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# Epidemiology and clinical researches in atherosclerosis and cardiovascular disease

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# Epidemiology and clinical researches in atherosclerosis and cardiovascular disease

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# Editorial: Epidemiology and clinical researches in atherosclerosis and cardiovascular disease

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KEYWORDS

atherosclerosis, cardiovascular disease, epimiology, cohort study, dementia

Editorial on the Research Topic
Epidemiology and clinical researches in atherosclerosis and cardiovascular disease

Atherosclerosis is a systemic disease and the common cause of heart attacks, strokes and peripheral vascular disease collectively referred to as cardiovascular diseases (CVD), which are the leading cause of global mortality and a major contributor to disability and rising health care costs. Additionally, a wealth of epidemiological data demonstrated that atherosclerosis risk factors, including (but not limited to) hypertension, diabetes, and hyperlipidemia are associated with other chronic diseases such as chronic kidney disease, cognitive decline and dementia (1–6). The huge and still growing burden of CVD and dementia on individuals, families, and health-care systems indicates an urgent need for prevention and treatment measures on atherosclerotic diseases. Preventing severe atherosclerosis progression is expected to decrease high cardiovascular and dementia event rate.

However, there still exist challenges to be addressed. These challenges include but are not limited to (1) early detect participants with high-risk of CVD; (2) identify novel indicators for progression and prognosis of atherosclerotic diseases; (3) comorbidities of atherosclerotic diseases; (4) new drugs and therapies on atherosclerosis and CVD.

This research topic aimed at creating a forum for high-quality epidemiology and clinical researches in the field of atherosclerosis and CVD. The issue currently includes 12 papers on guiding comprehensive care and practice in preventing and managing major atherosclerotic CVD, including coronary heart disease, stroke, and peripheral vascular disease, and other chronic diseases which are associated with atherosclerosis.

In this topic, Wang et al. conducted a cohort study to explore the association between non-HDL-C and arterial stiffness on a large-scale Chinese population (https://www.frontiersin.org/articles/10.3389/fcvm.2022.981028/full). The results highlighted non-HDL-C as a potential risk factor for arterial stiffness, in especially for younger people. The clinical benefits of lowering non-HDL-C concentration should be further considered in the future.

Gao et al. performed a systematic review and meta-regression analysis to investigate the impact of statins on CRP/hsCRP reduction on coronary plaque burden measured using total atheroma volume (TAV), percent atheroma volume (PAV), and plaque volume (PV) (https://www.frontiersin.org/articles/10.3389/fcvm.2022.989527/full). After adjusting for percent change of LDL-C, age, gender and study duration, this meta-regression analysis mainly found that the percent change of CRP/hsCRP was significantly associated with the change of TAV/PV. The results indicated that statins promote plaque regression, which may be associated to their capacity to reduce inflammation.

In a multi-ethnic longitudinal cohort study, Anbar et al. compared carotid atherosclerosis in Europeans (EA), South Asian (SA), and African Caribbean (AC) participants in the Southall and Brent Revisited (SABRE) study and they found that the prevalence of any plaque was comparable in EA and SA, although it was lower in AC. Total plaque area, numbers of plaques, plaque class, or greyscale median did not differ by ethnicity in individuals who had plaque (https://www.frontiersin.org/articles/10.3389/fcvm.2022.1002820/full). This study indicated that the similarity of plaque burden in SA and EA despite established differences in atherosclerotic CVD risk casts some doubt on the utility of carotid ultrasound as a means of assessing risk across these ethnic groups.

Sundquist et al. performed a population-based follow-up study to examine the role of mtDNA-CN in heart failure (HF) incidence and its role in the association between myocardial infarction (MI) and HF. In addition, this study also investigated the role of mtDNA-CN in overall and HF mortality (https://www.frontiersin.org/articles/10.3389/fcvm.2022.1012403/full). This study mainly found that low baseline mtDNA-CN is a molecular risk factor for HF incidence and may be a risk factor for overall and HF-related mortality.

In a cohort study published in this topic (https://www.frontiersin.org/articles/10.3389/fcvm.2022.1026597/full), Moon et al. examined the association between height loss and the prevalence of CVD using data from a sizable Korean cohort. The participants were divided into three groups based on their annual height loss: Group 1 (height loss: <0.3 cm/year), Group 2 (height loss: 0.3 to <0.6 cm/year), or Group 3 (height loss: ≥0.6 cm/year). The results indicated that the incidence of major adverse cardiac and cerebral event was substantially higher in Groups 2 and 3 than in Group 1. In the Korean population, the severity of height reduction was independently correlated with the occurrence of CVD.

Muhammad et al. conducted a longitudinal two-cohort analysis, and identified association between positive triglyceride-glucose (TyG) index and increased arterial stiffness and increased incidence of diabetes, CE, stroke, and all-cause and cardiovascular mortality (https://www.frontiersin.org/articles/10.3389/fcvm.2022.1035105/full). The results of this work represent preliminary evidence that TyG index can potentially be helpful in the identification of those at increased long-term risk of adverse health outcomes.

A classification tree analysis (CTA) model established by Ruan et al. in this topic identified four key correlates of depressive disorders: loneliness was the most salient, followed by arthritis, family relationship, and heart disease (https://www.frontiersin.org/articles/10.3389/fcvm.2022.1035203/full). Due to the potential for modification or treatment, these findings regarding the four main correlates of depressive disorders are clinically interesting. The clinical needs for collaborative multidisciplinary management services—which integrate social work outreach services to foster family relationships, mental health services to relive loneliness, and primary care services to manage arthritis and heart disease—are further indicated by the significant interactions between the four major factors.

In a cohort study, Ma et al. recruited 299 patients with new-onset non-valvular atrial fibrillation (AF) between 2013 and 2015 at the Department of Cardiovascular Medicine of the Southwest Hospital of the Army Medical University (Third Military Medical University) in Chongqing, China (https://www.frontiersin.org/articles/10.3389/fcvm.2022.1072164/full). The findings revealed that throughout the median follow-up period of 28 (IQR: 27, 29) months, IL-34 and IL-38 were independently associated with stroke and all-cause mortality in patients with AF. Additionally, IL-38 and NT-proBNP considerably increased the CHA2DS2-VASc score's capacity to predict AF-related all-cause death.

In another large-scale cohort study, Hua et al. found that participants with and without heart disease experienced similar changes in global cognitive scores during the pre-pandemic period, however, in comparison to the group without heart disease, the heart disease group experienced a greater decline in the global cognitive score during the pandemic period (https://www.frontiersin.org/articles/10.3389/fcvm.2022.1077800/full). The findings highlight the need for rapid cognitive monitoring and therapies for the population suffering from heart diseases.

Grabitz et al. focused on exploring the early indicators and rivers of cardiovascular disease in young athletes pursuing a career in competitive sports (https://www.frontiersin.org/articles/10.3389/fcvm.2023.1081675/abstract). They discovered an unexpectedly high rate of cardiovascular risk factors despite regular exercise and the absence of obesity. Their findings suggested that children and young adults, who initially appeared to be in good condition, require rigorous medical examinations. To further investigate potential negative impacts on vascular health, long-term monitoring of those who began engaging in excessive physical activity as children and young seems required.

In this topic, Ni et al. employed linkage disequilibrium score (LDSC) regression and a two-sample Mendelian randomization (MR) framework to systematically examine the causal interplay between key factors that influence vascular calcification and CVD, as well as longevity (https://www.frontiersin.org/articles/10.3389/fcvm.2023.1096662/full). The results provide evidence for a causal relationship between VK1 levels and CVD risk as well as a genetic genetic correlation between serum Ca and VD

and CVD risk. Cardiovascular risk can be decreased by maintaining appropriate serum Ca (2.376 mmol/L) and VD levels (46.8 nmol/L).

In the last article published in this topic, Wright et al. examined associations between a history of pregnancy loss and incident CVD among participants in the Women's Health Initiative Observational Study (https://www.frontiersin.org/articles/10.3389/fcvm.2023.1108286/full). In this cohort study of postmenopausal women aged 50–79, history of stillbirth was strongly associated with a risk of cardiovascular outcomes within 5 years of baseline. Additionally, history of pregnancy loss, and of stillbirth, may be a therapeutically effective marker of cardiovascular disease risk in women.

In conclusion, the articles published in this research topic provide additional evidence from epidemiology and clinical researches for current literature on atherosclerosis and cardiovascular disease. Nevertheless, incredible challenges on the prevention and treatment of atherosclerosis and cardiovascular disease need more attention following the aging of the population and the development of social economy. We thank the authors for their cutting-edge works, and also express our gratitude to all the reviewers for their generously devoted time and highly valuable comments. Finally, we hope that the reader will enjoy these articles.

## Author contributions

DG and WX drafted this manuscript, and all authors revised the manuscript. All authors contributed to the article and approved the submitted version.

## Conflict of interest

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

## Publisher's note



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# Age-specific association between non-HDL-C and arterial stiffness in the Chinese population

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**Background:** While some epidemiological studies have found correlations between non-high-density lipoprotein cholesterol (non-HDL-C) and arterial stiffness, there are still exist controversial and age-stratified analysis are scarce yet.

**Methods:** All individuals in this study were recruited in the Third Xiangya Hospital of Central South University from 2012 to 2016. Arterial stiffness was defined as brachial-ankle pulse wave velocity (baPWV) ≥1,400 cm/s. Association between non-HDL-C and arterial stiffness were explored using Cox proportional-hazards model. We also conducted subanalysis stratified by age. Furthermore, restricted cubic splines were used to model exposure-response relationships in cohort sample.

**Results:** This cohort study included 7,276 participants without arterial stiffness at baseline. Over a median follow-up of 1.78 years (IQR, 1.03–2.49), 1,669 participants have identified with incident arterial stiffness. In multivariable-adjusted analyses, higher non-HDL-C concentration was associated with incident arterial stiffness with an adjusted hazard ratio (HR) of 1.09 [95% confidence interval (CI), 1.02–1.17] per 1 mmol/L increase. Compared with the lowest tertile, the HR for arterial stiffness with respect to the highest tertile of non-HDL-C was 1.26 (95% CI, 1.07–1.48). The results were similar in the analysis of young participants (age <60 years).

**Conclusion:** Our study identified that non-HDL-C as a potential risk factor of arterial stiffness, especially for younger. The clinical benefits of decreasing non-HDL-C concentration should be further considered in the future.

KEYWORDS

**PWV, non-HDL-C, arterial stiffness, vascular health, age-specific**

# Introduction

Dyslipidemia have received increasing attention as the global burden of cardiovascular disease increases. A high serum cholesterol level has been shown to be a risk factor for cardiovascular disease (1, 2), with studies showing that the average cholesterol level is rising in Asian countries, which is of particular concern in China because the major rise in the Chinese population is in non-HDL-C (3). Notably, non-HDL-C is considered to have greater potential for cardiovascular disease (CVD) prognosis (4–6), and a stronger association with major CVD events among statin-treated patients compared with LDL-C (7). AHA/AHC and ESC/EAS guidelines have both recommended non-HDL-C for CVD risk estimation in 2019 (8, 9). However, it is unclear through which mechanism the effect of non-HDL-C on CVD is mediated.

Arterial stiffness plays a key role in CVD and mortality (10–12), and is recognized as a core characteristic of vascular aging. Pulse wave velocity is a commonly used method to measure arterial stiffness due to its advantages of convenience and non-invasiveness. In high-risk individuals, a 1 m/s increase in branchial-ankle pulse wave velocity (baPWV) raises the risk of a cardiovascular event by 12% (13). Currently, although most studies have reported that non-HDL-C is significantly correlated with PWV in young and old populations (6, 14–18), the clinical significance of non-HDL-C for arterial stiffness still remains controversial in the current available studies. Vallée et al. found a strong association between non-HDL-C and arterial stiffness (15), whereas a Chinese study showed inconsistent results in middle-aged and elderly people (17). Therefore, more studies are needed to verify the relationship between non-HDL-C and arterial stiffness in Chinese people, so as to explore whether the effect of non-HDL-C on CVD is mediated by vascular aging mechanism. In addition, no studies have evaluated the effects of non-HDL-C on arterial stiffness among different age groups.

Our research was conducted on a large-scale Chinese population. The purpose of this study was to examine the associations between non-HDL-C and arterial stiffness, and to simultaneously explore whether the effects of non-HDL-C were differed in different age group.

# Methods

## Study population

All individuals in this study were recruited in the Third Xiangya Hospital of Central South University between 2012 and 2016. Individuals lacking baPWV and non-HDL-C data, or with ineligible baPWV data, or <18 years of age were excluding. We included 67,116 participants with totally 84,853 person-exams. Furthermore, participants that underwent only one baPWV measurement were excluded in the study, excluding individuals with arterial stiffness at baseline (Supplementary Figure S1). The remaining individuals were comprised a cohort and remained for analyzed. Detailed information about the subjects can be found in our previous study (19). This cohort study is reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement (Supplementary Table S1).

## Clinical and laboratory assessments

Each participant completed a standardized health examination and a detailed questionnaire. Age, sex, height, and weight were recorded directly, and exercise, smoking, and drinking status were derived from the questionnaire. Physical activity was recorded as "Yes" if the subject reported exercising. Smoking was recorded as "Yes" if the subject reported smoking more than one cigarette per day on average. Drinking was recorded as "Yes" if the subject reported alcohol (beer, wine, or liquor) consumption at least two days per week on average.

Height and weight were measured in a standing position after having taken off shoes and clothes. BMI (body mass index) was calculated as weight (kg) divided by height (m) squared (i.e., $kg/m^2$). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with an electronic sphygmomanometer (Omron 9020) on the right upper arm. SBP and DBP were recorded as the average of two readings in the sitting position after a 10-min rest. If the two readings differed by >5 mmHg, a third measurement was performed and the average of all three readings was recorded.

Venous blood samples were collected after an overnight fast and then transferred into EDTA-containing vacuum tubes. Concentration of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) triglycerides (TG), and fasting blood glucose (FBG) were immediately analyzed at the clinical laboratory of the Third Xiangya Hospital with enzymatic methods (Hitachi 7600-110; Hitachi, Tokyo, Japan). Non-HDL-C was calculated as TC minus HDL-C.

## Measurement of baPWV

An automatic waveform analyzer (BP-203 RPE, Omron Healthcare, Dalian, China) was used to measure baPWV and ankle-branchial index (ABI) simultaneously [details could be found in our previous study (19)]. In brief, after 5 min of rest, one cuff was wrapped around each arm and ankle, after which the analyzer obtained a report including the baPWV and ABI of the left and right sides of the body. The baPWV was measured twice on both sides of the body, with the average recorded as the final value. An ABI <0.9 was considered to indicate severe peripheral arterial disease, which might lead to measurement

error (20). To decrease measurement bias, subjects with bilateral ABI <0.9 were excluded from analysis, and subjects with ABI <0.9 on one side of the body were only evaluated using the baPWV from the other side. Furthermore, subjects with an average difference in baPWV ≥1,000 cm/s on the left and right sides of the body were also excluded (21). The primary endpoint in this cohort study was incidence of arterial stiffness (defined as a individual with baPWV ≥1,400 cm/s). For individual with arterial stiffness, the endpoint time was defined as the time when arterial stiffness was first detected, and for people without arterial stiffness, endpoint time was defined as the time of the last valid measurement.

## Statistical analysis

Characteristics of participants were presented as the mean followed by the standard deviation (SD) in parentheses for continuous variables with normal distributions, or the median followed by the interquartile range (IQR) in parentheses for continuous variables with skewed distributions, or as percentages for categorical variables. Differences between groups were evaluated using the Kruskal–Wallis test for continuous variables, and the chi-square test for categorical variables. Two-tailed *P*-values of ≤0.05 were considered significant in all analyses. The Cox proportional-hazards model was applied to calculate the hazard ratio (HR) and 95% confidence interval (CI) of non-HDL-C for incident arterial stiffness with exposure both as a continuous variable (per 1 mmol/L increase) and as a categorical variable (tertiles). Model 1 was adjusted for age and sex. Model 2 was additionally adjusted for BMI, SBP, and fasting blood glucose. Model 3 was additionally adjusted for exercise, smoking status, and drinking status. We also performed analysis stratified by age (two categories: <60 years; ≥60 years). Additionally, restricted cubic splines were done to model the concentrations of non-HDL-C as a continuous variable for the different age groups (<60 and ≥60).

Furthermore, some sensitivity analyses were conducted to confirm the robust of our analysis: (1) excluding individuals with missing data; (2) excluding individuals with <1 year follow-up time.

All statistical analyses were performed using Stata software (version 16.0; StataCorp LLC, College Station, TX, USA) and R version 4.0.3 (The R Foundation for Statistical Computing).

# Results

## Study population

The cohort study consisted of 7,276 participants for analysis with median follow-up of 1.78 years, after excluding individuals with only one health exam. The median age of all subjects was 44 (IQR, 37–49) years, and 2,354 were female. Individuals with arterial stiffness tended to be older and to have higher BMI, SBP, DBP, FBG TG, TC, LDL-C, and non-HDL-C, and lower HDL-C levels than subjects without arterial stiffness. Additionally, individuals with arterial stiffness were more likely to be male, smoking, and drinking (Table 1).

## Longitudinal association between non-HDL-C and incident arterial stiffness

During the median follow-up of 1.78 year (range: 0.05 to 4.72 years; IQR: 1.03–2.49 years), 1,669 were diagnosed with arterial stiffness according to the definition baPWV ≥1,400 cm/s (Table 2). Compared with participants with the lowest tertile of non-HDL-C, the fully adjusted HRs for arterial stiffness risk of non-HDL were 1.26 (95%CI, 1.07–1.48) among those with the highest tertile of non-HDL-C with $P_{\text{for}-\text{trend}} = 0.005$ (in Model 3; Table 2). The fully adjusted HRs of arterial stiffness incidence risk for per 1 mmol/L increase in non-HDL-C were 1.09 (95% CI, 1.02–1.17, in Model 3) among total participants (Table 2). Stratified analysis by age and sex revealed that the risk of incident arterial stiffness was significantly higher among younger participants (<60 years; Table 2).

The associations between non-HDL-C and risk of incident arterial stiffness across the entire levels were shown in Figure 1A. Additionally, stratified analyses revealed that the risk of incident arterial stiffness associated with non-HDL-C was differed among different age group (Figures 1B,C). The risk of incident arterial stiffness was significantly higher among younger participants (<60 years).

## Sensitivity analyses

The association of non-HDL-C with an increased risk of incident arterial stiffness was still robust in sensitivity analyses. After successively excluding the participants with missing data and participants with <1 follow-up year, the adjusted HRs of non-HDL-C for arterial stiffness were consistent with the results from the main analyses (Supplementary Table S3). The baseline characteristic of the remaining participants was presented in Supplementary Table S2. In the sensitivity analysis after excluding individuals with less than 1 year follow-up, the results still robust (Supplementary Table S3).

# Discussion

In this cohort study, our results showed that higher non-HDL-C concentration was associated with incidence risk of arterial stiffness. The effect of non-HDL-C was still robust in

TABLE 1 Baseline characteristic of cohort.

| Parameter | Total $n = 7,276$ | Without arterial stiffness $n = 5,607$ | With arterial stiffness $n = 1,669$ | *P*-Value |
|---|---|---|---|---|
| Age (years) | 44 (37–49) | 42 (36–48) | 48 (42–55) | <0.001 |
| Sex (female, %) | 2,354 (32.35%) | 2,021 (36.04%) | 333 (19.95%) | <0.001 |
| baPWV (cm/s) | 1,261.50 (1,181.50–1,329.50) | 1,236.50 (1,161.00–1,307.00) | 1,328.50 (1,273.50–1,367.50) | <0.001 |
| SBP (mmHg) | 118 (110–128) | 116 (108–124) | 126 (118–134) | <0.001 |
| DBP (mmHg) | 74 (68–82) | 74 (68–80) | 80 (74–88) | <0.001 |
| Body mass index (kg/m$^2$) | 24.19 (22.02–26.31) | 23.94 (21.81–26.11) | 25.09 (23.05–26.85) | <0.001 |
| FBG (mmol/L) | 5.09 (4.75–5.46) | 5.05 (4.72–5.41) | 5.24 (4.87–5.69) | <0.001 |
| TG (mmol/L) | 1.34 (0.92–2.05) | 1.27 (0.89–1.95) | 1.57 (1.09–2.36) | <0.001 |
| HDL cholesterol (mmol/L) | 1.47 (1.23–1.76) | 1.50 (1.25–1.78) | 1.38 (1.17–1.66) | <0.001 |
| LDL cholesterol (mmol/L) | 2.58 (2.09–3.12) | 2.56 (2.07–3.10) | 2.68 (2.18–3.18) | <0.001 |
| TC (mmol/L) | 4.87 (4.32–5.49) | 4.83 (4.28–5.44) | 4.99 (4.44–5.60) | <0.001 |
| non-HDL cholesterol (mmol/L) | 3.36 (2.76–3.99) | 3.30 (2.70–3.93) | 3.56 (3.00–4.14) | <0.001 |
| Smoking status | | | | |
| No | 3,272 (62.62%) | 2,582 (64.18%) | 690 (57.40%) | <0.001 |
| Yes | 1,953 (37.38%) | 1,441 (35.82%) | 512 (42.60%) | |
| Drinking status | | | | |
| No | 3,056 (58.49%) | 2,427 (60.33%) | 629 (52.33%) | <0.001 |
| Yes | 2,169 (41.51%) | 1,596 (39.67%) | 573 (47.67%) | |
| Exercise | | | | |
| No | 1,476 (28.25%) | 1,166 (28.98%) | 310 (25.79%) | 0.031 |
| Yes | 3,749 (71.75%) | 2,857 (71.02%) | 892 (74.21%) | |

baPWV, brachial-ankle pulse wave velocity; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; FBG, fasting blood glucose; TG, Triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; TC, total cholesterol; data were presented as median (interquartile range, IQR) for continuous variables and percentage for dichotomous variables.

younger participants (<60 years old), while disappeared in older participants (≥60 years old). These findings may provide a clue that the potential target threshold of non-HDL-C and the intensity of lipid lowering should vary with age in future management decisions.

Arterial stiffness plays a central role in the vascular aging process of CVD. Furthermore, considering the strong correlation between the two, non-HDL-C may contribute to CVD through vascular aging. Previous studies, most of which were cross-sectional, reported that non-HDL-C significantly correlated with PWV in both the young and old (6, 14–18). However, these studies did not compare the effects of non-HDL-C on arterial stiffness between young and old subjects. In contrast to the previous studies, our study established a cohort to clarify the role of non-HDL-C, and to specifically compare its potentially different effects on younger and older subpopulations. We also used arterial stiffness defined by baPWV ≥1,400 cm/s as the dichotomous outcome. Our study further highlights the significant association between non-HDL-C and arterial stiffness. Surprisingly, non-HDL-C has different effects in different age groups. Non-HDL-C shows the strongest correlation with arterial stiffness in the young age group (<60). For the oldest age group (≥60), the effect disappeared altogether.

Non-HDL-C, including LDL, VLDL, lipoprotein(a), apolipoprotein B, and other effective components, has become a superior surrogate marker for treatment assessment. A meta-analysis including 233,455 subjects suggests that non-HDL-C is a more effective target for lipid-lowering therapy than LDL-C (22). Compared with LDL-C, non-HDL-C is also more strongly associated with atherosclerosis than LDL-C is (23). Furthermore, several studies have demonstrated that non-HDL-C is a better predictor of cardiovascular disease (4, 24). The Framingham Heart Study have found long term exposure to elevated non-HDL-C increases atherosclerotic cardiovascular disease (ASCVD) risk and mortality (5). Even for populations with low estimated 10-year ASCVD risk, non-HDL-C ≥160 mg/dl was associated with an 80% increased relative risk of CVD mortality (25). Patients with diabetes, metabolic syndrome, or obesity are also more likely to show elevated non-HDL-C in the presence of normal LDL-C, making non-HDL-C a better risk predictor for CVD (26, 27). In addition, non-HDL-C is calculated as TC minus HDL-C, without any additional cost or the need for prior fasting (28).

TABLE 2 The HRs of non-HDL-C concentration with the incidence risk of arterial stiffness.

| Stratified by | | Categorical | | | *P*-trend | Per 1.0 mmol/L↑ |
|---|---|---|---|---|---|---|
| | | Tertile 1 | Tertile 2 | Tertile 3 | | |
| Total | Case/*N* | 401/2,440 | 582/2,424 | 686/2,412 | – | 1,669/7,276 |
| | Model 1 | Reference | 1.26 (1.11, 1.44)*** | 1.47 (1.29, 1.66)*** | <0.001 | 1.16 (1.10,1.22)*** |
| | Model 2 | Reference | 1.18 (1.02, 1.36)* | 1.30 (1.13, 1.49)*** | <0.001 | 1.10 (1.04,1.17)** |
| | Model 3 | Reference | 1.15 (0.98, 1.35) | 1.26 (1.07, 1.48)** | 0.005 | 1.09 (1.02,1.17)** |
| <60 years | Case/*N* | 312/2,307 | 509/2,307 | 601/2,290 | – | 1,422/6,904 |
| | Model 1 | Reference | 1.30 (1.12,1.50)*** | 1.45 (1.26, 1.67)*** | <0.001 | 1.14 (1.08, 1.21)*** |
| | Model 2 | Reference | 1.25 (1.07, 1.47)** | 1.32 (1.13, 1.55)** | 0.001 | 1.09 (1.03,1.17)** |
| | Model 3 | Reference | 1.23 (1.03, 1.49)* | 1.30 (1.08,1.56)** | 0.005 | 1.09 (1.01, 1.17)* |
| ≥60 years | Case/*N* | 89/133 | 73/117 | 85/122 | – | 247/372 |
| | Model 1 | reference | 0.97 (0.71, 1.33) | 1.36 (1.00, 1.85)* | 0.047 | 1.19 (1.02, 1.38)* |
| | Model 2 | reference | 0.90 (0.64, 1.27) | 1.09 (0.78, 1.53) | 0.608 | 1.09 (0.93,1.28) |
| | Model 3 | reference | 0.83 (0.58, 1.21) | 1.00 (0.69, 1.45) | 0.998 | 1.02 (0.85, 1.22) |

Model 1: adjusted by age and sex. Model 2: Model 1 + adjusted by BMI, SBP, and fasting glucose. Model 3: Model 2 + adjusted by smoking status, drinking status, and exercise. Non-HDL cholesterol category: tertile 1, <2.98 mmol/L; tertile 2, 2.98–3.76 mmol/L; tertile 3, >3.76 mmol/L. Reference defined as tertile 1.
*P < 0.05.
**P < 0.01.
***P < 0.001.

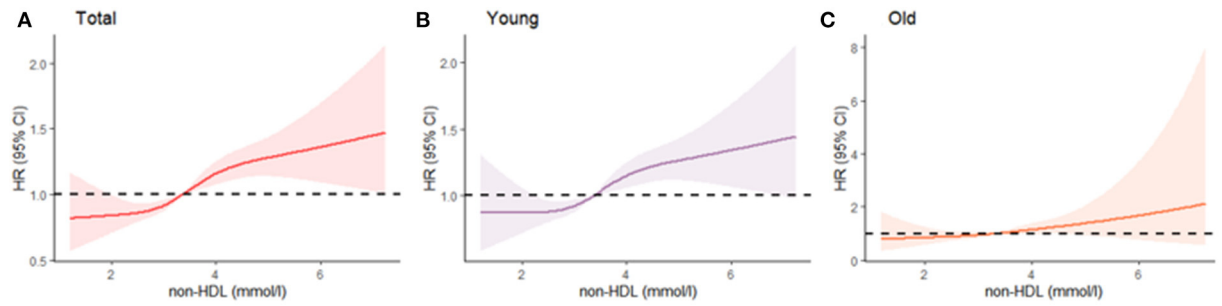


FIGURE 1
Age-specific continuous association of non-HDL-C and arterial stiffness **(A)** total; **(B)** young; **(C)** old. The Cox model used is adjusted for age and sex. Non-HDL-C was modeled using cubic splines. Median follow-up was 1.78 years.

Due to its convenience and greater predictive power, there are many guidelines, as well as a growing medical consensus, recommending non-HDL-C for clinical use (8, 9, 27). In mechanisms, dyslipidemia, especially a high level of non-HDL-C, was closely related to endothelial dysfunction (29). The important components of Non-HDL-C such as LDL and ApoB can cross the endothelial barrier and infiltrates specific areas of the arterial wall (30). Oxidative stress and chronic inflammation induced by long term exposure to high level of non-HDL also considered potential pathophysiological mechanisms in arterial stiffness. On the one hand, vascular inflammation causes arterial stiffness by stimulating proliferation of fibroblasts and smooth muscle cell (31, 32); on the other hand, inflammation and oxidative stress exacerbate endothelial dysfunction and impair arterial mechanical properties (33). These will ultimately lead to vascular aging and an increase in PWV.

It is necessary to discuss the age dependent association between non-HDL-C and arterial stiffness. As our study demonstrates, arterial stiffness is more often attributed to non-HDL-C in the young than in the elderly. Thus, young people are more likely to benefit from controlling non-HDL-C. For the elderly, non-HDL-C is not associated with arterial stiffness, further illustrating the etiological complexity of aging. Across life course, middle age was in the essential stage of arterial stiffness with a steeper increase in baPWV during this stage (19). This partly explains why non-HDL is more effective for participants <60. This age-specific effect may provide better guidelines for disease prevention and control. Our results depict a different risk curve for non-HDL-C concentration among the young

and old, respectively (Figure 1). Therefore, the application of appropriate thresholds for different age groups should yield better outcomes. Our findings also advocate that the prevention of arterial stiffness should be initiated as early as possible to reduce the lifetime risk of cardiovascular disease

There are several advantages in our study. First, we have the strength of a large number of participants. Second, we discovered effects of non-HDL-C among different age group. Participants were divided into two groups according to age. The study provides the novel findings that non-HDL-c performs more effectively in identifying individuals at increased arterial stiffness risk especially for young individuals. However, several limitations remain. First, arterial stiffness is a chronic process, while the follow-up time of participants in our study was short. To reduce the influence of the short follow-up time, we did a sensitivity analysis among participants with more than 1 year follow-up. Our results still indicated the adverse effect of non-HDL-C. Second, our analysis in final model only included a subset of the participants due to missing values. To addressed this issue, we limited the main analysis to participants with complete data in sensitivity analysis. Third, our study lacks the information about lipid lowering therapy, that may be related with the risk of arterial stiffness. Finally, the included participants were Chinese and most of these participants were come from central region of China, which may be limited in the generalizability of these results.

In conclusion, our study indicates that non-HDL-C carries a greater incidence risk for arterial stiffness, especially for younger individuals. Our study suggests that the target threshold for non-HDL-C should be different according to the age.

## Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: Privacy of participants. Requests to access these datasets should be directed at: ZH, hzter1985@163.com.

## Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board of The Third Xiangya Hospital of Central South University. The Ethics Committee waived the requirement of written informed consent for participation.

## Author contributions

Conceptualization: ZH and JL. Data curation: ZC. Formal analysis: JieW and RM. Investigation: JiaW and HY. Supervision: JL, RM, and ZH. Writing—original draft: JieW. Writing—review and editing: JL. 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/fcvm.2022.981028/full#supplementary-material

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# Depressive disorders in older Chinese adults with essential hypertension: A classification tree analysis

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**Background:** Although there has been accumulating evidence on the elevated risk of depression in hypertensive patients, data regarding depressive disorders in older adults with hypertension and the interplay between factors associated with depression in this population are very limited. Disentangling the mutual influences between factors may help illuminate the pathways involved in the pathogenesis of the comorbidity of depression in hypertension. This study investigated the prevalence of depressive disorders in older Chinese adults with hypertension and examined major correlates of depressive disorders and the interactions between correlates by using classification tree analysis (CTA).

**Methods:** In total, 374 older adults with essential hypertension were enrolled from seven urban and six rural primary care centers in Wuhan, China, and interviewed with the Chinese Mini-international Neuropsychiatric Interview 5.0. Family relationship and feelings of loneliness were assessed with standardized questions. A checklist was used to assess the presence of six major medical conditions: diabetes mellitus, heart disease, cerebrovascular disease, chronic obstructive pulmonary disease, chronic gastric ulcer, and arthritis.

**Results:** The 1-month prevalence rate of depressive disorders was 25.7%. The CTA model identified four major correlates of depressive disorders: loneliness was the most salient, followed by arthritis, family relationship, and heart disease. There were statistically significant interactions between loneliness and arthritis, loneliness and family relationship, and arthritis and heart disease.

**Conclusion:** Over one out of every four older Chinese adults with hypertension suffer from depressive disorders. Collaborative multidisciplinary management services are needed to reduce the burden of depression in hypertensive older adults, which may include social work outreach services to promote family relationship, mental health services to relive loneliness, and primary care services to manage arthritis and heart disease.

KEYWORDS

**depressive disorders, hypertension, older adults, classification tree analysis, interaction**

# Introduction

Although pathophysiological mechanisms underlying the hypertension-depression link are complex and still not fully understood, there has been strong and clear evidence on the elevated risk of depressive symptoms and disorders in hypertensive patients (1–4). For example, in China the prevalence rates of depressive symptoms and DSM-IV depressive disorders among outpatients with essential hypertension in large tertiary general hospitals are 47.6 and 16.6%, which are three and ten times as high as those in the general Chinese population, respectively (5, 6). The co-occurring depression has been associated with prolonged duration of hypertension, poor compliance with antihypertensive agents, and failure of adherence to lifestyle interventions, which in turn, complicates the management of hypertension and increases risk of cardiovascular complications (7–9). In this context, expanding our knowledge on the etiology and mechanisms of depression in hypertensive patients is clinically relevant, which may facilitate the effective management of hypertension and the prevention of hypertension-related complications.

The cause of depression in the general population is multifactorial in nature, which involves biological, psychological, and social factors and their interplays (10–12) and, accordingly, the cause of depression in hypertensive patients is no exception. In the literature, a range of factors associated with depression in hypertensive patients have been reported by many clinical studies in China and many other countries, including female sex, advanced age, a low level of educational attainment, marital status of divorced and widowed, low income, living alone, smoking, alcohol consumption, inadequate social support, a long duration of hypertension, and coexistence of major medical conditions (5, 6, 13–20). Nevertheless, nearly all the available studies focused on the main effects of these factors but none paid attention to how the factors work together to determine the depression risk, which may reveal the mechanisms of the mutual cross-talk between factors and how the combination of factors influences the risk of depression, and, in turn, inform the planning of mental health services. For example, if the influence of living alone on depression is conditional on the sex of an individual: statistically significant association of living alone with depression is evident only in women, providing mental health services to women who were living alone would be more cost-effective. In addition, a further limitation of the prior studies is no findings on the relative contributions of the identified factors to the risk of depression, because the statistical method adopted by previous studies, multiple logistic regression model, is often used to identify statistically significant correlates of depression, not clinically important correlates (21).

Because of the higher prevalence of hypertension in older adults than middle-aged and younger adults, older adults with hypertension are the main target population of interest of many previous studies examining depression in hypertension (13–15, 19, 20). These studies used a variety of self-rating scales of depressive symptoms (i.e., nine-item Patient Health Questionnaire and Zung's Self-rating Depression Scale) and reported a wide range of prevalence rates of depressive symptoms (12.8–61.0%) in hypertensive older adults (14, 19). However, because of no rigorous psychiatric interviews, the proportion of hypertensive older adults whose depressive symptoms are severe enough to meet the clinical diagnostic criteria of depressive disorders remains unknown (22).

To advance the literature in this area, this study was set out to investigate the prevalence of depressive disorders in older Chinese adults with hypertension, and, adopted classification tree analysis (CTA) to examine the major correlates of depressive disorders and identify the interaction between correlates. Unlike traditional binary logistic regression, CTA is a robust algorithm to identify clinically important factors associated with the outcome of interest and effectively detect factor interactions (23). Furthermore, another strength of CTA is its user-friendly way to show findings on factors associated with the outcome and their interactions, which can be easily applied to routine clinical and primary care practice by healthcare workers with limited statistical understanding (24).

# Materials and methods

## Sample

The study sample was 374 hypertensive older adults from a large-scale multi-center cross-sectional survey that examined mental health and quality of life among a representative sample of older adults receiving primary care in seven urban and six rural primary care centers in Wuhan, China, between October 2015 and November 2016. Older primary care patients who were 65 years or older, voluntary to join the study, and diagnosed with essential hypertension or taking antihypertensive medications were included in the current analysis. Details of the sampling and the recruitment of respondents have been published elsewhere (25–29).

The Ethics Committee of Wuhan Mental Health Center approved the study proposal before the formal survey (approval number WMHC-IRB-S065). All respondents and their guardians (when necessary) provided written informed consent form before the interview.

## Instruments and procedures

The study instrument was a questionnaire, which was administered in a face-to-face format by trained primary care physicians (PCPs). The validated Chinese version of the Mini-international Neuropsychiatric Interview (MINI) 5.0 was used to assess the presence of DSM-IV depressive disorders within the past month, including major depressive disorder, dysthymic disorder, and minor depressive disorder (30).

The demographic variables in the questionnaire were sex, age, education, marital status, self-rated financial status, and residence place. Social factors included living arrangement (alone or not alone), self-rated relationship with family members (good, fair, poor), and self-rated relationship with non-family associates (good, fair, poor). Lifestyle factor was currently smoking, which was defined as smoking 5 days per week or more within the last month (27). Psychological factor was feelings of loneliness, which was assessed with a single-item question: "How often do you feel lonely?" with five answer options: always, often, sometimes, seldom, and never. Participants who felt lonely "sometimes," "often," and "always" were those having feelings of loneliness (28). Clinical factors were the comorbid major medical conditions, which was assessed with a checklist and included diabetes mellitus, heart disease, cerebrovascular disease, chronic obstructive pulmonary disease, chronic gastric ulcer, and arthritis.

## Statistical analysis

IBM SPSS statistics software, version 24 (SPSS Inc., Chicago, IL, USA) was used to perform all the analyses. Two-sided $P < 0.05$ was statistically significant. Prevalence rates of depressive disorders and their three subtypes were calculated. By using Chi-square test, we compared the characteristics between respondents with and without depressive disorders to characterize respondents with depressive disorders.

To identify major correlates of depressive disorders and their potential interactions, the exhaustive Chi-squared automatic interaction detection (exhaustive CHAID) growing approach was used to perform the CTA. The target category of the outcome in the CTA was the presence of depressive disorders, and all demographic, social, lifestyle, psychological, and clinical variables were included as input variables. We set the maximum number of layers of growth beneath the root node at three and the minimum node sizes at 50 for parent nodes and 25 for child nodes. The CTA divided the study sample into branch-like segments by comparing Chi-square statistics of all possible categories in relation to depressive disorders and this process continued recursively until the tree was fully grown. These segments consisted of an inverted tree with a root node, internal nodes, and end nodes. Accordingly, the classification tree automatically identified correlates of depressive disorders from the root nodes to endnotes, in the order of importance, as well as the interactions between these correlates (23, 31).

