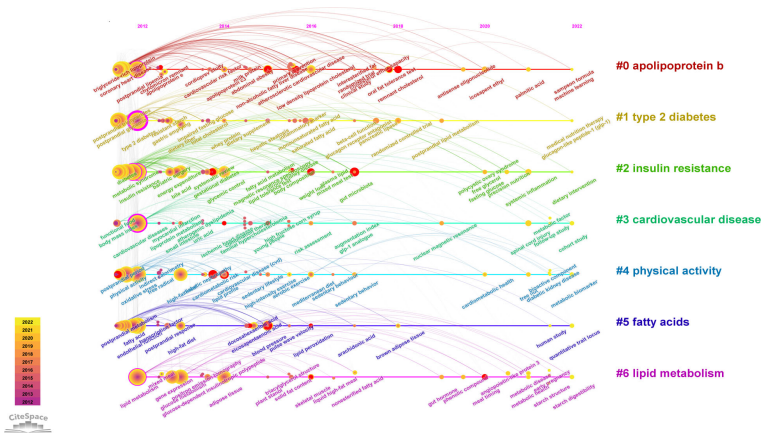


FIGURE 8
Timeline view of co-citation keywords.

University of Copenhagen in Denmark is the most influential center in this field, and exhibited the most active connections with other institutions. Professor Børge G Nordestgaard, who is affiliated to the University of Copenhagen, made the most outstanding contribution to this field. The dual-map overlaps and subject category list suggest this is a multidisciplinary area. Clinical medicine and its sub-groups, nutrition and food science, biochemistry and molecular biology complement each other in this field. In terms of journals, *Nutrients* is the most popular journal, with the largest number of publications on non-fasting lipid. Among the most co-citation journals, *American Journal of Clinical Nutrition* ranked the first.

## 4.2 Emerging trends, hotspots, and frontiers

Co-citation reference networks reveal the research structure in the non-fasting lipid field. If a reference is co-cited by numerous publications, it is considered a basis in the field. The top 20 references with the strongest bursts and cluster transformation illuminated the trends of the field. Among the co-citation references, four papers had a high BC, indicating that potential structural variations or revolutionary points may occur at these nodes (3, 31–33). Through analysis of context in references, we concluded that cluster labels in Figure 7 from #0 to #6 represent the following topics: #0 lipid-lowering strategies, #1 statin therapy, #2 high-fat meal, #3 metabolic response, #4 insulin resistance, #5 physical exercise, #6 fructose. The timeline view of co-cited keywords shows that hotspots in the non-fasting lipid area varied over time. Cluster analysis of keywords revealed the hot topics. Keywords with the strongest bursts represent alterations of frontiers. The #0 cluster label of co-occurrence keywords was Apo B, indicating that research related to Apo B recently received the most interest. Based on occurrence time, burst strength and maintenance period, “oral fat tolerance test”, “gut microbiota”, and “meal timing” keywords were the most noteworthy topics.

The co-cited references and keyword networks showed that research focus in this field has shifted from observational studies recognizing hazards of non-fasting lipid disorders in diverse diseases to investigating management methods and implementation of preventive strategies. Causal relationships among non-fasting dyslipidemia, CVD, and type 2 diabetes are still under investigation. The major aspects of management approaches include non-fasting lipid routine test and OFTT to identify potential postprandial dyslipidemia. Prevention strategies include lipid-lowering medications, lipid modification by anti-diabetic medications, and lifestyle interventions, such as physical exercise and diet changes. The association between gut microbiota and non-fasting lipid are being investigated.

### 4.2.1 Observational evidence

Two prospective cohort studies published in 2007 are milestones in this field. One of them provided powerful evidence on the importance of non-fasting rather than fasting TG as the crucial risk factor for cardiovascular events (2). The other study, which showed that elevated non-fasting TG levels are associated with an increased risk of myocardial infarction, ischemic heart disease, and death acted as a junction for researches about #1 statin therapy, #2 high-fat meal, #3 metabolic response, #4 insulin resistance, and #6 fructose from 2012 to 2016 (3). Elevated non-fasting TG levels have also been shown to be a risk factor for ischemic stroke (4). However, due to the complexity of TG metabolism, it has not been established whether TG causes CVD. When TG levels are elevated, TRL, chylomicrons and very low-density lipoprotein (VLDL) contains more TG or increases in number of particles. In 2016, it was proven that elevated TRL, which cannot be fully reflected in a fasting sample, is a causal risk factor for inflammation, CVD and mortality (34). Recently, mendelian randomization analysis showed that suppressed Apo B levels reflect the benefits of reducing TG and LDL-C concentrations for a low risk of coronary heart disease, complied with genetic variants (38). Based on increasing evidence, routine Apo B assessments should be considered in clinical practice. A previous meta-analysis reporting that diabetes and fasting blood glucose are risk factors for various vascular diseases formed the bond in researches on #1 statin therapy, #2 high-fat meal, #3 metabolic responses, #4 insulin resistance, and #6 fructose from 2012 to 2017 (32). The research article reported that it was fructose and not glucose that induces insulin resistance and dyslipidemia, which led to researches on #6 fructose with #4 insulin-resistance and #3 metabolic response from 2015 to 2017 (33). Research heat on #6 fructose faded in 2018.

### 4.2.2 Screening strategies

In the past decade, global guidelines and consensus have emphasized non-fasting lipid management. According to the 2011 European Society of Cardiology (ESC)/EAS guidelines for dyslipidemia management, high-density lipoprotein cholesterol (HDL-C), TC, Apo B, and Apo A1 levels can be assayed in non-fasting samples, whereas TG levels and LDL-C, which is calculated by the Friedewald formula should be assessed in fasting samples (39). The 2011 EAS guidelines for patients who achieved the LDL-C goal but still had high cardiovascular risks recommend that elevated TG, TRL and remnant cholesterol, and/or low HDL-C levels require further clinical management. The 2011 EAS guidelines also acted as the most influential bridge among researches on #4 insulin resistance, #2 high-fat meals, and #2 statin therapy from 2012 to 2019 (31). The 2013 American College of Cardiology/American Heart Association guidelines for cholesterol reduction to prevent atherosclerotic cardiovascular risk provided the non-fasting non-HDL-C and TG cut-off points for reappraisal. However, the guidelines preferred fasting lipid assessments (40). In 2016, the EAS/European Federation of Clinical Chemistry and Laboratory Medicine joint consensus provided a recommendation that non-fasting blood samples be used for routine screening of lipid profiles. Among the lipid profiles, TG, TC, and calculated concentrations of LDL-C, remnant cholesterol, and non-HDL-C are mostly affected

by non-fasting status, whereas concentrations of HDL-C, Apo A1, Apo B, and lipoprotein(a) are not affected. Thus, the guidelines provided the cut-off levels for situations where fasting lipids are required to complement routine measurements of non-fasting lipid (35). The Canadian guidelines for dyslipidemia management also recommended non-fasting lipid and lipoprotein examinations during routine screening (12). The 2019 ESC/EAS guidelines showed that in terms of screening for prognostic outcomes of cardiovascular risks, high TG levels of 0.3 mmol/L (27 mg/dL) in non-fasting samples had equivalent clinical significance to fasting samples (15). However, fasting samples are recommended for patients with metabolic syndromes, diabetes, severe dyslipidemia, and follow-up for patients with hypertriglyceridemia (15, 41).