# Results

The average age of the 374 hypertensive older adults was 72.9 years (standard deviation [SD]: 5.8, range: 65–93) and 41.7% were men. **Table 1** shows the characteristics of the whole sample and respondents with and without depressive disorders.

The 1-month prevalence rate of depressive disorders was 25.7%. The corresponding rates for major depressive disorder, dysthymic disorder, and minor depressive disorder were 13.6, 6.1, and 5.9%, respectively.

As displayed in **Table 1**, compared to respondents without depressive disorders, depressed respondents were more likely to be women, have an educational attainment of illiterate and primary school, rate their economic status as "poor," dwell in rural areas, rate their relationship with family members as "fair and poor," feel lonely, suffer from heart disease, suffer from cerebrovascular disease, suffer from chronic gastric ulcer, and suffer from arthritis ($P \leq 0.030$).

As shown in **Figure 1**, the CTA model had three layers of eight nodes, including five end nodes. Four major correlates of depressive disorders were identified: loneliness was the most salient, followed by arthritis, family relationship, and heart disease. Compared to respondents who were not lonely, lonely respondents were 1.9-fold more likely to have depressive disorders (40.9 vs. 21.0%, $P < 0.001$). Among the lonely respondents, relative to those who had good family relationship, those having fair and poor relationship were 1.8-fold more likely to have depressive disorders (58.6 vs. 32.2%, $P = 0.018$). Among respondents who were not lonely, relative to those having no arthritis, those having arthritis were 3.0-fold more likely to have depressive disorders (53.8 vs. 17.7%, $P < 0.001$). Among respondents who were not lonely and did not suffer from arthritis, relative to those having no heart disease, those having heart disease were 2.2-fold more likely to have depressive disorders (34.4 vs. 15.4%, $P = 0.008$). There were statistically significant interactions between loneliness and arthritis, loneliness and family relationship, and arthritis and heart disease.

# Discussion

In China, both hypertension and depressive disorders occur predominantly in the elderly population, and the comorbidity of depression further significantly contributes to the vulnerability of the elderly to hypertension (32).

TABLE 1 Characteristics of hypertensive older Chinese adults, split by the presence and absence of depressive disorders, n (%).

| Variable | | Total sample ($n$ = 374) | Without depressive disorders ($n$ = 278) | With depressive disorders ($n$ = 96) | $\chi^2$ | $P$ |
|---|---|---|---|---|---|---|
| Sex | Male | 156 (41.7) | 125 (45.0) | 31 (32.3) | | |
| | Female | 218 (58.3) | 153 (55.0) | 65 (67.7) | 4.713 | 0.030 |
| Age-groups | 65–74 years | 235 (62.8) | 172 (61.9) | 63 (65.6) | | |
| | 75 + years | 139 (37.2) | 106 (38.1) | 33 (34.4) | 0.431 | 0.512 |
| Education | Illiterate | 79 (21.1) | 54 (19.4) | 25 (26.0) | | |
| | Primary school | 106 (28.3) | 70 (25.2) | 36 (37.5) | | |
| | Middle school and above | 189 (50.5) | 154 (55.4) | 35 (36.5) | 10.365 | 0.006 |
| Marital status | Married | 269 (71.9) | 201 (72.3) | 68 (70.8) | | |
| | Others* | 105 (28.1) | 77 (27.7) | 28 (29.2) | 0.076 | 0.782 |
| Self-rated economic status | Good | 66 (17.6) | 56 (20.1) | 10 (10.4) | | |
| | Fair | 275 (73.5) | 207 (74.5) | 68 (70.8) | | |
| | Poor | 33 (8.8) | 15 (5.4) | 18 (18.8) | 18.376 | <0.001 |
| Residence place | Urban | 210 (56.1) | 168 (60.4) | 42 (43.8) | | |
| | Rural | 164 (43.9) | 110 (39.6) | 54 (56.3) | 8.065 | 0.005 |
| Living alone | No | 336 (89.8) | 250 (89.9) | 86 (89.6) | | |
| | Yes | 38 (10.2) | 28 (10.1) | 10 (10.4) | 0.009 | 0.923 |
| Self-rated family relationship | Good | 310 (82.9) | 241 (86.7) | 69 (71.9) | | |
| | Fair and poor** | 64 (17.1) | 37 (13.3) | 27 (28.1) | 11.043 | 0.001 |
| Self-rated non-family relationship | Good | 377 (74.1) | 212 (76.3) | 65 (67.7) | | |
| | Fair and poor** | 97 (25.9) | 66 (23.7) | 31 (32.3) | 2.716 | 0.099 |
| Feelings of loneliness | No | 286 (76.5) | 226 (81.3) | 60 (62.5) | | |
| | Yes | 88 (23.5) | 52 (18.7) | 36 (37.5) | 14.009 | <0.001 |
| Currently smoking | No | 320 (85.6) | 234 (84.2) | 86 (89.6) | | |
| | Yes | 54 (14.4) | 44 (15.8) | 10 (10.4) | 1.691 | 0.193 |
| Diabetes mellitus | No | 287 (76.7) | 216 (77.7) | 71 (74.0) | | |
| | Yes | 87 (23.3) | 62 (22.3) | 25 (26.0) | 0.559 | 0.455 |
| Heart disease | No | 319 (85.3) | 246 (88.5) | 73 (76.0) | | |
| | Yes | 55 (14.7) | 32 (11.5) | 23 (24.0) | 8.815 | 0.003 |
| Cerebrovascular disease | No | 337 (90.1) | 257 (92.4) | 80 (83.3) | | |
| | Yes | 37 (9.9) | 21 (7.6) | 16 (16.7) | 6.647 | 0.010 |
| Chronic obstructive pulmonary disease | No | 351 (93.9) | 264 (95.0) | 87 (90.6) | | |
| | Yes | 23 (6.1) | 14 (5.0) | 9 (9.4) | 2.328 | 0.127 |
| Chronic gastric ulcer | No | 359 (96.0) | 273 (98.2) | 86 (89.6) | | |
| | Yes | 15 (4.0) | 5 (1.8) | 10 (10.4) | 13.767 | <0.001 |
| Arthritis | No | 343 (91.7) | 262 (94.2) | 81 (84.4) | | |
| | Yes | 31 (8.3) | 16 (5.8) | 15 (15.6) | 9.144 | 0.002 |

*"Others" included never married, separated, divorced, widowed, cohabitating, and remarried.
**Because of the very small numbers of the category of "poor" relationship ($n$ < 10), "poor" and "fair" were merged into one category.

In the context of rapid aging in China, disentangling the complex relationship between factors associated with depression in hypertension may help illuminate the pathways involved in the pathogenesis of the comorbidity of depression, potentially leading the way for effective management of hypertension and effective public health interventions to reduce the disease burden of hypertension (33–37). The present study fills the knowledge gaps by providing empirical data on the prevalence rates of depressive disorders and their subtypes in the elderly population with hypertension and demonstrating major factors associated with depressive disorders and the interactions between these factors. To the best of our knowledge, this is the first study in China examining depressive disorders and testing the interplays

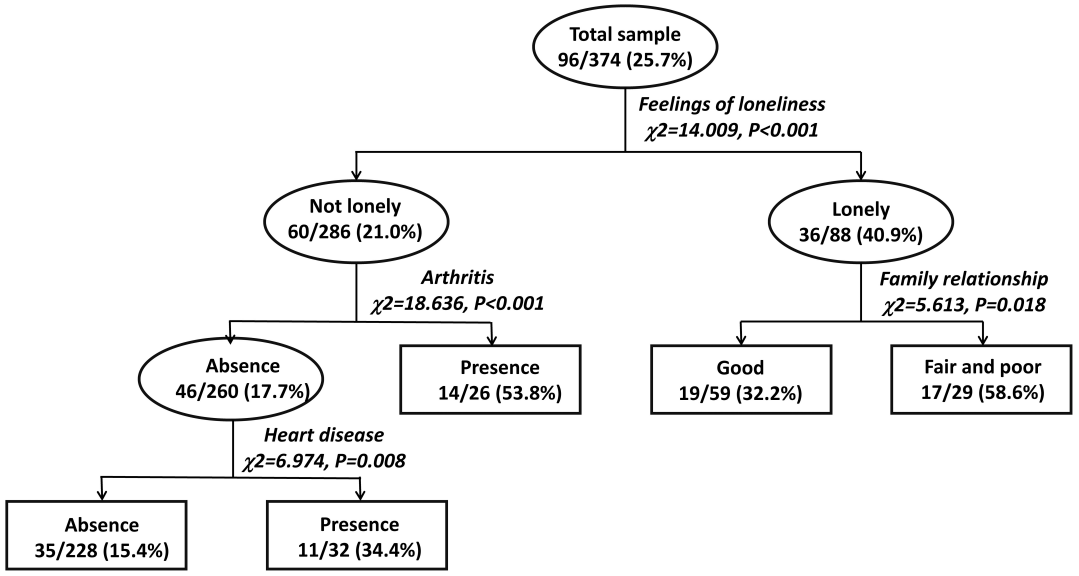


FIGURE 1
Classification tree analysis for major correlates of depressive disorders and the interactions of factors in older Chinese adults with hypertension.

between factors associated with depression in older adults with hypertension.

The main findings of this study are the 25.7% prevalence of depressive disorders in hypertensive older adults with major depressive disorder being the most common, four major correlates of depressive disorders with loneliness being the most prominent, and the significant interactions between loneliness and arthritis, loneliness and family relationship, and arthritis and heart disease.

In community-residing older Chinese adults, the 1-month prevalence rates of depressive disorders, major depressive disorder, dysthymic disorder, and mood disorder not otherwise specified (minor depressive disorder is a subtype of this category) were 5.5, 3.8–5.9, 3.9, and 3.0%, respectively (38–40). In older Chinese adults seeking treatment in primary care settings, the 1-month prevalence rates of depressive disorders, major depressive disorder, dysthymic disorder, and minor depressive disorder were 20.3, 10.2–11.3, 4.8, and 5.3%, respectively (26, 41). Therefore, in comparison to these prevalence estimates in older Chinese adults in both community and primary care settings, we found the higher risk of depressive disorders and their subtypes in Chinese patients with hypertension. In the literature, possible explanations for how hypertension results in or exacerbates depression include the mental health burden of suffering from hypertension and its negative impact on a person's quality of life, a low sense of self-worth, low self-esteem, and a loss of locus of control due to the negative psychological effect of hypertension, and structural changes in brain areas related to emotion as a result of pathophysiologic effects of hypertension on central nervous system (42).

Our findings on possible factors associated with depressive disorders in hypertensive older adults (**Table 1**) are largely consistent with those from previous studies (5, 6, 13–20). Nevertheless, only four of these factors were finally identified as major correlates of depression in the CTA, suggesting the considerable contributions of loneliness, arthritis, fair and poor family relationship, and heart disease to the elevated risk of depression.

Evidence from longitudinal studies has confirmed the vicious circle between loneliness and depression, that is, loneliness triggers depressive emotions, which create feelings of isolation and alienation and, in turn, result in loneliness (43–45). Accordingly, we replicated the significant loneliness-depression association in hypertensive older adults. In a population-based study of middle-aged adults, Dunlop and colleagues found that both arthritis and heart disease were significantly associated with major depression and the functional limitation caused by the two chronic illnesses can explain their associations with major depression (46). Similar to this study, we also found the significant association of depression with arthritis and heart disease in hypertensive older adults. We also speculate the functional limitation associated with the two major medical conditions might be the primary cause of depressive disorders. Unlike older adults in western countries, family harmony and intergenerational relationship play a pivotal role in the mental well-being of older Chinese adults due to the influence of Confucian culture (47, 48). In accordance with this perspective, fair and poor family relationship was significantly associated with depression in hypertensive older adults.

The three significant interactions between the four major correlates suggest that the factor *per se* not only directly

contributes to the risk of depression but also magnifies the negative effects of other factors on the risk of depression. The four factors work together may substantially increase the risk of depression in hypertensive older adults.

This study has several limitations. First, this is a cross-sectional study, so longitudinal studies are warranted to further ascertain the causal relationships between the four identified major correlates and depressive disorders. Second, our CTA is exploratory without evidence of external validity. More studies are needed to validate the findings in other cohorts of older adults with hypertension. Third, the sample size of this study is relatively small. Further, the sample of hypertensive older adults was recruited from primary care settings in Wuhan China. Hypertensive older adults from large general hospitals and other cities in China were not included. Therefore, there might be selection bias in our study sample. Fourth, other factors potentially associated with depression in hypertensive older adults such as personality, physical pain, blood pressure control status, stage of hypertension, and type of antihypertensive drugs were not measured.

In summary, over one out of every four older Chinese adults with hypertension suffer from depressive disorders, suggesting the high risk of depressive disorders in hypertensive older adults. Considering many negative outcomes associated with depression, mental health services for this patient population are urgently needed, which should include psychosocial support, periodic screening for depressive symptoms to ensure early recognition of older adults with depressive disorders, and early initiation of antidepressant treatment when necessary. Our findings on the four major correlates of depressive disorders are clinically interesting because feelings of loneliness, family relationship, arthritis, and heart disease are all potentially modifiable or treatable. The significant interplays between the four major factors further indicate the clinical needs for collaborative multidisciplinary management services for reducing the burden of depression in hypertension, which integrate social work outreach services to promote family relationship, mental health services to relive loneliness, and primary care services to manage arthritis and heart disease.

## Data availability statement

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

## Ethics statement

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

## Author contributions

JR: acquisition and analysis of data for the study, drafting the manuscript, and interpretation of data for the study. JR and Y-MX: design and acquisition of data for the study. B-LZ: drafting the manuscript, revising the manuscript for important intellectual content, and interpretation of data for the study. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

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

## Publisher's note

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# Association of height loss and cardiovascular disease: Data from a large Korean cohort

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**Background:** Height declines with age, and its degree differs among individuals. Despite epidemiologic evidence for the inverse relationship between adult height and cardiovascular disease (CVD) incidence, the clinical significance of height loss in CVD remains to be elucidated. Therefore, this study investigated the association between height loss and CVD incidence.

**Methods:** In total, 127,573 Korean participants were enrolled; their heights were monitored from 2002 to 2011. The annual height loss (cm/year) was the difference between the first and last height measurements within the observation period divided by the number of years. The participants were classified as Group 1 (height loss: <0.3 cm/year; *n* = 102,554), Group 2 (height loss: 0.3– < 0.6 cm/year; *n* = 17,324), or Group 3 (height loss: ≥0.6 cm/year; *n* = 7,695).

**Results:** The cumulative major adverse cardiac and cerebral event (MACCE: cardiac death, non-fatal myocardial infarction, and unplanned hospitalization for heart failure or stroke) incidence rate was 3.6% for Group 1, 4.5% for Group 2, and 5.2% for Group 3. Group 2 (hazard ratio [HR] = 1.27, 95% confidence interval [CI] = 1.17–1.37) and Group 3 (HR = 1.46, 95% CI = 1.32–1.62) had a significantly higher incidence of MACCE than Group 1. In the model adjusted for age, sex, comorbidities, income level, body mass index, smoking, and drinking status, the MACCE risk was higher in Group 2 (HR = 1.11, 95% CI = 1.07–1.20) and Group 3 (HR = 1.25, 95% CI = 1.13–1.39) than in Group 1.

**Conclusion:** The degree of height loss was independently associated with CVD occurrences in the Korean population.

KEYWORDS

**height loss, CVD, MACCE, aging, cardiovascular disease**

# Introduction

Growing evidence suggests that short height is associated with cardiovascular disease (CVD) occurrence (1–4), although a rationale for this epidemiological finding remains unclear. Thus, genetically determined height may be a non-modifiable indicator for increased CVD risk. The maximal stature of a given individual is determined in the late teen years (5). However, height declines with aging due to senile changes in the musculoskeletal system (6). The degree of height loss varies among individuals based on the severity of osteoporosis or sarcopenia, which are significant contributors to stature decrease and have a known association with mortality (7–9). Hence, it can be hypothesized that the degree of height loss is correlated with future CVD occurrence. This topic was investigated once in elderly British men two decades ago, and the authors observed that marked height loss (≥3 cm over the preceding two decades) was an independent CVD risk factor (10). However, data from different countries, eras, and ethnicity are needed to confirm this hypothesis because anthropometric parameters, such as height, are influenced by demographic characteristics and the socioeconomic status of the society. The Republic of Korea is suitable for the research for that purpose because it is homogenous as regards ethnicity, and there is a government-driven annual medical check-up/treatment data for all eligible Koreans. This study used a large Korean cohort to investigate the relationship between height loss and CVD prevalence.

# Materials and methods

## Study samples

The National Health Insurance Service (NHIS) of the Republic of Korea operates a mandatory public insurance program for all citizens and supports public health policy and research activities by developing and maintaining the National Health Information Database (11). This study was performed using NHIS data.

Eligible participants were ethnically Korean, ≥40 years, underwent medical examinations in the Republic of Korea between January 1, 2002, and December 31, 2015; 332,579 individuals were eligible. The exclusion was based on the following: 89,194 individuals had only one height measurement, 57,164 had unreliable height data (such as increasing height, a decrease of ≥20 cm, or an annual decrease rate of ≥10 cm/year), 689 had missing demographic data during the observation period (2002–2011), 27,599 had major adverse cardiac and cerebral events (MACCE) during the observation period, and 30,360 died before 2012, which was the beginning of the outcome monitoring period. As a result, 127,573 individuals were included in the analysis (**Figure 1**).

## Height and height loss measurements

Height was measured to the nearest 0.1 cm using a stadiometer with the participants standing upright. The observation period for measuring the decrease in height (measured in cm) was ten years (2002–2011). Each participant was included in the study at the time of the first height measurement recording. Heights were measured annually, and the interval between each height measurement was a year or more. The annual height decrease rate (cm/year) was calculated as the difference between the first and the last height measurement within the observation period divided by the number of years. According to annual height decrease rate, participants were classified as Group 1 (reference; height loss: <0.3 cm/year; $n$ = 102,554), Group 2 (height loss: 0.3– < 0.6 cm/year; $n$ = 17,324), or Group 3 (height loss: ≥0.6 cm/year; $n$ = 7,695; **Figure 1**).

## Outcome definitions and monitoring

Using the Korean Standard Classification of Diseases (KCD-7), which is based on the International Statistical Classification of Diseases and Related Health Problems, MACCE included cardiac death (acute myocardial infarction [MI] [I21], heart failure [I50, I130, I132, I110], cardiac arrest [I46], all with death), non-fatal MI, unplanned hospitalization for heart failure (HF), and stroke (10). Stroke included ischemic and hemorrhagic pathologies. The demographic variables of sex, age, income deciles (1st to 10th deciles), diabetes mellitus, dyslipidemia, hypertension, smoking (individuals who answered "I still smoke" to their smoking status were considered smokers), drinking (individuals who drank > two times per week were considered drinkers), and body mass index (BMI, kg/m$^2$) were collected. The BMI was calculated using body weight measured to the nearest 0.1 kg at enrolment. For stratified analysis, those ≥ 60 years were considered elderly (prone to height loss). Women ≥ 50 years were arbitrarily defined as "menopausal" (after which height prominently decreases) based on the previously reported mean menopausal age of Korean women (12).

The primary endpoint was MACCE occurrences between 2012 and 2015. Participants whose last height measurement was at least five years after the first measurement between 2002 and 2011 were included in this study. The incidence of MACCE was collectively followed-up between 2012 and 2015. The start date of MACCE incidences from the last height measurement differed for each participant; therefore, those who developed MACCE before 2012 were excluded from the data set.

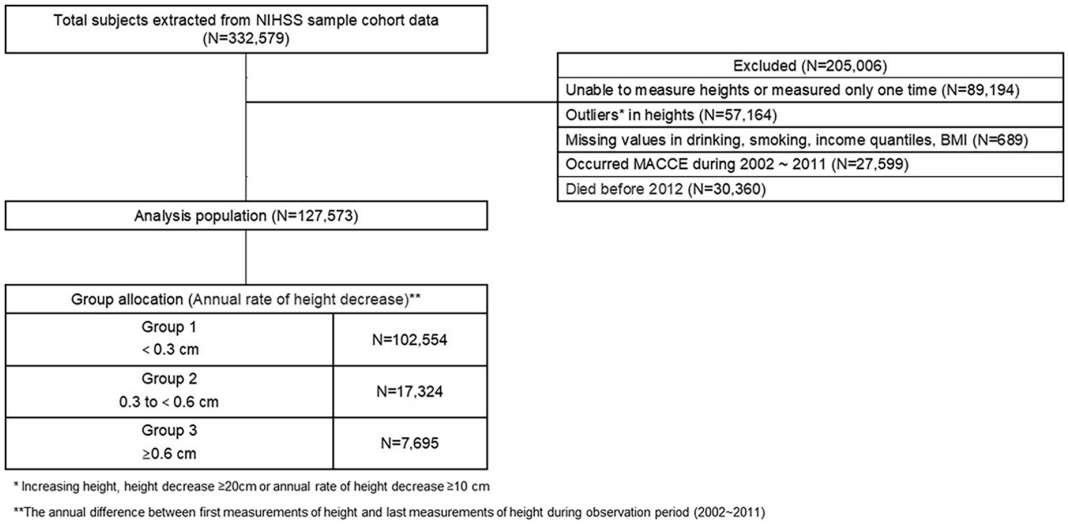


FIGURE 1
A flow diagram of the study population.

## Statistical analyses

Differences in demographic characteristics based on the annual height decrease rate were evaluated using the Chi-square test and the Kruskal–Wallis test. MACCE hazard ratios (HR) and 95% confidence intervals (CI) were calculated based on the annual height decrease rate using a Cox proportional-hazard model adjusted for sex, age, income deciles, diabetes, dyslipidemia, hypertension, smoking, drinking, and BMI. The proportional assumption of the Cox analysis was conducted for Cox proportional-hazard modeling. In univariate analysis, a Log-rank test was conducted to select significant variables. Subsequently, multiple analyses were conducted using Cox's proportional hazard model. Factors affecting height decreases were analyzed using multivariate linear regression and Kaplan–Meier survival curves and presented to estimate the cumulative MACCE incidence rates with time. Data presents the stratified analyses for the elderly and menopausal populations. All the analyses were performed using the SAS 9.4 software (SAS Institute, Cary, NC, USA) at a statistical significance level of $\alpha = 0.05$.

# Results

## Study population and baseline characteristics

The overall number of height measurements was 4.1 ± 2.0 (4.3 ± 2.0 times for Group 1, 3.5 ± 1.6 times for Group 2, and 2.8 ± 1.3 times for Group 3). The overall time gap between the first and the last height measurements was 69 ± 28 months (median: 72, interquartile range [IQR, 47–94]) and 73 ± 27 months (median: 78, IQR [51–96]) for Group 1, 58 ± 27 months (median: 57, IQR [32–77]) for Group 2, and 39 ± 27 months (median: 30, IQR [19–55]) for Group 3. Table 1 presents the demographic and clinical characteristic distributions based on the height decrease rate. There were 57,623 males (45.2%) and 69,950 females (54.8%). Group 1 had the highest proportion of males, and Group 3 had the highest proportion of females with a significant inter-group difference ($p < 0.0001$). The highest number of participants were in their 50 s, while the lowest were in their 90 s, with a significant inter-group difference ($p < 0.0001$). The highest number of participants was in the 10th decile of income, and the lowest number was in the 5th decile; this inter-group difference was also significant ($p < 0.0001$). Overall, 48, 488 participants (38.0%) had hypertension, and Group 2 had the highest proportion of patients with hypertension; the inter-group difference was significant ($p < 0.0001$). Further, 44,619 participants (35.0%) had dyslipidemia, and Group 1 had the largest number; the inter-group difference was also significant ($p < 0.0001$). In total, 20,004 participants (15.7%) were smokers, and Group 1 had the highest proportion; the inter-group difference was significant ($p = 0.0485$). Finally, Group 1 had the highest proportion of drinkers, and the inter-group difference was significant ($p < 0.0001$).

## Outcomes and height loss associations

Table 2 presents the cumulative MACCE incidence based on the ranges of the 4-year height decrease. The cumulative MACCE incidence rates were 3.6% for Group 1, 4.5% for Group

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TABLE 1 Study population baseline characteristics.

| | Total | Annual rate of height decrease† | | | *p*-value†† |
|---|---|---|---|---|---|
| | | Group 1 | Group 2 | Group 3 | |
| | (*N* = 127,573) | (*N* = 102,554) | (*N* = 17,324) | (*N* = 7,695) | |
| | *n* (%) | *n* (%) | *n* (%) | *n* (%) | |
| Sex | | | | | <0.0001 |
| Male | 57,623 (45.2) | 48,053 (46.9) | 6,780 (39.1) | 2,790 (36.3) | |
| Female | 69,950 (54.8) | 54,501 (53.1) | 10,544 (60.9) | 4,905 (63.7) | |
| Age | | | | | <0.0001 |
| 40–49 years | 17,826 (14.0) | 13,359 (13.0) | 2,835 (16.4) | 1,632 (21.2) | |
| 50–59 years | 55,529 (43.5) | 47,056 (45.9) | 6,121 (35.3) | 2,352 (30.6) | |
| 60–69 years | 34,497 (27.0) | 27,863 (27.2) | 4,725 (27.3) | 1,909 (24.8) | |
| 70–79 years | 17,434 (13.7) | 12,826 (12.5) | 3,129 (18.1) | 1,479 (19.2) | |
| 80–89 years | 2,264 (1.8) | 1,436 (1.4) | 507 (2.9) | 321 (4.2) | |
| ≥90 years | 23 (0.0) | 14 (0.0) | 7 (0.0) | 2 (0.0) | |
| Income deciles | | | | | <0.0001 |
| Decile 1 | 11,399 (8.9) | 8,870 (8.7) | 1,669 (9.6) | 860 (11.2) | |
| Decile 2 | 9,722 (7.6) | 7,619 (7.4) | 1,437 (8.3) | 666 (8.7) | |
| Decile 3 | 9,616 (7.5) | 7,609 (7.4) | 1,346 (7.8) | 661 (8.6) | |
| Decile 4 | 9,372 (7.4) | 7,375 (7.2) | 1,306 (7.5) | 691 (9.0) | |
| Decile 5 | 9,365 (7.3) | 7,488 (7.3) | 1,303 (7.5) | 574 (7.5) | |
| Decile 6 | 11,135 (8.7) | 8,950 (8.7) | 1,506 (8.7) | 679 (8.8) | |
| Decile 7 | 12,455 (9.8) | 9,980 (9.7) | 1,734 (10.0) | 741 (9.6) | |
| Decile 8 | 14,853 (11.6) | 12,006 (11.7) | 2,019 (11.7) | 828 (10.8) | |
| Decile 9 | 18,168 (14.2) | 14,803 (14.4) | 2,396 (13.8) | 969 (12.6) | |
| Decile 10 | 21,488 (16.8) | 17,854 (17.4) | 2,608 (15.1) | 1,026 (13.3) | |
| Hypertension | | | | | |
| Yes | 48,488 (38.0) | 38,632 (37.7) | 6,828 (39.4) | 3,028 (39.4) | <0.0001 |
| No | 79,085 (62.0) | 63,922 (62.3) | 10,496 (60.6) | 4,667 (60.7) | |
| Diabetes mellitus | | | | | 0.0769 |
| Yes | 29,468 (23.1) | 23,554 (23.0) | 4,101 (23.7) | 1,813 (23.6) | |
| No | 98,105 (76.9) | 79,000 (77.0) | 13,223 (76.3) | 5,882 (76.4) | |
| Dyslipidemia | | | | | <0.0001 |
| Yes | 44,619 (35.0) | 36,179 (35.3) | 5,949 (34.3) | 2,491 (32.4) | |
| No | 82,954 (65.0) | 66,375 (64.7) | 11,375 (65.7) | 5,204 (67.6) | |
| Smoke | | | | | 0.0485 |
| Yes | 20,004 (15.7) | 16,205 (15.8) | 2,617 (15.1) | 1,182 (15.4) | |
| No | 107,569 (84.3) | 86,349 (84.2) | 14,707 (84.9) | 6,513 (84.6) | |
| Drink | | | | | <0.0001 |
| Yes | 47,178 (37.0) | 38,978 (38.0) | 5,776 (33.3) | 2,424 (31.5) | |
| No | 80,395 (63.0) | 63,576 (62.0) | 11,548 (66.7) | 5,271 (68.5) | |
| Baseline height (cm) | | | | | <0.0001 |
| Mean | 161.27 | 161.47 | 160.44 | 160.43 | Group 1–Group 2[s], |
| Standard deviation | 8.39 | 8.37 | 8.35 | 8.59 | Group 1–Group 3[s] |
| Body mass index (kg/m$^2$) | | | | | 0.0144 |
| Mean | 23.97 | 23.95 | 24.04 | 24.01 | Group 1–Group 2[s] |
| Standard deviation | 2.98 | 2.95 | 3.09 | 3.19 | |

†Annual rate of height decrease: Group 1 (<0.3 cm), Group 2 (0.3 to <0.6 cm), and Group 3 (≥0.6 cm).

††Chi-square test and the Kruskal–Wallis test.

If there were missing values in sex, age, and income deciles, we imputed them using the closest ones from 2012. We regarded it as a Yes if there was at least one disease.

In case of smoke, drink, and body mass index, the values in 2012 were the criteria. If there were missing values in those variables, we imputed them by the values in 2011.

[s]Significant by *post hoc* test.

TABLE 2 The cumulative adverse cardiovascular and cerebral event incidence rates per the annual height decrease rate.

| | All | | | Male | | | Female | | |
|---|---|---|---|---|---|---|---|---|---|
| | Annual rate of height decrease†† | | | Annual rate of height decrease†† | | | Annual rate of height decrease†† | | |
| | Group 1 | Group 2 | Group 3 | Group 1 | Group 2 | Group 3 | Group 1 | Group 2 | Group 3 |
| | (*N* = 102,554) | (*N* = 17,324) | (*N* = 7,695) | (*N* = 48,053) | (*N* = 6,780) | (*N* = 2,790) | (*N* = 54,501) | (*N* = 10,544) | (*N* = 4,905) |
| **Cumulative incidence rate†** | ***n* (%)** | ***n* (%)** | ***n* (%)** | ***n* (%)** | ***n* (%)** | ***n* (%)** | ***n* (%)** | ***n* (%)** | ***n* (%)** |
| **MACCE** | | | | | | | | | |
| 1st year | 892 (0.9) | 205 (1.2) | 95 (1.2) | 501 (1.0) | 97 (1.4) | 42 (1.5) | 391 (0.7) | 108 (1.0) | 53 (1.1) |
| 2nd year | 1,817 (1.8) | 391 (2.3) | 183 (2.4) | 1,006 (2.1) | 176 (2.6) | 78 (2.8) | 811 (1.5) | 215 (2.0) | 105 (2.1) |
| 3rd year | 2,696 (2.6) | 580 (3.4) | 295 (3.8) | 1,473 (3.1) | 255 (3.8) | 124 (4.4) | 1,223 (2.2) | 325 (3.1) | 171 (3.5) |
| 4th year | 3,687 (3.6) | 785 (4.5) | 401 (5.2) | 2,042 (4.3) | 338 (5.0) | 169 (6.1) | 1,645 (3.0) | 447 (4.2) | 232 (4.7) |
| All | 9,092 (8.9) | 1,961 (11.3) | 974 (12.7) | 5,022 (10.5) | 866 (12.8) | 413 (14.8) | 4,070 (7.5) | 1,095 (10.4) | 561 (11.4) |
| **Cardiac death** | | | | | | | | | |
| 1st year | 24 (0.0) | 5 (0.0) | 6 (0.1) | 18 (0.0) | 3 (0.0) | 3 (0.1) | 6 (0.0) | 2 (0.0) | 3 (0.1) |
| 2nd year | 85 (0.1) | 12 (0.1) | 9 (0.1) | 58 (0.1) | 7 (0.1) | 6 (0.2) | 27 (0.1) | 5 (0.1) | 3 (0.1) |
| 3rd year | 149 (0.2) | 29 (0.2) | 23 (0.3) | 94 (0.2) | 16 (0.2) | 11 (0.4) | 55 (0.1) | 13 (0.1) | 12 (0.2) |
| 4th year | 245 (0.2) | 50 (0.3) | 42 (0.6) | 155 (0.3) | 27 (0.4) | 19 (0.7) | 90 (0.2) | 23 (0.2) | 23 (0.5) |
| All | 503 (0.5) | 96 (0.6) | 80 (1.1) | 325 (0.7) | 53 (0.8) | 39 (1.4) | 178 (0.3) | 43 (0.4) | 41 (0.8) |
| **Non-fatal MI** | | | | | | | | | |
| 1st year | 114 (0.1) | 31 (0.2) | 10 (0.1) | 84 (0.2) | 20 (0.3) | 6 (0.2) | 30 (0.1) | 11 (0.1) | 4 (0.1) |
| 2nd year | 232 (0.2) | 52 (0.3) | 21 (0.3) | 159 (0.3) | 30 (0.4) | 12 (0.4) | 73 (0.1) | 22 (0.2) | 9 (0.2) |
| 3rd year | 365 (0.4) | 75 (0.4) | 36 (0.5) | 252 (0.5) | 44 (0.7) | 22 (0.8) | 113 (0.2) | 31 (0.3) | 14 (0.3) |
| 4th year | 504 (0.5) | 100 (0.6) | 51 (0.7) | 351 (0.7) | 55 (0.8) | 31 (1.1) | 153 (0.3) | 45 (0.4) | 20 (0.4) |
| All | 1,215 (1.2) | 258 (1.5) | 118 (1.5) | 846 (1.8) | 149 (2.2) | 71 (2.6) | 369 (0.7) | 109 (1.0) | 47 (1.0) |
| **Stroke** | | | | | | | | | |
| 1st year | 737 (0.7) | 171 (1.0) | 72 (0.9) | 394 (0.8) | 79 (1.2) | 30 (1.1) | 343 (0.6) | 92 (0.9) | 42 (0.9) |
| 2nd year | 1,472 (1.4) | 323 (1.9) | 149 (1.9) | 781 (1.6) | 140 (2.1) | 60 (2.2) | 691 (1.3) | 183 (1.7) | 89 (1.8) |
| 3rd year | 2,154 (2.1) | 464 (2.7) | 227 (3.0) | 1,122 (2.3) | 193 (2.9) | 89 (3.2) | 1,032 (1.9) | 271 (2.6) | 138 (2.8) |
| 4th year | 2,876 (2.8) | 620 (3.6) | 296 (3.9) | 1,520 (3.2) | 255 (3.8) | 116 (4.2) | 1,356 (2.5) | 365 (3.5) | 180 (3.7) |
| All | 7,239 (7.1) | 1,578 (9.1) | 744 (9.7) | 3,817 (7.9) | 667 (9.8) | 295 (10.6) | 3,422 (6.3) | 911 (8.6) | 449 (9.2) |
| **Hospitalization for HF** | | | | | | | | | |
| 1st year | 56 (0.1) | 11 (0.1) | 8 (0.1) | 35 (0.1) | 6 (0.1) | 3 (0.1) | 21 (0.0) | 5 (0.1) | 5 (0.1) |
| 2nd year | 116 (0.1) | 27 (0.2) | 14 (0.2) | 69 (0.1) | 17 (0.3) | 6 (0.2) | 47 (0.1) | 10 (0.1) | 8 (0.2) |
| 3rd year | 177 (0.2) | 47 (0.3) | 29 (0.4) | 103 (0.2) | 24 (0.4) | 11 (0.4) | 74 (0.1) | 23 (0.2) | 18 (0.4) |
| 4th year | 270 (0.3) | 65 (0.4) | 42 (0.6) | 152 (0.3) | 28 (0.4) | 17 (0.6) | 118 (0.2) | 37 (0.4) | 25 (0.5) |
| All | 619 (0.6) | 150 (0.9) | 93 (1.2) | 359 (0.7) | 75 (1.1) | 37 (1.3) | 260 (0.5) | 75 (0.7) | 56 (1.1) |

MACCE, major adverse cardiovascular and cerebral events; MI, myocardial infarction; HF, heart failure.

†After observing the degrees of height reduction for 10 years from 2002, we calculated the cumulative incidence rate of MACCE from January 2012 annually.

††Annual rate of height decrease: Group 1 (<0.3 cm), Group 2 (0.3 to <0.6 cm), and Group 3 (≥0.6 cm).



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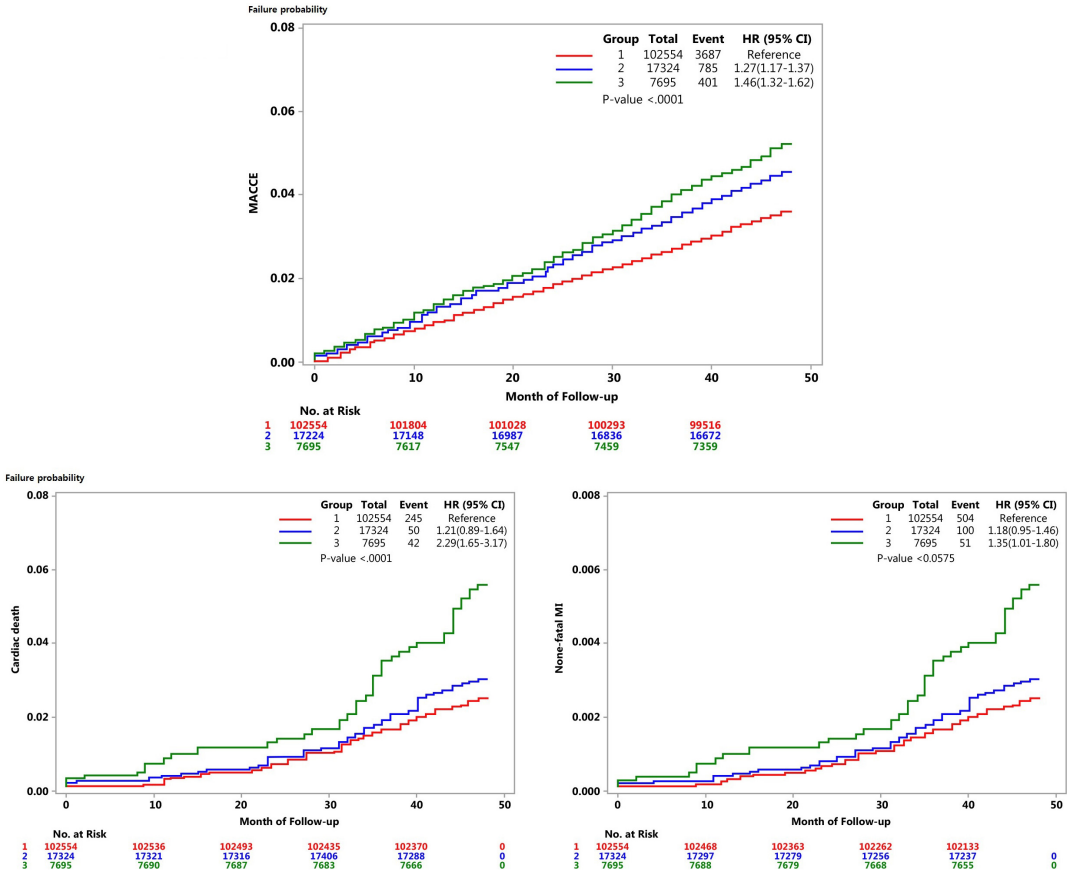


FIGURE 2
Kaplan–Meier curves for major adverse cardiovascular and cerebral events.

TABLE 3 Hazard ratios of adverse cardiovascular and cerebral events per the annual height decrease rate.

| | Annual rate of height decrease†† | | | | |
|---|---|---|---|---|---|
| | Group 1 | Group 2 | | Group 3 | |
| Cardiovascular disease | (ref.) | HR | 95% CI | HR | 95% CI |
| **Crude model** | | | | | |
| MACCE | 1 | 1.27 | 1.17, 1.37 | 1.46 | 1.32, 1.62 |
| Cardiac death | 1 | 1.21 | 0.89, 1.64 | 2.29 | 1.65, 3.17 |
| Non-fatal MI | 1 | 1.18 | 0.95, 1.46 | 1.35 | 1.01, 1.80 |
| Stroke | 1 | 1.28 | 1.18, 1.40 | 1.38 | 1.22, 1.56 |
| Hospitalization for HF | 1 | 1.43 | 1.09, 1.87 | 2.08 | 1.50, 2.87 |
| **Adjusted model†** | | | | | |
| MACCE | 1 | 1.11 | 1.07, 1.20 | 1.25 | 1.13, 1.39 |
| Cardiac death | 1 | 0.96 | 0.71, 1.30 | 1.67 | 1.20, 2.33 |
| Non-fatal MI | 1 | 1.12 | 0.90, 1.39 | 1.30 | 0.97, 1.73 |
| Stroke | 1 | 1.12 | 1.02, 1.22 | 1.18 | 1.04, 1.33 |
| Hospitalization for HF | 1 | 1.15 | 0.88, 1.51 | 1.56 | 1.12, 2.17 |

MACCE, major adverse cardiovascular and cerebral events; MI, myocardial infarction; HF, heart failure.
†Sex, age, income deciles, diabetes, dyslipidemia, hypertension, smoke, alcohol use, and body mass index were adjusted.
††Annual rate of height decrease: Group 1 (<0.3 cm), Group 2 (0.3 to <0.6 cm), and Group 3 (≥0.6 cm).
Cox proportional hazard model.

TABLE 4 The cumulative incidence rates of major adverse cardiovascular and cerebral events per the annual height decrease rate in patients aged ≥ 60 years.

| | All | | | Male | | | Female | | |
|---|---|---|---|---|---|---|---|---|---|
| | Annual rate of height decrease†† | | | Annual rate of height decrease†† | | | Annual rate of height decrease†† | | |
| | Group 1 | Group 2 | Group 3 | Group 1 | Group 2 | Group 3 | Group 1 | Group 2 | Group 3 |
| | (*N* = 42,139) | (*N* = 8,368) | (*N* = 3,711) | (*N* = 20,341) | (*N* = 3,365) | (*N* = 1,353) | (*N* = 21,798) | (*N* = 5,003) | (*N* = 2,358) |
| **Cumulative incidence rate†** | ***n* (%)** | ***n* (%)** | ***n* (%)** | ***n* (%)** | ***n* (%)** | ***n* (%)** | ***n* (%)** | ***n* (%)** | ***n* (%)** |
| **MACCE** | | | | | | | | | |
| 1st year | 637 (1.5) | 165 (2.0) | 83 (2.2) | 355 (1.8) | 75 (2.2) | 32 (2.4) | 282 (1.3) | 90 (1.8) | 51 (2.2) |
| 2nd year | 1,295 (3.1) | 306 (3.7) | 148 (4.0) | 706 (3.5) | 135 (4.0) | 58 (4.3) | 589 (2.7) | 171 (3.4) | 90 (3.8) |
| 3rd year | 1,923 (4.6) | 457 (5.5) | 244 (6.6) | 1,033 (5.1) | 194 (5.8) | 98 (7.2) | 890 (4.1) | 263 (5.3) | 146 (6.2) |
| 4th year | 2,646 (6.3) | 620 (7.4) | 329 (8.9) | 1,439 (7.1) | 258 (7.7) | 133 (9.8) | 1,207 (5.5) | 362 (7.2) | 196 (8.3) |
| All | 6,501 (15.4) | 1,548 (18.5) | 804 (21.7) | 3,533 (17.5) | 662 (19.7) | 321 (23.7) | 2,968 (13.6) | 886 (17.7) | 483 (20.5) |
| **Cardiac death** | | | | | | | | | |
| 1st year | 22 (0.1) | 5 (0.1) | 6 (0.2) | 16 (0.1) | 3 (0.1) | 3 (0.2) | 6 (0.03) | 2 (0.04) | 3 (0.1) |
| 2nd year | 69 (0.2) | 11 (0.1) | 8 (0.2) | 47 (0.2) | 6 (0.2) | 5 (0.4) | 22 (0.1) | 5 (0.1) | 3 (0.1) |
| 3rd year | 122 (0.3) | 24 (0.3) | 21 (0.6) | 77 (0.4) | 11 (0.3) | 10 (0.7) | 45 (0.2) | 13 (0.3) | 11 (0.5) |
| 4th year | 201 (0.5) | 43 (0.5) | 38 (1.0) | 123 (0.6) | 20 (0.6) | 16 (1.2) | 78 (0.4) | 23 (0.5) | 22 (0.9) |
| All | 414 (1.0) | 83 (1.0) | 73 (2.0) | 263 (1.3) | 40 (1.2) | 34 (2.5) | 151 (0.7) | 43 (0.9) | 39 (1.6) |
| **Non-fatal MI** | | | | | | | | | |
| 1st year | 77 (0.2) | 25 (0.3) | 8 (0.2) | 55 (0.3) | 16 (0.5) | 4 (0.3) | 22 (0.1) | 9 (0.2) | 4 (0.2) |
| 2nd year | 148 (0.4) | 41 (0.5) | 15 (0.4) | 95 (0.5) | 24 (0.7) | 6 (0.4) | 53 (0.2) | 17 (0.3) | 9 (0.4) |
| 3rd year | 232 (0.6) | 58 (0.7) | 29 (0.8) | 145 (0.7) | 33 (1.0) | 15 (1.1) | 87 (0.4) | 25 (0.5) | 14 (0.6) |
| 4th year | 318 (0.8) | 77 (0.9) | 38 (1.0) | 207 (1.0) | 40 (1.2) | 18 (1.3) | 111 (0.5) | 37 (0.7) | 20 (0.9) |
| All | 775 (1.8) | 201 (2.4) | 90 (2.4) | 502 (2.5) | 113 (3.4) | 43 (3.1) | 273 (1.2) | 88 (1.7) | 47 (2.1) |
| **Stroke** | | | | | | | | | |
| 1st year | 529 (1.3) | 139 (1.7) | 62 (1.7) | 284 (1.4) | 62 (1.8) | 22 (1.6) | 245 (1.1) | 77 (1.5) | 40 (1.7) |
| 2nd year | 1,063 (2.5) | 253 (3.0) | 121 (3.3) | 568 (2.8) | 107 (3.2) | 46 (3.4) | 495 (2.3) | 146 (2.9) | 75 (3.2) |
| 3rd year | 1,559 (3.7) | 367 (4.4) | 187 (5.0) | 822 (4.0) | 149 (4.4) | 70 (5.2) | 737 (3.4) | 218 (4.4) | 117 (5.0) |
| 4th year | 2,091 (5.0) | 487 (5.8) | 243 (6.6) | 1,112 (5.5) | 197 (5.9) | 94 (7.0) | 979 (4.5) | 290 (5.8) | 149 (6.3) |
| All | 5,242 (12.4) | 1,246 (14.9) | 613 (16.5) | 2,786 (13.7) | 515 (15.3) | 232 (17.2) | 2,456 (11.3) | 731 (14.6) | 381 (16.2) |
| **Hospitalization for HF** | | | | | | | | | |
| 1st year | 44 (0.1) | 8 (0.1) | 8 (0.2) | 26 (0.1) | 4 (0.1) | 3 (0.2) | 18 (0.1) | 4 (0.1) | 5 (0.2) |
| 2nd year | 90 (0.2) | 22 (0.3) | 13 (0.4) | 48 (0.2) | 14 (0.4) | 6 (0.4) | 42 (0.2) | 8 (0.2) | 7 (0.3) |
| 3rd year | 135 (0.3) | 41 (0.5) | 25 (0.7) | 71 (0.4) | 21 (0.6) | 11 (0.8) | 64 (0.3) | 20 (0.4) | 14 (0.6) |
| 4th year | 208 (0.5) | 59 (0.7) | 37 (1.0) | 107 (0.5) | 25 (0.7) | 17 (1.3) | 101 (0.5) | 34 (0.7) | 20 (0.9) |
| All | 477 (1.1) | 130 (1.6) | 83 (2.2) | 252 (1.2) | 64 (1.8) | 37 (2.7) | 225 (1.1) | 66 (1.4) | 46 (2.0) |

MACCE, major adverse cardiovascular and cerebral events; MI, myocardial infarction; HF, heart failure.

† After observing the degrees of height reduction for 10 years from 2002, we annually calculated cumulative incidence rate of MACCE from January of 2012.

†† Annual rate of height decrease: Group 1 (<0.3 cm), Group 2 (0.3–<0.6 cm), and Group 3 (≥0.6 cm).



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TABLE 5 The hazard ratios of major adverse cardiovascular and cerebral events per the annual height decrease rate in patients aged ≥ 60 years.

| | Annual rate of height decrease†† | | | | |
|---|---|---|---|---|---|
| | Group 1 | Group 2 | | Group 3 | |
| Cardiovascular disease | (ref.) | HR | 95% CI | HR | 95% CI |
| **Crude model** | | | | | |
| **MACCE** | 1 | 1.19 | 1.09, 1.30 | 1.43 | 1.28, 1.67 |
| Cardiac death | 1 | 1.08 | 0.78, 1.50 | 2.15 | 1.52, 3.04 |
| Non-fatal MI | 1 | 1.22 | 0.95, 1.57 | 1.36 | 0.97, 1.90 |
| Stroke | 1 | 1.18 | 1.07, 1.30 | 1.33 | 1.17, 1.52 |
| Hospitalization for HF | 1 | 1.43 | 1.07, 1.91 | 2.03 | 1.43, 2.87 |
| **Adjusted model†** | | | | | |
| **MACCE** | 1 | 1.10 | 1.00, 1.20 | 1.26 | 1.12, 1.41 |
| Cardiac death | 1 | 0.97 | 0.67, 1.30 | 1.71 | 1.20, 2.43 |
| Non-fatal MI | 1 | 1.20 | 0.93, 1.54 | 1.30 | 0.92, 1.82 |
| Stroke | 1 | 1.09 | 0.99, 1.20 | 1.18 | 1.03, 1.35 |
| Hospitalization for HF | 1 | 1.22 | 0.91, 1.63 | 1.56 | 1.09, 2.22 |

MACCE, major adverse cardiovascular and cerebral events; MI, myocardial infarction; HF, heart failure.
†Sex, age, income deciles, diabetes, dyslipidemia, osteoporosis, hypertension, smoke, drink, BMI were adjusted.
††Annual rate of height decrease: Group 1 (<0.3 cm), Group 2 (0.3 to <0.6 cm), and Group 3 (≥0.6 cm).
Cox proportional hazard model.

2, and 5.2% for Group 3. The rates were 4.3% in Group 1, 5.0% in Group 2, and 6.1% in Group 3 among men, and 3.0% in Group 1, 4.2% in Group 2, and 4.7% in Group 3 among women.

Figure 2 presents the Kaplan–Meier survival curves for the MACCE incidence rates based on the extent of height decrease. The cumulative MACCE incidence rate was highest in Group 3 (HR = 1.46, 95% CI = 1.32–1.62), followed by Group 2 (HR = 1.27, 95% CI = 1.17–1.37) and Group 1 (Reference); the inter-group difference was significant ($p < 0.0001$). All the MACCE components, including cardiac death (HR = 2.29, 95% CI = 1.65–3.17), non-fatal MI (HR = 1.35, 95% CI = 1.01–1.80), and stroke (HR = 1.38, 95% CI = 1.22–1.55) occurred more frequently in Group 3 than in Group 1.

Table 3 presents the Cox proportional-hazard model results for the annual height decrease rate and MACCE incidences. In the crude model, the MACCE and cardiac death risks were 1.46 (95% CI = 1.32–1.62) and 2.29 (95% CI = 1.65–3.17) times higher in Group 3 than in Group 1, respectively, and the inter-group difference was significant, considering the 95% CI. In the adjusted model, the risks of MACCE (HR = 1.25, 95% CI = 1.13–1.39), cardiac death (HR = 1.67, 95% CI = 1.20–2.33), stroke (HR = 1.18, 95% CI = 1.04–1.33), and hospitalization for HF (HR = 1.56, 95% CI = 1.12–2.17) were higher in Group 3 than in Group 1; the inter-group difference was significant. The subgroup analysis of the elderly population (≥60 years) demonstrated similar results (Tables 4, 5). In females, height loss increased dramatically after menopause. The analysis of premenopausal versus menopausal groups demonstrated that MACCE rarely occurred in premenopausal women (Tables 6, 7), and height loss was significantly associated with MACCE in postmenopausal women (≥50 years).

## Height loss determinants

Table 8 presents the multivariate linear regression results on the demographic and clinical characteristics and the annual height decrease. Significant variables affecting the decrease in height included sex, age, low-income, hypertension, dyslipidemia, smoking, drinking, BMI, and baseline height ($p < 0.0001$). Diabetes did not affect the annual height decrease. Particularly, sex (female), age ≥ 60 years, low-income, hypertension, smoking and baseline height positively affect the decrease in height.

# Discussion

In this current research using Korean big data, we observed that the degree of height loss was independently associated with an increased risk of MACCE in a dose-dependent manner. Subgroup analyses of the elderly and postmenopausal women, who have a greater decline in height, also demonstrated this association. These results parallel the British males' data ($n$ = 4,213) published approximately two decades ago and supported the hypothesis that loss of height is more than a common aging process and is a marker for increased CVD risk (10). Compared to that study, the foremost strength of this study is the large sample size. In addition, our study

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TABLE 6 The cumulative incidence rates of major adverse cardiovascular and cerebral events per the annual height decrease rate in female patients based on menopausal status.

| | No | | | Yes | | |
|---|---|---|---|---|---|---|
| | Annual rate of height decrease†† | | | Annual rate of height decrease†† | | |
| | Group 1 | Group 2 | Group 3 | Group 1 | Group 2 | Group 3 |
| | (*N* = 9,070) | (*N* = 1,939) | (*N* = 1,098) | (*N* = 45,431) | (*N* = 8,605) | (*N* = 3,807) |
| **Cumulative incidence rate†** | ***n* (%)** | ***n* (%)** | ***n* (%)** | ***n* (%)** | ***n* (%)** | ***n* (%)** |
| **MACCE** | | | | | | |
| 1st year | 14 (0.2) | 0 (0.0) | 0 (0.0) | 377 (0.8) | 108 (1.3) | 53 (1.4) |
| 2nd year | 31 (0.3) | 1 (0.1) | 2 (0.2) | 780 (1.7) | 214 (2.5) | 103 (2.7) |
| 3rd year | 50 (0.6) | 4 (0.2) | 6 (0.6) | 1,173 (2.6) | 321 (3.7) | 165 (4.3) |
| 4th year | 68 (0.8) | 7 (0.4) | 7 (0.6) | 1,577 (3.5) | 440 (5.1) | 225 (5.9) |
| All | 163 (1.8) | 12 (0.6) | 15 (1.4) | 3,907 (8.6) | 1,083 (12.6) | 546 (14.3) |
| **Cardiac death** | | | | | | |
| 1st year | 0 (0.0) | 0 (0.0) | 0 (0.0) | 6 (0.0) | 2 (0.0) | 3 (0.1) |
| 2nd year | 2 (0.0) | 0 (0.0) | 0 (0.0) | 25 (0.1) | 5 (0.1) | 3 (0.1) |
| 3rd year | 6 (0.1) | 0 (0.0) | 1 (0.1) | 49 (0.1) | 13 (0.2) | 11 (0.3) |
| 4th year | 7 (0.1) | 0 (0.0) | 1 (0.1) | 83 (0.2) | 23 (0.3) | 22 (0.6) |
| All | 15 (0.2) | 0 (0.0) | 2 (0.2) | 163 (0.4) | 43 (0.5) | 39 (1.0) |
| **Non-fatal MI** | | | | | | |
| 1st year | 1 (0.0) | 0 (0.0) | 0 (0.0) | 29 (0.1) | 11 (0.1) | 4 (0.1) |
| 2nd year | 3 (0.0) | 0 (0.0) | 0 (0.0) | 70 (0.2) | 22 (0.3) | 9 (0.2) |
| 3rd year | 4 (0.0) | 0 (0.0) | 0 (0.0) | 109 (0.2) | 31 (0.4) | 14 (0.4) |
| 4th year | 6 (0.1) | 0 (0.0) | 0 (0.0) | 147 (0.3) | 45 (0.5) | 20 (0.5) |
| All | 14 (0.2) | 0 (0.0) | 0 (0.0) | 355 (0.8) | 109 (1.3) | 47 (1.3) |
| **Stroke** | | | | | | |
| 1st year | 13 (0.1) | 0 (0.0) | 0 (0.0) | 330 (0.7) | 92 (1.1) | 42 (1.1) |
| 2nd year | 26 (0.3) | 1 (0.1) | 1 (0.1) | 665 (1.5) | 182 (2.1) | 88 (2.3) |
| 3rd year | 41 (0.5) | 3 (0.2) | 3 (0.3) | 991 (2.2) | 268 (3.1) | 135 (3.6) |
| 4th year | 56 (0.6) | 6 (0.3) | 4 (0.4) | 1,300 (2.9) | 359 (4.2) | 176 (4.6) |
| All | 136 (1.5) | 10 (0.5) | 8 (0.7) | 3,286 (7.2) | 901 (10.5) | 441 (11.6) |
| **Hospitalization for HF** | | | | | | |
| 1st year | 0 (0.0) | 0 (0.0) | 0 (0.0) | 21 (0.1) | 5 (0.1) | 5 (0.1) |
| 2nd year | 0 (0.0) | 0 (0.0) | 1 (0.1) | 47 (0.1) | 10 (0.1) | 7 (0.2) |
| 3rd year | 1 (0.0) | 1 (0.1) | 3 (0.3) | 73 (0.2) | 22 (0.3) | 15 (0.4) |
| 4th year | 1 (0.0) | 1 (0.1) | 3 (0.3) | 117 (0.3) | 36 (0.4) | 22 (0.6) |
| All | 2 (0.0) | 2 (0.1) | 7 (0.6) | 258 (0.6) | 73 (0.9) | 49 (1.3) |

MACCE, major adverse cardiovascular and cerebral events; MI, myocardial infarction; HF, heart failure.

† After observing the degrees of height reduction for 10 years from 2002, we annually calculated cumulative incidence rate of MACCE from January of 2012.

†† Annual rate of height decrease: Group 1 (<0.3 cm), Group 2 (0.3 to <0.6 cm), and Group 3 (≥0.6 cm).

included approximately 54.8% of female participants that were not included in the British data. Homogeneity in the study sample is also noteworthy, as all data were obtained from one country in a relatively short time frame, and the cohort was composed of one ethnicity: Korean. Adult height and its loss are influenced by many factors. As regards confounders affecting height and its loss, the study sample was relatively free from the effects of ethnicity and the chronological change in national socioeconomic status. Based on our study results, patients with significant height decreases should be monitored for CVD occurrence in clinical practice because it might represent an increased risk of CVD. Additionally, considering that determinants of height loss included older age, female sex, hypertension, smoking, baseline height, and low-income level, more attention should be paid to such individuals. The prevention and treatment of senile changes in the musculoskeletal system could also be helpful for cardiovascular health.

TABLE 7 The hazard ratios of major adverse cardiovascular and cerebral events per the annual height decrease rate in female patients based on menopausal status.

| | No | | | | | Yes | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Annual rate of height decrease†† | | | | | Annual rate of height decrease†† | | | | |
| | Group 1 | Group 2 | | Group 3 | | Group 1 | Group 2 | | Group 3 | |
| Cardiovascular disease | (ref.) | HR | 95% CI | HR | 95% CI | (ref.) | HR | 95% CI | HR | 95% CI |
| **Crude model** | | | | | | | | | | |
| **MACCE** | 1 | 0.48 | 0.22, 1.05 | 0.85 | 0.39, 1.85 | 1 | 1.49 | 1.34, 1.65 | 1.72 | 1.50, 1.98 |
| Cardiac death | 1 | 0.00 | – | 1.18 | 0.15, 9.59 | 1 | 1.46 | 0.92, 2.32 | 3.17 | 1.98, 5.07 |
| Non-fatal MI | 1 | 0.00 | – | 0.00 | – | 1 | 1.62 | 1.16, 2.26 | 1.63 | 1.02, 2.59 |
| Stroke | 1 | 0.50 | 0.22, 1.16 | 0.59 | 0.21, 1.62 | 1 | 1.47 | 1.31, 1.65 | 1.63 | 1.39, 1.91 |
| Hospitalization for HF | 1 | 4.68 | 0.29, 74.77 | 24.88 | 2.58, 238.48 | 1 | 1.63 | 1.12, 2.36 | 2.25 | 1.43, 3.54 |
| **Adjusted model†** | | | | | | | | | | |
| **MACCE** | 1 | 0.47 | 0.22, 1.03 | 0.83 | 0.38, 1.82 | 1 | 1.22 | 1.09, 1.35 | 1.27 | 1.11, 1.47 |
| Cardiac death | 1 | 0.00 | – | 1.13 | 0.14, 9.22 | 1 | 1.00 | 0.63, 1.59 | 1.74 | 1.08, 2.81 |
| Non-fatal MI | 1 | 0.00 | – | 0.00 | – | 1 | 1.33 | 0.95, 1.87 | 1.20 | 0.74, 1.92 |
| Stroke | 1 | 0.49 | 0.21, 1.14 | 0.57 | 0.21, 1.58 | 1 | 1.22 | 1.09, 1.37 | 1.24 | 1.06, 1.46 |
| Hospitalization for HF | 1 | 4.57 | 0.29, 73.28 | 23.69 | 2.45, 229.50 | 1 | 1.15 | 0.79, 1.67 | 1.30 | 0.82, 2.07 |

MACCE, major adverse cardiovascular and cerebral events; MI, myocardial infarction; HF, heart failure.

† Age, income deciles, diabetes, dyslipidemia, osteoporosis, hypertension, smoke, drink, body mass index were adjusted.

†† Annual rate of height decrease: Group 1 (<0.3 cm), Group 2 (0.3 to <0.6 cm), and Group 3 (≥0.6 cm). Cox proportional hazard model.



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For decades, the inverse relationship between adult height and CVD has been repeatedly reported in epidemiological investigations. However, no single theory could explain the mechanism for the phenomenon. Although we and our other colleagues have claimed that dyslipidemia, arterial stiffening represented by a higher pulse wave velocity, or diastolic dysfunction of the heart serve as links between short height and CVD occurrences and outcomes (13–17), entirely satisfactory explanations are still lacking. In this study, we revealed that height loss is associated with increased CVD risk, and we reckon this is clinically meaningful because (1) An individual's maximum height is genetically determined and is a non-modifiable factor; (2) Conversely, loss of height resulting from senile bone, muscle, and joint changes, is affected by environmental or personal factors, and thus, might be modifiable. The pathophysiology of osteoporosis and sarcopenia, which are responsible for senile height loss, is poorly understood and likely multifactorial; nonetheless, some factors can be targeted for intervention, such as physical inactivity, alcohol use, tobacco use, vitamin D deficiency, or an unhealthy diet. Osteoporosis, characterized by reduced bone density and increased fracture risk, is the primary contributor to height loss by reducing vertebral body strength and inducing the loss of mineral content and trabecular connectivity. Notably, a decrease in stature from osteoporosis and fracture is often remarkable and is likely to reach several centimeters (10, 18). CVD and osteoporosis, which often coexist, are public health challenges with multiple epidemiological links, pathophysiological mechanisms, and economic consequences (19–22). Studies have demonstrated that cardiovascular morbidity and mortality are associated with bone fractures and reduced bone mineral density which is related to vascular calcification, a well-known cardiovascular risk factor (19–22). Additionally, sarcopenia and poor muscle strength, as part of aging, are associated with bone loss and diseased bone structure, eventually resulting in height loss. Osteoporosis and sarcopenia are predictors of mortality and are, at least theoretically, preventable and treatable conditions through environmental modifications and medical interventions (9).

The mechanism relating to the loss of height and subsequent CVD remains unclear. However, the effect of height decrease, a predictor of vertebral fracture, on the respiratory and gastrointestinal systems is noteworthy. Vertebral fractures induce thoracic deformity or pain and disturb pulmonary function subsequently. Conversely, percutaneous vertebroplasty and kyphoplasty for vertebral fractures improve lung function. Further, lung capacity and gastrointestinal function decrease as height declines, potentially leading to less exercise, effort intolerance, early satiety, and poor nutritional status, resulting in sarcopenia and senile fragility (23–28). Weight loss and leanness, attributed to the loss of skeletal muscle mass in the elderly, are risk factors for CVD (the so-called "obesity paradox") (29–32). Moreover, a musculoskeletal deformity significantly affects psychological conditions, such as depression and anxiety and the quality of life. From a different perspective, however, bone loss and poor bone quality (important determinants of height decline) share common pathophysiological mechanisms with CVD, such as oxidative stress, inflammation, abnormal homocysteine levels, and metabolic risk factors (such as hypertension, diabetes, and dyslipidemia) (33). CVD prevalence increases with age, and the same is true for bone loss and muscle mass decrease: these phenomena might result from shared mechanisms. Hence, from that viewpoint, loss of height might merely be a co-finding or a bystander with CVD rather than a primary cause. Further study is warranted to shed light on the pathophysiological link between loss of height and CVD.

TABLE 8 Factors associated with affecting the annual height decrease.

| | Estimate | Standard error | *T*-value | *P*-value |
|---|---|---|---|---|
| Intercept | −0.284 | 0.029 | −9.91 | <0.0001 |
| Sex (female) | 0.072 | 0.003 | 23.73 | <0.0001 |
| Age (>60) | 0.051 | 0.002 | 25.11 | <0.0001 |
| Low-income | 0.021 | 0.002 | 10.99 | <0.0001 |
| Hypertension | 0.007 | 0.002 | 3.60 | 0.0003 |
| Diabetes | 0.002 | 0.002 | 0.98 | 0.3267 |
| Dyslipidemia | −0.017 | 0.002 | −8.31 | <0.0001 |
| Smoking | 0.025 | 0.003 | 8.86 | <0.0001 |
| Drinking | −0.008 | 0.002 | −3.51 | 0.0005 |
| BMI (<25) | −0.011 | 0.002 | −5.91 | <0.0001 |
| Baseline height | 0.003 | 0.0002 | 15.02 | <0.0001 |

$R^2$ = 0.0111, Adj $R^2$ = 0.0110, *F*-value = 142.93, *P*-value ≤0.0001.
Multivariate linear regression.

## Limitations

The current investigation was performed based on KCD-7. Thus, biomarkers known as cardiovascular risk factors, such as laboratory findings, were unavailable for analysis. Further, each patient's medication history was unavailable. The main contributor to height loss is osteoporosis; however, we could not objectively assess each patient's bone mass density beyond the KCD-7 code. Hence, further study is warranted to clarify the potential pathophysiological association between senile changes in the musculoskeletal system and MACCE. In addition, unreliable height data were excluded from the analysis, and the real change in height cannot be fully distinguished from possible measurement errors. This limitation was due to the retrospective nature of this study. Annual height decrease rate is the main parameter in analysis; however, it could be too crude to represent senile changes in the musculoskeletal system

meticulously. In addition, no consideration was given to the potential for non-linear height reduction in data analysis. This was another limitation of this study because it is plausible that serious adverse health events or a period of poor nutrition could lead to a significant loss of height.

## Conclusion

The degree of height loss was independently associated with the occurrence of MACCE in a Korean population, although it has yet to be determined if there is a causal relationship between the two.

## Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: Restrictions apply to the availability of the raw data, which were used under a policy of the NHIS. The data and materials other than the raw data underlying the study are available from the corresponding author, WCK, on reasonable request. Requests to access these datasets should be directed to WCK, kangwch@gilhospital.com.

## Ethics statement

The studies involving human participants were reviewed and approved by Gachon University Gil Medical Center (IRB No. GFIRB2019-304). The patients/participants provided their written informed consent to participate in this study.

## Author contributions

JGM and WCK designed the study and prepared the manuscript as submitted. JGM analyzed and interpreted the data. WCK supervised the project. All authors reviewed and critically revised the manuscript, read and approved the final manuscript.

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## Conflict of interest

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

## Publisher's note



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# Baseline mitochondrial DNA copy number and heart failure incidence and its role in overall and heart failure mortality in middle-aged women

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Heart failure (HF) is a leading cause of death in both men and women. However, risk factors seem to differ for men and women and significant gaps in sex-specific knowledge exist. Mitochondria are critical for cardiomyocytes and in this study, we investigated the role of baseline mitochondrial DNA copy number (mtDNA-CN) in HF incidence in middle-aged women and its possible role in the association between myocardial infarction (MI) and HF. Finally, we also investigated whether baseline mtDNA-CN was associated with overall and HF mortality. Baseline levels of mtDNA-CN were quantified by droplet digital PCR in a population-based follow-up study of middle-aged (50–59 years) Swedish women ($n = 2,508$). The median follow-up period was 17 years. Levels of mtDNA-CN were associated with age, BMI, alcohol, smoking, education, physical activity and lipid biomarkers. Multivariable Cox regression analysis adjusted for potential confounders showed that each standard deviation decrease of baseline mtDNA-CN was associated with higher incidence of HF (HR = 1.34; 95% CI=1.11–1.63). Similar results were obtained when mtDNA-CN levels were categorized into quartiles with lowest vs. highest quartile showing the highest risk of HF incidence (HR = 2.04 95% CI=1.14; 3.63). We could not detect any role of mtDNA-CN in the association between MI and HF incidence. Lower baseline mtDNA-CN levels were associated with both overall (HR = 1.27; 95% CI=1.10–1.46) and HF mortality (HR = 1.93; 95% CI=1.04–3.60); however, in multivariable analysis adjusted for potential confounders, the higher risks of HF mortality were no longer significant (HR=1.57; 95% CI=0.85–2.90). In conclusion, low baseline mtDNA-CN is an easily quantifiable molecular risk factor for HF incidence and may be a risk factor for overall and HF-related mortality.

KEYWORDS

**mitochondrial copy number, mitochondrial dysfunction, risk assessment, heart failure, mortality**

# Introduction

Heart failure (HF) is a growing public health problem characterized by frequent hospitalization, poor quality of life and a higher mortality rate. Despite a decline in the overall risk of cardiovascular diseases (CVD), HF rates have remained unchanged (1). Between the ages of 65 and 85 years, it is estimated that the incidence of HF doubles in men with each 10-year increase, whereas the rate of incidence of HF triples in the same time frame among women (2).

MI is known to be the most common cause of HF in both men and women; however, pathophysiological factors associated with the progression of MI to HF seem to be different in men and women (3). Recent epidemiological studies further support the sex differences for HF type. For example, HF with preserved ejection fraction (HFpEF), which is associated with high morbidity and mortality and lacks any proven therapy, is the most common HF phenotype among women, compared to men who are predisposed to HF with reduced ejection fraction (HFrEF) (4). The higher risk of HFrEF in men compared to women has been attributable to their predisposition to macrovascular coronary heart disease, whereas coronary microvascular dysfunction/endothelial inflammation has been postulated to play a major role in HFpEF (5), which is more common in women. However, initiation and progression of inflammatory responses within the heart remain poorly defined.

Mitochondria are critical for normal functioning of cardiomyocytes and its dysfunction can lead to pathophysiological consequences in heart tissue and beyond (6). Damaged mitochondria are normally removed by autophagy/lysosome system and cardiomyocytes (7). Mitochondrial DNA that escapes autophagy leads to inflammatory responses in cardiomyocytes and may induce myocarditis, and dilated cardiomyopathy (8). Mitochondrial copy number (mtDNA-CN) is a surrogate marker of mitochondrial dysfunction (9) and therefore it can be a useful clinical biomarker for risk assessment of several diseases. However, it is important to note that mtDNA-CN depends on cell-type and the disease in question (10). For example, using whole blood, we and others have shown that mtDNA-CN is decreased in people with CVDs (11) whereas both low and high mtDNA-CN have been shown in people with T2D (12, 13) and cancer (14), probably depending on study design, severity of the disease and methods used.

Nevertheless, mtDNA-CN is easily quantifiable in a large-scale manner due to current high-throughput and accurate methods such as droplet digital PCR. It can thus be used for absolute quantification of mtDNA-CN in blood (15) where higher mtDNA-CN is a biomarker of better mitochondrial function and vice versa. Mitochondrial dysfunction is associated with aging and can affect cellular functions and thereby result in a variety of cardiovascular diseases such as MI, cardiomyopathy, heart failure, and arrhythmias (11, 16, 17). Despite the improvements in therapy, the absolute mortality for HF remains approximately 50% within 5 years after diagnosis (18). It is therefore important to identify novel risk factors and biomarkers that could lead to better treatment approaches, prevention, and risk stratification (19). Even though mitochondria play an important role in normal function and mitochondrial dysfunction is associated with CVD and its risk factors, its role in HF, especially in women, is not well established.

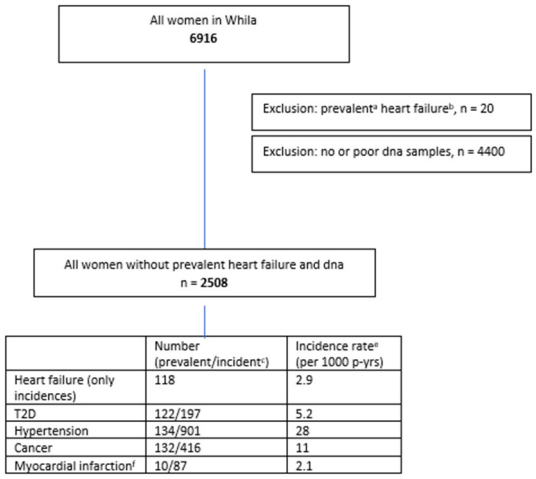
The primary prevention of HF in women should involve targeted, sex-specific strategies, however, despite the sex differences, women are underrepresented (20–25% of cohorts) in most of the clinical and epidemiological studies (5). Furthermore, molecular and clinical risk factors for HF in middle-aged women are not well established. We could find only two studies on the role of mtDNA-CN and HF, an observational study (including 66% males) followed for a median follow-up of only 17 months, demonstrated that lower mtDNA-CN was associated with higher risk of HF (20) and another follow-up study on both men and women showed that lower baseline mtDNA-CN was associated with higher HF incidence (21).

We have recently shown that lower baseline mtDNA-CN levels are associated with MI incidence in middle-aged women (11). In this study, we examined the role of mtDNA-CN in HF incidence and its role in the association between MI and HF. In addition, we also investigated the role of mtDNA-CN in overall and HF mortality. Mitochondrial DNA-CN was quantified in whole blood samples obtained from a well-defined population-based study on middle-aged women with a median follow-up of 17 years. We used a well optimized ddPCR based method for accurate and absolute quantification of mtDNA-CN.

# Materials and methods

## Study population

Women health in Lund area (WHILA) is a well-characterized population-based follow-up study. All women aged 50–65 years (born between 1935 and 1945) who living in southern country (Region Skåne) of Sweden were invited to participate in a health survey. From Dec 1995 to Feb 2000, a total of 6,916 women (out of 10,766, the total population of women in in the five southern municipalities in 1995) underwent a physical examination and answered a questionnaire. The questionnaire that was distributed to all participants has been described previously (22). Participants were followed from the day of screening until death, or if no event occurred until May 31st, 2015. The primary end point for the present study is a first occurrence of fatal or non-fatal HF, death from any cause. Participants were




| | Number (prevalent/incident[c]) | Incidence rate[e] (per 1000 p-yrs) |
|---|---|---|
| Heart failure (only incidences) | 118 | 2.9 |
| T2D | 122/197 | 5.2 |
| Hypertension | 134/901 | 28 |
| Cancer | 132/416 | 11 |
| Myocardial infarction[f] | 10/87 | 2.1 |

FIGURE 1
Heart failure diagnoses were found in primary health care register (60%), hospital discharge register (33%), outpatient register (7%) and death register (0.3%). [a]Prevalent is defined as first diagnose date before or at baseline. [b]Heart failure icd-codes = I50, I110, 428, 427.00, 427.10, 434.10, and 434.20. [c]Incident is defined as first diagnose date after baseline. [d]Cumulative incidence = number of cases/number of individulas at baseline. [e]Incidence rate = number of cases/total person-years of observation during study. [f]Mycardial infarction icd-codes = 121, 410, 410, 420.10, and 420.17.

followed from the day of screening until primary endpoint or death, or if no event occurred until May 31st, 2015. However, the blood samples for DNA extraction were collected midway through this study (from October 1997), therefore, approximately half of the participants' blood samples were not available for mtDNA-CN quantification and after exclusion of samples with poor quality of DNA and prevalent HF ($n$ = 20), 2,508 participants were included in the present study (Figure 1).

## Variables

Age at screening, BMI biomarkers of lipid metabolism, blood pressure and glucose were used as continuous variables. These variables were measured as described previously (23). Educational level was categorized according to the number of years of education as follows: <= 9 years as low, 10–12 years as middle and > 12 years as higher education. Physical activity was defined according to the questionnaire with a score between 1 and 6 into low and high activity. Participants with the score 1–3 were categorized as low activity at home (1= hardly do anything at all, 2= mostly sedentary, 3= light physical exertion). High activity at home was categorized with a score between 4 and 6 (4= strenuous exercise 1–2 h/week, 5= strenuous exercise at least 3 h/week, 6= hard regular exercise).