### 4.2.3 Postprandial metabolic responses

Almost 40 years ago, Zilversmit hypothesized that atherogenesis might occur over a postprandial period when chylomicrons and/or remnants are elevated (42). Postprandial responses represent a typical non-fasting state (43). Based on available evidence, appropriate strategies should be developed to screen for and treat postprandial dysmetabolism to prevent CVD and diabetes (44). The OFTT is used to identify patients with postprandial lipidemia. However, this approach should be standardized. In 2011, experts established guidelines on non-fasting and postprandial TG levels, including classified concentrations, a practical project of OFTT, and treatment recommendations (16). However, evidence of elevated postprandial TG is not conclusive. In 2019, OFTT trials were summarized in a series of metabolic diseases and non-fasting TG is still considered as evidence for postprandial hypertriglyceridemia measurements (10). Implementable schemes and the benefits of fat overload have not been fully established. Metabolic responses of fat overload tests that had been adjusted to body weight versus standardized high-fat meals were compared in a randomized crossover study, suggesting that postprandial TG levels of healthy participants fluctuate in an acceptable scope with both interventions, supporting the feasibility of standardized meals in both scientific studies and clinical practice (45). Apo C3 reflects the risk of postprandial TG disorders (46). Instead of fat distribution, postprandial TG levels may reflect early fat metabolic disturbances (47). Chinese people with fasting TG levels in the range of 1.0 to 1.7 mmol/L may benefit most from OFTT to identify postprandial dyslipidemia (48). The triglyceride-insulin-glucose-glucagon-like peptide-1 model was built to assess glucose, insulin, and incretin responses following a high-fat meal (49). The OFTT can help patients identify insulin sensitivity impairments, insulin resistance, beta-cell dysfunctions and secretion disorders of gut hormones, including glucose-dependent insulinotropic polypeptides and glucagon-like peptide-1 (21, 50).

Apo B is considered to be an indicator of atherogenic lipoproteins. Particularly, Apo B48 is an emerging hotspot that is increasingly being studied. After a meal, TGs are packaged into chylomicrons particles and transported to various tissues. Apo B48 which is synthesized by the intestines is a unique biomarker of postprandial intestinal chylomicron metabolites, which indicates the absorption of TG (51). Observational studies have reported that elevated fasting Apo B48 remnants associated with fat intolerance can predict postprandial Apo B48 disorder and increase the risk of CVD in adolescents with obesity (52). Postprandial Apo B48 contributes to subclinical atherosclerosis in patients with rheumatoid arthritis (53). The intestinal FGF15/19-SHP-TFEB pathway is activated after a meal, which mediates lipophagy and regulates postprandial TG and chylomicron via Apo B48. This study revealed a novel target for postprandial dyslipidemia treatment (54). Statin therapy can prevent the production of Apo B48 in intestines and promote the catabolism of Apo B48 to reduce postprandial TG synthesis (55). Semaglutide can decrease the production of postprandial Apo B48 levels (56). T2DM patients treated with liraglutide showed decreased levels of Apo B48, swelling of chylomicrons, reduced chylomicrons and remnant formation (57). Diet composition can also affect Apo B48 levels. A comparison of two common saturated fat of mixed meals, higher palmitic-acid intake found lower Apo B48 levels than higher stearic-acid intake (58).

### 4.2.4 Medication regimens

Evidence from many studies has confirmed that LDL-C causes atherosclerotic cardiovascular disease (37). The guidelines show that statin therapy should be the preferred treatment to reduce LDL-C levels compared with other lipids-lowering strategies because it can prevent and improve the prognosis of cardiovascular events (7, 12, 15). Elevated TG cannot be underestimated, especially in a non-fasting state (59).The results of the IMPROVE-IT trial revealed that the combination of ezetimibe and statin therapy was more effective and did not cause adverse effects (60). In 2017, evolocumab combined with statin therapy was found to reduce the risk of cardiovascular events in the FOURIER trial (61). In 2019, the results of REDUCE-IT trial showed that icosapent ethyl decreased the risk of ischemic events among patients with hypertriglyceridemia receiving statin therapy (36). As shown in the co-citation reference networks from 2019 to 2022, #1 statin therapy was changed to #0 lipid-lowering strategies, suggesting that other drugs for dyslipidemia treatment are under investigation.

For the cluster #4 insulin resistance, the main research topic was the cardiovascular protective effects of anti-diabetic medications due to improved postprandial lipids metabolism, particularly, incretin-based drugs including dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-1 receptor agonists (62, 63). Some of these medications have been proven to reduce TRL significantly (57, 64).

### 4.2.5 Lifestyle interventions

Lifestyle interventions included physical exercise and chrononutrition. Research on the application of #5 physical exercise to reduce postprandial lipidemia increased in the 2013-2014 and 2018-2020 period. From the cluster analysis of keywords, we found that oxidative stress was highly linked to physical exercise. Endurance exercise can have a beneficial impact on postprandial TG levels, however, it does not seem to affect markers of oxidative

stress (65). Regular physical exercise even walking can improve postprandial lipidemia and blood glucose (66, 67). Aerobic exercise has similar effects with statin therapy in terms of reducing postprandial TG levels as reported in a previous meta-analysis (68). In 2017, scientists won the Nobel Prize for elucidating the biological clock. Since then, research on the circadian variation of lipids has experienced bursts into hotspots. Meal timing strongly affects postprandial lipid profiles (69). Nighttime food intake may also increase the risk of dyslipidemia and hence potential of CVD due to postprandial TG metabolic dysfunction (70). Feeding time restricted within eight hours for five days can increase the fat oxidation rate in both fasting and non-fasting state, but cannot affect postprandial lipemia (71).

#### 4.2.6 Gut microbiota

Variation in intestinal microbiota composition and drugs targeted at gut microbiota have been investigated from animals to human beings (9). In animal studies, a high-fat/high-fructose diet causes gut microbial alteration in hamsters leading to metabolic syndrome (24). Extracts of fermented green tea were reported to reduce high-fat diet-induced hypertriglyceridemia in hamsters by decreasing the proportion of phylum Firmicutes in gut microbiota (72). In human studies, the PREMOTE trial showed that the combination of berberine and probiotic improved postprandial dyslipidemia among T2DM patients, providing a novel therapy targeting gut microbiota (73). Importantly, the PREDICT 1 trial built a machine-learning model to evaluate metabolic responses to food intake involving meal nutrients, meal timing, physical exercise, diurnal rhythm, and gut microbiota. This model can function as an algorithmic tool that allows for the accurate customization of nutritional strategies in the treatment of illnesses (25).

### 4.3 Advantages and limitations

This is the first bibliometric analysis to provide a credible account of the current state of research on non-fasting lipids. The findings of this study are expected to shed light on new directions and reveal ideas for further investigations. However, there are still some limitations. First, owing to the nature of the bibliometric software, we only analyzed data from the WoSCC (SCI-E) database. Second, only English-language papers were analyzed. Therefore, this study may not fully represent the entire spectrum of this field. Third, some new ideas may have been overlooked because the most recently published papers often have lower citations.