Alcohol consumption was assessed by the questionnaire as described previously (24). Smoking information was obtained by questionnaire and was categorized as current smokers and no or former smokers.

All explanatory variables, except age, weight and height, MI, HF, T2D and mortality were self-reported in the validated questionnaire (25). Age was taken from the population register, while weight, height, and T2D were obtained from a clinical investigation. MI, HF, and mortality information were collected from the Swedish nationwide and regional registers, which includes, primary health care diagnoses, the hospital discharge register, the outpatient register and the death register. HF cases were found mainly from the primary health care register (60%), followed by the hospital discharge register (33%), outpatient register (7%) and a few cases from the death register (0.3%) (Figure 1).

## Quantification of MtDNA copy number by droplet digital PCR

A full description of the method is provided in the Supplementary information. Briefly, DNA was extracted from whole blood and quantification of mtDNA-CN was performed by droplet digital PCR based method, as also described previously (15) and data were analyzed using QuantaSoft™ Software, which determines the numbers of droplets that were positive and negative for each fluorophore in each sample. The fraction of positive droplets was then fitted to a Poisson distribution in QuantaSoft™ Software to determine the absolute copy number in units of copies/µl. DNA preparation and PCR experiments were performed in separate designated rooms and each run included negative and positive controls. No significant hazards or risks are associated with the reported work.

## Statistical analysis

Prevalent diseases were defined as diagnosed at baseline or before inclusion, while incident disorders were defined as first diagnosis during follow-up. All participants with a mitochondrial DNA measure and no prevalent heart failure (HF) were followed from baseline to incident HF, death or end of study, whichever came first.

We used univariate linear regression models to examine the association between mtDNA-CN and clinical risk factors. To estimate hazard ratios for the association between mtDNA-CN and clinical risk factors and time to incident HF, we used univariate Cox proportional hazards models. The association between mtDNA-CN and HF was then adjusted for the clinical variables significantly associated with both mtDNA-CN and HF, including age, BMI, smoking, education, systolic blood pressure, triglycerides, high-density lipoprotein

TABLE 1 Baseline characteristics for all individuals with a mtDNA-CN measure, stratified by no heart failure and incident heart failure.

| | Total ($n = 2,508$) | No incident HF ($n = 2,390$) | Incident HF ($n = 118$) | $p$-value |
|---|---|---|---|---|
| MtDNA-CN, median (IQR) | 117 (35) | 117 (35) | 106 (29) | 0.0002 |
| Min-max | 32.4–226 | 32.8–226 | 32.4–178 | |
| Missing | 0% | 0% | 0% | |
| Age, median (IQR) | 57 (4) | 57 (4) | 58 (5) 0% | 0.002 |
| Missing | 0% | 0% | | |
| BMI, median (IQR) | 25 (5) | 25 (5) | 26 (7) | 0.03 |
| Missing | 0% | 0% | 0% | |
| Smoker, % | | | | |
| Yes or former/no | 20/80 | 20/80 | 27/73 | 0.045 |
| Missing | 2% | 2% | 0.9% | |
| Alcohol consumption[a], % | | | | |
| Low/medium/high | 26/61/14 | 25/61/14 | 33/50/17 | 0.06 |
| Missing | 5% | 5% | 7% | |
| Education, % | | | | |
| <= 9 years, 10–12, | 55/16/30 | 54/16/30 | 69/14/17 | 0.003 |
| > 12 | 1% | 2% | 0.9% | |
| Missing | | | | |
| Activity at home[b], % | | | | |
| Low/medium/high | 5/91/4 | 5/91/5 | 8/89/3 | 0.32 |
| Missing | 2% | 2% | 2% | |
| Systolic blood pressure, mean (SD) | 132 (17) | 132 (17) | 138 (20) 0% | 0.0001 |
| Missing | 0.08% | 0.08% | | |
| Diastolic blood pressure, mean (SD) | 85 (9.0) | 85 (8.9) | 87 (9.1) | 0.005 |
| Missing | 0.04% | 0.04% | 0% | |
| Total cholesterol, mean (SD) | 6.0 (1.1) | 6.0 (1.1) | 5.9 (1.1) | 0.20 |
| Missing | 0.08% | 0.08% | 0% | |
| Triglycerides, median (IQR) | 1.5 (1.0) | 1.5 (1.0) | 1.7 (1.1) | 0.08 |
| Missing | 0.04% | 0.04% | 0% | |
| HDL, mean (SD) | 1.8 (0.5) | 1.8 (0.4) | 1.7 (0.5) | 0.03 |
| Missing | 0.04% | 0.04% | 0% | |
| LDL, mean (SD) | 3.4 (1.0) | 3.4 (1.0) | 3.4 (1.0) | 0.91 |
| Missing | 11% | 11% | 12% | |

[a]Low alcohol = 0 grams per day, medium = 0.1–11.9 and high ≤ 12 grams per day.
[b]Activity at home was categorized from a 6-point scale.
Low: 1 = hardly anything at all or 2 = mostly sedentary.
Medium: 3 = lighter physical exertion or 4 = strenuous exercise 1–2 h/week.
High: 5 = strenuous exercise at least 3 h/week or 6 = hard regular exercise.

(HDL) and prevalent type two diabetes (T2D). MtDNA-CN was in these Cox regression models reversed to estimate HR for decrease in mtDNA-CN and standardized to get a comparable scale. We also categorized mtDNA-CN into quartiles where we used the lowest quartile as the reference in the models. The same method was used to examine the association between mtDNA-CN and mortality, both overall and due to HF. In these models the adjusting variables were age, smoking, alcohol consumption, education, physical activity, and HDL. We used Schoenfeld residuals to test the proportionality assumption in the Cox regression models. During the follow-up time, several competing events, which potentially can prevent HF from happening, were defined. To consider the informative censoring nature of these competing events, we therefore also analyzed the association between mtDNA-CN and HF by using competing risk analysis. The competing events were cancer, myocardial infarction (MI) and death. We calculated subdistributional hazard ratios using

TABLE 2 Univariate linear regression models examining association between mtDNA-CN and clinical risk factors.

| Outcome: MtDNA–CN | $\beta$ | $p$–value | 95% CI |
|---|---|---|---|
| Age at baseline (years) | −0.5 | 0.01 | −0.9; −0.1 |
| BMI (kg/m$^2$) | −0.3 | 0.03 | −0.5; −0.03 |
| Smoker (yes or former vs. no) | −8.0 | < 0.0001 | −0.1; −5.4 |
| Alcohol consumption (grams per day) | 2.5 | 0.006 | 0.7; 4.2 |
| Education (<= 9 years vs. > 9 years) | −3.4 | 0.002 | −5.5; −1.3 |
| Activity at home (low vs. medium or high)[a] | −8.3 | 0.001 | −13; −3.4 |
| Systolic blood pressure (mmHg) | −0.1 | 0.02 | −0.1; −0.01 |
| Diastolic blood pressure (mmHg) | −0.04 | 0.52 | −0.2; 0.1 |
| Total cholesterol (mg/dL) | 0.7 | 0.18 | −0.3; 1.6 |
| Triglycerides (mg/dL) | −1.8 | 0.001 | −2.9; −0.7 |
| HDL (mg/dL) | 4.8 | < 0.0001 | 2.5; 7.1 |
| LDL (mg/dL) | 0.8 | 0.14 | −0.3; 2.0 |
| Prevalent T2D (yes vs. no) | −7.2 | 0.004 | −12; −2.3 |
| Prevalent cancer (yes vs. no) | 2.6 | 0.28 | −2.1; 7.2 |
| Prevalent MI (yes vs. no) | −3.2 | 0.71 | −19; 13 |

[a]Low = hardly anything at all or mostly sedentary, medium or high = lighter physical exertion, strenuous exercise 1–2 h/week, strenuous exercise at least 3 h/week or hard regular exercise.

the method of Fine and Gray (26). We also plotted a cumulative incidence graph that quantifies the probability of HF accounting for competing risks. This graph is shown for quartiles of mtDNA-CN.

To investigate the role of mtDNA-CN and MI in the association with HF we used three different methods. First, we examined the possibility of MI or mtDNA-CN acting as confounders. Second, we used interaction analyses to estimate MI and mtDNA-CN as effect modifiers in the risk of future HF. Last, we examined the mediating effect of MI and mtDNA-CN where we separated the total effect into direct and indirect effects. In order to investigate the robustness of our results we performed a sensitivity analyses after having excluded women with prevalent MI. Statistical analyses were performed by using STATA version 16 (StataCorp LP).

# Results

## Baseline characteristics and MtDNA-CN levels in HF

In total 2,508 women with mtDNA-CN measures and no prevalent HF were included in the study. Participants diagnosed with HF during the follow-up (incidence) were older and had higher BMI, higher prevalence of smoking, lower level of education, higher systolic and diastolic blood pressure, and lower HDL levels. Prevalent T2D, prevalent MI and prevalent cancer were more common in participants with HF than in participants with no HF during the follow-up (Table 1).

## Association between MtDNA-CN and clinical risk factors

Univariate linear regression analysis was performed to investigate the association between mtDNA-CN and clinical risk factors. Higher levels of mtDNA-CN were inversely associated with age, BMI, smoking, education level, physical activity at home, systolic blood pressure, triglycerides and prevalent T2D. Higher levels of mtDNA-CN were positively associated with alcohol consumption and HDL. Diastolic blood pressure, total cholesterol, LDL, prevalent cancer and prevalent MI were not significantly associated with mtDNA-CN (Table 2).

## Association between MtDNA-CN and incident HF and other risk factors

Unadjusted Cox regression analysis showed that there was a significant association between baseline levels of mtDNA-CN and future risk of HF. The proportionality assumption was tested using Schoenfeld residuals and no violation of the proportionality assumption was found ($p$ = 0.62), which justified the use of the hazard models. Low mtDNA-CN (1 standard deviation decrease) was associated with a 44% higher HF incidence (HR = 1.44; 95% CI = 1.20–1.74). Categorization of mtDNA-CN into quartiles showed a dose-dependent effect on the associations between mtDNA-CN and HF. Participants in the lowest quartile of mtDNA-CN had 2.6-times the hazard of developing HF compared to participants in the highest quartile (Table 3). Among risk factors, prevalent MI was the strongest risk factor for HF (HR = 11.2; 95% CI = 4.13–30.3). Age at baseline, BMI, smoking, education level, systolic and diastolic blood pressure, triglycerides and prevalent T2D were also associated with increased risk of HF incidence whereas higher HDL levels were associated with lower HF incidence (Table 3). In the multivariable model, variables which were significantly associated with both mtDNA-CN (Table 2) and HF incidence (Table 3 univariate analysis) were included. Lower mtDNA-CN levels were significantly associated with higher HF incidence even after adjusting for potential confounders. A dose dependent effect was observed when mtDNA-CN levels were stratified in quartiles (Table 3). The potential effect of baseline mtDNA-CN on the probability of HF as first event in the presence of competing events such as cancer, MI and death was analyzed. Cumulative incidence function after adjusting for age, BMI, smoking, education, systolic blood pressure, triglycerides and HDL showed that lower mtDNA-CN levels were associated with higher cumulative HF incidence compared

TABLE 3 Cox regression models examining effect of mtDNA-CN and other risk factors on risk for incident heart failure.

| | Univariate | | | Adjusted[a] | | |
|---|---|---|---|---|---|---|
| **Outcome: time to heart failure** | **HR** | ***p*-value** | **95% CI** | **HR** | ***p*-value** | **95% CI** |
| MtDNA-CN (decrease, std)[b] | 1.44 | < 0.0001 | 1.20; 1.74 | 1.34 | 0.003 | 1.11; 1.63 |
| MtDNA-CN quartiles[c] | | | | | | |
| $Q_3$ vs. $Q_4$ | 1.44 | 0.18 | 0.84; 2.47 | 1.18 | 0.56 | 0.68; 2.04 |
| $Q_2$ vs. $Q_4$ | 1.91 | 0.01 | 1.14; 3.20 | 1.63 | 0.07 | 0.96; 2.74 |
| $Q_1$ vs. $Q_4$ | 2.60 | 0.001 | 1.47; 4.58 | 2.04 | 0.02 | 1.14; 3.63 |
| Age at baseline (years) | 1.11 | 0.001 | 1.04; 1.19 | | | |
| BMI ($kg/m^2$) | 1.05 | 0.02 | 1.01; 1.09 | | | |
| Smoker (yes or former vs. no) | 1.55 | 0.04 | 1.03; 2.32 | | | |
| Alcohol consumption (grams per day) | 0.89 | 0.46 | 0.66; 1.21 | | | |
| Education (<= 9 years vs. > 9 years) | 1.90 | 0.001 | 1.28; 2.81 | | | |
| Activity at home (low vs. medium or high) | 1.67 | 0.14 | 0.85; 3.30 | | | |
| Systolic blood pressure (mmHg) | 1.02 | < 0.0001 | 1.01; 1.03 | | | |
| Diastolic blood pressure (mmHg) | 1.03 | 0.004 | 1.01; 105 | | | |
| Total cholesterol (mg/dL) | 0.90 | 0.22 | 0.75; 1.07 | | | |
| Triglycerides (mg/dL) | 1.22 | 0.01 | 1.05; 1.43 | | | |
| HDL (mg/dL) | 0.63 | 0.02 | 0.42; 0.94 | | | |
| LDL (mg/dL) | 1.02 | 0.87 | 0.84; 1.24 | | | |
| Prevalent T2D (yes vs. no) | 2.13 | 0.02 | 1.14; 3.96 | | | |
| Prevalent cancer (yes vs. no) | 1.96 | 0.03 | 1.05; 3.65 | | | |
| Prevalent MI (yes vs. no) | 11.2 | < 0.0001 | 4.13; 30.3 | | | |

[a]Adjusted for age, bmi, smoking, education, systolic, triglycerides, HDL and prevalent T2D.
[b]MtDNA-CN has been reversed and standardized (HR for a one standard deviation decrease in mtDNA-CN).
[c]$Q_1$ = 32.4–88, $Q_2$ = 89–110, $Q_3$ = 111–130, $Q_4$ = 131–226.

to higher mtDNA-CN (Figure 2). Fine and Gray method was used to calculate subdistribution hazard ratio (SHR) and 1 SD decrease in mtDNA-CN was associated with a 32% increase in SHR (SHR = 1.32; 95% CI = 1.03–1.71) in the presence of competing risks and adjusted for age, BMI, smoking, education, systolic blood pressure, triglycerides and HDL (Table 4).

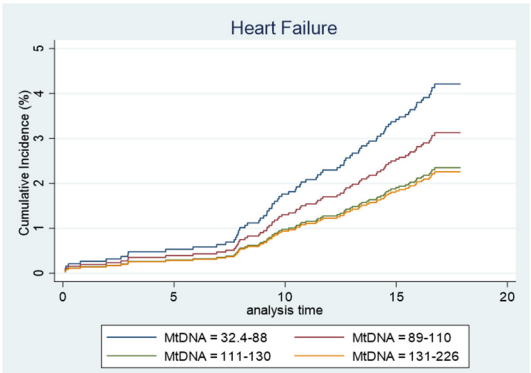



FIGURE 2
Cumulative incidence function after adjusted (for age, bmi, smoking, education, systolic, triglyceride, and HDL) competing risk regression where cancer, MI, and death are considered competing events.

## The role of MtDNA-CN and MI in the association with incident HF

We also investigated whether mtDNA-CN and MI had any confounding, modifying or mediating effect on their associations with incident HF. To disentangle this effect we analyzed potential confounding, interaction and mediation effects. We found that MI slightly reduced the effect of mtDNA-CN on HF, and therefore controlled for MI in the analyses of the association between mtDNA-CN and HF. Neither mtDNA-CN nor MI had any modifying effect on the association with incident HF (interaction term analysis). The mediation analysis showed that although mtDNA-CN affects the risk of MI (OR = 1.48) and MI *via* a risk of future HF (OR = 8.0), the indirect effect of MI on the association between mtDNA-CN and HF was small and did not reach statistical significance (OR = 1.06; *p*-value = 0.14) (Supplementary Table 1 and Figure 3).

TABLE 4 Effect of mtDNA on heart failure using competing-risks regression model by a subdistribution hazard approach (fine and gray).

| | Univariate | | | Adjusted[a] | | |
|---|---|---|---|---|---|---|
| **Outcome: time to heart failure** | **SHR[b]** | ***p*-value** | **95% CI** | **SHR** | ***p*-value** | **95% CI** |
| MtDNA (decrease, std)[c] | 1.40 | 0.008 | 1.09; 1.80 | 1.32 | 0.03 | 1.03; 1.71 |
| MtDNA quartiles[d] | | | | | | |
| $Q_3$ vs. $Q_4$ | 1.23 | 0.54 | 0.64; 2.34 | 1.04 | 0.91 | 0.54; 2.02 |
| $Q_2$ vs. $Q_4$ | 1.56 | 0.16 | 0.84; 2.91 | 1.39 | 0.30 | 0.74; 2.61 |
| $Q_1$ vs. $Q_4$ | 2.34 | 0.01 | 1.19; 4.58 | 1.88 | 0.07 | 0.96; 3.71 |

[a]Adjusted for age, bmi, smoking, education, systolic, triglycerides and hdl.
[b]Subdistributional hazard ratio where cancer, MI and death are considered competing events.
[c]MtDNA has been reversed and standardized (HR for a one standard deviation decrease in mtDNA).
[d]$Q_1 = 32.4–88$, $Q_2 = 89–110$, $Q_3 = 111–130$, $Q_4 = 131–226$.

## Baseline MtDNA-CN and risk of overall and HF related mortality

During the follow-up, 195 deaths from all causes (overall mortality) and 11 deaths related to HF were observed. In multivariable models adjusted for potential confounders, lower baseline mtDNA-CN (1 SD decrease) was associated with significantly higher risk of overall mortality (HR = 1.20; 95% CI = 1.03–1.39). Similar results were observed when mtDNA-CN was categorized into quartiles (Table 5). Lower baseline levels of mtDNA-CN were also associated with higher risks of HF related mortality (HR = 1.93; 95% CI= 1.04–3.60); however, the statistical significance was lost when adjusted for potential confounders (HR = 1.57; 95% CI = 0.85–2.90), Table 5). The proportionality assumption for the model examining HF mortality was not violated ($p$-value = 0.52).

## Sensitivity analyses

Since the number of prevalent MI differed between women with incident and no incident HF (Table 1), we also performed a sensitivity analysis to examine if our results were robust. After exclusion of prevalent MI, the associations between mtDNA-CN and HF in Tables 3–5 only showed minor changes when women with prevalent MI were excluded (data not shown). Thus, the sensitivity analysis further confirmed that our results were robust, and our conclusions remain the same.

# Discussion

Lower mtDNA-CN at baseline is associated with higher risk of HF incidence, independent of potential confounders in a well characterized cohort of middle-aged women followed for a median follow-up of 17 years. Furthermore, we also showed that low mtDNA-CN at baseline was associated with both overall and HF mortality.

HF is a debilitating disease associated with higher morbidity and mortality both in men and women (27). Due to the complexity of the disease conventional risk factors may not accurately predict HF and identification of non-conventional biomarkers which can easily be quantified can assist in better prediction of HF (28). Incidence of HF differ according to sex and is attributed to differences in pathophysiology, risk factors, age and cardiac ejection fraction (29). HF with preserved ejection fraction is more common in women than in men and accounts for at least half of the cases of HF in women (30). Furthermore, women with HF may have a higher probability of HF related readmission than men (3). Levels of mtDNA-CN has been suggested as a biomarker of MI incidence, a major risk factor for HF, in a previous study of ours on middle-aged women (11) and by other researchers in both men and women (31). Most cardiac biomarkers used today do not take the sex differences into account. Therefore, sex-specific reference ranges for cardiac biomarkers used routinely in clinical practice has been proposed (32). Considering the important role of mitochondria in normal functioning of cardiomyocytes, it is not surprising that mitochondrial dysfunction may lead to abnormal functioning of cardiomyocytes, which may eventually result in HF. In agreement with our results, a follow-up study of both men and women showed an inverse association between mtDNA-CN and HF incidence (21). Another study of both men and women and a relatively shorter follow-up of 17 months also showed an inverse relationship between mtDNA-CN and HF risk (20). However, none of the above studies had a stratification according to sex; therefore, it is difficult to conclude whether mtDNA-CN had similar effects on both men and women. Nevertheless, our results demonstrate that lower baseline mtDNA-CN may be a risk factor for HF incidence in middle-aged women.

To evaluate the prognostic performance of a biomarker for an event of interest, it is important to consider potentially competing events whose occurrence could preclude the primary event of interest (33). For example, as shown also in this study, MI is one of the major risk factors of HF (34) and we have previously shown that lower mtDNA-CN is associated with

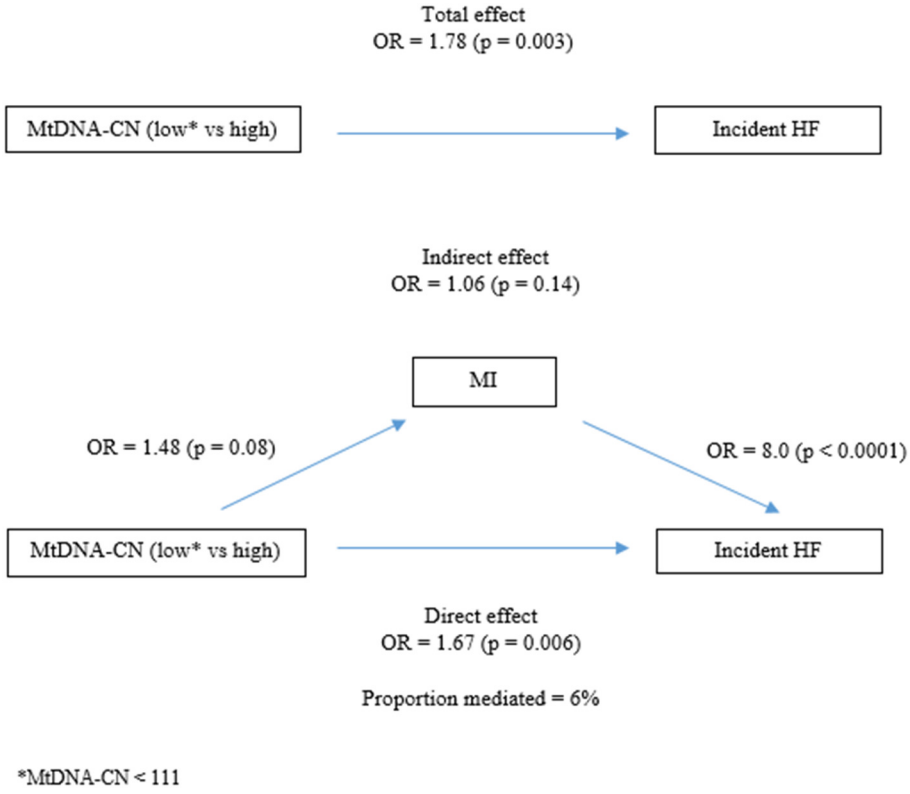



FIGURE 3
Mediation analysis.

TABLE 5 Cox regression models examining effect of mtDNA-CN on overall mortality and mortality due to heart failure.

| | Univariate | | | Adjusted[a] | | |
|---|---|---|---|---|---|---|
| **Outcome: time to death (any)[b]** | **HR** | ***p*-value** | **95% CI** | **HR** | ***p*-value** | **95% CI** |
| MtDNA-CN (decrease, std)[c] | 1.27 | 0.001 | 1.10; 1.46 | 1.20 | 0.02 | 1.03; 1.39 |
| MtDNA-CN quartiles[d] | | | | | | |
| $Q_3$ vs. $Q_4$ | 2.02 | 0.001 | 1.35; 3.03 | 1.87 | 0.004 | 1.23; 2.85 |
| $Q_2$ vs. $Q_4$ | 1.77 | 0.007 | 1.16; 2.68 | 1.50 | 0.07 | 0.97; 2.33 |
| $Q_1$ vs. $Q_4$ | 2.22 | 0.001 | 1.39; 3.55 | 1.87 | 0.01 | 1.14; 3.05 |
| Outcome: time to death due to heart failure[e] | | | | | | |
| MtDNA-CN (decrease, std) | 1.93 | 0.04 | 1.04; 3.60 | 1.57 | 0.15 | 0.85; 2.90 |

[a]Adjusted for age, smoking, alcohol consumption, education, activity and HDL.
[b]Number of deaths = 195.
[c]MtDNA-CN has been reversed and standardized (HR for a one standard deviation decrease in mtDNA-CN).
[d]$Q_1$ = 32.4–88, $Q_2$ = 89–110, $Q_3$ = 111–130, $Q_4$ = 131–226.
[e]Number of deaths due to heart failure = 11 (found in death register, all causes ICD10 = I50 or I110).

future risk of MI in women (11); hence, can be a competing risk. Furthermore, hypertension and T2D play an important role in the pathophysiology of coronary artery disease more in women than in men; thus, they also are direct or indirect important risk factors of HF in women (2, 35). In our study, mtDNA-CN levels were strongly associated with both systolic blood pressure and T2D. We calculated the cumulative incidence by competing risk regression analysis, where cancer, MI and death were considered competing events. Our results demonstrated that mtDNA-CN is associated with HF incidence independent of the competing risks included in the model. Although MI is a major risk factor for HF, not all MI patients develop HF. Considering the

association of mtDNA-CN with both MI and HF incidence, we hypothesized that mtDNA-CN may be a mediating factor between MI and HF or that MI could be a mediating factor between mtDNA-CN and HF. However, we could not find any mediating effect of mtDNA-CN on the association between MI and HF. The mediating role of MI in the association between mtDNA-CN and HF was small and non-significant.

MtDNA-CN is associated with all-cause mortality (36) and higher cardiovascular disease related mortality (20), which is consistent with our study. One of the possible explanations for this association is that the changes in mtDNA-CN influence nDNA methylation at specific loci and result in differential expression of specific genes that may impact disease and mortality *via* altered cell signaling (37). However, the number of deaths due to HF in this study was quite low ($n = 11$) and therefore, this needs to be confirmed in future studies.

### Strength and limitations

This study has several strengths and limitations that must be recognized in the interpretation of our results. The main strength of the study is that it is based on a well characterized population-based follow-up cohort of middle-aged women where an absolute quantification of mtDNA-CN was quantified by a well optimized ddPCR method. Moreover, all diagnoses were collected from a questionnaire and/or Swedish health registers, which provided almost complete information on diagnoses during a long follow-up. We did not have the information on ejection fraction of the participants included in this study, which precluded our possibilities to conduct separate analyses for the different types of HF. Finally, mtDNA-CN was measured only at baseline as follow-up samples were not collected; therefore, we do not know the status of mitochondrial function at the time of diagnosis.

In conclusion, our results demonstrate that mtDNA-CN, an easily quantifiable biomarker in blood, is a molecular risk factor for incident HF, independent of potential confounders and competing events.

## Data availability statement

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

## Ethics statement

The studies involving human participants were reviewed and approved by the regional Ethical Committee at Lund University approved the study (approval nos. 95/174, 2011/494 and 2015/6) and written informed consent was given by all the participants in the study after full explanation of the purpose and nature of all procedures.

## Author contributions

KS, JS, and AM conceived, designed the study, performed the data analysis, and interpretation. KS and KP performed the statistical analysis. AM and JS collected the samples and clinical data. KS and AM wrote the first draft. KS, JS, KP, and AM revised the article, and approved the final version. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships 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/fcvm.2022.1012403/full#supplementary-material

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# C-reactive protein and coronary atheroma regression following statin therapy: A meta-regression of randomized controlled trials

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**Objective:** Several clinical trials have indicated that statins stabilize and reverse atherosclerotic plaque. However, different studies have provided inconsistent findings regarding mechanisms and influencing factors of plaque regression under statin therapy. Apart from lipid-lowering effect, statins have pleiotropic effects including anti inflammation in humans. In this study, meta-analysis and meta-regression were used to determine the effects of statin medications on coronary plaque volume. Meanwhile, to assess whether statins promote plaque regression effect was related to their anti-inflammatory ability, the impact of CRP/hsCRP reduction during statin therapy on plaque regression was investigated.

**Methods:** Up to June 15, 2022, a systematic PubMed, EMBASE, and Cochrane search was performed for randomized controlled trials that assessed treatment effect using total atheroma volume (TAV), percent atheroma volume (PAV), or plaque volume (PV). Only CRP/hsCRP and LDL-C values reported before and after treatment were considered.

**Results:** 12 studies (2,812 patients with heart and/or vascular disease) fulfilled the inclusion criteria and were included in the systematic review. A meta-analysis of 15 statin-treated arms reported a significant reduction in change of TAV/PV [standardized mean difference (SMD): −0.27, 95% confidence intervals (−CI): −0.42, −0.12, $p < 0.001$], compared with the control arms. Another meta-analysis of 7 trials also found that patients in the intervention group had a significant reduction in change of PAV (SMD: -0.16, 95% CI: −0.29, −0.03, $p = 0.019$), compared with those in the control group. Meta-regressionanalysis revealed that the percent change of CRP/hsCRP was

significantly associated with SMD in change of TAV/PV after adjusting for percent change of LDL-C, age, gender and study duration. Meta-regression analysis showed that percent change of CRP/hsCRP statistically influenced SMD in change of PAV, when percent change of CRP/hsCRP was included separately. However, the percent change of CRP/hsCRP was not significantly associated with SMD of PAV change after adjusting for all covariates.

**Conclusion:** In conclusion, statin therapy is beneficial for plaque regression. Statins promote plaque regression, which might be associated to their anti-inflammatory ability.

KEYWORDS

**statins, regression of atherosclerosis, C-reactive protein, randomized controlled trial, meta-analysis**

## Introduction

Cardiovascular diseases are considered the leading causes of death worldwide. Among them, coronary heart disease (CHD) has garnered considerable attention due to its high prevalence and burden. The pathological basis of CHD is atherosclerosis, which is characterized by the accumulation of lipids and cholesterol in the artery's subintima and progressive chronic inflammation of the fibrotic plaque on the wall of great and medium arteries (1). Assessment of coronary artery plaques provides clinical information regarding the progression of disease and the risk of experiencing future adverse cardiovascular events (2). In recent studies, indicators including total atheroma volume (TAV), percent atheroma volume (PAV), or plaque volume (PV) have been widely used to assess plaque burden (3).

Coronary plaque regression has a significant positive correlation with low density lipoprotein cholesterol (LDL-C). As important lipid-lowering drugs, several studies have demonstrated that statin drugs promote coronary atheroma stabilization and regression in patients with acute coronary events or stable coronary disease (4). Among those studies, recent clinical studies have demonstrated that statins can reduce plaque burden by demonstrating a reduction in TAV, PAV, and PV (5). Currently, statins are widely used to prevent atherosclerotic cardiovascular disease (ASCVD). Numerous studies have shown that statins are effective in reducing LDL-C, and the risk of death and recurrent coronary and cardiovascular events in those with a history of ASCVD (6). Meanwhile, statin therapy is a first-line treatment for the primary prevention of ASCVD in patients with elevated low-density lipoprotein cholesterol levels ($\geq$ 190 mg/dL), those with diabetes mellitus, those who are 40–75 years of age, and those determined to be at sufficient ASCVD risk after a clinician–patient risk discussion (7).

As the mechanism of vascular inflammation is gradually elucidated, numerous evidences have demonstrated that C-reactive protein (CRP) and high-sensitivity C-reactive protein (hsCRP) may play direct pathogenic roles in atherosclerosis (8, 9). Initially, statin drugs were used primarily to reduce blood lipids. With the deepening of research, its non-lipid-lowering effects, such as the anti-inflammatory effect of statins on the coronary plaque volume, have become the focus of recent studies. Ridker et al. discovered that rosuvastatin (20 mg/d) and placebo were administered to randomly selected healthy people with elevated hs-CRP but no evidence of hyperlipidemia. After an average follow-up of 1.9 years, the hs-CRP level in the treatment group decreased by 37% compared with that in the control group, implying that statins may have anti-atherosclerosis functions *via* anti-inflammatory mechanisms (10). Numerous clinical trials, such as the Air Force/Texas Coronary Atherosclerosis Prevention (AFCAPS/TexCAPS) study, the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial, and the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction 22 (PROVE IT-TIMI 22) trial, have demonstrated that statins reduce hsCRP levels independently of lowering LDL-C levels. In a trial with canakinumab for atherosclerotic disease, the rate of cardiovascular event recurrence was significantly lower in the treated group than in the placebo group, implying that reducing inflammation without affecting lipid levels can reduce cardiovascular disease risk (11).

Statin therapy was shown to be beneficial in reducing CRP/hsCRP. However, few studies have attempted to investigate the relationship between the degree of CRP/hsCRP reduction associated with changes in coronary plaque burden during statins treatment. To answer the question of whether the CRP/hsCRP lowering effect of statins could delay or reverse the progression of atherosclerosis, we conducted this study.

The aim of the present study was to provide a systematic review and meta-regression analysis to examine the impact of statins on CRP/hsCRP reduction on coronary plaque burden assessed with TAV, PAV, and PV. At the same time, we analyzed the joint effects of LDL-C and CRP/hsCRP changes on plaques.

## Methods

This work followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) and amendments to the Quality of Reporting of Meta-analyses (QUOROM) statement (12, 13).

## Search strategy and study selection

For this meta-analysis, we conducted a search in PubMed, EMBASE and the Cochrane Library to identify studies relevant to this topic from their inception to June 15, 2022. The study selection was performed independently by 2-group investigators (CLL, YJM as group 1, and RH, DJ as group 2) using highly sensitive strategy. Disagreements were resolved by consensus with a senior author (WXX). Here we show the search strategy of PubMed: "[(statin) OR (hydroxy-methyl-glutaryl-CoA) OR (HMG-COA) OR (pravastatin) OR (lovastatin) OR (simvastatin) OR (Atorvastatin) OR (fluvastatin) OR (Rosuvastatin) OR (Pitavastatin)] AND [(intravascular ultrasound) OR (IVUS) OR (plaque) OR (atheroma)] AND [(intravascular ultrasound) OR (IVUS) OR (coronary)] AND (Clinical Trial[ptyp])." **Supplementary Table 1** shows details of the search syntax.

## Selection criteria

Studies were included according to the following criteria: (a) randomized controlled trials (RCTs); (b) investigating the impact of statin therapy on plaque volume using IVUS; (c) reporting at least one of the following data: TAV, PV, and PAV; (d) with a follow-up longer than or equal to 6 months; (e) reporting LDL-C at baseline and the end of the study or reporting data of percent change of LDL-C; (d) reporting CRP or hsCRP before and after statin treatment (or percent change of CRP/hs-CRP).

Exclusion criteria included the following: (a) duplicate publication or secondary analyses of the same study population; (b) lack of sufficient information on baseline or follow-up IVUS data, LDL-C data, and CRP/hsCRP data.

## Data extraction quality appraisal

The data were extracted from each study using standard tables. The extracted data included the following: study characteristics (the first author, title, publication time, number of patients, country, and study duration), patient characteristics (age and sex), intervention, control, method characteristics (randomization, blind implementation, and follow-up loss), and patient outcomes. For patient outcomes, we extracted TAV, PAV, or PV data as measured using IVUS technique, LDL-C data, CRP, hsCRP data (including values at baseline and endpoint) and other useful information.

After data extraction, we conducted statistical analysis to calculate change of TAV, change of PV, change of PAV, percent change of LDL-C, percent change of CRP, and percent change of hsCRP. Articles reported mean values and standard deviation (SD) of change of TAV/PV/PAV, the original number was entered. Some studies (14–17) did not report SD values, which were filled by using the SD of the baseline data of the control group. 1 study (18) provided standard error (SE) rather than SD, and then SD value was calculated based on SE value. If the IVUS efficacy endpoints were reported as medians, with distribution-free 95% confidence intervals (CI), the median reported in the original text was extracted, and SD was calculated by formula.

In terms of LDL-C, if the article reported percent change of LDL-C, the original number was entered; otherwise, percent change of LDL-C was calculated using the following formula:

$$\begin{aligned} &\textit{percent change of LDL} - \textit{C}\ (\%) \\ &= \frac{\textit{follow up value} - \textit{baseline value}}{\textit{baseline value}} \times 100\% \end{aligned}$$

Percent change of CRP and percent change of hsCRP were calculated using the same approach. **Supplementary Table 2** shows details of data extraction.

Two independent authors (RH and DRG) assessed the risk of bias in each included study. According to Cochrane's indications, un-blinded, independent reviewers evaluated the quality of included studies using pre-specified forms (risk of bias table), including seven examined fields: random sequence generation (selection bias); allocation sequence concealment (selection bias); blinding of participants and personnel (performance bias); blinding of outcome assessment (detection bias); incomplete outcome data (attrition bias); selective outcome reporting (reporting bias); and other potential sources of bias.

## Data analysis and synthesis

Continuous variables were expressed as mean ± *SD*, whereas categorical variables were expressed as *n* (%). Heterogeneity among individual studies was assessed with the Q-test and

quantified with the $I^2$ statistic (range: 0–100%). $I^2$ represents the proportion of the total variance that can be attributed to heterogeneity of true study effects (19). The heterogeneity was regarded as low if $I^2 \leq 25\%$, as moderate if $I^2$ in the range of 26–74% and as high if $I^2 \geq 75\%$ (20). When a study is gathered from the published literature, the random-effects model is generally a more plausible match. For the random-effects model allows the true effect size may vary from study to study. In addition, the standard error of the summary effect and the confidence intervals for the summary effect are wider under the random-effects model than under the fixed-effect model (21). Thus, we performed meta-analysis to pool estimates using random effects model. Meta-analysis with continuous outcome variables was performed, and the effect of statin therapy (vs. control) on change of TAV, PV, and PAV at the end of follow-up was estimated as standardized mean difference (SMD) and 95% CI. If $p < 0.05$ and the 95% CI did not include zero, the point estimate of SMD was considered statistically significant. To avoid double-counting of subjects and consequent unit-of-analysis error in trials with more than one treatment arm, the control group was evenly divided (where possible) (10). Since the units ($mm^3$) of change of TAV and change of PV were the same, we combined these two indicators for data synthesis.

To explore the link between the dependent variable and the covariate, meta-regression is often used. We hypothesized that the included studies may have shown differences according to the percent change of CRP/hsCRP, percent change of LDL-C, age, gender and study duration of the patients. To evaluate the possible impact of these factors on the results of the meta-analysis, we established model with the change of TAV/PV or change of PAV as the dependent variable. In particular, change in TAV/PV was our primary outcome, and change in PAV was the secondary outcome.

Funnel plot analysis and Begg's and Egger's tests were performed to evaluate potential publication bias. Sensitivity analysis was conducted to assess the stability of studies. Sensitivity analysis was conducted using leave-one-out method, i.e., removing one study each time and repeating the analysis. Statistical analyses were carried out using meta packages in *R* version 4.1.2 (2021-11-01) and risk of bias was evaluated with Review Manager (RevMan 5.3; Cochrane Collaboration).

# Result

## Flowchart of included studies

The initial literature search retrieved 1,313 articles. After the removal of duplicates, the titles and abstracts of 805 articles were carefully checked, leading to the exclusion of 666 articles for failing to meet the inclusion criteria. Initially, 139 articles were selected, and their full texts were evaluated. Of them, 124 articles were excluded: 22 because CRP/hsCRP levels were not reported, 12 because plaque evaluation (TAV, PAV, or PV) was not performed, 50 because they were not RCTs, 31 because statins were not used, and 9 because of repeated trials. A total of 15 articles entered the third round of evaluation. One was excluded due to a discrepancy between the number of participants receiving statins and the number of people participating in IVUS measurements (22). And two were excluded because of data quality: in one study, CRP was reported, but the indicators of the control group declined significantly (23); in another study, the *SD* at baseline and follow-up varied greatly and the reported difference value was inconsistent with the calculated difference value (24). Overall, this analysis included 12 trials (14–18, 25–31) **Figure 1** summarizes the study selection process.

## Characteristics of included studies

The study characteristics are reported in **Table 1**. A total of 2,812 subjects were included in the 12 eligible studies. Included studies were published between 2004 and 2016 and were reported from China, the USA, Korea and Japan. The largest study had a population size of 1,039 subjects while the smallest study recruited 30 subjects. The mean age of the participants ranged from 55.8 to 67.0 years.

12 trials with 16 treatment arms were included. 8 treatment arms used atorvastatin (dose range: 10–80 mg/day; duration of treatment: 24–72 weeks), 6 treatment arms used rosuvastatin (dose range: 10–40 mg/day; duration of treatment: 44–104 weeks), 1 treatment arm used pravastatin (dose: 20 mg/day; duration of treatment: 24 weeks), and 1 treatment arm used pitavastatin (dose: 4 mg/day; duration of treatment: 32 weeks).

IVUS was used in all studies to evaluate plaque volume. In addition to 1 study (24) 11 studies reported change of TAV/PV, and 7 studies reported change of PAV. As described in the data extraction section, percent change of CRP/hsCRP and percent change of LDL-C were reported in all studies.

Overall, random sequence generation was observed in 6 studies, 4 of them reported allocation concealment. 3 trials were double-blinded, and 8 studies performed blinded assessments of the outcomes. Moreover, 2 studies existed incomplete outcome data because of a high attrition rate. **Supplementary Figure 1** shows details of the risk of bias assessment.

## Effect of statin therapy on change of TAV/PV

11 trials ($n$ = 2,696) including 15 comparisons reported change of TAV/PV. Compared with control arms, our meta-analysis showed that 15 treatment arms revealed a significant decrease in change of TAV/PV (SMD: –0.27, 95% CI: –0.42, –0.12, $p < 0.001$), with a moderate heterogeneity ($Q$ = 27.55,

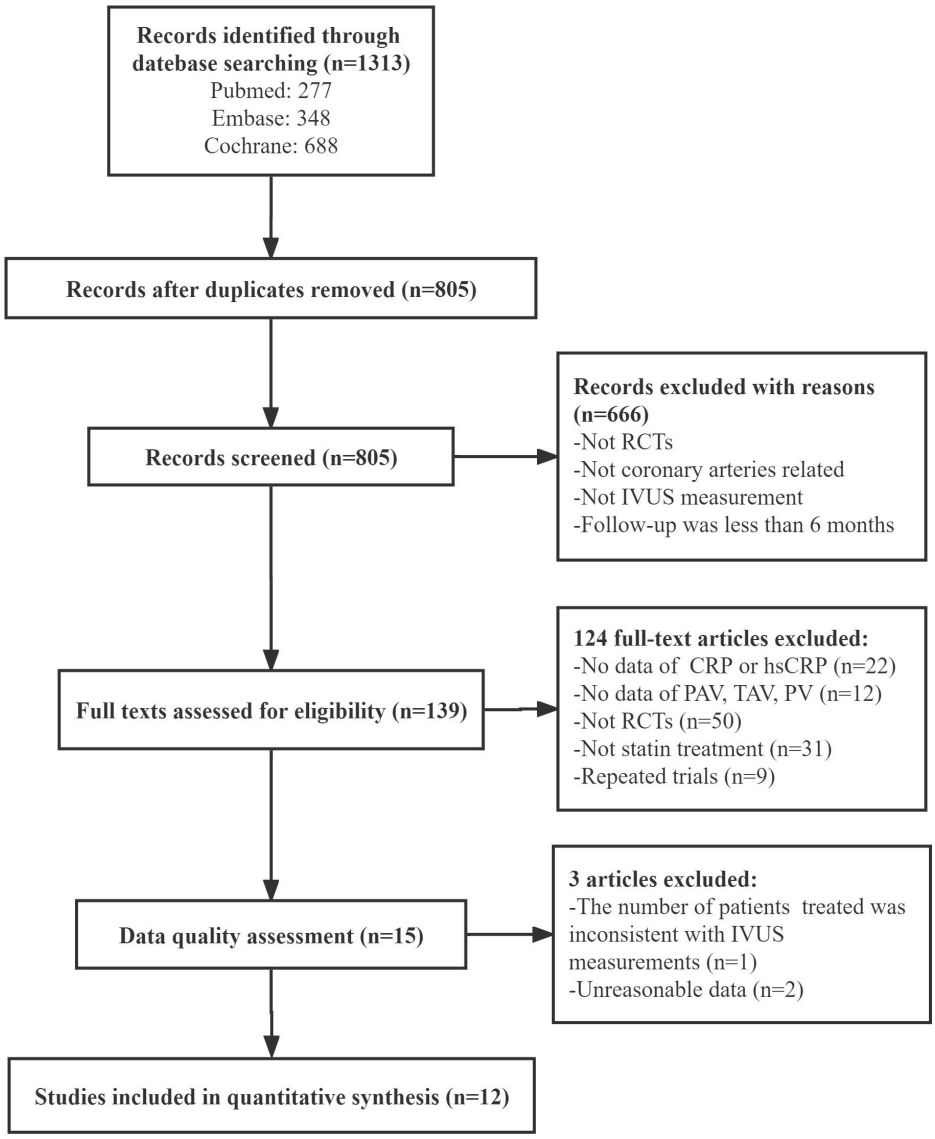


FIGURE 1
Flowchart for study. RCTs, randomized controlled trials; IVUS, intravenous ultrasound; CRP, C-reactive protein; hsCRP, high-sensitivity C-reactive protein; TAV, total atheroma volume; PAV, percent atheroma volume; PV, plaque volume.

$df$ = 17, $p$ = 0.02, $I^2$ = 49.2%). Figure 2 presents the combined results of the 15 head-to-head comparisons in this meta-analysis.

## Effect of statin therapy on change of percent atheroma volume

7 studies ($n$ = 2,295) reported change of PAV. Heterogeneity test of data from 7 studies shown moderate heterogeneity ($Q$ = 10.19, $df$ = 6, $p$ = 0.12, $I^2$ = 41.1%) and random effect model was adopted. Compared with those in the control group, this meta-analysis indicated that patients in the intervention group have a significant reduction in change of PAV (SMD: –0.16, 95% CI: –0.29, –0.03, $p$ = 0.019). Figure 3 presents the combined results of 7 studies in this meta-analysis.

## Meta-regression for standardized mean difference in change of TAV/PV

Meta-regression was then employed to test whether the percent change of CRP/hsCRP was associated with the change of TAV/PV. The results of the meta-regression analysis are given in Table 2. Model 1 demonstrates that the impact of percent change of CRP/hsCRP on change of TAV/PV was statistically significant

TABLE 1 Main characteristics and findings of included studies.

| References | Country | Study duration | Therapy[a] (mg/d) | Participants (*n*) | Age (years) | Male (%) | CRP /hsCRP | Percent change of CRP/hsCRP (%) | Percent change of LDL-C (%) | Change of TAV/PV ($mm^3$) | Change of PAV (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Hong et al. (27) | Korea | 12 months | Ros 20 | 16 | 60 ± 8 | 75 | hsCRP | −94.35 | −46.38 | −5.62 ± 7.71 | −0.80 ± 1.27 |
| | | | A to 40 | 14 | 62 ± 90 | 43 | | −93.85 | −43.31 | −4.74 ± 8.51 | −0.57 ± 1.15 |
| Hong et al. (28) | Korea | 11 months | Ros 20 | 65 | 59 ± 10 | 75 | hsCRP | −80 | −49.18 | −4.4 ± 7.3 | −0.73 ± 2.05 |
| | | | A to 40 | 63 | 58 ± 10 | 73 | | −89.25 | −40.17 | −3.6 ± 6.8 | −0.19 ± 2.10 |
| Kawasaki et al. (15) | Japan | 6 months | A to 20 | 18 | 66 ± 8.7 | 70.6 | CRP | −65 | −39 | −3.8 ± 32.2 | / |
| | | | Pra 20 | 17 | 67 ± 7.8 | 72.2 | | −18 | −32 | −1.6 ± 32.1 | / |
| | | | Diet | 17 | 66 ± 6.4 | 82.4 | | 17 | −2 | 0 ± 29.9 | / |
| Nicholls et al. (29) | The USA, et al | 26 months | Ros 40 | 520 | 57.4 ± 8.6 | 72.9 | CRP | −35.29 | −47.83 | −6.39 ± 13.96 | −1.22 ± 3.61 |
| | | | A to 80 | 519 | 57.9 ± 8.5 | 74.4 | | −33.33 | −41.45 | −4.42 ± 15.81 | −0.99 ± 3.49 |
| Nissen et al. (30) | The USA | 18 months | A to 80 | 253 | 55.8 ± 9.8 | 71 | CRP | −36.4 | −46.3 | −0.9 ± 20.69 | 0.2 ± 3.25 |
| | | | Pra 40 | 249 | 56.6 ± 9.2 | 73 | | −5.2 | −25.2 | 4.4 ± 23.75 | 1.6 ± 4.03 |
| Nozue et al. (31) | Japan | 8 months | Pit 4 | 58 | 66 ± 9 | 90 | hsCRP | −75 | −41 | / | −0.2 ± 3.4 |
| | | | Pra 20 | 61 | 67 ± 11 | 77 | | −75 | −29 | / | 0.2 ± 4.8 |
| Park et al. (18) | Korea | 12 months | Ros 40 | 152 | 62.6 ± 9.3 | 71 | hsCRP | −52.38 | −43.87 | −14.72 ± 29.59 | −0.88 ± 4.93 |
| | | | Ros 10 | 73 | 61.8 ± 8.9 | 77 | | −47.83 | −27.90 | −13.63 ± 21.87 | −0.85 ± 3.25 |
| Takayama et al. (16) | Japan | 12 months | Ros 20 | 18 | 65.1 ± 10.1 | 72 | hsCRP | −65 | −50 | −3.1 ± 33.5 | / |
| | | | Ros 2.5 | 19 | 63.8 ± 8.5 | 83 | | −60 | −30 | 1.2 ± 33.5 | / |
| Hiro et al. (25) | Japan | 8–12 months | A to 20 | 127 | 62.4 ± 10.6 | 81.1 | hsCRP | −95.4 | −35.8 | −10.6 ± 10.6 | −6.3 ± 6.1 |
| | | | Pit 4 | 125 | 62.5 ± 11.5 | 82.4 | | −97.3 | −36.2 | −8.2 ± 8.9 | −5.7 ± 6.3 |
| Hong et al. (26) | Korea | 12 months | Ros 10 | 50 | 59 ± 9 | 74 | CRP | −57.14 | −44.83 | −3.6 ± 7.2 | / |
| | | | Sim 20 | 50 | 58 ± 10 | 80 | | −29.41 | −34.45 | −1.8 ± 5.7 | / |
| Zhang et al. (17) | China | 9 months | A to 80 | 50 | 64.5 ± 13.8 | 62 | hsCRP | −66.36 | −40.91 | −1.5 ± 9.33 | / |
| | | | A to 20 | 50 | 65.5 ± 6.2 | 58 | | −37.41 | −24.58 | 8.36 ± 9.33 | / |
| Guo et al. (14) | China | 6 months | A to 10 | 47 | 62.64 ± 12.00 | 85.1 | hsCRP | 11.59 | −22.11 | −0.02 ± 13.76 | / |
| | | | A to 20 | 45 | 59.18 ± 8.48 | 80.0 | | 0.39 | −31.16 | 2.29 ± 13.76 | / |
| | | | A to 40 | 43 | 58.91 ± 12.90 | 95.3 | | −13.94 | −36.21 | −6.37 ± 13.76 | / |
| | | | A to 80 | 39 | 58.95 ± 9.68 | 87.2 | | −41.15 | −36.04 | −11.48 ± 13.76 | / |
| | | | Placebo | 54 | 62.07 ± 8.51 | 88.9 | | 35.50 | 1.02 | 2.63 ± 13.76 | / |

[a]Ros, rosuvastatin; Ato, atorvastatin; Pra, pravastatin; Pit, pitavastatin; Sim, simvastatin.





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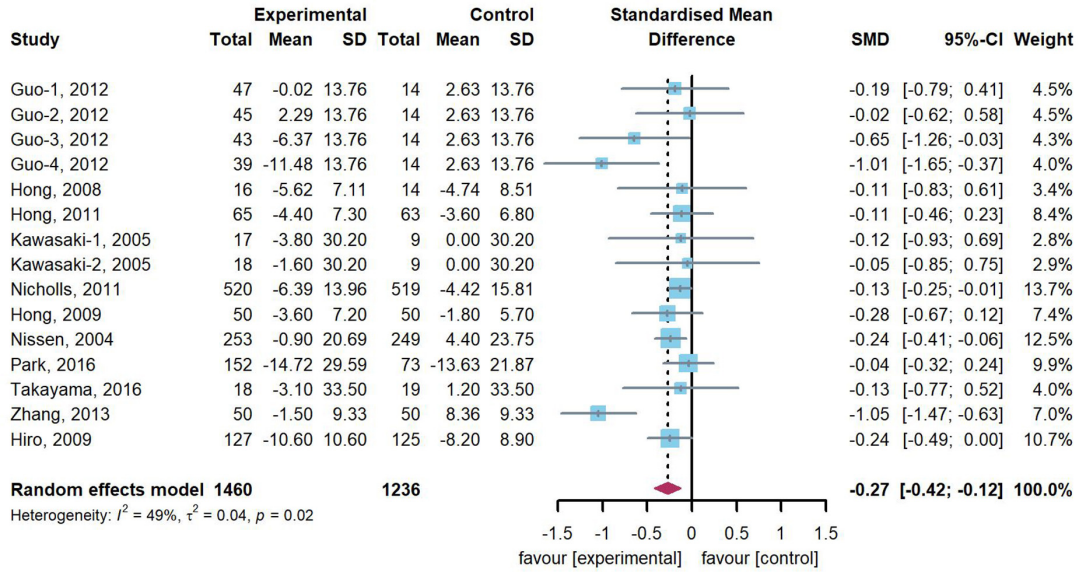


FIGURE 2
Forest plot of change of TAV/PV. A meta-analysis of 15 statin-treated arms reported a significant reduction in change of TAV/PV [standardized mean difference (SMD): −0.27, 95% confidence intervals (CI): −0.42, −0.12], compared with the control arms.

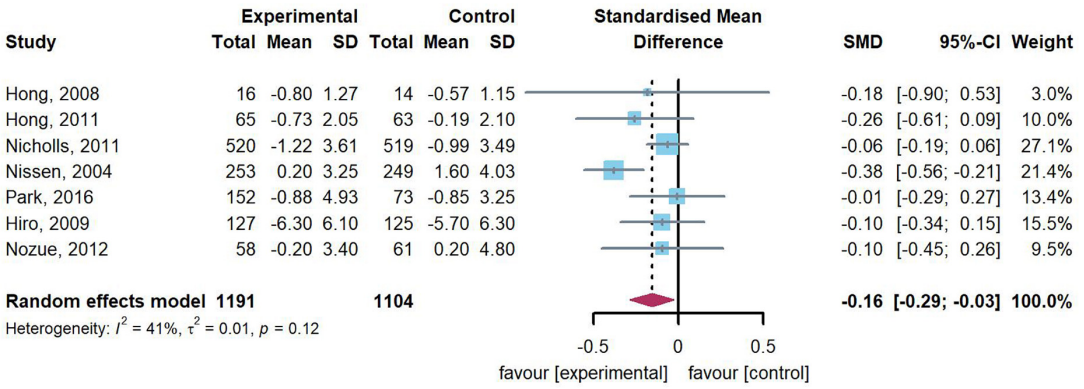


FIGURE 3
Forest plot of change of PAV. A meta-analysis of 7 studies reported a significant reduction in change of PAV [standardized mean difference (SMD): −0.16, 95% confidence intervals (CI): −0.29, −0.03], compared with the control.

($p$ = 0.024). The regression coefficient of this independent variable was β = 0.0064 (95% CI: 0.0009–0.0120). Model 2 analyzed the influence of percent change of LDL-C on change of TAV/PV. The results showed that percent change of LDL-C had no significant effect on change of TAV/PV ($p$ = 0.268). Model 3 incorporates percent changes of CRP/hsCRP and LDL-C. Only percent change of CRP/hsCRP was associated with change of TAV/PV (β = 0.0119, 95% CI: 0.0017–0.0221, $p$ = 0.022). In Model 4, we entered percent change of CRP/hsCRP, percent change of LDL-C, age, gender and study duration. Among them, only percent change of CRP/hsCRP statistically influenced the dependent variable ($p$ = 0.046).

## Meta-regression for standardized mean difference in change of percent atheroma volume

Similarly, we performed another meta-regression to explore how the percent change of CRP/hsCRP affects change of PAV. The results of the meta-regression analysis are given in Table 3. Model 1 used the percent change of CRP/hsCRP as an independent variable. The results indicated that the percent change of CRP/hsCRP (β = 0.0086, 95% CI: 0.0022–0.0150) affects PAV change ($p$ = 0.009). When the percent change of CRP/hsCRP was higher, change of PAV was greater.

TABLE 2 Meta-regression analysis for SMD in change of TAV/PV.

| Variables | Model 1 | Model 2 | Model 3 | Model 4 |
|---|---|---|---|---|
| | β (95% CI) | β (95% CI) | β (95% CI) | β (95% CI) |
| Intercept | −0.1463 (−0.2982, 0.0057) | −0.1513 (−0.4122, 0.1095) | −0.2419* (−0.4510, −0.0329) | −0.6063 (−5.5278, 4.3152) |
| Percent change of CRP/hsCRP[a] | 0.0064* (0.0009, 0.0120) | – | 0.0119* (0.0017, 0.0221) | 0.0116* (0.0002, 0.0230) |
| Percent change of LDL–C | – | 0.0075 (−0.0058, 0.0208) | −0.0129 (−0.0333, 0.0075) | −0.0135 (−0.0375, 0.0104) |
| Age | – | – | – | −0.0057 (−0.0690, 0.0577) |
| Gender | – | – | – | 0.0074 (−0.0133, 0.0281) |
| Study duration | – | – | – | 0.0092 (−0.0237, 0.0421) |

[a] *$p < 0.05$.

Model 2 shows that the percent change of LDL-C was not significantly associated with PAV change ($p = 0.066$). In Model 3 (both percent change of CRP/hsCRP and percent change of LDL-C were included as independent variables) and Model 4 (independent variables including percent change of CRP/hsCRP, percent change of LDL-C, age, gender and study duration), multivariable meta-regression analyses did not reveal any significance between independent variables and the change of PAV.

## Publication bias and sensitivity analysis

Although Begg's rank correlation ($p = 0.7290$) and Egger's linear regression ($p = 0.2323$) tests were not significant, the funnel plot was asymmetric, implying potential publication bias in reporting the effect of statin therapy on change of TAV/PV. Regarding the impact of statin therapy on change of PAV, the number of studies was insufficient to conduct Begg's test and Egger's tests. However, the funnel plot also indicated potential publication bias. Funnel plots are presented in **Supplementary Figures 2, 3**.

Sensitivity analysis by excluding one study each time confirmed that the pooled estimate was consistent among studies with balanced weight. Additional sensitivity analyses are presented in **Supplementary Figures 4, 5**.

# Discussion

This meta-analysis comprised RCTs using IVUS to measure coronary plaque burden and reporting results of TAV, PAV, or PV changes. The present meta-analysis demonstrated that (1) quantitative synthesis revealed a decrease in TAV/PV and PAV levels after statin treatment compared with the control. All studies included in the meta-analysis were RCTs, further confirming that statins are effective drugs for reducing the volume of atherosclerotic plaque in coronary arteries; (2) Meta-regressions showed that the percent change of CRP/hsCRP reduction was associated with a significant reduction in change of TAV/PV after statin therapy. After adjusting for percent change of LDL-C, age, gender and study duration, this association still existed. These findings indicate that the reduction in CRP/hsCRP levels might play an important role in the beneficial effects of statins on the progression of the atherosclerotic plaque. To the best of our knowledge, this study firstly investigated the association between CRP/hsCRP change and atherosclerotic plaque reduction using meta-regressions analyses.

Statins are HMG-COA reductase inhibitors. They reduce CHD incidence due to their lipid-regulating and extra-lipid-regulating effects and are important drugs for the primary and secondary prevention of CHD (32, 33). The benefits of statins have been demonstrated to be based on stabilization and/or reversal of atherosclerotic plaque (34–37). Particularly since the introduction of IVUS technology, numerous studies have used it as an important tool for studying coronary plaque. IVUS has recently become the main tool to study the effects of statins on coronary atherosclerotic plaque, and the data obtained by IVUS served as the primary endpoint in several studies (38, 39).

Recent studies suggest that LDL-C accumulates abnormally in the vascular wall due to endothelial cell dysfunction. In addition, LDL-C can be converted into oxidized low-density lipoprotein cholesterol (oxLDL-C), eventually promoting plaque progression (40). This implies that LDL-C change is a potential factor affecting plaque regression. A *post hoc* analysis found that statin therapy was associated with regression of coronary atherosclerosis when LDL-C was substantially reduced and high density lipoprotein cholesterol was increased by more than 7.5% (41). As a result, we separately included percent change of LDL-C as an independent variable to establish a simple linear regression model, and the results showed that LDL-C change did not influence the result. Moreover, when the percent change of CRP/hsCRP, percent change of LDL-C, age, gender and study duration were simultaneously taken as independent variables to establish the regression model, only the percent change of CRP/hsCRP had a significant impact on TAV/PV. These results indicated that in the included RCTs studies using statins as intervention drugs, the ability of statins to reduce TAV/PV is probably affected by their

TABLE 3 Meta-regression analysis for SMD in change of PAV.

| Variables | Model 1 | Model 2 | Model 3 | Model 4 |
|---|---|---|---|---|
| | β (95% CI) | β (95% CI) | β (95% CI) | β (95% CI) |
| Intercept | −0.0833 (0.1784, 0.0117) | −0.0217 (−0.1926, 0.1493) | -0.0735 (−0.2504, 0.1034) | -2.6636 (−6.5456, 1.2183) |
| Percent change of CRP/hsCRP[a] | 0.0086** (0.0022, 0.0150) | – | 0.0079 (−0.0043, 0.0201) | 0.0039 (−0.0113, 0.0190) |
| Percent change of LDL-C | – | 0.0127 (−0.0008, 0.0261) | 0.0015 (−0.0209, 0.0238) | 0.0010 (−0.0256, 0.0277) |
| Age | – | – | – | 0.0458 (−0.0213, 0.1129) |
| Gender | – | – | – | −0.0060 (−0.0405, 0.0285) |
| Study duration | – | – | – | 0.0160 (−0.0051, 0.0371) |

[a] $^{**}p < 0.01$.

effect of reducing CRP/hsCRP instead of reducing LDL-C. The greater the reduction in CRP/hsCRP from baseline after statin treatment, the greater the reduction in TAV/PV. After adjusting for covariates (percent change of LDL-C, age, gender, and study duration), this association still existed. A previous study that analyzed the effect of pitavastatin treatment on changes of plaque volume had similar findings to our study. It demonstrated that TAV and PAV decreased more significantly in patients with reduction in hs-CRP $\geq$ 1 mg/dl than in those with reduction in hs-CRP $<$ 1 mg/dl (42).

Various factors influence the degree of plaque regression under statin therapy. For instance, the statin drug type (43), plaque composition (44), and patient's age and gender (45). In addition, clinical trials using IVUS demonstrated a linear relationship between LDL-C levels and reductions in atheroma burden under statin treatment (46). Despite the well-established causal role of LDL-C in the pathogenesis of atherosclerosis, our findings do not seem to support a reduction in TAV/PV relying on LDL-C levels. Recent investigations have demonstrated that changes in LDL-C levels are unrelated to plaque progression/regression following ezetimibe treatment (47). This is consistent with our research conclusions. However, the percent change of CRP/hsCRP was not significantly associated with SMD in change of PAV after adjusting for the percent change of LDL-C, age, gender and study duration. This could be because only seven trials were included in the regression analysis. The instability of research outcomes is caused by insufficient research data and an excessive number of independent variables.

It has previously been shown that anti-inflammatory therapy alone is beneficial for plaque regression (48). Considering the pleiotropic nature of statins, CRP/hsCRP is an important indicator of the anti-inflammatory effect of statins. Our findings imply that statins promote plaque regression, which is associated with their anti-inflammatory ability. And the effect of plaque regression may not be affected by their ability to regulate LDL-C.

At present, the main mechanisms of plaque formation include vascular endothelial dysfunction, intimal hyperplasia, lipid accumulation, and inflammatory response. Arterial inflammation plays an important role in the initiation and progression of atherosclerosis. Consistent with growing evidence that atherosclerosis is an inflammatory condition and many inflammatory cells, especially macrophages and foam cells can produce a variety of cytokines that may stimulate the hepatic expression of the CRP gene and up-regulate CRP production in the liver (49, 50). Therefore, elevated CRP, elevated hsCRP and changes of some other inflammatory markers may be potentially related to the risk of atherosclerosis development (50, 51). It is thought that the roles of CRP in the development of atherosclerotic plaque are complicated (52). Recent evidence propose that CRP and type oxidized LDL-C after being converted into foam cells stimulate tissue factor before thrombus formation, endothelial cell expression of adhesion molecules, and vascular endothelial dysfunction, all of which contribute to unstable atherosclerotic plaque (53, 54). In addition, several studies have suggested that atherosclerotic plaques also express CRP, and induce macrophage activation (55). Simultaneously, the expression and release of inflammatory factors are regulated to accelerate atherosclerotic plaque formation (56). Other studies also found that smooth muscle cells of atherosclerotic lesions could produce CRP and the locally produced CRP could participate in atherogenesis and the development of cardiovascular complications directly (50, 57). These associations between CRP and atherosclerosis suggest that inhibition of CRP may represent a therapeutic modality for the treatment of cardiovascular disease (49).

In addition to their cholesterol-lowering effects, recent clinical trials have established that the advantages of statins are based on their pleiotropic properties, such as reducing inflammation, stabilizing plaque, improving vascular endothelial function, suppressing vascular smooth muscle proliferation, and so on (58). And the ability to reduce inflammatory markers such as CRP and hsCRP is also included (59). Statins block CRP production by a variety of mechanisms (60). On the one hand, statins suppress CRP production by reducing IL-6, which is involved in stimulating CRP production by liver cells. On the other hand, statins reduce the production of inflammatory mediators from atherosclerotic plaques due

to the decrease in LDL-C and consequently oxLDL-C (59, 61). Moreover, a direct interaction between statin molecules and CRP was found *in silico* evidence (62). Clinical trials also tried to confirm that the effects of statins on lowering CRP/hsCRP levels were beneficial to the prognosis of coronary plaque volume. For instance, an intervention trial evaluating rosuvastatin revealed that rosuvastatin reduced hs-CRP levels by 37% and hs-CRP are indicators of successful treatment with statins (63).

Despite the large body of evidence associating CRP with atherosclerotic lesions in previous studies, there is a lack of a direct correlation between its concentration and the extension of atherosclerosis as determined by imaging techniques (8). Our study indicates that the anti-inflammatory effects of statins may have a positive effect on atherosclerotic plaque regression as measured by the IVUS technique. This result suggests that CRP/hsCRP may be a potential therapeutic target in the process of atherosclerosis during statin therapy. Therefore, future research should continue to further study the effect of statin therapy on anti-inflammatory, including reducing serum CRP/hsCRP levels directly.

This study also has some limitations. First of all, we only searched 3 commonly used databases. It is possible that some studies in other databases and gray literature are overlooked. However, given that PubMed, EMBASE, and the Cochrane library are three most common databases used for meta-analysis and systematic review, our results should be a representative sample (64–66). Second, although the studies included in this meta-analysis were all RCTs and the quality of evidence was relatively higher, not all studies were double-blind trials. It is possible that performance bias is introduced. The meta-regression analysis (SMD in change of PAV as the dependent variable) was performed with 7 trials, which might lead to insufficient statistical power. In addition, this research adopted aggregate study-level data rather than individual-patient-level data. Individual-patient-level data may reflect the actual allocation plan of the subjects and improve the accuracy and integrity of the data. If future research could establish regression model based on individual-patient-level data to analyze the relationship between CRP/hsCRP levels and plaque regression, our research results could be further verified.

## Conclusion

In conclusion, our mete-analysis indicated that statins could significantly reduce plaque load measured by TAV/PV and PAV. Further meta-regression revealed that the percent change of CRP/hsCRP was significantly associated with the reduction in plaque volume. However, the percent change of LDL-C was not significantly associated with TAV/PV change or PAV change. Our results support that CRP/hsCRP decrease is crucial in the reduction of TAV/PV during statin treatment. Statins could promote plaque regression through their anti-inflammatory ability and that their ability to reduce plaque volume might be unaffected by their ability to reduce LDL-C. This finding will provide new avenues for future research on plaque regression.

## Data availability statement

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

## Author contributions

RH, YM, and WX conceived and designed the study. RH, DJ, YM, CL, and SW performed the statistical analysis. DG, RH, and WX drafted and revised the manuscript. WX and QM were responsible for the integrity of the work as a whole. 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/fcvm.2022.989527/full#supplementary-material

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# Triglyceride-glucose (TyG) index is a predictor of arterial stiffness, incidence of diabetes, cardiovascular disease, and all-cause and cardiovascular mortality: A longitudinal two-cohort analysis

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**Background:** Triglyceride-glucose (TyG) index is a useful low-cost marker of insulin resistance. We aimed to evaluate the association between TyG index and arterial stiffness, incidence of diabetes, adverse cardiovascular outcomes, and all-cause and cardiovascular mortality in two large prospective Swedish cohorts, the Malmö Diet and Cancer Study-Cardiovascular Cohort (MDCS-CV) and the Malmö Preventive Project (MPP).

**Methods:** Association between baseline TyG index and arterial stiffness, measured by carotid femoral pulse wave velocity (c-f PWV), was assessed using linear regression and general linear models, adjusting for covariates. Cox proportional hazard regression was used to assess the association between TyG index and incidence of diabetes, coronary events (CE), stroke, atrial fibrillation (AF), heart failure, and all-cause and cardiovascular mortality.

**Results:** After multivariable adjustment, baseline TyG index was significantly associated with increased arterial stiffness (β for c-f PWV = 0.61, $p$ = 0.018). Participants in the highest quartile of TyG index vs. lowest quartile had an increased incidence of diabetes (HR: 3.30, 95% CI: 2.47–4.41), CE (HR: 1.53, 95% CI: 1.41–1.68), stroke (HR: 1.30, 95% CI: 1.18–1.44), all-cause mortality (HR: 1.22, 95% CI: 1.16–1.28), and cardiovascular mortality (HR: 1.37, 95% CI: 1.26–1.49) after adjustment for covariates. Per unit increase in TyG index was

associated with increased heart failure risk. No significant association was observed for incident AF.

**Conclusion:** Elevated TyG index is positively associated with increased arterial stiffness and increased incidence of diabetes, CE, stroke, and all-cause and cardiovascular mortality. The results suggest that TyG index can potentially be useful in the identification of those at increased long-term risk of adverse health outcomes.

KEYWORDS

**arterial stiffness, cardiovascular disease, cardiovascular mortality, diabetes, insulin resistance, mortality, triglyceride-glucose index**

# Introduction

Hyperglycemia is known to be a main driver for the development of diabetes and cardiovascular diseases (CVD). Insulin is closely related to glucose and lipid metabolism. Insulin resistance (IR), reflecting the insensitivity state of the peripheral tissue toward insulin, leads to defective glucose uptake, decreased glycogenesis and dyslipidemia (1). IR has been shown to have a close relationship with adverse cardiovascular and metabolic outcomes (1, 2). Thus, identifying those with IR could be of value for early risk stratification and clinical management.

Triglyceride-glucose index (TyG) is a surrogate marker for IR and is readily calculated using fasting measurements of plasma triglyceride and glucose, which are routine clinical lab investigations. TyG index has shown to be correlated with euglycemic-hyperinsulinemic clamp test, which is considered the gold standard for determining insulin sensitivity (3), but it is a costly and invasive method requiring trained staff. Another frequently used approach to determine IR is using the homeostasis model assessment of insulin resistance (HOMA-IR), which requires insulin levels for calculation. However, as insulin levels are not routinely measured in the clinical setting, the wider application of this measure outside research settings is limited. TyG index has been shown to correlate with HOMA-IR (4) and is therefore a useful surrogate tool to identify those with IR.

Triglyceride-glucose index has shown to have a close association with a range of adverse health risk factors such as obesity and hypertriglyceridemia (5–7), indicating its potential impact on cardiometabolic health. Moreover, the role of TyG index in CVD has been explored in those with diabetes but not widely so in the general population. We aimed to investigate whether TyG index is associated with increased risk of diabetes, arterial stiffness, and CVD including coronary events (CE), stroke, atrial fibrillation (AF), heart failure, and all-cause as well as cardiovascular mortality in the general population.

# Materials and methods

## Study population

Data for this analysis was used from the Malmö Diet and Cancer Study-Cardiovascular Cohort (MDCS-CV) and the Malmö Preventive Project (MPP), two prospective population-based cohort studies from an urban area in the south of Sweden.

The MDCS study is a large prospective cohort, comprising of men and women from the city of Malmö, Sweden (8). From this cohort, a random sample of participants were re-invited between November 1991 and February 1994 to investigate the epidemiology of carotid artery abnormalities. This sub-cohort was called the MDCS-CV and comprised of 6,103 participants (2,572 men and 3,531 women) (9). Between May 2007 and January 2012, a re-examination of participants from the sub-cohort was carried out. Follow-up data from 3,734 participants (76% participation rate) who attended the re-examination was attained (10). Measurements of carotid femoral pulse wave velocity (c-f PWV) was carried out during the re-examination and data was available for 3,056 participants (10). Participants with missing data on triglycerides, glucose and key covariates ($n$ = 359) were excluded, resulting in a final study population of 2,697 subjects.

For analysis for incident diabetes, we excluded participants with, use of antidiabetic medication, self-reported diabetes, or with fasting blood glucose $\geq$6.1 mmol/L [corresponding to a fasting plasma glucose cut-off of 7.0 mmol/L indicating a diagnosis of diabetes ($n$ = 558)]. Participants with missing values of triglycerides and glucose ($n$ = 569) and for other covariates ($n$ = 405) were also excluded. The final study population was 4,571.

The study population for the analyses in MDCS-CV is shown in **Supplementary Figure 1**.

The MPP consisted of 22,444 men and 10,902 women (33,346 participants in total) who were recruited by pre-specified birth year groups from Malmö city to

take part in a health examination. Participants with missing data on triglycerides and glucose ($n$ = 225) and other key covariates ($n$ = 161) were excluded. We also excluded cases of prevalent cardiovascular disease, i.e., CE, stroke, AF, and heart failure, for each of their respective analysis. The study population flow chart for the analyses in MPP is illustrated in **Supplementary Figure 2**.

The Regional Ethics Review Board in Lund approved the study (LU 51-90, LU 532-2006, LU 85-2004, and LU 2011-412) and the participants provided written informed consent in MDCS-CV and verbal informed consent in MPP. The study was conducted in accordance with the Helsinki Declaration.

## Baseline examinations

### MDCS-CV

The baseline examinations comprised of physical examination, blood sample collection, and a self-administered questionnaire. Information about smoking habits, leisure-time physical activity, and use of antihypertensive medication was collected from the questionnaire. A total physical activity score was calculated by multiplying the duration of specific activities by the corresponding intensity coefficient. Smoking status was categorized into three groups: former smokers, non-smokers, and current smokers. Blood pressure (mmHg) was measured once, after 10 min of rest, while the subject was in supine position using a mercury-column sphygmomanometer. Waist circumference (cm) was determined midway between the lowest rib margin and the iliac crest. Height (cm) was measured by using a fixed stadiometer. A calibrated balance-beam scale was used to measure weight (kg), with the participants wearing light clothing and no shoes. BMI was calculated as kg/m$^2$. Blood glucose (mmol/L), triglycerides (mmol/L) and high-density lipoprotein cholesterol (HDL-C; mmol/L) were determined from fasting blood samples, using standardized procedures at the Department of Clinical Chemistry, Skåne University Hospital. Low-density lipoprotein cholesterol (LDL-C; mmol/L) was calculated using Friedewald's formula. Insulin (mIU/L) was analyzed by using radioimmunoassay. C-reactive protein (CRP; mg/L) was determined with a Tina-quant CRP latex assay (Roche Diagnostics, Basel, Switzerland) (10). HOMA2-IR was calculated with the use of a HOMA2-IR calculator (11).

### MPP

Blood samples were taken after an overnight fast and were analyzed using standard procedures at the Department of Clinical Chemistry, Malmö University Hospital to determine triglycerides, cholesterol, and glucose. Fasting blood glucose was analyzed using two methods during the different study times: the glucose-oxidase method (1974–1977) or the hexokinase-oxidase method (1977–1992). As the two methods provide similar results, no conversion factor was used. Height (m) was measured standing without shoes using a fixed stadiometer. Weight (kg) was measured on a balance beam scale with light indoor clothing. BMI was calculated as kg/m$^2$. Blood pressure (mmHg) was measured twice after 10 min rest in the supine position using a sphygmomanometer. Information about smoking status, anti-hypertensive medication, alcohol intake and physical activity was gathered from a self-administered questionnaire. Smoking status was categorized into two categories; current smokers, and non-smokers and ex-smokers. Prevalent diabetes information was retrieved using self-reported diabetes at baseline, prior diagnosis of diabetes in hospital or other registers, or fasting whole blood glucose $\geq$6.1 mmol/L at baseline (corresponding to a plasma glucose of $\geq$7.0 mmol/L) (10).