## 5 Conclusions

This bibliometric analysis of non-fasting lipid from 2012 to 2022 was conducted to visualize trends, hotspots and frontiers. The study of lipids in a non-fasting state is highly important in various fields including nutrition, endocrinology, cardiology, pharmacology, molecular biology, and sports medicine. In this field, researchers have switched from observing hazards of non-fasting dyslipidemia to elucidating the causal relationship of non-fasting dyslipidemia in diseases, especially cardiovascular diseases and diabetes. The guidelines and expert consensus are constantly being updated regarding the recommendations for non-fasting lipid assessment. The investigation of standardized OFTT to identify potential postprandial dyslipidemia is ongoing. The lipid-lowering strategies, statin therapy, high-fat meal, insulin resistance, physical exercise, and fructose are hotspots. Identification of optimal lipid-lowering strategies should be intensified with a focus on lipid-lowering drugs, lipid-reducing effects of anti-diabetic drugs (especially incretin-based drugs), physical exercise and nutrition therapies. Apo B48, gut microbiota, and chrononutrition are the frontiers in the research field of non-fasting lipid.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

## Author contributions

YH: Conceptualization, Methodology, Formal analysis, Data Curation, Visualization, Writing - Original Draft. ZA: Validation, Formal analysis, Data Curation. XH and YG: Validation, Supervision. GS: Conceptualization, Supervision, 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.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1136048/full#supplementary-material

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# Association of alpha-aminoadipic acid with cardiometabolic risk factors in healthy and high-risk individuals

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**Introduction:** Plasma levels of the metabolite alpha-aminoadipic acid (2-AAA) have been associated with risk of type 2 diabetes (T2D) and atherosclerosis. However, little is known about the relationship of 2-AAA to other cardiometabolic risk markers in pre-disease states, or in the setting of comorbid disease.

**Methods:** We measured circulating 2-AAA using two methods in 1) a sample of 261 healthy individuals (2-AAA Study), and 2) in a sample of 134 persons comprising 110 individuals with treated HIV, with or without T2D, a population at high risk of metabolic disease and cardiovascular events despite suppression of circulating virus, and 24 individuals with T2D without HIV (HATIM Study). We examined associations between plasma 2-AAA and markers of cardiometabolic health within each cohort.

**Results and discussion:** We observed differences in 2-AAA by sex and race in both cohorts, with higher levels observed in men compared with women, and in Asian compared with Black or white individuals ($P<0.05$). There was no significant difference in 2-AAA by HIV status within individuals with T2D in the HATIM Study. We confirmed associations between 2-AAA and dyslipidemia in both cohorts, where high 2-AAA associated with low HDL cholesterol ($P<0.001$) and high triglycerides ($P<0.05$). As expected, within the cohort of people with HIV, 2-AAA was higher in the setting of T2D compared to pre-diabetes or normoglycemia ($P<0.001$). 2-AAA was positively associated with body mass index (BMI) in the 2-AAA Study, and with waist circumference and measures of visceral fat volume in HATIM (all $P<0.05$). Further, 2-AAA associated with increased liver fat in persons with HIV ($P<0.001$). Our study confirms 2-AAA as a marker of cardiometabolic risk in both healthy individuals and those at high cardiometabolic risk, reveals relationships with adiposity and hepatic steatosis, and highlights important differences by sex and race. Further studies are warranted to establish molecular mechanisms linking 2-AAA to disease in other high-risk populations.

KEYWORDS

2-AAA, diabetes, cardiometabolic disease, HIV, biomarker

# Introduction

Cardiometabolic diseases, including diabetes and cardiovascular disease (CVD) are increasing in prevalence globally and represent a major contributor to mortality (1). Known risk factors include obesity, dyslipidemia, dysregulated glucose metabolism and inflammation (2). However, after accounting for these risk factors there remains a high degree of variability in disease susceptibility. There is a clear need for more refined biomarkers of cardiometabolic risk to improve our understanding of the underlying disease mechanisms and to improve prediction and treatment of at-risk individuals.

Cardiometabolic diseases are characterized by changes in metabolism that may contribute to disease pathophysiology, or may act as biomarkers of disease progression (3). Circulating metabolites that associate with disease states can shed light on underlying disease etiology, biological mechanisms, and may have clinical utility for prediction (4). Strategies to identify individuals at high cardiometabolic risk and to modulate disease processes in these individuals before onset of overt disease, would have significant impact in reducing mortality, morbidity, and healthcare costs. For this approach to be successful, early biomarkers of disease that predict at-risk individuals are required, as well as discovering novel pathways for therapeutic targeting. To this end, studying both healthy individuals, as well as individuals with conditions that place them at higher risk of cardiometabolic diseases, may provide an important model to identify novel physiologic relationships.

The metabolite alpha-aminoadipic acid (2-AAA) is associated with the development of type 2 diabetes (T2D) (5) and atherosclerosis (6), potentially identifying at-risk individuals before development of other known risk markers (7). Relatively little is known about the function of 2-AAA, or potential mechanisms linking 2-AAA to disease. 2-AAA is derived from the breakdown of the essential amino acid lysine, and is primarily metabolized within mitochondria, with potential involvement in oxidative stress (8, 9). Elevated 2-AAA is associated with increased insulin secretion, obesity, and dysregulated mitochondrial metabolism (5, 7, 10–13). This makes 2-AAA an interesting novel candidate in cardiometabolic disease biology. However, the relationships between 2-AAA and other cardiometabolic risk markers have not been well-described.

The purpose of this study was to characterize the association between 2-AAA and other demographic and circulating markers in a sample of healthy individuals, as well individuals at high risk of metabolic and cardiovascular disease. As chronic viral infections, including treated human immunodeficiency virus (HIV), predispose individuals to a higher incidence of cardiometabolic disease and earlier onset, these conditions can serve as an models of exaggerated or accelerated risk to further identify important physiologic relationships (14–17). Here, we assess the relationship of 2-AAA with range of cardiometabolic disease conditions and risk factors among healthy individuals and those with treated HIV infection.

# Materials and methods

## Study populations

Samples and data from two independent studies are included here. Participants of both studies were recruited from the same geographic area (Nashville, TN, and surrounding areas), and study procedures completed at Vanderbilt University Medical Center.

## Determinants of 2-AAA: screening study

Healthy adults (non-pregnant and non-lactating women and men, age 18-45 years) were recruited to complete a single study visit as part of a cross-sectional study at Vanderbilt University Medical Center between November 2018 and June 2021. Exclusion criteria included body mass index (BMI) >30 kg/m$^2$, active use of tobacco products, active use of prescription medications (apart from hormonal birth control), and diagnosis of diabetes mellitus, cardiovascular disease, renal disease, liver disease, or bleeding disorders. Data for 261 individuals who completed study procedures (vital signs, anthropometric measurements), provided a fasting blood sample, and had sufficient plasma available for 2-AAA measurement are included in the current analysis. All participants provided written, informed consent, and the study was approved by the Vanderbilt University Institutional Review Board.

## The HIV, adipose tissue immunology, and metabolism study

Adults with human immunodeficiency virus (HIV, N=112) were recruited from the Vanderbilt Comprehensive Care Clinic between August 2017 and November 2019. Participants were on combination antiretroviral therapy (ART) for ≥18 months, with a minimum of 12 months of sustained suppression of plasma viremia at enrollment and had no known inflammatory or rheumatologic conditions. Exclusion criteria were self-reported heavy alcohol use (>11 drinks/week), known cirrhosis, active hepatitis B or C, cocaine or amphetamine use, and use of corticosteroids or growth hormones. By design and to enrich for the presence of cardiometabolic disease, the cohort enrolled approximately equal numbers of individuals who were normoglycemic (HbA1c < 5.7 or fasting blood glucose (FBG) < 100 mg/dL); pre-diabetes (HbA1c 5.7%-6.4% and/or FBG 100-126 mg/dL); and diabetes (HbA1c ≥ 6.4%, and/or FBG ≥ 126 mg/dL or on diabetes medication). To allow for direct comparison of 2-AAA levels with HIV-negative individuals, the study also recruited individuals with diabetes but without HIV (N=24). Participants provided written, informed consent, and the study was approved by the Vanderbilt University Institutional Review Board (ClinicalTrials.gov Identifier: NCT04451980).

## Measurement of 2-AAA

In the *2-AAA Study*, plasma levels of 2-AAA were quantified by liquid chromatography mass spectrometry (LCMS) at the Vanderbilt Mass Spectrometry Core. Samples were spiked with internal standard (Arginine-15N4, Sigma Aldrich), extracted with methanol, and derivatized with dansyl chloride (Sigma Aldrich) prior to analysis. The dansyl derivative of 2-AAA ([M+H]+ 395.1271) was measured by targeted selected ion monitoring (SIM) using a Vanquish ultrahigh performance liquid chromatography (UHPLC) system interfaced to a QExactive HF quadrupole/orbitrap mass spectrometer (Thermo Fisher Scientific). Data acquisition and quantitative spectral analysis were conducted using Thermo-Finnigan Xcaliber version 4.1 and Thermo-Finnigan LCQuan version 2.7, respectively. Calibration curves were constructed by plotting peak area ratios (2-AAA/Arg-15N4) against analyte concentrations for a series of 2-AAA standards. Electrospray ionization source parameters were tuned and optimized using an authentic 2-AAA reference standard (Sigma Aldrich) derivatized with dansyl chloride and desalted by solid phase extraction prior to direct liquid infusion.

In the *HATIM Study*, plasma 2-AAA was measured as part of a metabolomics panel, at the Southeast Center for Integrated Metabolomics (SECIM) at the University of Florida, using previously described methods (18, 19). Briefly, plasma samples were spiked with internal standards solution. Proteins were precipitated using 8:1:1 Acetonitrile: Methanol: Acetone (Fisher Scientific, San Jose, CA), and the supernatant dried under a gentle stream of nitrogen at 30°C (Organomation Associates, Inc., Berlin, MA). Samples were reconstituted with injection standards solution. LC-MS untargeted metabolomics was performed on a Thermo Q-Exactive Orbitrap mass spectrometer equipped with a Dionex UPLC system (Thermo, San Jose, CA). Percent relative standard deviation of internal standard peak areas were calculated to evaluate extraction and injection reproducibility. Mzmine 2 was used to identify features, deisotope, align features and perform gap filling. The data was searched against SECIM internal retention time metabolite library. All adducts and complexes were identified and removed from the data set. Ion counts from features mapping to alpha-aminoadipic acid in positive ion mode were summed for analysis. Because measurement of 2-AAA was conducted at different sites, studies were analyzed separately.

## Lipid and biomarker measurement

In the *2-AAA Study*, serum lipids were profiled at the Vanderbilt Lipid Laboratory. Briefly, total cholesterol and triglycerides (TG) were measured by standard enzymatic assays. High-density lipoprotein (HDL) was measured with the enzymatic method after precipitation of VLDL and LDL using polyethylene glycol reagent (PEG). LDL cholesterol was calculated using the Friedewald equation (20). In the *HATIM Study*, fasting plasma HDL, LDL, and TG were measured using the selective enzyme hydrolysis method (Abbott, Chicago, IL). In the 2-AAA Study, fasting glucose was measured at the study visit by finger prick (AimStrip Plus Blood Glucose Meter, Germaine Laboratories Inc., San Antonio TX). In the HATIM Study, insulin was measured by radioimmunoassay (Millipore Cat. # PI-13K). The assay utilizes $^{125}$I -labeled insulin and a double antibody/PEG technique to determine serum insulin levels. The assay was modified by the Vanderbilt Hormone and Analytical Services Core to improve the sensitivity to 1uU/ml(0.04ng/ml). Glucose and hemoglobin A1c (HbA1c) were measured in fasting blood samples at the Vanderbilt Clinical Chemistry Laboratory.

## Body composition analysis

In the *HATIM Study*, individuals underwent computed tomography (CT) imaging using a Siemens Somatom Force multidetector scanner (Erlangen, Germany) to acquire chest, abdominal and liver images, as described (21, 22). Briefly, separate non-contrast electrocardiogram-gated thorax (top of the aortic arch through the lung base) and abdominal (diaphragm to lumbosacral junction) scans were performed using a scanning protocol and image interpretation approach previously described (23–25). Abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) volumes were measured within a 10-mm block of images consisting of eight images, 1.25-mm thick, at the L4-5 vertebrae using Osirix software. Pericardial adipose tissue (PAT) volume was measured within a 45-mm block of images spanning 15 mm above and 30 mm below the superior extent of the left main coronary artery, which includes the adipose tissue located around the epicardial coronary arteries (left main coronary, left anterior descending, right coronary, and circumflex arteries) as well as the epicardial and PAT around the coronary arteries (26, 27). Images at T12-L1 were used to identify the liver below the right diaphragm corresponding to superior aspects of the right and medial lobes or hepatic segments 4a, 7, and 8 using the Couinaud classification system. Three regions of interest within homogenous portions of the liver at three levels were identified and liver density was averaged from the nine total regions. Tissue radiodensity was quantified using the Hounsfield Units scale where water has a value of 0 HU and air has a value of -1000 HU.

## Statistical analysis

Plasma 2-AAA was assessed for normality of distribution through visualization, and testing for skewness and kurtosis, and was found to follow a normal distribution in both the 2-AAA and HATIM studies. Two individuals were considered outliers for 2-AAA in HATIM (>3 SD from the mean) and were removed prior to analysis. Associations between 2-AAA and continuous variables were analyzed using linear regression models and correlation analysis. Analyses between 2-AAA and discrete variables were analyzed by T-test or ANOVA. Models were adjusted for sex and race in both studies and for additional covariates in HATIM (smoking, diabetes group). Inclusion of age in the model did not alter the results, and was not included in the base models. Models were further adjusted for other risk factors as indicated in the

corresponding results sections, including BMI, cholesterol, HDL, LDL, TG, fasting glucose. P<0.05 was considered statistically significant, and Bonferroni P<0.05 considered statistically significant for *post hoc* multiple testing correction. Analyses were completed and results visualized using IBM SPSS Statistics version 28 (IBM, Armonk NY) and GraphPad Prism version 9.4.1 (GraphPad Software, San Diego, CA).