### TyG index

For analysis in both cohorts, TyG index was calculated using the following formula (5, 12): Ln(fasting triglycerides (mg per dl) $\times$ fasting glucose (mg per dl))/2.

## Endpoint ascertainment

### MDCS-CV

#### Arterial stiffness measurement

Arterial stiffness was measured in 2007–2012 as c-f PWV using an applanation tonometry technique (SphygmoCor, Atcor Medical, Australia) and has been described in detail previously (13). The participants were asked to rest in a supine position for 5 min, after which pulse curves from the carotid and femoral arteries were obtained using a pressure-sensitive probe. The distance was measured from the suprasternal notch to the umbilicus and from the umbilicus to the measuring point at the femoral artery, subtracting the distance between the suprasternal notch and the measuring point at the carotid artery. The time from the peak of the R-wave on the electrocardiogram to the foot of the pulse wave at the carotid and femoral arteries was automatically calculated by using the simultaneously registered electrocardiogram (13). Every participant had a varying number of successful measurements (ranging between one and five). The aim was to achieve three measurements in each (possible in 86.7% of the case subjects). Mean c-f PWV was calculated from these measurements. The formula (2 $\times$ diastolic pressure + systolic pressure) / 3 was used to calculate mean arterial pressure (MAP) (13).

#### Incidence of diabetes

All participants free of diabetes ($n$ = 4,571) were followed from the baseline measurements until first incidence of diabetes,

emigration from Sweden, death, or the end of follow-up (31 December 2020), whichever came first. Both local and national registers were used to identify incident diabetes cases, and have been explained in detail previously (14). Briefly, incident diabetes information was collected from six sources: the Swedish National Diabetes Register, the regional Diabetes 2000 register of the Scania region, the Swedish Inpatient Register, the Swedish Outpatient Register, the Malmö HbA1c register and the nationwide Swedish Drug Prescription Register. New cases of diabetes were diagnosed according to established criteria (fasting plasma glucose concentration ≥7.0 mmol/L resulting from two repeated tests on separate occasions) in the Swedish National Diabetes Register and the Diabetes 2000 register. In the Malmö local HbA1c register, subjects were diagnosed with diabetes if they had at least two HbA1c recordings ≥42 mmol/mol (6.0%), based on the Swedish Mono-S-based standardization system [corresponding to 53 mmol/mol (7.0%), according to the U.S. National Glycohemoglobin Standardization Program]. In the Swedish inpatient and outpatient registers, a senior physician diagnosed diabetes. A filled prescription of insulin or glucose lowering medication (Anatomical Therapeutic Chemical Classification System code A10) was required for a diagnosis of diabetes in the nationwide prescription register (13).

### MPP

#### Incidence of cardiovascular disease and mortality

Participants in MPP were followed from baseline examinations until diagnosis of studied outcome, emigration from Sweden or end of follow-up (31 December 2019), whichever came first. For analysis of each of the cardiovascular outcomes, the respective prevalent cases were excluded. Endpoints were ascertained by data linkages to local and national registers. The studied outcomes were incident CE (ICD-9 codes: 410–414), incident stroke (ICD-9 codes: 430, 431, 434, 436 and 23 unknown cases), incident AF (ICD-9 codes: 427D), and incident heart failure (ICD-9 codes: 428, ICD-10 codes: 428 and I11.0). Information about all-cause and cardiovascular mortality was retrieved from the national Swedish cause of death register. Cardiovascular mortality as underlying cause of death was defined as ICD-9 codes: 390–459 or ICD-10 codes: C00-D48.

## Statistical analysis

C-reactive protein and HOMA-IR in the MDCS-CV were naturally log-transformed due to their skewed distribution. Descriptive data for the study population was reported as means ± SD, median (25th–75th percentiles) or proportions (percentage), as appropriate. The differences across the baseline characteristics were tested using $\chi^2$ for categorical variables and Analysis of Variance (ANOVA) for continuous variables.

Quartiles of TyG index were created with the lowest quartile (Q1) as the reference category. Linear regression and univariate general linear models were used to explore the association between TyG index and c-f PWV as the dependent variable. Cox proportional hazard regression was used to estimate the hazard ratios (HRs) with corresponding 95% confidence intervals (CIs) to assess the association across the quartiles of TyG index and incidence of diabetes, CE, stroke, AF, heart failure, and all-cause and cardiovascular mortality. Time to follow-up was used as time scale. Proportional hazard assumptions were tested by incorporating the time-dependent effects of covariates. Some deviation from assumption was observed in case of incident CE, stroke and heart failure. The analyses were, therefore, conducted again for follow-up time intervals before and after the median. However, this showed that the effect of this deviation on HRs was minor. Adjustments were done for potential confounders, which were selected based on factors in the literature that are known to affect the association between TyG index and the various health outcomes. For incident diabetes, three incremental models were fitted: *Model 1* was adjusted for age and sex. *Model 2* was further adjusted for waist, systolic blood pressure, HDL-C, LDL-C, smoking status, anti-hypertensive medication, and lipid-lowering medication. Finally, *Model 3* was additionally adjusted for CRP. In the analysis for c-f PWV, additional adjustments for age at the time of measurement of c-f PWV, MAP, heart rate, and prevalent diabetes were carried out.

For the analysis in MPP, the multivariable analyses included adjustments for age, sex, BMI, smoking status, total cholesterol, diabetes, anti-hypertensive medication, physical activity, and alcohol habits in a series of models. Kaplan–Meier curves were used to plot the incidence of all health outcomes across the quartiles of TyG index.

We also conducted several sensitivity analysis. Information for insulin and HOMA-IR was available for the MDCS-CV cohort. In a sensitivity analysis, we repeated the analyses using HOMA-IR in place of TyG index as a surrogate marker for IR to test the association with arterial stiffness. As obesity is closely related to diabetes and IR, we explored the associations stratified for BMI to test the predictive ability of TyG index in non-obese participants as well. BMI cut-off values recommended by WHO were used to categorize participants into three categories, i.e., normal weight (BMI <25), overweight (BMI ≥25 to <30), and obese (BMI ≥30 kg/m$^2$) (15). We also examined the association by testing for interaction for age, sex, and BMI. Furthermore, in an exploratory analysis, the study population was stratified for age, sex and BMI for all health outcomes. We also calculated area under the curve (AUC) for TyG index in relation to CVD mortality, all-cause mortality and incident diabetes.

All analyses were performed using SPSS version 27 (IBM Corp., Armonk, NY, USA). A two-tailed $p$-value of <0.05 was regarded as statistically significant.

# Results

## Baseline characteristics

The baseline characteristics of the participants in the MDCS-CV and MPP are presented in **Supplementary Tables 1, 2**, respectively. The mean age of the MDCS-CV and the MPP cohorts at baseline were 58 and 46 years, respectively. In MPP, participants with higher TyG index were more likely to be men, had higher systolic blood pressure, less physically active, more likely to be smokers, and had a higher BMI compared with other participants.

## Arterial stiffness

The mean follow-up time from baseline examinations to the c-f PWV measurement was 16.9 ± 1.5 years. There was a significant association between baseline TyG index and c-f PWV ($\beta = 0.61$, $p = 0.018$), after adjustments in the final *Model 3* (**Supplementary Table 3**). When the quartiles of TyG index were compared, c-f PWV was significantly higher for the participants in the fourth quartile vs. first quartile (11.0 vs. 10.93 m/s) ($p < 0.001$) in *Model 1*. The association remained significant after adjustment for potential confounders as shown in **Table 1**. In an additional sensitivity model, when further adjustments were done for physical activity, the results remained essentially unchanged (results not shown). When the analyses was conducted using HOMA-IR in place of TyG index, no significant association was observed (results not shown).

## Incidence of diabetes

During a mean follow-up time of 21.2 ± 7.4 years in the MDCS-CV, there were 754 cases of incident diabetes. HRs for incident diabetes are presented in **Table 2**. After all the adjustments in *Model 3*, higher TyG index was associated with a significantly higher risk of diabetes incidence (HR: 3.30, 95% CI: 2.47–4.41) ($p$ for trend <0.001). The HR for incident diabetes per unit increase in TyG index was 5.53 (95% CI: 3.78–8.09) in the Model 3. The results remained changed when further adjustments were done for physical activity (results not shown). There was no significant interaction with age and BMI but significant interactions with sex were observed. In the analysis stratified by BMI, increasing TyG index quartiles were significantly associated with greater risk of incidence of diabetes across all BMI categories. Overall, HR for incident diabetes for participants per unit increase in TyG index was 7.13 (95% CI: 3.75–13.58) for normal weight, 4.54 (95% CI: 2.55–8.08) for over-weight, and 5.39 (95% CI: 2.28–12.72) for obese participants. The age, sex, and BMI stratified HR are presented in **Figure 1**. The Kaplan–Meier estimates for incident diabetes were plotted in **Figure 2**.

TABLE 1 Association between quartiles of TyG-index and c-f PWV ($n$ = 2,697).

| TyG index | Q1 | Q2 | Q3 | Q4 | $p$ for trend |
|---|---|---|---|---|---|
| Participants, $n$ | 675 | 672 | 675 | 675 | |
| Model 1 | 10.93 | 10.43** | 10.45** | 11.00*** | < 0.001 |
| Model 2 | 10.27 | 10.53* | 10.45 | 10.74*** | 0.001 |
| Model 3 | 10.33 | 10.55* | 10.43 | 10.67* | 0.039 |
| Model 4 | 10.33 | 10.55* | 10.42 | 10.68* | 0.030 |

Model 1: Adjusted for sex, MAP, heart rate and age at follow-up, and age at baseline. Model 2: Adjusted for sex, MAP, heart rate and age at follow-up, baseline age, smoking habits, systolic blood pressure, waist circumference, diabetes, use of anti-hypertensive medication, and use of lipid-lowering medication. Model 3: Adjusted for sex, MAP, heart rate and age at follow-up, baseline age, smoking habits, systolic blood pressure, waist circumference, diabetes, use of anti-hypertensive medication, use of lipid-lowering medication, HDL, and LDL. Model 4: Model 3 + CRP.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

## Incidence of CVD

The results of the Cox proportional hazard regression to assess the association between TyG index and various cardiovascular outcomes are reported in **Table 3**. Significant association was observed between TyG index and incident CE and stroke in the highest quartile and per unit increase in TyG index, and with heart failure for per unit increase in TyG index after adjusting for potential confounders. No association was observed with AF. Compared to the reference category (Q1), HRs (95% CI) for incident CE and stroke for individuals in the fourth quartile of TyG index were 1.53 (1.41–1.68) and 1.30 (1.18–1.44), respectively, after adjustments in the final *Model 3* (**Table 3**). A significant interaction was observed between sex and TyG index for incident CE. No significant interaction was observed between age, sex, BMI, and TyG index in relation to stroke or heart failure. Kaplan–Meier curves for incident CVD outcomes are shown in **Figure 2**. Stratified results for the CVD outcomes are presented in **Figure 3**.

## All-cause and cardiovascular mortality

Triglyceride-glucose index was significantly associated with all-cause and cardiovascular mortality. After multivariable adjustment, the HR for all-cause mortality for the participants in the fourth quartile (Q4) of TyG index vs. Q1 was 1.22 (95% CI: 1.16–1.28) (**Table 3**). Per unit increase in TyG index, the HR was 1.42 (1.34–1.52) for the final multivariable adjusted model. For cardiovascular mortality, the risk was significantly increased with higher TyG index (HR for Q4 vs. Q1: 1.37, 95% CI: 1.26–1.49). Significant interactions were observed for sex and BMI, but not for age for all-cause

TABLE 2 Incidence of diabetes in relation to quartiles of TyG index in MDC-CC ($n$ = 4,571, incident diabetes, $n$ = 754).

| TyG index | Q1 | Q2 | Q3 | Q4 | $p$ for trend | Per unit |
|---|---|---|---|---|---|---|
| Participants, $n$ | 1,147 | 1,140 | 1,140 | 1,144 | | |
| Incidence of diabetes, $n$ (per 1,000 person-years) | 75 (2.67) | 149 (5.75) | 215 (8.68) | 315 (13.84) | | |
| Model 1 | 1 | 2.19 (1.66–2.90)*** | 3.36 (2.58–4.38)*** | 5.47 (4.23–7.08)*** | <0.001 | 12.18 (8.97–16.55)*** |
| Model 2 | 1 | 1.85 (1.39–2.45)*** | 2.44 (1.84–3.22)*** | 3.27 (2.44–4.37)*** | <0.001 | 5.36 (3.66–7.84)*** |
| Model 3 | 1 | 1.86 (1.40–2.46)*** | 2.43 (1.84–3.21)*** | 3.30 (2.47–4.41)*** | <0.001 | 5.53 (3.78–8.09)*** |

Model 1: Age and sex. Model 2: Age, sex smoking status, waist, antihypertensive medication, lipid-lowering medication, HDL, LDL, and systolic blood pressure. Model 3: Model 2 + CRP. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

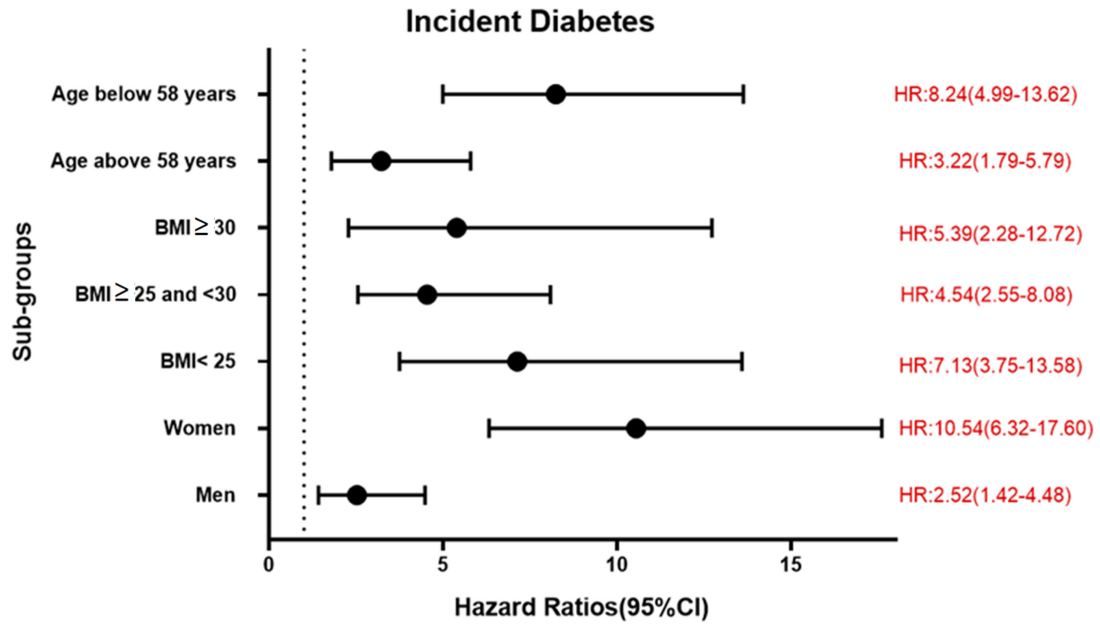


FIGURE 1
Association between per unit increase of TyG index and incident diabetes in different sub-groups in MDCS-CV.

mortality, and only with BMI for cardiovascular mortality. When stratified for BMI categories, mean age and gender, the results remained significant for both men and women and across all categories of BMI and age after adjustments. Similar results were observed for cardiovascular mortality (**Figure 3**). **Figure 2** shows Kaplan–Meier curves for all-cause and cardiovascular mortality.

The AUC of TyG index for CVD mortality, all-cause mortality, and incident diabetes was 0.59, 0.59, and 0.71, respectively (all $p < 0.001$).

## Discussion

In this large population-based study based on two cohorts, TyG index was an independent predictor for increased arterial stiffness, incident diabetes, CE, stroke, and heart failure. We also found that TyG index was associated with increased risk of all-cause and cardiovascular mortality in the general population. There was, however, no significant association with incident AF.

Although diabetes is a major risk factor for CVD, disturbances in metabolism in advance of diabetes may have a role to promote CVD. Triglycerides have been shown to be associated with arterial stiffness (15). Arterial stiffness has also shown to be elevated in individuals with metabolic syndrome (16). Few studies have looked at the association between TyG index and arterial stiffness, when for example brachial-ankle PWV (baPWV) has been used as a measure of arterial stiffness (17). Yan et al. assessed the longitudinal relationship between TyG index and baPWV but were limited by the size of the study population (18). The results of our study show that IR may lead to higher c-f PWV in the general population. In a cross-sectional study, Wang et al. (19) found an association between TyG index and baPWV in patients with type 2 diabetes,

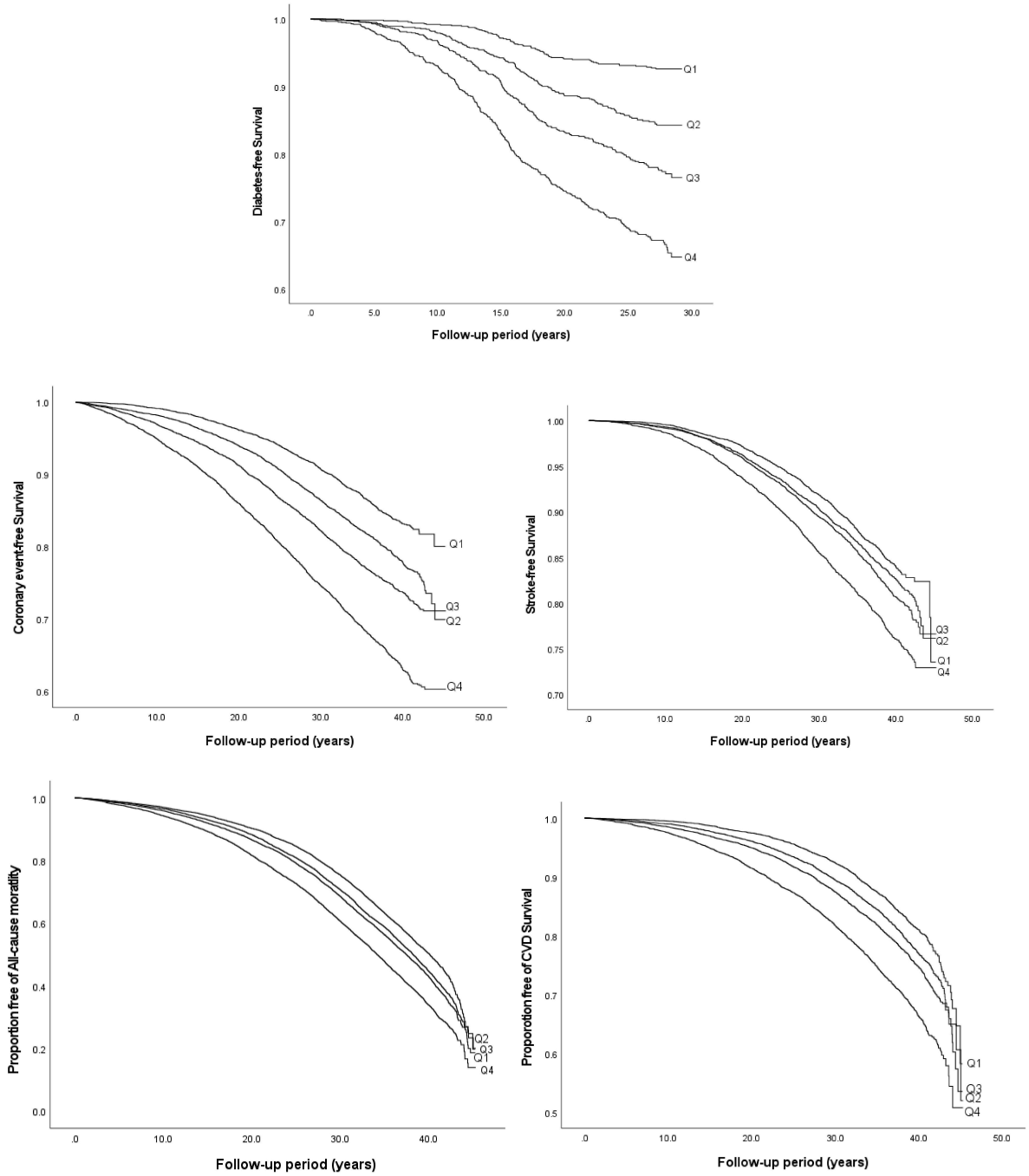


FIGURE 2
Kaplan–Meier curves of incident diabetes, CVD, all-cause, and cardiovascular mortality by quartiles of TyG index.

but not with HOMA-IR. Similarly, we found TyG index to be associated with arterial stiffness after follow-up whereas no significant association was observed between HOMA-IR and arterial stiffness. Although vascular aging is a natural phenomenon, the process is accelerated with increasing age, obesity and in the presence of diabetes as well as IR. One mechanism linked with increased arterial stiffness in an IR state is endothelial cell dysfunction and vascular smooth muscle cell stiffness (20), of which hyperglycemia is a key driving factor. As arterial stiffness is an established determinant of cardiovascular events and all-cause mortality (21), association of TyG index would be of interest to improve risk stratification. In our study, the difference in c-f PWV between the fourth quartile vs. first quartile of TyG index, though statistically significant,

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TABLE 3 Incidence of CVD events and all-cause as well as CV mortality in relation to quartiles of TyG index in MPP.

| TyG index | Q1 | Q2 | Q3 | Q4 | *p* for trend | Per unit |
|---|---|---|---|---|---|---|
| **Cardiac events (6,402/32,838)** | | | | | | |
| Participants, *n* | 8,224 | 8,198 | 8,205 | 8,211 | | |
| Incidence of CE, *n* (per 1,000 person-years) | 988 (3.80) | 1,379 (5.43) | 1,717 (6.90) | 2,318 (10.07) | | |
| Model 1 | 1 | 1.34 (1.24–1.46)*** | 1.68 (1.55–1.82)*** | 2.40 (2.22–2.59)*** | <0.001 | 3.25 (2.99–3.54)*** |
| Model 2 | 1 | 1.18 (1.09–1.28)*** | 1.34 (1.24–1.46)*** | 1.55 (1.42–1.68)*** | <0.001 | 1.73 (1.56–1.91)*** |
| Model 3 | 1 | 1.17 (1.08–1.27)*** | 1.33 (1.23–1.44)*** | 1.53 (1.41–1.68)*** | <0.001 | 1.72 (1.55–1.90)*** |
| **Stroke (4,385/32,920)** | | | | | | |
| Participants, *n* | 8,228 | 8,240 | 8,211 | 8,241 | | |
| Incidence of stroke, *n* (per 1,000 person-years) | 909 (3.50) | 1,026 (4.01) | 1,114 (4.41) | 1,336 (5.61) | | |
| Model 1 | 1 | 1.14 (1.04–1.25)*** | 1.27 (1.16–1.39)*** | 1.65 (1.52–1.80)*** | <0.001 | 2.12 (1.90–2.36)*** |
| Model 2 | 1 | 1.07 (0.98–1.17) | 1.13 (1.03–1.24)** | 1.31 (1.19–1.44)*** | <0.001 | 1.53 (1.35–1.74)*** |
| Model 3 | 1 | 1.07 (0.98–1.17) | 1.13 (1.03–1.24)** | 1.30 (1.18–1.44)*** | <0.001 | 1.52 (1.33–1.72)*** |
| **Atrial fibrillation (6,950/32,917)** | | | | | | |
| Participants, *n* | 8,221 | 8,232 | 8,232 | 8,214 | | |
| Incidence of AF, *n* (per 1,000 person-years) | 1,613 (6.34) | 1,766 (7.02) | 1,753 (7.01) | 1,813 (7.68) | | |
| Model 1 | 1 | 1.10 (1.03–1.18)** | 1.11 (1.04–1.19)** | 1.26 (1.18–1.35)*** | <0.001 | 1.45 (1.32–1.58)*** |
| Model 2 | 1 | 1.01 (0.94–1.08) | 0.95 (0.89–1.02) | 0.95 (0.88–1.02) | 0.063 | 0.97 (0.88–1.08) |
| Model 3 | 1 | 1.02 (0.95–1.09) | 0.97 (0.90–1.04) | 0.96 (0.89–1.04) | 0.142 | 0.99 (0.89–1.11) |
| **Heart failure (2,105/32,831)** | | | | | | |
| Participants, *n* | 8,223 | 8,197 | 8,203 | 8,208 | | |
| Incidence of heart failure, *n* (per 1,000 person-years) | 425 (1.65) | 503 (2.00) | 521 (2.11) | 656 (2.88) | | |
| Model 1 | 1 | 1.22 (1.16–1.51)** | 1.32 (1.16–1.51)*** | 1.92 (1.69–2.17)*** | <0.001 | 2.74 (2.35–3.20)*** |
| Model 2 | 1 | 1.04 (0.92–1.19) | 1.01 (0.88–1.16) | 1.14 (0.99–1.31) | 0.112 | 1.33 (1.11–1.60)** |
| Model 3 | 1 | 1.04 (0.91–1.18) | 1.00 (0.88–1.15) | 1.12 (0.97–1.29) | 0.166 | 1.30 (1.08–1.56)** |
| **All-cause mortality (17,916/32,960)** | | | | | | |
| Participants, *n* | 8,236 | 8,248 | 8,250 | 8,226 | | |
| Incidence of all-cause mortality, *n* (per 1,000 person-years) | 3,612 (13.59) | 4,321 (16.37) | 4,629 (17.62) | 5,354 (21.61) | | |
| Model 1 | 1 | 1.18 (1.13–1.24)*** | 1.28 (1.22–1.34)*** | 1.61 (1.54–1.68)*** | <0.001 | 2.07 (1.96–2.19*** |
| Model 2 | 1 | 1.09 (1.04–1.14)*** | 1.12 (1.07–1.18)*** | 1.25 (1.19–1.31)*** | <0.001 | 1.47 (1.38–1.57)*** |
| Model 3 | 1 | 1.08 (1.04–1.13)*** | 1.12 (1.07–1.17)*** | 1.22 (1.16–1.28)*** | <0.001 | 1.42 (1.34–1.52)*** |
| **Cardiovascular (CV) mortality (6,160/32,960)** | | | | | | |
| Participants, *n* | 8,236 | 8,248 | 8,250 | 8,226 | | |
| Incidence of CV mortality, *n* (per 1,000 person-years) | 1,077 (4.05) | 1,390 (5.27) | 1,610 (6.13) | 2,083 (8.40) | | |
| Model 1 | 1 | 1.25 (1.15–1.35)*** | 1.44 (1.33–1.56)*** | 2.00 (1.86–2.16)*** | <0.001 | 2.85 (2.61–3.11)*** |
| Model 2 | 1 | 1.12 (1.03–1.21)*** | 1.21 (1.12–1.31)*** | 1.40 (1.29–1.52)*** | <0.001 | 1.74 (1.57–1.92)*** |
| Model 3 | 1 | 1.11 (1.02–1.20)*** | 1.19 (1.10–1.29)*** | 1.37 (1.26–1.49)*** | <0.001 | 1.68 (1.52–1.86)*** |

Model 1: Age and sex. Model 2: Age, sex, BMI, systolic blood pressure, cholesterol, smoking status, diabetes, and antihypertensive medication. Model 3: Model 2 + physical activity and alcohol.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

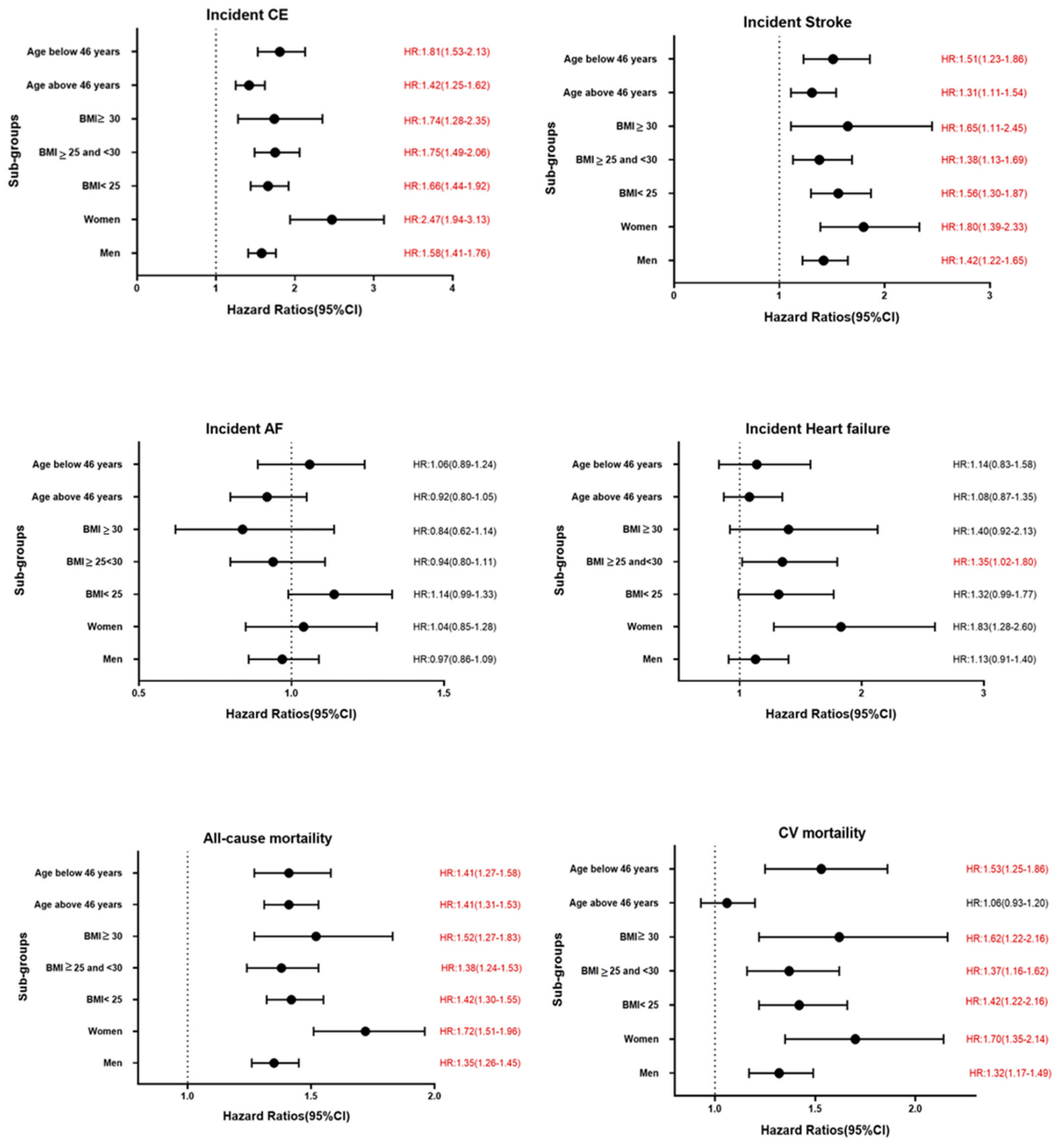


FIGURE 3
Association between per unit increase of TyG index and various CVD outcomes in different sub-groups in MPP. Significant results are presented in red.

was marginal. However, it shows how the high risk in those with high TyG index develops over time, and hence may be of clinical relevance.

Insulin resistance is a key pathological feature of type 2 diabetes. It is closely related to obesity and precedes the development of diabetes (22). The results of our study show that TyG index was associated with a higher risk of incident diabetes, even among normal weight individuals. Hence, the TyG index can be a useful predictor for incident diabetes even in non-obese individuals. This confirms the findings of a recent meta-analysis that demonstrated an association of TyG index with risk of future diabetes (23). Pancreatic β-cell dysfunction is another pathological feature of type 2 diabetes. There also exists a complex relation between IR and β-cell dysfunction (24). In brief, IR and β-cell dysfunction are pivotal characteristics of future type 2 diabetes. Thus, TyG index, a surrogate marker for IR, could be of considerable clinical use to identify those at risk of developing type 2 diabetes. Moreover, obesity is a major

driving feature of IR, but stratifying the study population by BMI showed that TyG index retained predictive value across all strata, and therefore, is independent of obesity.

Epidemiological data has shown that TyG index is associated with CVD in high-risk populations. Previously it was observed that IR is associated with adverse cardiovascular events in patients with coronary artery disease (25), hypertensive patients (26), or non-ST elevation acute coronary syndrome (27). Our results are in line with previous studies, which demonstrated association of TyG index with adverse cardiovascular events (11, 28). A recent meta-analysis reported an independent association between higher TyG index with an increased risk of incidence of atherosclerotic CVD (29). Increased risk for heart failure was found for per unit increase in TyG index. Though association between IR and heart failure is well accepted (30), results in the general population are limited. One study explored relationship between TyG index as a marker for IR and heart failure, but these authors did not observe a significant association (31). However, a recent study from the Atherosclerosis Risk in Communities study (ARIC) showed a significant association between TyG index and incident HF (32).

We did not find any association of TyG index with AF in our study population. Previous studies looking at this association have been carried out in high-risk patient groups. One study showed an association of TyG index with AF in patients undergoing septal myectomy for hypertrophic obstructive cardiomyopathy (33), or in ST-segment elevation myocardial infarction (34). It is possible that comorbidities may have an important role in the observed association in these studies.

Finally, TyG index was significantly associated with all-cause mortality in our study. In a previous study, Liu et al. (35) demonstrated the association of TyG index with all-cause and cardiovascular mortality. Conversely, in a study by Kim et al., the association between TyG index and all-cause mortality was observed for men but not for women (36). A significant association was also observed between TyG index and cardiovascular mortality in our study. This confirms and extends the results of a previous cross-sectional study where association between TyG index and all-cause and cardiovascular mortality was observed (35). The possible mechanism behind this association remains unclear. It can be speculated that TyG index is closely associated with adverse health risk factors such as obesity, hypertension, and diabetes, which can be contributing factors. It might be that TyG index reflects unhealthy risk factors in general that are strongly linked to mortality risk.

There are several strengths of our study. We used two large cohort studies with prospective design and a long follow-up time linked to national registers of high quality. We were able to explore the longitudinal association between TyG index and arterial stiffness using c-f PWV. Incidence of diabetes and CVD were retrieved through linkages to registers that have been validated (37). A few limitations of the study also need to be considered. Residual confounding cannot be ruled out as this is an observational study. However, information was available for important covariates, which allowed adjustments for parameters. Potentially, another limitation is that covariates were measured once at baseline and might have changed over time. However, in that case it would be more likely to bias our study toward the null.

## Conclusion

In conclusion, the findings of this observational study indicate TyG index to be associated with increased risk of incidence of diabetes, CE, stroke, HF, all-cause mortality and cardiovascular mortality, and with elevated arterial stiffness. As TyG index is a measure of IR, our results indicate that IR is implicated in the risk of future cardiometabolic disorders. TyG index can be easily applied as a useful marker for cardiometabolic risk stratification in the general population. As the TyG index is derived from routine clinical lab investigations, our findings highlight the clinical implications of using TyG index as a marker for IR to routinely screen those at high risk for several adverse health outcomes.

## Data availability statement

The data sets presented in this article are not publicly available because they were used under license for the current study. The data sets are however available from the authors upon reasonable request and with permission of Lund University.

## Ethics statement

The studies involving human participants were reviewed and approved by the Regional Ethics Review Board in Lund (LU 51-90, LU 532-2006, LU 85-2004, and LU 2011-412) and the participants provided written informed consent in MDCS-CV and verbal informed consent in MPP. The study was carried out in accordance with the Helsinki Declaration.

## Author contributions

IFM and SZ designed the study and contributed to the final analysis. IFM performed the statistical analysis and drafted the

manuscript. IFM, XB, PN, and SZ contributed to interpretation of the data and critical revision of the manuscript, and reviewed and edited the manuscript. SZ was the guarantor of this work and, as such, had access to all study data and took responsibility for the data integrity and accuracy of the data analysis. All authors approved the final version of the manuscript.

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## Conflict of interest

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

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.1035105/full#supplementary-material

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# Prognostic value of interleukin-34 and interleukin-38 in patients with newly diagnosed atrial fibrillation

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**Background:** Interleukin (IL)-34 and IL-38 are associated with cardiovascular disease (CVD). However, their involvement in atrial fibrillation (AF) and AF-associated adverse events remains uncertain. Therefore, we aimed to investigate their association with various AF prognostic factors in a cohort study and assessed their predictive value for the prognosis of patients with AF.

**Methods:** Patients with new-onset non-valvular AF were consecutively enrolled between 2013 and 2015 at the Department of Cardiovascular Medicine of the Southwest Hospital of the Army Medical University (Third Military Medical University) in Chongqing, China. The endpoints included stroke and all-cause mortality. The baseline levels of plasma IL-34, IL-38, NT-proBNP, high-sensitivity cardiac troponin T (hs-cTnT), and GDF-15 were measured and their correlation with AF-related adverse events were analyzed in a Cox proportional-hazards regression model. The *C*-statistic, net reclassification improvement (NRI), and integrated discrimination improvement (IDI) were used to evaluate the performance of the AF prognostic models. Decision curve analysis (DCA) was used to evaluate the clinical net benefit of the original and modified models.

**Results:** A total of 299 patients with new-onset AF were enrolled. During the median follow-up time of 28 (IQR: 27, 29) months, the higher levels of IL-34 were associated with a lower risk of stroke, and the higher levels of IL-38 were associated with an increased risk of all-cause death (all adjusted $P < 0.05$). In addition, elevated hs-cTnT and NT-proBNP concentrations were associated with a higher risk of stroke and all-cause mortality (all adjusted $P < 0.05$). Furthermore, the $CHA_2DS_2$-VASc score combined with IL-38 and NT-proBNP significantly improved the *C*-statistic, IDI, and NRI (all $P < 0.01$). There was

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no statistically significant difference (all $P > 0.05$) in the discrimination power between the preference models and the ABC (age, biomarkers, and clinical history) score for the two prognostic outcomes.

**Conclusion:** Our results suggested that IL-34 and IL-38 were independently associated with stroke and all-cause mortality in patients with AF. Moreover, adding IL-38 and NT-proBNP to the $CHA_2DS_2$-VASc score significantly improved its predictive ability of AF-related all-cause death. Finally, the preference model performed equally well as the ABC score in predicting AF prognosis.