# Results

The characteristics of the participants of the 2-AAA Study are shown in Table 1. Characteristics of the participants of the HATIM Study are shown in Table 2. Participants of the 2-AAA study were 72% female, and 74% white, with an average age of 28 years. Participants of the HATIM study were 67% male, and 54% white, with an average age of 48 years. Plasma 2-AAA in persons with HIV (PWH) with diabetes (ion count $312x10^4 \pm 75x10^4$) was slightly higher than that in HIV-negative with diabetes (ion count $271x10^4 \pm 74x10^4$), but the difference was not statistically significant (P=0.08).

## Plasma 2-AAA levels are higher in men than in women, and higher in Asian individuals

There was a significant difference in plasma 2-AAA by sex in the 2-AAA Study, with higher levels in men than in women (plasma 2-AAA 95.99 ± 33.7 vs. 68.43 ± 27.7 ng/ml, P<0.0001; Figure 1A). A similar difference by sex was observed in the HATIM Study samples, with higher levels in men than women (plasma 2-AAA ion count $281x10^4 \pm 73\ x10^4$ vs. $242\ x10^4 \pm 65\ x10^4$ ion count, P=0.004; Figure 1C). Because other risk factors also differ by sex, we performed stepwise linear regression models including risk factors (age, BMI, fasting glucose, cholesterol, HDL, LDL, TG), and found that the associations with sex remained significant (P<0.001 *2-AAA Study*, P<0.02 *HATIM Study*). We observed a significant difference by self-reported race in the 2-AAA Study (Overall P=0.002; Figure 1B), with individuals self-identifying as Asian having borderline significantly higher plasma 2-AAA (95.68 ± 35.5 ng/ml) compared with individuals self-identifying as Black or African American (72.26 ± 30.0 ng/ml, P=0.05), or white (72.73 ± 30.7 ng/ml, P=0.007). This was not attributable to differences in sex distribution, age, or risk factors between groups. In fact, Asian individuals in the 2-AAA Study had significantly lower BMI (P=0.018) and systolic blood pressure (P=0.005) than other individuals. Interestingly, there was also an overall difference by self-reported race in the HATIM sample (P=0.014; Figure 1D), with a trend towards higher levels of 2-AAA in Asian (2-AAA ion count $359\ x10^4 \pm 45\ x10^4$) compared to Black (2-AAA ion count $249\ x10^4 \pm 65\ x10^4$) and white (2-AAA ion count $279x10^4 \pm 75x10^4$) individuals, although there were only three individuals self-identifying as Asian in this sample, so the differences did not reach statistical significance in *post hoc* tests. There was no association between 2-AAA and age in either dataset (2-AAA Study $r^2 = 0.028$, P=0.65; HATIM $r^2 = 0.092$, P=0.26).

## Plasma 2-AAA levels associate with dyslipidemia in healthy individuals and PWH

Higher plasma 2-AAA was associated with lower HDL cholesterol (2-AAA Study $r^2 = -0.267$, P<0.001; HATIM $r^2 = -0.579$, P<0.001; Figures 2A, B), and higher triglycerides (2-AAA Study $r^2 = 0.246$, P=0.027; HATIM $r^2 = 0.526$, P=0.007; Figures 2C, D). There was no significant association with LDL cholesterol.

TABLE 1 Characteristics of the participants of the 2-AAA Screening Study.

| | Male (N=72) | Female (N=189) |
|---|---|---|
| | Mean (SD) | Mean (SD) |
| **Age (years)** | 28.96 (6.92) | 27.76 (7.2) |
| **Race (N [%] Black, white, Asian, other)** | 3, 55, 8, 6<br>[4, 77, 11, 8%] | 14, 139, 25, 11<br>[7, 74, 13, 6%] |
| **BMI (kg/m$^2$)** | 24.77 (2.9) | 22.94 (2.9) |
| **Systolic Blood Pressure (mmHg)** | 120.57 (13.5) | 111.26 (10.7) |
| **Diastolic Blood Pressure (mmHg)** | 73.80 (9.5) | 69.67 (8.1) |
| **Glucose (mg/dL)** | 90.0 (7.6) | 91.18 (8.2) |
| **Total cholesterol (mg/dL)** | 166.56 (30.7) | 167.94 (32.4) |
| **HDL (mg/dL)** | 53.47 (10.7) | 64.67 (13.1) |
| **LDL (mg/dL)** | 95.56 (24.9) | 87.41 (24.5) |
| **TG (mg/dL)** | 87.44 (37.5) | 79.29 (37.1) |
| **2-AAA (ng/ml)** | 95.99 (33.8) | 68.44 (27.7) |

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TABLE 2 Characteristics of participants of the HATIM Study.

| | PWH | | | | | | HIV-negative | |
|---|---|---|---|---|---|---|---|---|
| | Insulin sensitive | | Pre-Diabetes | | Diabetes | | Diabetes | |
| | Male (N=33) | Female (N=8) | Male (N=27) | Female (N=7) | Male (N=24) | Female (N=11) | Male (N=6) | Female (N=18) |
| | Mean (SD) | Mean (SD) | Mean (SD) | Mean (SD) | Mean (SD) | Mean (SD) | Mean (SD) | Mean (SD) |
| **Age (years)** | 42.15 (11.7) | 46.37 (7.7) | 44.40 (12.2) | 49.14 (10.2) | 52.62 (9.4) | 48 (12.0) | 51.33 (12.1) | 57.61 (9.6) |
| **Race (N [%] Black, white, Asian, other)** | 10, 22, 1, 0 [30, 67, 3, 0%] | 5, 2, 0, 1 [63, 25, 0, 12%] | 11, 14, 1, 1 [40, 52, 4, 4%] | 4, 3, 0, 0 [57, 43, 0, 0%] | 10, 14, 0, 0 [42, 58, 0, 0%] | 7, 1, 0, 3 [64, 9, 0, 27%] | 1, 5, 0, 0 [17, 83, 0, 0%] | 6, 11, 1, 0 [33, 61, 6, 0%] |
| **BMI (kg/m$^2$)** | 30.78 (3.8) | 34.21 (5.8) | 33.28 (6.3) | 34.03 (6.1) | 34.76 (7.25) | 40.01 (9.8) | 38.16 (8.8) | 37.31 (5.1) |
| **Waist circumference (cm)** | 100.39 (12.6) | 104 (12.5) | 105.40 (13.9) | 102.86 (14.8) | 115.21 (12.6) | 114.7 (17.5) | 126.31 (20.4) | 114.7 (13.85) |
| **Total cholesterol (mg/dL)** | 174.63 (38.3) | 186.6 (23.0) | 175.7 (35.1) | 223.71 (38.5) | 173.75 (33.1) | 179.45 (41.3) | 183.66 (85.8) | 171.44 (27.9) |
| **LDL (mg/dL)** | 102.12 (34.1) | 110.3 (18.8) | 110.77 (44.9) | 129 (35) | 91.39 (28.9) | 95.2 (33.9) | 86.83 (27.0) | 102.0 (27.1) |
| **HDL (mg/dL)** | 47.06 (18.2) | 54.1 (15.9) | 41.22 (13.3) | 65.43 (23.6) | 37.96 (10.5) | 49.72 (12.9) | 36.33 (10.1) | 47.55 (8.8) |
| **TG** | 127.27 (82.9) | 111.8 (64.2) | 154.89 (83.4) | 146.86 (56.8) | 250.29 (224.9) | 189.27 (145.4) | 327.16 (470.9) | 109.33 (45.4) |
| **Glucose (mg/dL)** | 87.0 (9.5) | 89.38 (6.0) | 111.55 (14.0) | 112.42 (8.1) | 203.6 (88.4) | 156 (59.7) | 164.33 (78.5) | 128.61 (34.9) |
| **Insulin (uU/mL)** | 19.19 (23.0) | 18.39 (19.4) | 55.06 (59.2) | 30.9 (24.9) | 38.58 (21.2) | 36.62 (19.3) | 46.99 (29.4) | 28.92 (16.3) |
| **HOMA-IR** | 4.23 (4.9) | 4.24 (4.8) | 14.91 (15.6) | 9.10 (7.7) | 22.63 (19.1) | 16.49 (14.8) | 17.78 (13.0) | 8.73 (6.5) |
| **Hemoglobin A1c (%)** | 5.1 (0.46) | 5.16 (0.23) | 5.52 (0.5) | 5.52 (0.3) | 8.18 (2.3) | 7.10 (1.8) | 7.81 (2.0) | 6.85 (0.93) |
| **Liver attenuation (HU)** | 61.44 (7.5) | 63.87 (3.4) | 62.17 (9.1) | 61.3 (12.9) | 53.03 (14.6) | 57.54 (11.5) | 48.25 (11.6) | 45.62 (19.0) |
| **Plasma 2-AAA (ion count)** | 2441358 (623282) | 1898487 (225878) | 2769029 (548804) | 2059280 (355932) | 3270163 (715556) | 2794366 (739755) | 3141535 (969840) | 2576285 (622577) |

## Higher plasma 2-AAA levels associate with diabetes status in PWH

There were significant differences in plasma 2-AAA by diabetes status within PWH in the HATIM sample (P<0.001, Figure 3). Individuals with diabetes had significantly higher levels of 2-AAA (ion count $312x10^4 \pm 75x10^4$) than both the insulin sensitive (ion count $233x10^4 \pm 60x10^4$, P<0.001) and the pre-diabetic (ion count $262x10^4 \pm 58x10^4$, P=0.005) groups in models adjusted for sex, race, BMI and smoking status.

## Plasma 2-AAA associates with elevated fasting glucose, insulin, and HbA1c in PWH

Across all PWH individuals in HATIM, plasma 2-AAA was associated with increased fasting glucose ($r^2$ = 0.576, P<0.001), fasting insulin ($r^2$ = 0.623, P<0.001), HOMA-IR ($r^2$ = 0.538, P<0.001) and hemoglobin A1c ($r^2$ = 0.580, P<0.001). In secondary analyses split by diabetes status, 2-AAA associated with glucose and HbA1c only in the individuals with diabetes (glucose $r^2$ = 0.52, HbA1c $r^2$ = 0.58; both P<0.0001 for diabetes, vs P>0.5 for insulin sensitive and pre-diabetes), but 2-AAA was associated with insulin in both people with and without diabetes ($r^2$ = 0.27, P<0.02 insulin sensitive, $r^2$ = 0.5, p<0.001 pre-diabetes, $r^2$ = 0.26, P<0.002 diabetes). In the 2-AAA Study, a small number of people (n=25) had evidence of potential impaired fasting glucose (IFG, defined as glucose >100mg/dL but <125 mg/dL). While plasma 2-AAA levels were slightly higher within the individuals with IFG (82.5 vs. 75.4 ng/ml), this difference did not reach statistical significance.

## Elevated plasma 2-AAA levels associate with differences in anthropometrics, adipose tissue, and liver density

We found a significant association between plasma 2-AAA and higher BMI in the 2-AAA Study ($r^2$ = 0.275, P<0.001, model adjusted for sex and race), but this was not significant in HATIM. However, in HATIM, higher plasma 2-AAA was significantly associated with increased waist circumference ($r^2$ = 0.219, P<0.001), as well as greater visceral adipose tissue volume ($r^2$ = 0.225, P<0.001), but not with measures of subcutaneous or pericardial adipose tissue. In HATIM, 2-AAA was negatively associated with liver density ($r^2$ = 0.192, P=0.003; Figure 4). Lower liver density is a marker of higher proportion of ectopic fat in the liver.

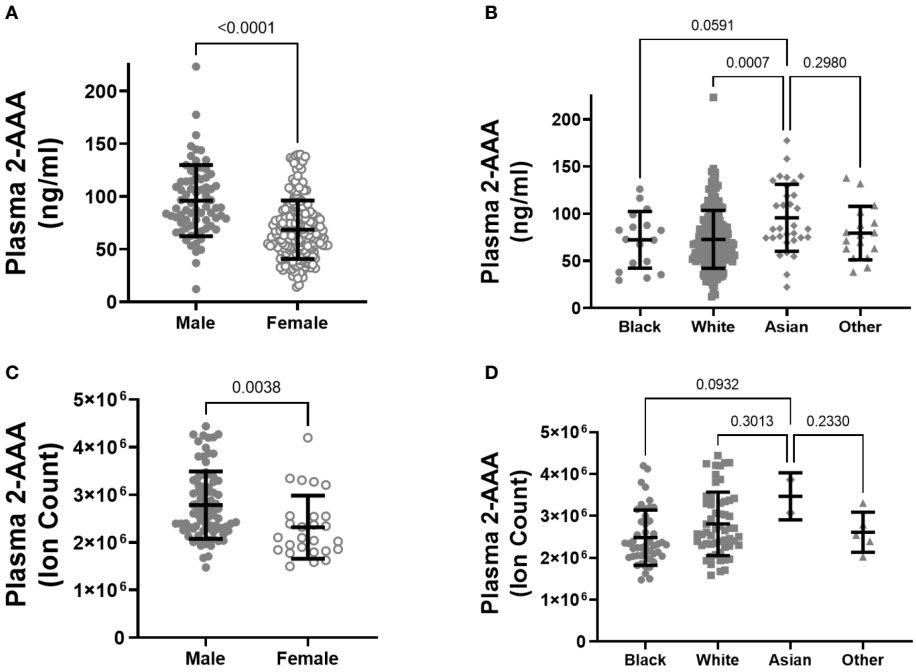


FIGURE 1
Plasma 2-AAA is significantly higher in men than women in the 2-AAA **(A)** and HATIM Study **(C)**. 2-AAA is higher in Asian compared to Black or white individuals in the 2-AAA Study **(B)** with a similar trend in the HATIM Study **(D)**. Data are expressed as ng/ml for data from the 2-AAA Study and ion counts for the HATIM study.