KEYWORDS

**interleukins, prognosis, CHA2DS2-VASc score, ABC score, atrial fibrillation**

# 1. Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia which has become a major public health concern worldwide, and its prevalence is projected to increase further in the coming years (1). The onset of AF and its development is usually accompanied by many adverse events, which can lead to disability and mortality (2). Therefore, prediction and risk stratification of prognosis is particularly essential for better monitoring and management of AF. Risk stratification patterns recommended by current AF guidelines are mainly based on clinical risk factors, such as the $CHA_2DS_2$-VASc score (3). However, the identification power of $CHA_2DS_2$-VASc score is still limited in estimating the "truly high-risk" subjects, with a *C*-statistic of only 0.6 (4). Multiple studies have found biomarkers carrying rich prognostic information in AF (5–7). Hijazi et al. developed a new biomarker-based model in 2016, the ABC (age, biomarkers, and clinical history) score, which yielded a higher *C*-statistic than the $CHA_2DS_2$-VASc score (8).

Circulating interleukins (ILs) are a class of cytokines involved in information transmission, activation, and inflammatory responses, which have been intensively investigated in cardiovascular disease (CVD) over the last few years (9–11). Among them, IL-34 has been shown associated with CVD risk (12, 13), and IL-38 may be a key regulator of CVD (14). At present, relatively little is known about the role of IL-34 and IL-38 in AF-associated adverse events. In addition, the question remains to be answered whether IL-34 and IL-38 can improve the predictive power of the existing risk stratification scheme ($CHA_2DS_2$-VASc score) for AF-related prognosis.

Therefore, we aimed to explore the associations between ILs and stroke and all-cause death and to evaluate their predictive value in AF outcomes. Furthermore, we compared the prognostic predictive power of $CHA_2DS_2$-VASc score combined with IL-34 or IL-38 to that of the ABC score, to find new prognosis-associated biomarkers of AF and improve prognostic assessment of AF patients.

# 2. Materials and methods

## 2.1. Study design and populations

We conducted a cohort study. Patients aged greater than or equal to 18 years with new-onset AF were consecutively enrolled between December 2013 and August 2015 at the Department of Cardiovascular Medicine of the Southwest Hospital of the Army Medical University (Third Military Medical University) in Chongqing, China. The diagnostic criteria of AF were based on the definition by the 2020 ESC Guidelines (15). Patients were excluded if they met one of the following criteria: moderate-to-severe mitral stenosis, artificial valve replacement, malignant tumors, acute and chronic inflammatory diseases, connective tissue diseases, and/or infections. The study complied with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Southwest Hospital of the Army Medical University. Informed consents have been obtained from all subjects.

## 2.2. The $CHA_2DS_2$-VASc and ABC scores

The $CHA_2DS_2$-VASc score was calculated using clinical information [heart failure, hypertension, diabetes, vascular disease, aged 65–74 years old, female accounted for one point. Age $\geq$ 75 years old, and stroke history/transient ischemic attack (TIA)/thromboembolism history accounted for two points] (3). The ABC-stroke score was calculated according to the study of Hijazi et al. [age, the levels of troponin (Tn) T/I, NT-proBNP,

prior stroke/systemic embolism (SE), and 1-year stroke risk incidence rate of individual] (8). Also, the ABC-death score was calculated according to the study of Hijazi et al. (16) (age, the levels of Tn T/I, NT-proBNP, GDF-15, the history of heart failure, and 1-year mortality risk incidence rate of individual).

## 2.3. Measurement of the levels of biomarkers and interleukins

Fasting blood samples (5 ml) were drawn from all participants (within 48 h after admission and before any treatments). Plasma fractions were obtained immediately by centrifugation at 2,000 rpm/min for 15 min at 4°C and stored in a −80°C freezer until use.

NT-proBNP and high-sensitivity cardiac troponin T (hs-cTnT) levels were measured using an electro-chemiluminescence immunoassay (Cobas e601, Rocha Diagnostics, Manheim, Germany). The detection range of NT-proBNP and hs-cTnT were 5–35,000 pg/ml and 3–10,000 ng/L, respectively. GDF-15 levels were assessed by enzyme-linked immunosorbent assay (ELISA) kit (RayBiotech, Norcross, GA, USA), and the analytical range was 2–800 pg/ml, the inter-assay and intra-assay coefficient of variation were less than 10 and 12%, respectively. Measurements of IL-34 and IL-38 were performed with Bio-plex Pro$^{TM}$ plex xMAP array technology (Bio-Rad Corporation, Hercules, CA, USA) based on Luminex 200 system (Luminex Corporation, Austin, TX, USA), and the concentrations were calculated by fluorescence intensities from the corresponding standard curves, based on Bio-Plex Manager$^{TM}$ 6.1 (Bio-Rad) software. Each sample was tested once. The standard and reference products were used for quality control, all of which showed coefficients of variation of less than 12.1%.

## 2.4. Endpoint events and follow-up

The primary endpoint was stroke (ischemic and hemorrhagic) and the secondary endpoint was all-cause death. The follow-up time for each individual was recorded from the date of entry to the date of death, or the end of the trial. The patient's physical conditions or causes of death were verified annually through electronic medical records, reports of close relatives, and national death registration system.

## 2.5. Statistical analyses

The Shapiro–Wilk test was used to test if continuous variables follow a normal distribution. Continuous variables were presented by mean ± SD or median with interquartile ranges (*IQRs*). Categorical variables were presented as numbers and percentages. The optimal cut-off values of biomarkers were determined by X-tile software (Yale University, New Haven, CT, USA) (17). To avoid multiple cut-off values in this study, we considered stroke and all-cause death as a composite endpoint.

To investigate the associations between biomarkers and AF prognosis, biomarkers were initially examined by univariate Cox proportional-hazard analysis. Then, the variables with $P < 0.10$ in univariate Cox proportional-hazard analysis were incorporated into the multivariate Cox proportional-hazard analysis by the forward and likelihood ratio (LR) method. In addition, we used the same method (forward and LR) for sensitivity analysis to verify the robustness of the above results. Sensitivity analysis was performed with adjustment for the AF-related covariates: warfarin, statins, and angiotensin receptor inhibitors (ARB) and the components of the $CHA_2DS_2$-VASc score. The variance inflation factor (VIF) was used to examine the multicollinearity of the variables.

The value of *C*-index (Harrell's C) (18), integrated discrimination improvement (IDI), and net reclassification improvement (NRI) were calculated to evaluate the predictive power of the models in discrimination and reclassification (19). Decision curve analysis (DCA) as described by Vickers et al. (20) was used to quantify the clinical net benefit of each model and to visually compare models.

A two-sided *p*-value $< 0.05$ was considered statistically significant. All analyses were performed with SPSS statistical software version 25.0 (SPSS Inc., Chicago, IL, USA.) and R software version 4.1.2 (R Project for Statistical Computing, Vienna, Austria).

# 3. Results

## 3.1. Baseline characteristics of study participants

A total of 299 participants underwent this trial (**Supplementary Figure 1**). Among them, 132 (44.15%) were female, the median age was 66 years (*IQR*: 58, 73). 25 (8.36%) patients were lost to follow-up. The demographic and clinical characteristics of the participants at baseline were presented in **Table 1**. During the median follow-up time of 28 (*IQR*: 27, 29) months, we identified 26 strokes (4.12 per 100 person-years) and 30 deaths (4.48 per 100 person-years).

## 3.2. Association of biomarkers with AF prognosis

The optimal cut-off values of biomarkers were determined by the X-tile software based on follow-up time, composite endpoint event (stroke and all-cause death). Optimal cut-off

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TABLE 1 Demographic and clinical data of atrial fibrillation patients at baseline.

| Characteristics | Value ($n$ = 299) |
|---|---|
| **Age (years)** | 66 (58, 73) |
| <65 | 130 (43.48%) |
| 65–74 | 101 (33.78%) |
| ≥75 | 68 (22.74%) |
| **Gender** | |
| Male | 167 (55.85%) |
| Female | 132 (44.15%) |
| **BMI (kg/m$^2$)** | 23.9 (21.8, 26.2) |
| <24.9 | 185 (61.87%) |
| 25.0–29.9 | 92 (30.77%) |
| ≥30.0 | 22 (7.36%) |
| **Education** | |
| Junior middle school or below | 244 (81.61%) |
| High school or above | 55 (18.39%) |
| **The income per head (10,000 yuan/year)** | |
| <2.5 | 148 (49.50%) |
| ≥2.5 | 151 (50.50%) |
| **AF types** | |
| Paroxysmal AF | 100 (33.44%) |
| Chronic AF | 199 (66.56%) |
| Smoking | 100 (33.44%) |
| Alcohol consumption | 94 (31.44%) |
| **History of comorbidities** | |
| Hypertension | 153 (51.17%) |
| Diabetes mellitus | 60 (20.07%) |
| CAD | 124 (41.47%) |
| Cardiomyopathy | 35 (11.71%) |
| HF | 99 (33.11%) |
| TIA or previous stroke | 41 (13.71%) |
| Vascular disease | 18 (6.02%) |
| **Concomitant treatment** | |
| Antiarrhythmic therapy | 146 (48.83%) |
| ACEI | 107 (35.79%) |
| ARB | 29 (9.70%) |
| Beta-blockers | 119 (39.80%) |
| Warfarin | 96 (32.11%) |
| Statins | 144 (48.16%) |
| Ablation | 71 (23.7%) |
| LAAC | 13 (4.3%) |
| **Echocardiography parameters** | |
| LVEF (%) | 59 (50, 64) |
| LA diameter (mm) | 44.00 (40.00, 51.00) |
| **Biomarkers** | |
| NT-proBNP (pg/ml) | 1,083.90 (447.30, 2,154.00) |
| ≤3,580.17 | 255 (85.28%) |
| >3,580.17 | 44 (14.72%) |
| hs-cTnT (ng/ml) | 11.00 (7.00, 25.00) |
| ≤11.00 | 155 (51.84%) |
| >11.00 | 144 (48.16%) |
| GDF-15 (pg/ml) | 1,028.52 (742.12, 1,485.00) |
| ≤1,813.62 | 250 (83.61%) |
| >1,813.62 | 49 (16.39%) |
| **Interleukin** | |
| IL-34 (pg/ml) | 537.03 (13.93, 791.94) |
| ≤138.47 | 90 (30.10%) |
| >138.47 | 209 (69.90%) |
| IL-38 (pg/ml) | 17.75 (6.32, 33.07) |
| ≤58.25 | 268 (89.63%) |
| >58.25 | 31 (10.37%) |

Continuous variables were presented as median with interquartile ranges (IQRs). Categorical variables were presented as numbers and percentages. BMI, body mass index; AF, atrial fibrillation; CAD, coronary artery disease; HF, heart failure; TIA, transient ischemic attack; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin-renin blockers; LAAC, percutaneous left atrial appendage closure; LVEF, left ventricular ejection fraction; LA, left atrium; NT-proBNP, N-terminal fragment B-type natriuretic peptide; hs-cTnT, high-sensitivity cardiac troponin T; GDF, growth differentiation factor; IL, interleukin.

values of IL-34, IL-38, NT-proBNP, hs-cTnT, and GDF-15 were 138.47 pg/ml, 58.25 pg/ml, 3,580.17 pg/ml, 11.00 ng/ml, and 1,813.62 pg/ml, respectively (**Supplementary Table 1**).

We incorporated variables with $P < 0.10$ in univariate Cox regression (**Supplementary Table 2**) into the multivariate Cox regression model. In addition, the treatments (warfarin, statins, and ARB) and the components of the $CHA_2DS_2$-VASc score were further adjusted in the sensitive analysis and no differences were found. The collinearity was ignored in this study as the VIFs of variables were less than 2.0 in all models. We found that IL-34 [hazard ratio (HR): 0.36, 95% confidence interval (CI): 0.17–0.78, $P = 0.010$] and hs-cTnT (HR: 3.09, 95% CI: 1.33–7.19, $P = 0.009$) were independently correlated with the risk of stroke. For all-cause mortality, patients with higher levels of IL-38 (HR: 3.11, 95% CI 1.16–8.29, $P = 0.024$) and NT-proBNP (HR: 2.77, 95% CI 1.13–6.78, $P = 0.025$) had an increased risk of all-cause death (**Table 2**). Although higher GDF-15 level was related to a higher risk of all-cause death in unadjusted analysis, we found that it was not an independent risk factor for AF-related prognosis in the adjusted model.

TABLE 2 Associations between biomarkers concentrations and events during follow-up in atrial fibrillation (AF) patients.

| Endpoint | Biomarkers | Model* | | |
|---|---|---|---|---|
| | | Beta coefficients | Adjusted HR (95% CI) | *P*-value |
| Stroke | IL-34 (>138.47 pg/ml) | −1.019 | 0.36 (0.17−0.78) | **0.010** |
| | hs-cTnT (>11.00 ng/ml) | 1.130 | 3.09 (1.33−7.19) | **0.009** |
| All-cause mortality | IL-38 (>58.25 pg/ml) | 1.133 | 3.11 (1.16−8.29) | **0.024** |
| | NT-proBNP (>3,580.17 pg/ml) | 1.020 | 2.77 (1.13−6.78) | **0.025** |

Bold indicates $P < 0.05$. HR, hazard ratio; CI, confidence interval; ILs, interleukins; NT-proBNP, N-terminal fragment B-type natriuretic peptide; hs-cTnT, high-sensitivity cardiac troponin T. *Cox proportional-hazard model for stroke adjusted for age, diabetes, stroke/TIA/thromboembolism, left atrium (LA), and use of angiotensin-converting enzyme inhibitors (ACEI); for all-cause mortality adjusted for age, body mass index (BMI), types of AF, heart failure, left ventricular ejection fraction (LVEF), LA, hs-cTnT, and growth differentiation factor (GDF)-15.

TABLE 3 Discrimination and reclassification for stroke, all-cause mortality, or cardiovascular death.

| | Discrimination | | | Reclassification | | | |
|---|---|---|---|---|---|---|---|
| | C-statistic (95% CI) | Improvement in C-statistic (95% CI) | *P*-value | IDI (%) (95% CI) | *P*-value | NRI (%) (95% CI) | *P*-value |
| **Stroke** | | | | | | | |
| $CHA_2DS_2$-VASc | 0.71 (0.63, 0.79) | | | | | | |
| $CHA_2DS_2$-VASc + IL-34 | 0.72 (0.64, 0.81) | 0.01[#] (−0.04, 0.05) | 0.533 | 0.2[#] (−0.2, 1.7) | 0.498 | 20.3[#] (−28.7, 68.2) | 0.557 |
| $CHA_2DS_2$-VASc + hs-cTnT | 0.73 (0.66, 0.81) | 0.02[#] (−0.03, 0.07) | 0.303 | 0.3[#] (0.0, 1.8) | 0.070 | 52.6[#] (0.0, 57.9) | **0.010** |
| $CHA_2DS_2$-VASc + IL-34 + hs-cTnT | 0.75 (0.66, 0.83) | 0.04[#] (−0.04, 0.09) | 0.253 | 0.8[#] (0.0, 4.2) | 0.060 | 18.9[#] (−23.5, 69.3) | 0.219 |
| ABC-stroke | 0.73 (0.63, 0.82) | −0.02[a] (−0.10, 0.08) | 0.663 | | | | |
| **All-cause mortality** | | | | | | | |
| $CHA_2DS_2$-VASc | 0.70 (0.63, 0.77) | | | | | | |
| $CHA_2DS_2$-VASc + IL-38 | 0.72 (0.65, 0.80) | 0.02[#] (−0.03, 0.07) | 0.376 | 1.2 (−0.1, 6.4) | 0.080 | 40.2[#] (−20.6, 79.4) | 0.090 |
| $CHA_2DS_2$-VASc + NT-proBNP | 0.78 (0.71, 0.85) | 0.08[#] (0.02, 0.14) | **0.014** | 3.4[#] (0.0, 12.2) | 0.050 | 35.8[#] (−4.2, 72.9) | 0.090 |
| $CHA_2DS_2$-VASc + IL-38 + NT-proBNP | 0.80 (0.73, 0.87) | 0.10[#] (0.03, 0.17) | **0.005** | 3.7[#] (1.1, 13.3) | **<0.001** | 77.6[#] (21.9, 82.7) | **<0.001** |
| ABC-death | 0.83[b] (0.76, 0.89) | 0.02[b] (−0.04, 0.10) | 0.472 | | | | |

Bold indicates $P < 0.05$, the improvement of the C-statistic is statistically significant.
IDI, integrated discrimination improvement; NRI, net reclassification improvement; CI, confidence interval; ILs, interleukins; NT-proBNP, N-terminal fragment B-type natriuretic peptide; hs-cTnT, high-sensitivity cardiac troponin T.
[#]Comparison with $CHA_2DS_2$-VASc; [a]Comparison with $CHA_2DS_2$-VASc + IL-34 + hs-cTnT; [b]Comparison with $CHA_2DS_2$-VASc + IL-38 + NT-proBNP.

## 3.3. Incremental prognostic value of $CHA_2DS_2$-VASc combined with biomarkers for AF

We assessed the predictive capabilities of the $CHA_2DS_2$-VASc model combined with biomarkers (Table 3). For all-cause death risk stratification, the $CHA_2DS_2$-VASc integrated IL-38 and NT-proBNP, achieved a *C*-statistic of 0.80 (95% CI 0.73−0.87), which had a significant improvement over the original model (*C*-statistic: 0.70, 95% CI 0.63−0.77, $P = 0.005$), and the reclassification ability was significantly better (IDI: 3.7%, 95% CI: 1.1−13.3%, $P < 0.001$; NRI: 77.6%, 95% CI: 21.9−82.7%, $P < 0.001$). On the other hand, the $CHA_2DS_2$-VASc score combined with IL-34 and hs-cTnT for stroke risk stratification achieved highest *C*-statistic of 0.75 (95% CI 0.66−0.83), which did not show a significant statistical difference from other scores. The DCA visualized the clinical net benefit of the original and modified $CHA_2DS_2$-VASc score (Figure 1). For predicting stroke, the clinical net benefit of modified $CHA_2DS_2$-VASc score adding IL-34 and hs-cTnT was slightly better than other scores (Figure 1A). For predicting all-cause death, adding IL-38 and NT-proBNP to $CHA_2DS_2$-VASc had the best clinical net benefit when the threshold probability was between 9 and 35% (Figure 1B).

# 4. Discussion

In this study, we first explored the association of IL-34 and IL-38 with stroke and all-cause mortality in patients with new-onset AF and found that low levels of IL-34 were independent predictors of stroke, while high concentrations of IL-38 were

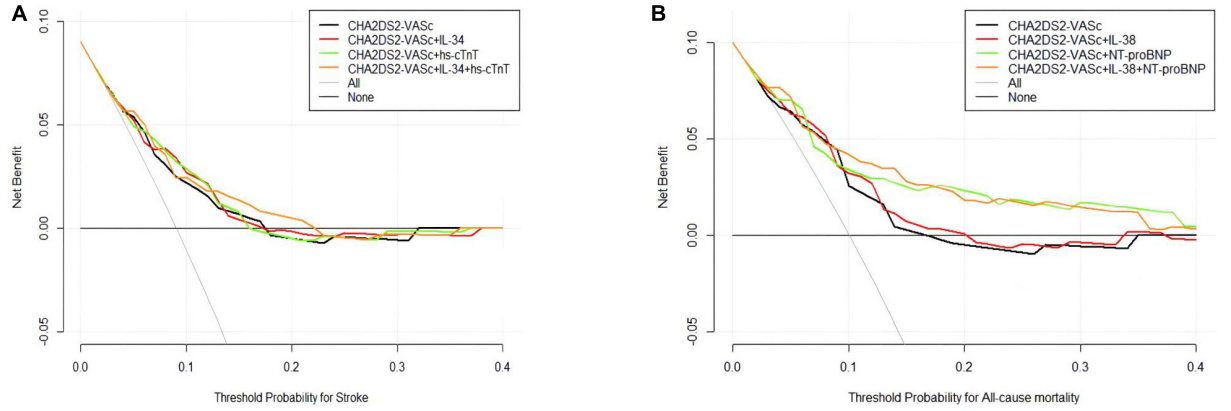


FIGURE 1
Decision curve analysis (DCA) of the original and modified $CHA_2DS_2$-VASc score. **(A)** Stroke model added hs-cTnT and/or IL-34; **(B)** all-cause mortality model added IL-38 and/or NT-proBNP. The black lines (original scores) and other lines (modified scores) represent the net benefit of each model graphically. The horizontal axis represents the threshold probability of the target adverse event risk. The longitudinal axis shows the net benefit. ILs, interleukins; NT-proBNP, N-terminal fragment B-type natriuretic peptide; hs-cTnT, high-sensitivity cardiac troponin.

correlated with all-cause death, which goes beyond earlier findings. In addition, we evaluated whether the addition of IL-34 and IL-38 could improve the predictive performance of the existing prognostic prediction model ($CHA_2DS_2$-VASc score). We showed significant improvement for the all-cause death risk prediction when IL-38 and NT-proBNP were added to the original model ($CHA_2DS_2$-VASc score) Finally, the preferred models in two adverse events predictive models performed equally well as the ABC score.

The pathophysiology of AF is complex and yet to be explored. Several studies have depicted correlations between AF and different inflammatory markers and mediators (21, 22). Tn elevation was initially seen as a sensitive indicator of myocardial damage and infarction. Recent data has shown that cardiac troponin I (cTnI) and T (cTnT) provide important prognostic information in anticoagulated patients with AF for predicting all-cause mortality, cardiac death, stroke or SE (23), which was consistent with our findings that hs-cTnT was independently related to stroke. NT-proBNP, which is degraded by pro-BNP, has been used as a biomarker for heart failure and renal insufficiency. In recent years, its prognostic values have also been applied to AF. The concentrations of NT-proBNP increased during states of hemodynamic stress such as in heart failure, acute coronary syndrome, and arrhythmias including AF (22). In a sub-study of RE-LY, increased concentrations of NT-proBNP were generally associated with the risk of stroke and mortality (24). The same conclusion was obtained in our study. GDF-15 is a member of the transforming growth factor-β (TGF-β) superfamily, which plays an important role as an inflammatory marker in tumor pathogenesis, and ischemic, and metabolic diseases. In addition, it has been widely studied in the field of CVDs. It is not expressed in healthy adult myocardium but significantly expressed in cardiomyocytes, adipocytes, macrophages, endothelial cells, and vascular smooth muscle cells after myocardial lesion (25). In our study, GDF-15 was a risk predictor for mortality on top of clinical characteristics. However, NT-proBNP, hs-TnT, and GDF-15 partly reflected the same processes, which was myocardial dysfunction and cardiovascular comorbidity (7). This might explain why in the adjusted model GDF-15 did not appear to be an independent risk indicator for all-cause death when other biomarkers and clinical characteristics have been taken into account.

Interleukin (IL)-34 as a novel ligand of CSF-1R was defined in 2008 (26), and its biology and function have been broadly studied. In physiologic cases, they perform critical roles in the development of microglia and Langerhans cells (LCs). They also play crucial roles in pathological conditions, such as inflammatory diseases. Over the past few years, its role in CVD has also been investigated and found to be significantly increased in patients with coronary heart disease (CHD), and its increase is positively correlated with the level of high-sensitivity C-reactive protein (hs-CRP), the results of which give evidence for IL-34 as a pro-inflammatory cytokine (27). Moreover, studies have shown that IL-34 may be an important predictor of CVD, heart failure hospitalization, and all-cause mortality in patients with chronic heart failure (CHF) (28). Concurrently, we found that it can be used as an independent predictor for stroke events in AF patients, which may provide a new insight connecting AF to stroke outcomes beyond other biomarkers. However, it was noteworthy that AF patients with lower IL-34 levels have a higher risk of stroke, which seemed counterintuitive to its pro-inflammatory role. On the one hand, the levels of IL-34 were measured only once at the baseline, failing to evaluate the impact of dynamic changes of them on the prognosis. Therefore, the effects of IL-34 level fluctuation on the outcomes of AF

need to be further explored in the future. On the other hand, some previous studies also reported that elevated IL-34 may act as a protective factor. Esaki et al. (29) identified decreased expression of IL-34 in atopic dermatitis (AD) compared to non-lesional AD and normal epidermis. The study by Mizuno et al. (30) demonstrated that *in vitro*, microglia treated with IL-34 attenuated the neurotoxic effects of oligomeric amyloid-β (oAβ), which mediates synaptic dysfunction and neuronal damage in Alzheimer's disease. Moreover, intracerebroventricular administration of IL-34 improves deficits in associative learning. In addition, IL-34 also has a protective role in some cancer, such as non-small cell lung cancer (31), colorectal cancer (32), breast cancer (33, 34), and lung cancer (32), hematologic malignancies (32), and head and neck cancer (33, 34). Therefore, the issue of whether IL-34 is beneficial or harmful in stroke-attacked AF patients merits further study.

Interleukin (IL)-38 (IL-1F10 or IL-1HY2) belongs to IL-36, one of the family members of IL-1. Its primary biological function is to block the activation of the IL-36R signaling pathway and influence the proinflammation function of IL-36, which is similar to IL-36Ra (35). IL-38 is expressed in the thymus, heart, placenta, and fetal liver in healthy conditions (36), whereas in disease it is predominantly expressed in settings in IL-1-driven inflammatory response, such as CVD (37). Previous study indicated that the expression of the IL-38 gene was increased in peripheral blood mononuclear cells (PBMCs) of patients with ST-segment elevation myocardial infarction (STEMI) and had been positively correlated with CRP, cTnI, and NT-proBNP (37).

The $CHA_2DS_2$-VASc score, the main method recommended by current guidelines for predicting stroke outcomes in AF, is derived from the $CHADS_2$ score [congestive heart failure, hypertension, age $\geq$ 75 years, diabetes mellitus, previous stroke (double weight)], and it is better at identifying "truly low risk" ($CHA_2DS_2$-VASc score = 0–1) than the $CHADS_2$ score (38). However, as research continues to unfold, it has been found that there was still room for improvement in terms of predicting the "truly high-risk" patients since the *C*-statistic of the $CHA_2DS_2$-VASc score was only 0.60 (95% CI 0.57−0.64) (3). The discovery that biomarkers provide rich information for AF prognosis has led to new strategies to improve prognosis stratification. Hijazi et al. developed an ABC score based on age, biomarkers and clinical history, which had a *C*-statistic of 0.65 (95% CI: 0.61−0.69) and were well-validated both internally and externally (8). In our findings, the *C*-statistic of stroke prediction model integrated IL-34 and/or hs-cTnT were all superior to the original model, and NRI of the $CHA_2DS_2$-VASc score combined with hs-cTnT had a significantly improved. Furthermore, when NT-proBNP was added to the $CHA_2DS_2$-VASc score for predicting all-cause mortality, the *C*-statistic was significantly improved in the new model. In addition, when IL-38 and NT-proBNP were simultaneously added to the $CHA_2DS_2$-VASc score, the *C*-statistic increased, and NRI, and IDI both showed significant improvements of the new model regarding prognostic prediction value. Finally, we also compared the new model with the largest C-statistic to the ABC score and found that they have comparable discrimination capacity, which indicated that our improvements to the current clinical model were acceptable.

## 5. Limitations

There were some limitations of our study. First, we selected participants from a single center which lacks external validity. Therefore, large-scale prospective validation is required in multicenter studies. Second, our follow-up time was relatively short, and the observation of end-point events was insufficient, so it is still necessary to extend the follow-up time to obtain more accurate results. Finally, further studies are needed on the exact biological mechanism of IL-34 and IL-38 in the pathology of AF.

## 6. Conclusion

In conclusion, serum IL-34 and IL-38 may serve as biomarkers of prognostic evaluation for stroke and all-cause mortality in patients with AF. The addition of IL-38 improved the predictive power of the existing model ($CHA_2DS_2$-VASc score) and increases the net clinical benefit. The new model showed comparable prognostic value to the ABC score. Our findings may help with clinical management and prognostic prediction of patients with AF.

## Data availability statement

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

## Ethics statement

The studies involving human participants were reviewed and approved by the Army Medical University. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

LZ, ZZ, and YL designed this study. JM, NW, and ZY drafted the article and analyzed the data. YC and CL were in charge

of data collection. WX did the critical revision of article. All authors have 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/fcvm.2022.1072164/full#supplementary-material

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# Carotid atherosclerosis in people of European, South Asian and African Caribbean ethnicity in the Southall and Brent revisited study (SABRE)

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**Background:** Atherosclerotic cardiovascular disease (ASCVD) risk differs by ethnicity. In comparison with Europeans (EA) South Asian (SA) people in UK experience higher risk of coronary heart disease (CHD) and stroke, while African Caribbean people have a lower risk of CHD but a higher risk of stroke.

**Aim:** To compare carotid atherosclerosis in EA, SA, and AC participants in the Southall and Brent Revisited (SABRE) study and establish if any differences were explained by ASCVD risk factors.

**Methods:** Cardiovascular risk factors were measured, and carotid ultrasound was performed in 985 individuals (438 EA, 325 SA, 228 AC). Carotid artery plaques and intima-media thickness (cIMT) were measured. Associations of carotid atherosclerosis with ethnicity were investigated using generalised linear models (GLMs), with and without adjustment for non-modifiable (age, sex) and modifiable risk factors (education, diabetes, hypertension, total cholesterol, HDL-C, alcohol consumption, current smoking).

**Results:** Prevalence of any plaque was similar in EA and SA, but lower in AC (16, 16, and 6%, respectively; $p < 0.001$). In those with plaque, total plaque area, numbers of plaques, plaque class, or greyscale median did not differ by ethnicity; adjustment for risk factors had minimal effects. cIMT was higher in AC than the other ethnic groups after adjustment for age and sex, adjustment for risk factors attenuated this difference.

**Conclusion:** Prevalence of carotid artery atherosclerotic plaques varies by ethnicity, independent of risk factors. Lower plaque prevalence in in AC is consistent with their lower risk of CHD but not their higher risk of stroke. Higher cIMT in AC may be explained by risk factors. The similarity of plaque burden in SA and EA despite established differences in ASCVD risk casts some doubt on the utility of carotid ultrasound as a means of assessing risk across these ethnic groups.

KEYWORDS

**ethnicity, cardiovascular disease, atherosclerosis, carotid artery, medical imaging**

# Introduction

Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of mortality and morbidity worldwide (1). There are marked differences in ASCVD risk in different ethnic groups, even within the same country. For example the risk of coronary heart disease (CHD) is ∼1.7-fold higher in migrants from the Indian subcontinent than in people of European origin in UK (2). In contrast, people of African-Caribbean ethnicity in the UK have markedly elevated risk of stroke, but their risk of CHD is lower in comparison with Europeans or migrants from the Indian subcontinent (2). In all ethnic groups, established risk factors [e.g., blood pressure (BP), total cholesterol, high-density lipoprotein cholesterol (HDL-C), diabetes, education, alcohol consumption, and tobacco smoking] predict risk of ASCVD (3, 4), although some the prevalence of some risk factors, such as dysglycaemia, smoking and adiposity differ by ethnicity (5, 6). Previous work suggests differences in these factors only partially explain ethnic differences in ASCVD risks (2).

Detailed phenotyping of subclinical atherosclerosis may provide more insights into ethnic differences in ASCVD risk. Ultrasonography is a reliable and non-invasive technique that is widely used to assess atherosclerosis in the carotid artery (7). In addition to measurement of common carotid artery intima-media thickness (cIMT) and quantification of atherosclerotic plaques (8, 9), this method can also provide some information on plaque composition and vulnerability (10–12).

Based on the existing evidence in relation to ethnic difference in ASCVD risk, we therefore hypothesised that, in comparison with Europeans, South Asian people would have a greater burden of carotid atherosclerosis and that African Caribbean people would have similar or lower levels. We also aimed to investigate whether plaque characteristics differed by ethnicity and the potential role of established ASCVD risk factors in differences observed between ethnic groups. Individuals studied were participants in the third follow-up visit of the South and Brent Revisited (SABRE) study, a multi-ethnic longitudinal cohort that has been followed up for over 30 years.

# Materials and methods

## Participants and study design

Detailed information about the Southall and Brent Revisited (SABRE) study has been published in previously (13, 14).In brief, SABRE is a longitudinal study that recruited European (EA), South Asian (SA), and African Caribbean (AC) participants living in West and North London in 1988–1991, when they were aged 40–69 years. Participants' ethnicity was determined by interviewers based on grand-parental origin and confirmed by participants. Surviving participants who remained in the study have undergone follow-up clinic-based investigations at 20-years (visit 2: 2008–2011) and 25-year (visit 3: 2014–2018). For the latter visit the partners of the original participants were also invited to attend. The current study included 991 individuals (437 European, 326 South Asian, 228 African Caribbean) from visit 3 (**Figure 1**). Ethical approval for the study was obtained from Ealing, Hounslow and Spelthorne, Parkside, and University College London Research Ethics Committees and all participants provided written informed consent.

## Clinical investigations

Participants were invited to a clinic appointment and were asked to refrain from alcohol, smoking, and caffeine for ≥12 h before attendance, and not to take their medication on the morning of the clinic visit. Information was recorded on age, sex, health behaviours, medical history, and medication (14). Height and weight were measured using a standardised protocol and body composition was measured using a Tanita BC 418 body composition analyser. Seated brachial BP was measured using an appropriately sized cuff using an automatic Omron 705 IT after 5–10 min rest according to ESH guidelines (15). The average of the second and third recordings was used as the estimate of clinic BP. Diabetes mellitus was defined according to the 1999 WHO guidelines (16), or physician diagnosis or receipt of anti-diabetes medications. Hypertension was defined as physician-diagnosed hypertension or participant-reported hypertension or receipt of BP-lowering medication. Smoking was classified into current or not. Alcohol consumption was categorised according to UK guidelines into none, ≤14 units per week or >14 units per week. Blood and urine samples were taken, and whole blood, serum, EDTA plasma and urine stored at −80°C prior to analysis. Glycosylated haemoglobin (HbA1c) was measured on an automated platform (c311, Roche Diagnostics, Burgess Hill, UK), serum total cholesterol, HDL-C and triglycerides were measured using enzymatic methods (Roche/Hitachi cobas c system). Low density lipoprotein cholesterol (LDL-C), All assays used the manufacturers calibration and quality control material.

## Ultrasound measurements

Ultrasound scans were performed by an experienced sonographer using a GE Vivid I Ultrasound system equipped with a 6-13 mHz broadband linear array transducer (12L-RS). The common carotid artery (CCA), internal carotid artery (ICA), external carotid artery (ECA) was assessed along the long- and short-axes bilaterally. Two-dimensional-images, spectral-Doppler imaging, Colour and Power Doppler were also recorded. Adequate quality of ECG signals and ultrasound images was ensured throughout the examination. A cine loop of at least five cardiac cycles at three angles (lateral, posterior, and anterior) as well as one 8-bit greyscale image captured at the R wave for each angle were acquired. cIMT and carotid lumen diameter was measured from the best visualised image over a 10 mm segment in the CCA according to the American Society of Echocardiography Carotid Intima-Media Thickness Task Force Consensus Statement (17). Plaque was defined according to the Mannheim consensus (8, 9), as a focal lesion that encroached into the carotid artery lumen by ≥0.5 mm or ≥50% of the surrounding cIMT value or had a thickness >1.5 mm as measured from the media-adventitia interface to the intima-lumen boundary. Carotid stenosis >50% was assessed by visual inspection of the B-mode ultrasound scan, using Colour and Power Doppler imaging as needed, and quantified according to NASCET criteria (18). All quantitative analyses were performed offline using validated software (AMS II) (19) that included automated measurement of plaque area, categorisation of plaque based on the Grey-Weale score (10), plaque size and estimation of grey-scale median (GSM) (20, 21). Repeatability and reproducibility of cIMT and plaque characteristics have been reported previously (22).

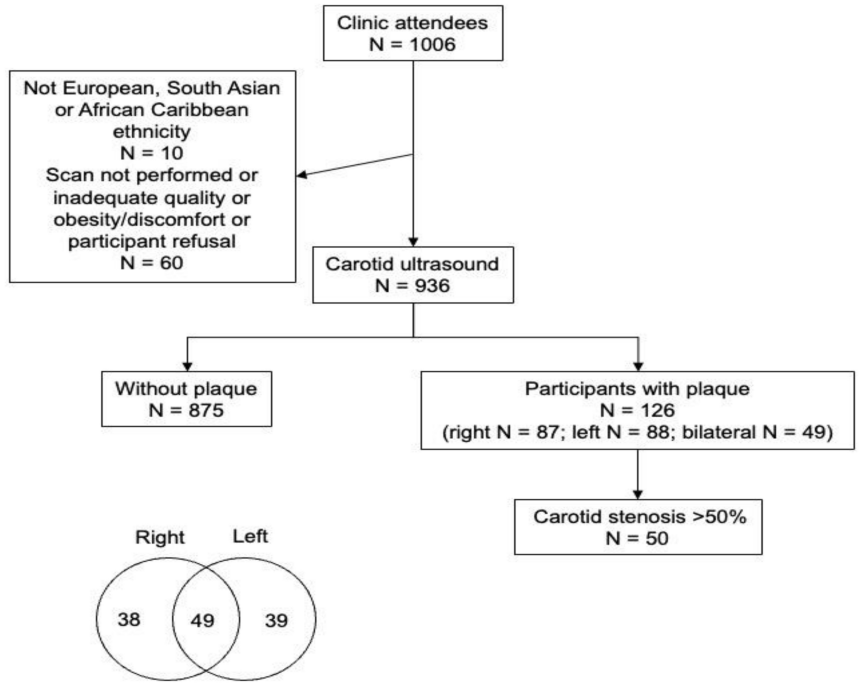


FIGURE 1
Diagram of participant flow including location of plaques as a Venn diagram.

## Statistical analysis

Statistical analyses were performed with Stata v.17.1 (StataCorp, College Station, TX, USA). Continuous data for the sample were summarised as means and standard deviations (SD) or median (interquartile range) for skewed data, categorical data as counts and percentages. Normality was assessed through frequency histograms, QQ plots and Shapiro-Wilk tests. Comparisons between ethnic groups were made using generalized linear modelling (GLM). Two models were used to provide further insight into ethnic differences: (Model 1) non-modifiable risk factors (age and sex); (Model 2) Model 1 plus established modifiable risk factors (diabetes, hypertension, total cholesterol, HDL-C, years of education, alcohol consumption, current smoking, statin medication). Choice of covariates was based on *a priori* knowledge (4, 23). Additional sensitivity analyses were performed where diabetes was replaced by HbA1c, or where systolic BP or body mass index (BMI) (or waist hip ratio) were added to models (these models showed negligible differences from the original models and the results are not presented). The possibility of effect modification by sex was looked for in all models by including a sex × ethnicity interaction term, if this was not statistically significant both sexes were pooled for analysis, otherwise it was planned that results for both sexes would be analysed separately.