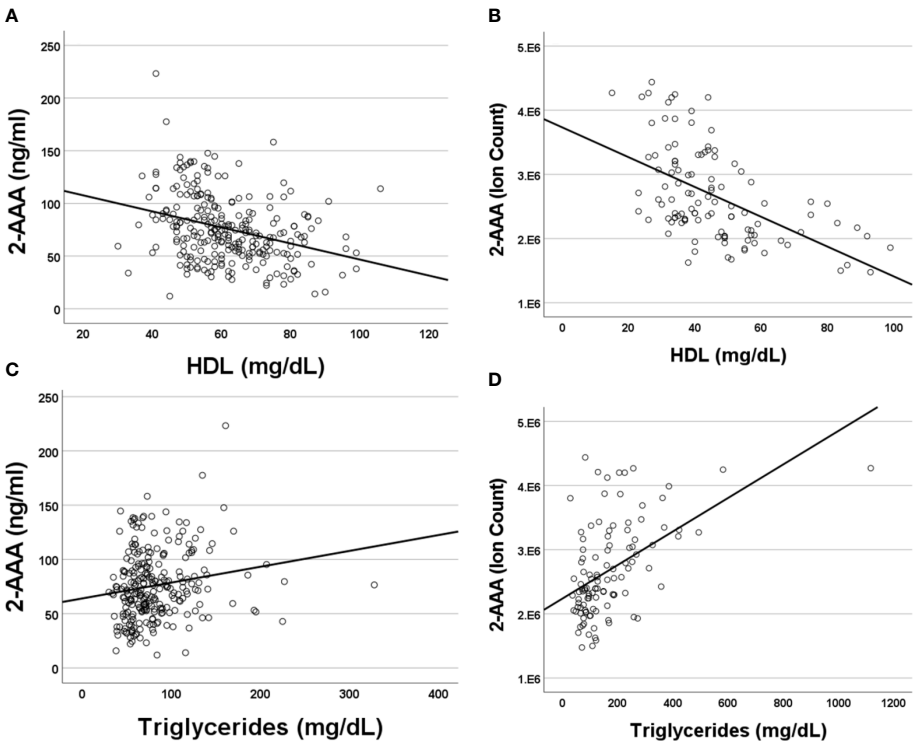


FIGURE 2
Plasma 2-AAA associates with lower HDL cholesterol and higher Triglycerides in the 2-AAA **(A, C)** and HATIM **(B, D)** studies. Data are expressed as ng/ml for data from the 2-AAA Study and ion counts for the HATIM study.

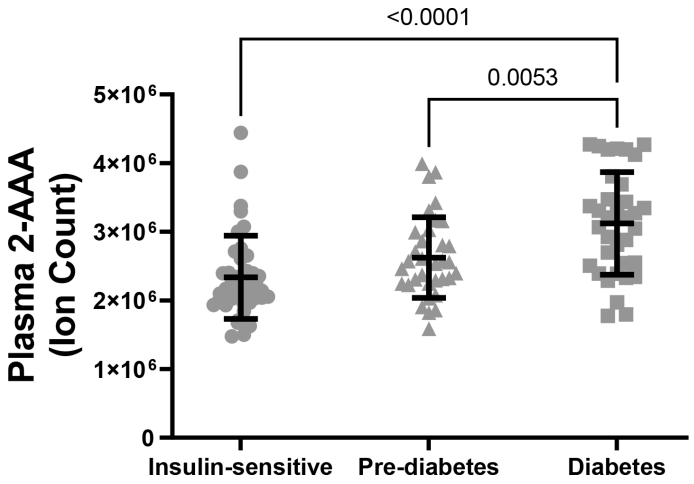


FIGURE 3
Plasma 2-AAA was significantly higher in PWH and diabetes, compared with PWH who were insulin sensitive or with pre-diabetes.

## Discussion

We measured plasma 2-AAA in two independent samples of individuals across the spectrum of healthy (no diagnosed diseases) to high cardiometabolic risk (diabetes and treated HIV infection). 2-AAA was elevated in diabetes but did not appear to be significantly elevated based on HIV status. We found that plasma 2-AAA is elevated in men compared with women, and in Asian compared with other self-identified ancestries. These associations are consistent in both healthy individuals and PWH. We confirmed associations between 2-AAA and both low HDL and high TG, and between 2-AAA and diabetes. We report novel relationships between 2-AAA and visceral adipose tissue measured by CT, and between 2-AAA and higher liver fat. Our data highlight consistent relationships between 2-AAA and cardiometabolic risk markers across two different sample groups, and further confirm 2-AAA as an important candidate biomarker with broad relevance for additional prognostic and therapeutic consideration.

Plasma 2-AAA levels differed by sex, an association that has been reported previously in Mexican young adults (28). Men have relatively higher risk of CVD than pre-menopausal women, yet the mechanisms underlying this difference are not fully understood (1). We further report differences by self-reported race, with Asian individuals having higher 2-AAA than other groups. Individuals of Asian ancestry have relatively higher risk of T2D and some CVD compared with individuals of European ancestry with the same risk factor profile (29, 30). The mechanisms underlying this are incompletely understood, and the risk factors for CVD in Asians may differ when compared with European ancestry (31). While the original discovery of 2-AAA as a diabetes metabolite was in

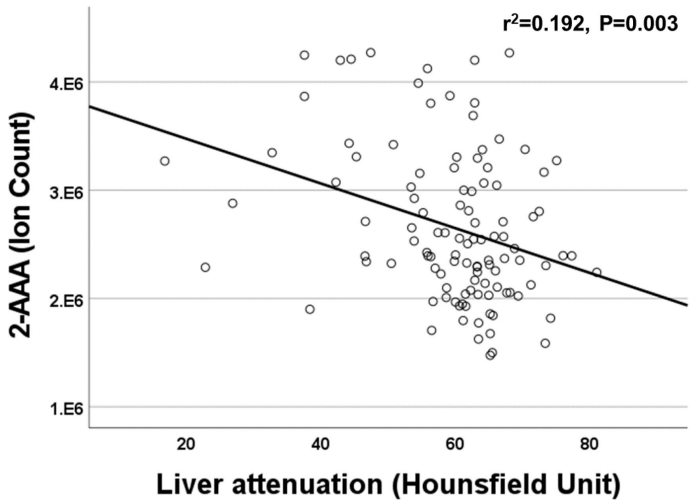


FIGURE 4
Plasma 2-AAA was negatively associated with liver attenuation in the HATIM Study of PWH.

European ancestry (5), 2-AAA has also been reported to associate with T2D in Chinese individuals (32). Whether differences in 2-AAA may play a role in mediating the relative increased risk in men compared with women, and Asian compared with other ancestries, remains to be determined.

We previously reported that plasma 2-AAA associates with both lower HDL cholesterol and higher triglycerides (33). We replicated those associations in the current study, establishing that this relationship is consistent across multiple different samples, including in a cohort of persons with HIV. Based on genetic evidence, 2-AAA drives the decrease in HDL (33). While low HDL cholesterol is consistently associated with increased cardiometabolic risk (34, 35), interventions to alter HDL have shown no benefit (36). This could be due to differences in HDL composition or function, or due to a causal biomarker that is upstream of HDL. This raises the intriguing hypothesis that elevated 2-AAA, rather than low HDL per se, may be driving increased cardiometabolic risk. However, careful mechanistic studies are required to interrogate this further.

2-AAA was originally discovered as a predictor of diabetes, and is associated with increased insulin secretion in animal models and cells (5). In the setting of experimental hyperglycemia in overweight and obese, but otherwise healthy individuals, 2-AAA was significantly decreased following 24 hours of hyperglycemia (37). 2-AAA has been shown to be reduced in the acute setting in response to insulin infusion (38). We found that 2-AAA was significantly higher in PWH who have diabetes, than in PWH who were insulin sensitive or pre-diabetic. This is similar to what has been reported in HIV-negative individuals (5, 39), and suggests that the relationship between 2-AAA and diabetes is consistent across different settings, including against the background of well-controlled HIV infection, a population at increased risk of cardiometabolic disease (17). We found no significant difference in plasma 2-AAA levels based on HIV status in the HATIM cohort within the subset of individuals with diabetes, further suggesting that 2-AAA is a useful biomarker of cardiometabolic risk in multiple at-risk populations. 2-AAA was associated with increased fasting glucose, fasting insulin, and hemoglobin A1c in the HATIM study. However, the association between 2-AAA and glucose was only significant in individuals with diabetes; 2-AAA was not associated with fasting glucose in insulin sensitive individuals in the 2-AAA Study or HATIM, or in individuals with pre-diabetes in HATIM. In contrast, 2-AAA was associated with higher insulin in individuals with and without diabetes. This distinction between the glycemic and insulin axis is consistent with the hypothesis that 2-AAA is an early marker or driver of hyperinsulinemia and is associated with elevated insulin before the development of overt hyperglycemia or diabetes. These data further support a mechanism where elevated 2-AAA precedes the onset of hyperglycemia, and associates with hyperinsulinemia even in individuals who appear insulin sensitive. Associations between 2-AAA and hyperglycemia are likely secondary to insulin resistance. However, further in-depth studies are required to assess potential reciprocal regulation of 2-AAA and insulin.

2-AAA was positively associated with BMI in the 2-AAA study, but not in the HATIM study. However, there was a significant association between 2-AAA and waist circumference in HATIM. This may suggest that the relationship between 2-AAA and adiposity is modulated by HIV-associated effects on adipose distribution (40). Previous studies have also highlighted an association between 2-AAA and obesity, including both BMI and waist circumference (7, 11, 41, 42). While one study has found that 2-AAA is protective against obesity and diabetes in mice (43), these findings are in contrast to all other studies, and may be related to specific metabolic anomalies in the mouse model used (13, 44, 1). In our study, 2-AAA associated with increased visceral fat in HATIM, but not subcutaneous or pericardial fat. These data are consistent with a previous study, where 2-AAA was associated with metabolically unhealthy central obesity, compared with metabolically healthy peripheral obesity (45). Thus, 2-AAA may relate specifically to pathogenic adipose tissue dysfunction, rather than to obesity itself.

Plasma 2-AAA associated with lower liver density, which corresponds to higher liver fat, and is considered a measure of hepatic steatosis. Previous data in mice found an association between 2-AAA and liver mass (10), however, to our knowledge our study describes this for the first time in humans. Elevated 2-AAA may thus be a risk factor for hepatic steatosis and development of fatty liver disease, however, whether this is independent of associations with BMI, visceral fat and circulating lipids remains to be determined.

Our study had several strengths. We analyzed plasma 2-AAA in two separate samples of well-phenotyped individuals, recruited from the same geographic area, to the same academic medical center. Our studies included both healthy individuals, and PWH across the diabetes spectrum, allowing us to assess whether the relationship between 2-AAA and cardiometabolic risk markers is consistent in the settings of chronic viral-induced inflammation as well as in individuals without diagnosed disease. 2-AAA was not measured in many previous metabolomic studies, and is not consistently detected or reported on popular metabolomics panels (e.g. Metabolon). Thus, the importance of this metabolite in cardiometabolic health may be under-appreciated. We used a targeted assay in the 2-AAA study to quantify 2-AAA, providing important data on circulating levels in healthy individuals. To our knowledge, this is the first study to measure associations between 2-AAA and metabolic disease in PWH. PWH suffer a disproportionate burden of diabetes, hypertension, fatty liver, and dyslipidemia compared to HIV negative persons (46–49), and allows for validation of the relevance of 2-AAA to disease within the setting of a highly-inflammatory exaggerated phenotype. Our study also had some limitations. Plasma 2-AAA was measured using a different method in HATIM compared with the 2-AAA study, limiting our ability to directly compare levels of 2-AAA in PWH compared with healthy individuals. However, we were able to compare levels between PWH and HIV-negative within a subset of individuals with diabetes. We also had limited sample size to fully characterize the differences by race across both samples, with small

numbers of Black individuals in the 2-AAA study and small numbers of Asian individuals in the HATIM study.

In conclusion, our study establishes differences in plasma 2-AAA by sex and race, confirms associations between 2-AAA and dyslipidemia in both healthy individuals and PWH with or without diabetes, and highlights novel relationships between 2-AAA and liver fat and visceral adipose tissue. Further mechanistic and longitudinal studies are required to establish whether 2-AAA is causally linked to cardiometabolic disease.

## Data availability statement

The data presented in the study are deposited in the Figshare repository, accession number 10.6084/m9.figshare.24074379.

## Ethics statement

The studies involving human participants were reviewed and approved by Vanderbilt University Institutional Review Board (IRB). The patients/participants provided their written informed consent to participate in this study.

## Author contributions

Study design: SD, CG, JK, JF. Acquisition, analysis and interpretation of data: SD, CG, HS, OA, CW, MC, AD, HS, SN, JT, JC, ML, JB, JK, JF. Drafted the manuscript: SD, CG, JF. Revised the manuscript and approved publication: SD, CG, HS, OA, CW, MC, AD, HS, SN, JT, JC, ML, JB, JK, JF. 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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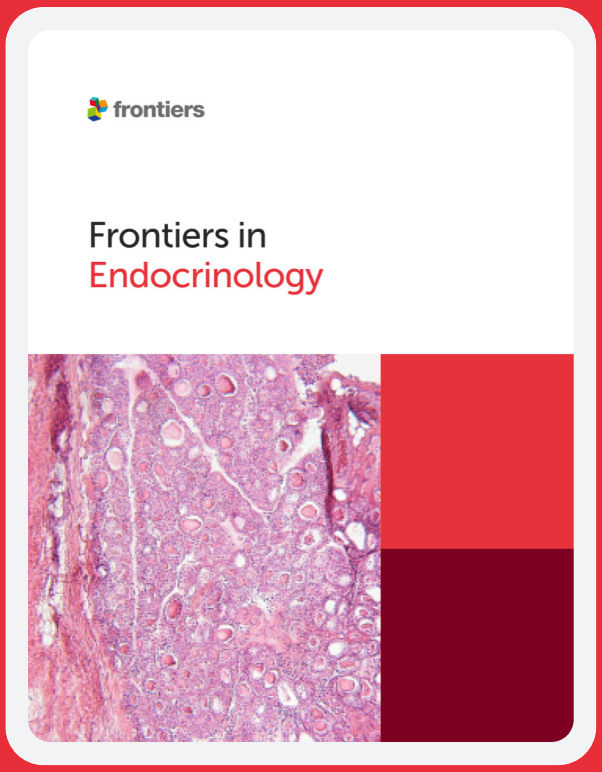


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