Dichotomous variables (e.g., presence of plaque or presence of carotid stenosis >50%) were modelled using GLM with a binomial and log family and link function. Ordered categorical variables with fewer than six categories [median plaque grade (manual and automatic)] were analysed using ordered logistic regression and the proportional-odds assumption was tested using an approximate likelihood ratio test. If the proportional-odds assumption was not met data were fit with partial proportional odds models using generalised ordinal logistic regression (gologit2) (24). Numbers of plaques were modelled using negative binomial models as data were expected to be over-dispersed (this was confirmed using the likelihood ratio test for alpha = 0). Risk ratios, or marginal probabilities and 95% confidence intervals (CI) were estimated from these models. Multiple linear regression models were used for continuous measures (total area of plaques, lowest GSM of all plaques, cIMT) and marginal means and CI estimated. If regression models showed evidence of heteroskedasticity, robust standard errors were calculated. Assumptions of linearity were checked by examination of residuals and if necessary, it was planned that non-linear models would be constructed using fractional polynomials. The primary analysis was a complete case analysis which is valid under the assumption that missingness was independent of outcomes. As a sensitivity analysis, models using full information maximum likelihood which is valid under the missing at random (MAR) assumption were also examined for linear models. Inference was based on a combination of *p*-values, effect sizes and CI, no adjustment was made for multiple comparisons.

# Results

**Table 1** shows the characteristics of the sample stratified by ethnicity. Participants were aged between 40 and 69 years and comprised 437 EA (mean age 74 years, 62% male), 326 SA (mean age 73.2 ± 6.3 years, 59.3% male), and 228 AC (mean age 71 years, 35.6% male). On average SA were slightly younger than EA and AC were younger than both EA and SA, and there were more women in the AC sample. AC and SA people were shorter, had higher systolic BP and more diabetes and hypertension than EA. Compared with EA, SA had a higher prevalence of known CHD, more years of education, lower heart rate, lower BMI, were shorter and were less likely to be current

TABLE 1 Characteristics of sample by ethnicity.

| Variables | Ethnicity | | | | | | | | | ANOVA or Chi$^2$ $p$-values |
|---|---|---|---|---|---|---|---|---|---|---|
| | EA | | | SA | | | AC | | | |
| | *N* | Mean/% | (SD) | N | Mean/% | (SD) | N | Mean/% | (SD) | |
| Age, years | 437 | 74.4 | (6.10) | 326 | 73.20* | (6.3) | 228 | 70.5* | (7.91) | <0.001 |
| Male sex | 274 | 49.3% | | 194 | 59.3% | | 85 | 37.0%* | | <0.001 |
| Systolic blood pressure, mmHg | 437 | 138.67 | (18.3) | 325 | 143.63* | (18.62) | 228 | 142.26* | (16.68) | <0.001 |
| Diastolic blood pressure, mmHg | 437 | 78.84 | (10.8) | 325 | 78.48 | (10.6) | 228 | 81.73*† | (10.25) | <0.001 |
| Heart rate, bpm | 437 | 68.18 | (11.33) | 325 | 65.76* | (10.24) | 228 | 68.12† | (11.35) | 0.001 |
| Height, cm | 437 | 167.99 | (8.7) | 326 | 162.28* | (8.86) | 228 | 164.12*† | (7.79) | <0.001 |
| BMI, kg/m$^2$ | 437 | 28.01 | (4.53) | 326 | 26.42* | (3.91) | 228 | 29.94*† | (5.30) | <0.001 |
| HbA1c, mmol/mol | 423 | 38.61 | (8.0) | 314 | 43.27* | (9.37) | 220 | 39.98† | (11.04) | <0.001 |
| Years of education | 377 | 11.9 | (3.53) | 223 | 13.5* | (3.77) | 153 | 12.01† | (3.69) | <0.001 |
| Diabetes mellitus | 53 | 13% | | 88 | 28%* | | 54 | 27%* | | <0.001 |
| CHD | 53 | 13% | | 59 | 19%* | | 13 | 6%*† | | <0.001 |
| Stroke | 8 | 2% | | 3 | 1% | | 4 | 2% | | 0.558 |
| Hypertension | 209 | 49% | | 210 | 66%* | | 152 | 69%* | | <0.001 |
| Alcohol consumption | | | | | | | | | | <0.001 |
| None | 107 | 24% | | 170 | 52% | | 99 | 43% | | |
| ≤14 units per week | 262 | 60% | | 150 | 46% | | 129 | 56% | | |
| >14 units per week | 71 | 16% | | 7 | 3% | | 2 | 1% | | |
| Current smoker | 16 | 4% | | 3 | 1%* | | 8 | 3%† | | 0.051 |
| Statin use | 210 | 48% | | 209 | 64%* | | 85 | 37%*† | | <0.001 |
| Presence of carotid plaque(s) | 74 | 17% | | 55 | 17% | | 14 | 6%*† | | <0.001 |
| Carotid stenosis >50% | 22 | 5% | | 23 | 7% | | 5 | 2.2%† | | 0.025 |
| cIMT, mm | 407 | 0.89 | (0.20) | 311 | 0.89 | (0.23) | 212 | 0.91 | (0.20) | 0.73 |
| Total plaque area per individual with plaque, mm$^2$ | 74 | 34.4 | (29.2) | 55 | 33.6 | (27.5) | 14 | 29.4 | (27.6) | 0.84 |
| Number of plaques per individual with plaque | 74 | 1.5 | (1.1) | 55 | 1.5 | (0.8) | 14 | 1.1 | (0.86) | 0.35 |
| Minimum GSM of all plaques | 63 | 86.9 | (35.5) | 52 | 80.1 | (28.8) | 10 | 79.8 | (12.7) | 0.48 |
| Class (manual) of largest plaque | 55 | 2.84 | (0.46) | 46 | 2.87 | (0.40) | 7 | 2.86 | (0.38) | 0.93 |
| Class (auto) of largest plaque | 63 | 2.32 | (0.53) | 52 | 2.19 | (0.40) | 10 | 2.3 | (0.48) | 0.37 |

BMI, body mass index; CHD, coronary heart disease; cIMT, carotid artery intima-media thickness; GSM, greyscale median; HbA1c, glycated haemoglobin; SD standard deviation. $p$-values were calculated using Chi$^2$ tests and logistic regression for categorical variable and ANOVA for continuous variables, Wald tests were used for individual comparisons. *$p < 0.05$ compared with Europeans, †$p < 0.05$ compared with South Asians.

smokers, and less likely to consume high quantities of alcohol, while AC had a lower prevalence of CHD, higher BMI, higher diastolic BP, more diabetes and hypertension and were less likely to consume high quantities of alcohol.

Carotid artery intima-media thickness was similar by ethnicity in an unadjusted model, but plaques were more frequent in EA and SA than AC (Table 1); however, there was no difference between EA and SA (Table 1). Plaques were more common in men than women but there was no evidence that sex modified the ethnic differences in plaque prevalence (Figure 2). There were no marked differences in distribution of plaques by ethnicity: 57.5% of Europeans had plaques in the left carotid artery, 47.6% in the right carotid artery and 44.1% had plaques bilaterally. A total of 42.5% of South Asians had plaques in the left carotid artery, 44.1% in the right carotid artery and 32.7% had plaques bilaterally. A total of 10% of African Caribbean's had plaques in left of carotid artery, 8.1% in the right carotid artery and

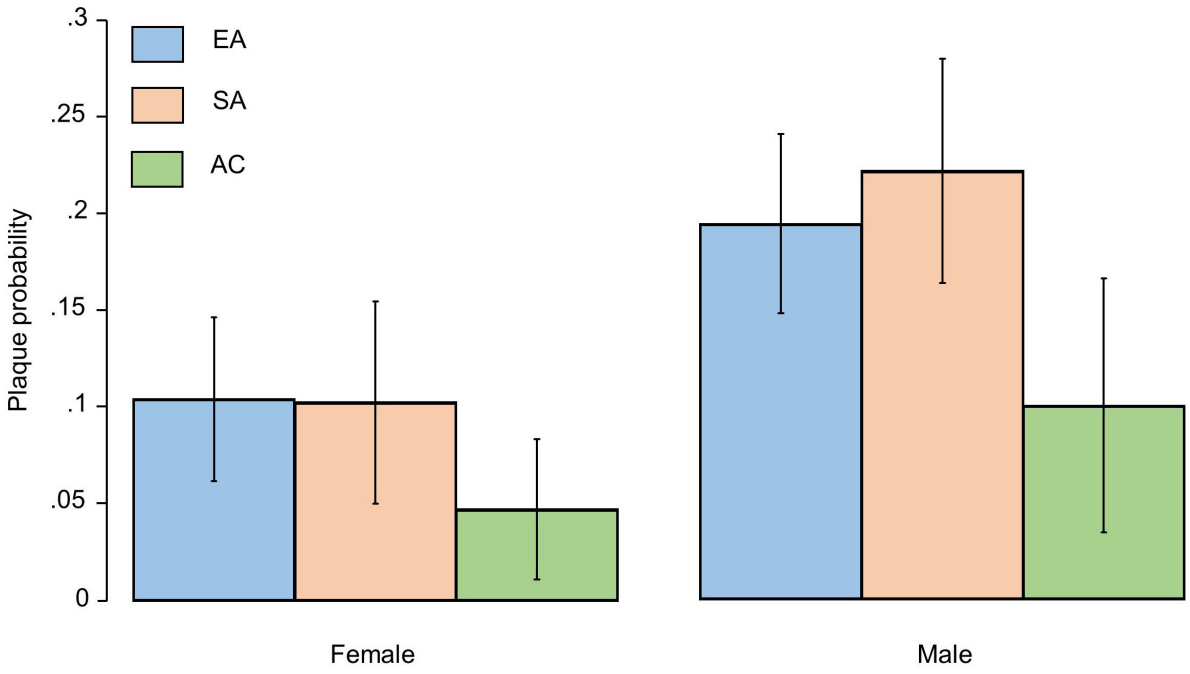


FIGURE 2
Bar plot showing marginal probabilities with 95% confidence intervals (adjusted for age) of having a plaque by sex and ethnicity. EA, European; SA, South Asian, and AC, African Caribbean.

TABLE 2 Marginal probabilities of having any plaques by ethnicity with and without adjustment for risk factors.

| Prevalence of plaque by ethnicity | | | *p*-value (individual comparisons) | | | *p*-value LR test |
|---|---|---|---|---|---|---|
| EA | SA | AC | EA vs. SA | EA vs. AC | SA vs. AC | |
| Unadjusted | | | | | | |
| 0.16 (0.13, 0.20) | 0.16 (0.12, 0.20) | 0.06 (0.03, 0.09) | 0.992 | <0.001 | <0.001 | <0.001 |
| Model 1 | | | | | | |
| 0.15 (0.12, 0.18) | 0.16 (0.12, 0.20) | 0.07 (0.03, 0.11) | 0.626 | 0.008 | 0.004 | 0.006 |
| Model 2 | | | | | | |
| 0.16 (0.12, 0.19) | 0.19 (0.14, 0.24) | 0.08 (0.03, 0.13) | 0.241 | 0.068 | 0.014 | 0.036 |

Data are marginal probabilities (95% confidence intervals). Model 1: age, sex adjusted, Model 2: Model 1 + years of education, diabetes, total cholesterol, HDL, alcohol consumption, current smoking, hypertension, statin use. LR, likelihood-ratio.

23.0% had plaques bilaterally (Figure 1). In comparison with EA, the risk ratios for having any plaque in SA after adjustment for non-modifiable, or non-modifiable plus modifiable risk factors were 1.08 (0.79, 1.46); $p = 0.65$ and 0.98 (0.66, 1.41); $p = 0.89$, respectively. For AC the comparable risk ratios were 0.48 (0.28, 0.82); $p = 0.013$ and 0.53 (0.268, 1.047); $p = 0.068$. The marginal probabilities of having one or more plaques in each ethnic group are shown in Table 2 with and without adjustment. The probability of having one or more plaques was similar in EA and SA but was lower by ∼50% in AC. Statistical adjustment had little effect on these estimates, although the estimated CI of the fully adjusted model were wider, probably as a result of the reduced sample size of the complete case analysis for non-modifiable plus modifiable risk factors ($n = 727$). Compared with EA, the risk ratio in SA for having a stenosis >50% was 1.56 (0.88, 2.76); $p = 0.12$ and 1.70 (0.81, 3.52); $p = 0.15$. For AC the risk ratios were 0.61 (0.23, 1.61); $p = 0.32$ and 0.65 (0.25, 1.70); $p = 0.38$. The limited number of stenoses in the sample ($n = 50$) made these estimates very imprecise and scope for inference was limited.

After adjustment for age and sex cIMT was higher in AC than EA or SA but there was no difference in cIMT between EA and SA (Table 3). Further adjustment for risk factors attenuated differences by ethnicity (Table 4).

In individuals with plaque, a comparison of plaque area, average number of plaques, minimum greyscale median, and plaque class is shown in Tables 3, 4 following adjustment for risk factors. Total plaque area was similar in all ethnic groups, as was plaque class and echogenicity as assessed by GSM.

## Discussion

We found ethnic differences in the prevalence of carotid plaque in a population-based sample of people in UK. People of AC

TABLE 3 Ethnic differences in cIMT and plaque characteristics with adjustment for age and sex (Model 1).

| Variables | Ethnicity | | | *p*-value (individual comparisons) | | | *p*-value LR test |
|---|---|---|---|---|---|---|---|
| | EA | SA | AC | EA vs. SA | EA vs. AC | SA vs. AC | |
| cIMT, mm | 0.88 (0.86, 0.90) | 0.89 (0.87, 0.92) | 0.93 (0.90, 0.96) | 0.285 | 0.002 | 0.033 | 0.003 |
| Total plaque area per individual with plaque, $mm^2$ | 40.6 (33.4, 46.6) | 35.2 (27.9, 42.5) | 44.5 (27.5, 61.4) | 0.343 | 0.624 | 0.323 | 0.473 |
| Number of plaques per individual with plaque | 1.76 (1.56, 1.96) | 1.64 (1.41, 1.86) | 1.62 (1.10, 2.13) | 0.416 | 0.604 | 0.945 | 0.667 |
| Minimum GSM of all plaques | 87.44 (79.60, 95.29) | 79.57 (70.89, 88.25) | 78.58 (58.64, 98.52) | 0.187 | 0.414 | 0.929 | 0.350 |
| Category of largest plaque | | | | 0.507 | 0.820 | 0.580 | 0.741 |
| Class 1 | 0 | 0 | 0 | | | | |
| Class 2 | 0.20 (0.10, 0.30) | 0.15 (0.06, 0.25) | 0.23 (−0.08, 0.55) | | | | |
| Class 3 | 0.78 (0.68, 0.87) | 0.82 (0.73, 0.90) | 0.75 (0.46, 1.03) | | | | |
| Class 4 | 0.02 (−0.01, 0.05) | 0.03 (−0.01, 0.07) | 0.02 (−0.02, 0.06) | | | | |
| Size class of largest plaque | | | | 0.121 | 0.849 | 0.296 | 0.247 |
| Mild | 0.01 (−0.01, 0.03) | 0.02 (−0.01, 0.05) | 0.01 (−0.01, 0.03) | | | | |
| Moderate | 0.66 (0.54, 0.77) | 0.77 (0.67, 0.87) | 0.63 (0.34, 0.92) | | | | |
| Severe | 0.33 (0.22, 0.45) | 0.21 (0.10, 0.31) | 0.36 (0.06, 0.67) | | | | |

All data are marginal means (95% confidence intervals) or probabilities (95% confidence intervals). BMI, body mass index; CHD, coronary heart disease; cIMT, carotid artery intima-media thickness; GSM, greyscale median; HbA1c, glycated haemoglobin; SD standard deviation; LR, likelihood-ratio.

TABLE 4 Ethnic differences in cIMT and plaque characteristics with adjustment for non-modifiable and modifiable risk factors (Model 2).

| Variables | Ethnicity | | | *p*-value (individual comparisons) | | | *p*-value LR test |
|---|---|---|---|---|---|---|---|
| | EA | SA | AC | EA vs. SA | EA vs. AC | SA vs. AC | |
| cIMT, mm | 0.88 (0.86, 0.90) | 0.89 (0.86, 0.92) | 0.92 (0.88, 0.95) | 0.700 | 0.091 | 0.188 | 0.220 |
| Total plaque area per individual with plaque, $mm^2$ | 41.3 (33.9, 48.7) | 34.2 (26.6, 43.1) | 44.5 (26.5, 62.4) | 0.285 | 0.742 | 0.332 | 0.402 |
| Number of plaques per individual with plaque | 1.85 (1.63, 2.07) | 1.56 (1.32, 1.81) | 1.57 (1.03, 2.10) | 0.111 | 0.332 | 0.996 | 0.196 |
| Minimum GSM of all plaques | 87.41 (79.00, 95.83) | 78.62 (69.23, 88.02) | 75.80 (55.36, 96.23) | 0.204 | 0.306 | 0.805 | 0.300 |
| Category of largest plaque | | | | 0.335 | 0.852 | 0.747 | 0.616 |
| Class 1 | 0 | 0 | 0 | | | | |
| Class 2 | 0.23 (0.11, 0.35) | 0.15 (0.04, 0.25) | 0.20 (−0.13, 0.52) | | | | |
| Class 3 | 0.75 (0.64, 0.86) | 0.82 (0.72, 0.92) | 0.78 (0.49, 1.06) | | | | |
| Class 4 | 0.02 (−0.01, 0.05) | 0.03 (−0.02, 0.08) | 0.02 (−0.03, 0.07) | | | | |
| Size class of largest plaque | | | | 0.498 | 0.884 | 0.607 | 0.748 |
| Mild | 0.02 (−0.01, 0.04) | 0.02 (−0.01, 0.06) | 0.01 (−0.02, 0.05) | | | | |
| Moderate | 0.68 (0.56, 0.79) | 0.74 (0.61, 0.87) | 0.65 (0.30, 1.00) | | | | |
| Severe | 0.31 (0.19, 0.43) | 0.24 (0.10, 0.38) | 0.34 (−0.04, 0.71) | | | | |

All data are marginal means (95% confidence intervals) or probabilities (95% confidence intervals). BMI, body mass index; CHD, coronary heart disease; cIMT, carotid artery intima-media thickness; GSM, greyscale median; HbA1c, glycated haemoglobin; SD standard deviation; LR, likelihood-ratio.

ethnicity had a lower occurrence of carotid plaque than the other ethnic groups, while the burden of plaque in EA and SA was similar. The lack of difference between EA and SA was surprising considering the large excess of cardiovascular disease reported in SA and our data for carotid plaque prevalence are not consistent with previous estimates of excess CHD risk in SA (2). In those with

plaque, plaque characteristics differed little between ethnic groups, in particular there was no evidence of SA having evidence of more lipid-rich or vulnerable plaques. Ethnic differences in plaque prevalence were unexplained by disparities in ASCVD risk factors. It therefore remains unclear why the AC group had a lower prevalence of carotid plaques than the other ethnic groups, however, this observation is consistent with previous work, including in SABRE, showing lower risk of CHD in people of AC ethnicity in UK, which was unexplained by conventional ASCVD risk factors (25, 26). cIMT also differed by ethnicity, after adjustment for age and sex, cIMT was higher in AC compared with the other ethnic groups, which could be consistent with their higher risk of stroke, but is inconsistent with their lower risk of CHD; this difference was attenuated after adjustment for non-modifiable and modifiable risk factors and is likely to be attributable to differences in ASCVD risk factors.

Better understanding and assessment of the prevalence of atherosclerosis and its relationship to cardiovascular risk factors in different ethnic groups is important. Such relationships may also provide insights into the pathogenesis of atherosclerosis in all ethnic groups. Our failure to identify factors explaining ethnic differences in carotid atherosclerotic plaque despite adjustment for ASCVD risk factors suggests that important determinants of ethnic differences in atherosclerosis susceptibility remain to be identified. Mechanisms related to population migration (27), socio-economic disadvantage (28) and racism (28, 29) seem plausible explanations, but given the differences observed between minority ethnic groups in this study this question merits further study. We cannot exclude genetic differences between populations of difference ancestry but currently there is little or no evidence to suggest that genetics makes a major contribution to ethnic differences in susceptibility to ASCVD (30, 31).

Previous studies have examined ethnic differences in carotid atherosclerosis, although few have included SA people. A UK community-based study found higher cIMT and lower prevalence of plaque in AC compared with EA (32) and this difference remained after adjustment for conventional ASCVD risk factors. Another UK-based study observed marginally higher cIMT in EA compared with SA despite higher prevalence of ASCVD in SA (33). In the US, the Multi-Ethnic Study of Atherosclerosis found that cIMT was higher in people of African American ethnicity, but the risk of new plaque formation was lower in African American, Hispanic and Chinese ethnicities compared with White Americans after adjustment for traditional ASCVD risk factors (34). The Diabetes Heart Study also found that African American people with T2DM had higher cIMT but lower prevalence of carotid plaque compared with those of European ancestry (35). In contrast, the Northern Manhattan Stroke study found similar maximum internal carotid artery plaque thickness (MICPT) in stroke-free African- and European- ethnicity individuals but lower MICPT in people of Hispanic ethnicity (36). A recent individual participant meta-analysis that compared the association of ASCVD risk factors with cIMT in different ethnicities from a range of countries, reported that high cIMT levels was highest amongst African American populations, similar in Asian, White and Hispanic people and lowest in African populations. In keeping with our findings, adjustment for risk factors only marginally attenuated these differences (37). Overall, despite some inconsistencies the results of these previous studies appear broadly consistent with our findings.

As has been observed in some previous studies (32–34), cIMT corresponded poorly with known risk differentials for ASCVD, especially CHD, in the ethnic groups. Plaque prevalence was consistent with the known lower risk of CHD in AC, but not with the elevated risk of ASCVD in SA or the elevated risk of stroke in AC (38, 39). This raises questions about the reliability of cIMT and plaque as a screening tool for early detection of atherosclerosis *across* different ethnic groups. For cIMT it has previously been suggested that arterial wall remodelling in response to haemodynamic stresses might complicate interpretation (40), but it is not obvious that this could explain the ethnic discordance between ASCVD risk and plaque prevalence, given the latter is generally considered a better predictor of ASCVD risk (41).

This study has limitations and strengths: it is cross-sectional so causal conclusions cannot be made. Participants were drawn from a randomly selected population-based cohort but possible bias due to non-participation, attrition, missing data and residual confounding by unmeasured or imprecisely measured variables cannot be excluded. As might be expected in a population-based sample, the frequency of carotid plaque was quite low, particularly in AC, which may have limited our ability to detect small differences in plaque prevalence or characteristics, nevertheless the precision of the estimates was sufficient to exclude disparities in plaque prevalence consistent with CVD risk differentials in South Asians. Our categorisation of ethnicity is crude and may obscure important differences within ethnic groups; (42), however, our categories reflect the original study design and correspond to the broad ethnic groups in used by the UK classification scheme (43). AC participants mostly migrated between 1950 and 1960 (i.e., around the ages of 20 to 30), while most of the SA participants arrived in the UK in the 1970's (i.e., around 40 years old) and limited data was available about exposures, including childhood exposures and healthcare provision, that occurred prior to migration or extent of acculturation after migration. We included a comprehensive set of risk factors for ASCVD, but we acknowledge that including these risk factors, which potentially act as mediators of ethnic differences, could introduce bias (44). The study's strengths are first and foremost its community-based methodology and that it compares people of different ethnicities in the same location. SA and AC participants make up the majority of British first-generation migrants and, unlike in some countries, universal healthcare, free at the point of use, is available in UK. This may lessen, though not abolish disadvantages in health access (45). All examinations were conducted according to a strict approach, resulting in a comprehensive phenotyping of this older age sample.

## Conclusion

In people resident in UK, EA and SA have a higher burden of atherosclerotic plaques in carotid arteries than AC, while in contrast cIMT was higher in AC than other ethnicities. These differences were unexplained by ASCVD risk factors. The disparity between these findings and the known risks of ASCVD in these ethnic groups raises questions about the utility of carotid ultrasound as a tool to predict risk in multi-ethnic populations.

## Data availability statement

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

## Ethics statement

The studies involving human participants were reviewed and approved by the Ealing, Hounslow and Spelthorne, Parkside, and University College London Research Ethics Committees. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

RA and ADH had full access to all the data in the study. RA performed the statistical analyses and wrote the first draft of the manuscript. All authors contributed to study design and interpretation and approved the final manuscript.

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## Conflict of interest

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

## Publisher's note



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# The genetic correlation and causal association between key factors that influence vascular calcification and cardiovascular disease incidence

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**Background:** Serum calcium (Ca), vitamin D (VD), and vitamin K (VK) levels are key determinants of vascular calcification, which itself impacts cardiovascular disease (CVD) risk. The specific relationships between the levels of these different compounds and particular forms of CVD, however, remain to be fully defined.

**Objective:** This study was designed to explore the associations between these serum levels and CVDs with the goal of identifying natural interventions capable of controlling vascular calcification and thereby protecting against CVD pathogenesis, extending the healthy lifespan of at-risk individuals.

**Methods:** Linkage disequilibrium score (LDSC) regression and a two-sample Mendelian randomization (MR) framework were leveraged to systematically examine the causal interplay between these serum levels and nine forms of CVD, as well as longevity through the use of large publically accessible Genome-Wide Association Studies (GWAS) datasets. The optimal concentrations of serum Ca and VD to lower CVD risk were examined through a restrictive cubic spline (RCS) approach.

**Results:** After Bonferroni correction, the positive genetic correlations were observed between serum Ca levels and myocardial infarction (MI) ($p$ = 1.356E−04), as well as coronary artery disease (CAD) ($p$ = 3.601E−04). Negative genetic correlations were detected between levels of VD and CAD ($p$ = 0.035), while elevated VK1 concentrations were causally associated with heart failure (HF) [odds ratios (OR) per 1-standard deviation (SD) increase: 1.044], large artery stroke (LAS) (OR per 1-SD increase: 1.172), and all stroke (AS) (OR per 1-SD increase: 1.041). Higher serum Ca concentrations (OR per 1-SD increase: 0.865) and VD levels (OR per 1-SD increase: 0.777) were causally associated with reduced odds of longevity. These findings

remained consistent in sensitivity analyses, and serum Ca and VD concentrations of 2.376 mmol/L and 46.8 nmol/L, respectively, were associated with a lower CVD risk ($p < 0.001$).

**Conclusion:** Our findings support a genetic correlation between serum Ca and VD and CVD risk, and a causal relationship between VK1 levels and CVD risk. The optimal serum Ca (2.376 mmol/L) and VD levels (46.8 nmol/L) can reduce cardiovascular risk.

KEYWORDS

**serum calcium, vitamin D, vitamin K, cardiovascular disease, risk factor**

## Introduction

The rate of global population aging continues to accelerate (1), contributing to elevated risks of a range of age-related disorders and diseases that ultimately impair function and increase the risk of mortality (2). The World Health Organization (WHO) has established cardiovascular disease (CVD) as the most prominent global cause of death, contributing to 17.9 million deaths per year on average (3). Epidemiological research has revealed a range of factors that are related to CVD risk, including nutrient intake, alcohol consumption, exercise, and smoking (4–8). The ability to prevent CVD and to facilitate a healthier aging process is thus strongly dependent on the identification and mitigation of early CVD-related risk factors.

In recent work, vascular calcification has been identified as a common finding in patients with various forms of CVD including atherosclerosis, coronary artery disease (CAD), myocardial infarction (MI), heart failure (HF), and ischemic stroke (IS), suggesting a possible relationship between calcium (Ca) deposition and these conditions (9–12). In addition, serum concentrations of Ca, vitamin D (VD), and vitamin K (VK) are closely related to vascular calcification incidence, ultimately impacting CVD development. In particular, elevated serum Ca has been shown to contribute to direct increases in vascular calcification and CVD risk. However, many prior studies assessing the relationship between CVD and serum Ca levels have yielded inconsistent findings (13).

Vitamin D plays an important role in regulating the endocrine system and whole-body Ca homeostasis (14), with VD deficiencies contributing to a range of CVD risk factors and higher mortality rates among CVD patients (15). Even so, recent randomized controlled trial data suggests that VD supplementation does not offer any benefit with respect to CVD (16). There is thus a clear need for further research aimed at clarifying the nature of any protective benefits provided by VD in CVD. VK similarly functions as a key regulator of Ca homeostasis, impacting the cardiovascular system *via* activating matrix Gla protein, which can prevent calcification. When inactive, this protein is associated with a range of CVD-related risk factors including increases in insulin resistance, vascular calcification, valvular calcification, arterial stiffness, and HF indices that all contribute to higher rates of CVD-related death (17). However, definitive population-level causal evidence regarding the relationship between VK and CVD is currently lacking.

Therefore, based on the direct and indirect effects of serum Ca, VD, and VK concentrations on vascular calcification, and the fact that vascular calcification has become a common cause of various types of CVD, in this study, single nucleotide variant (SNV)-based genetic correlation analyses and a two-sample Mendelian randomization (MR) framework were leveraged to conduct a comprehensive analysis of the causal relationships among serum Ca, VD, VK, and a range of CVD outcomes [including CAD, MI, HF, atrial fibrillation (AF), all stroke (AS), all IS (AIS), small vessel stroke (SVS), large artery stroke (LAS), and cardioembolic stroke (CES)]. In addition, CVDs and longevity are in essence the result of interaction between genetics and environment. Studies have shown that different alleles of the same gene locus affect homeostasis of vascular microenvironment through regulation of expression, which may lead to two opposite outcomes: CVDs and longevity (18). Longevity and CVD are both interconnected and opposites (19). Therefore, in our study, besides the normal control, longevity was also used as a negative control to compare with CVD. The goal of these analyses was to identify interventions with the potential to reduce the morbidity or mortality associated with CVD, contributing to healthier aging and a longer life (2).

## Materials and methods

### Study design

Linkage disequilibrium score (LDSC) regression analyses enable the examination of SNV-associated heritability and coheritability between traits. MR analyses permit the evaluation of possible causal relationships between two traits based upon Mendel's law of independent inheritance, offering an opportunity for a natural randomized control trial (RCT) (20). LDSC and MR approach complement one another as strategies for exploring how to traits are related to one another. A restrictive cubic spline (RCS) strategy was also used with appropriate multivariate regression analyses as a

Abbreviations: AF, atrial fibrillation; AIS, all ischemic stroke; AS, all stroke; Ca, calcium; CAD, coronary artery disease; CARDIoGRAMplusC4D, coronary artery disease genetics; CES, cardioembolic stroke; CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; CI, confidence interval; CVD, cardiovascular disease; HERMES, Heart Failure Molecular Epidemiology for Therapeutic Targets; HF, heart failure; IS, ischemic stroke; IVW, inverse-variance-weighting; LAS, large artery stroke; LDSC, linkage disequilibrium score; LVEF, left ventricular ejection fraction; MI, myocardial infarction; MR, Mendelian randomization; MR-PRESSO, MR Pleiotropy RESidual Sum and Outlier; ORs, odds ratios; RCS, restrictive cubic spline; RCT, randomized control trial; SD, standard deviation; SNPs, single nucleotide polymorphisms; SNV, single nucleotide variant; SVS, small vessel stroke; TDI, Townsend deprivation index; WHO, World Health Organization; VD, vitamin D; VK, vitamin K; 25[OH]D, serum 25-hydroxyvitamin D.

means of examining relationships between exposures and outcomes to define optimal threshold values for exposures of interest (21) (**Supplementary Figure 1**). As the analyses performed herein were based upon publically available datasets, no further ethical oversight or informed consent were necessary.

## Outcome data source

The primary outcomes for this analysis were CVDs and longevity, with outcome data sources being provided in detail in **Table 1**. AF-related data were derived from a study performed by Nielsen et al. (22), with paroxysmal or permanent AF and atrial flutter included in the definition of AF (60,620 cases, 970,216 controls), while summary statistics for HF were derived from the largest published Genome-Wide Association Studies (GWAS) meta-analysis performed by the HF Molecular Epidemiology for Therapeutic Targets (HERMES) Consortium analyzing individuals of European ancestry (47,309 cases, 930,014 controls) (23). Participants in this study were individuals diagnosed with HF of any etiological basis determined based upon left ventricular ejection fraction (LVEF) (24). Summary-level CAD data were derived from the CAS Genetics (CARDIoGRAMplusC4D) Consortium (122,733 cases, 424,528 controls) (24). Stroke summary statistics for individuals of European ancestry (67,162 cases, 454,450 controls) including 67,162 AS, 60,341 AIS, 6,688 LAS, 9,006 CES, and 11,710 SVS cases, were derived from the MEGASTROKE consortium aimed at reducing bias resulting from population stratification (25). Summary-level MI data were derived from the CARDIoGRAMplusC4D (60,801 cases, 123,504 controls), MIGen, and CARDIoGRAM Exome consortia (42,335 cases, 78,240 controls), and ESP EOMI (4,703 cases, 5,090 controls) datasets (26).

Longevity analyses were performed with summary statistics derived from a recent GWAS meta-analysis of individuals of European ancestry in ∼20 population- or family based cohorts in Europe and the USA (27). Cases ($n$ = 11,262) were individuals who lived to an age above the 90 or 99th percentile age based on cohort life tables from census data for the appropriate country, sex, and birth cohort. Controls ($n$ = 25,483) were individuals who died at or before the 60th percentile age or whose age at the last follow-up visit was at or before the 60th percentile age.

## Data sources and variant selection

Data pertaining to serum Ca (28), VD (29), and VK (30) concentrations for the included sources of exposure data are summarized in **Table 1**. Serum Ca and VD levels were derived from the UK Biobank Resource (Project #73697). All participants in the UK Biobank had provided informed consent, with oversight from the North West Multi-Centre Research Ethics Committee (11/NW/0382). VK1-related data were derived from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium Nutrition Working Group (30). The largest GWAS study focused mainly on individuals of European ancestry was used to select genetic variants related to modifiable risk factors.

Instrumental variables for modifiable risk factors were determined with the Plink software through the use of the clump procedure. Considering that VK did not screen out independent sites at the threshold $p < 5E{-}08$, single nucleotide polymorphisms (SNPs) linked to risk factors were selected at the selected threshold for possible genome-wide significance ($p < 1E{-}05$). While for serum Ca and VD, the causal correlation didn't change much when we used more stringent threshold (**Supplementary Table 2**). When linkage disequilibrium ($r^2 > 0.1$) for SNPs was evident for a given trait, SNPs that were most strongly associated with the exposure of interest based on the smallest measured $p$-value were selected. SNPs not included in CVD- or longevity-focused GWAS datasets were not included in this study. For the selected outcomes, the number of SNPs chosen as instrumental variables ranged from 8 to 448. These variants explained from 0.021 to 0.529% of phenotypic variation (**Supplementary Table 3**). To synchronize data between exposure and outcome GWAS, estimates of SNP effects were flipped with unrelated alleles and effects.

## LDSC regression analyses

Cross-trait LDSC analyses were used to evaluate genetic correlations between pairs of phenotypes and genome-wide SNPs (31). LD scores for individual SNPs were determined in accordance with genotypes for common SNPs [minor allele frequency (MAF) > 0.01, Hardy–Weinberg equilibrium $p > 1 \times 10^{-5}$] over a 10 Mb window when evaluating data derived from 503 European individuals included in the 1000 Genomes Project. The exact number of SNPs used in the genetic correlation analyses in each pair were shown in the **Supplementary Table 1**. LDSC analyses were then performed through the use of a weighted linear model *via* regressing $Z$-statistic products for two traits on LD scores across all variants throughout the genome. The resultant regression slow should provide an unbiased tool for estimating genetic correlations even if some individuals overlap between two GWASs. Bonferroni correction was used to correct for multiple testing, with a two-sided significance level of 0.0056 being established (0.05 divided by the nine included outcomes). Those associations exhibiting a $p$-value between 0.05 and 0.0056 were thought to be suggesting of a possible association. LDSC packages (32) in R version 4.0.2 were used for all analyses.

## MR estimates

Causal estimates for the impact of genetically predicted serum Ca, VD, and VK levels on outcome variables were assessed with an inverse-variance-weighting (IVW) approach using a fixed-effects model. Weighted median, MR-Egger regression, and MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) strategies were also employed to improve the reliability and robustness of study conclusions. The weighted median approach assumes that a minimum of 50% of available information is based upon valid Ivs (32). The MR-Egger approach offers validity despite permitting the presence of invalid variants, but can yield wide confidence interval (CI) values (33). The MR-PRESSO approach enables researchers to detect and correct for any analyses detected through IVW linear regression analyses (34). Bonferroni correction of these results was used as above, with a $p$-value < 0.0056 as the significance threshold and a $p$-value between 0.05 and 0.0056 being indicative of a possible association. Odds ratios (ORs) are given for every 1 standard

TABLE 1 Genome-Wide Association Studies (GWAS) data sources.

| Phenotypes | GWAS data source | Sample size | Ancestry | Covariates | Objective |
|---|---|---|---|---|---|
| Calcium | UK Biobank (UKB) (26) | 313,387 Individuals | European | Genotype principal components (PCs) (the top 40 PCs of the UK Biobank-provided genotype-based global PCs), age indicator variables (one for each integer age), sex, 5-year age indicators by sex interactions, self-identified ethnicity, self-identified ethnicity by sex interactions, fasting time (one indicator per fasting time, except a single indicator for >18 h and for 0 or 1 h), estimated sample dilution factor (icosatiles), assessment center indicators, genotyping batch indicators, icosatiles of time of sampling during the day, month of assessment (indicators for each month of participation, with the exception that all of 2006 and August through October of 2010 were assigned a single indicator), and day of assay (one indicator per day assay was performed). | Exposure (LDSC regression, MR, and RCS) |
| Vitamin D | UK Biobank (UKB) (27) | 417,580 Individuals | European | Age at time of assessment, sex, assessment month, assessment center, supplement-intake information, genotyping batch and the first 40 ancestry PCs as covariates. | Exposure (LDSC regression, MR, and RCS) |
| Vitamin K1 | CHARGE Consortium Nutrition Working Group cohorts (28) | 2,138 Individuals | European | Age, sex, and study-specific covariates, including population stratification by PC analysis (PCA) and clinical site. | Exposure (LDSC regression and MR) |
| AF | The Nord-Trøndelag Health Study (HUNT), deCODE, the Michigan Genomics Initiative (MGI), DiscovEHR, UK Biobank, and the AFGen Consortium (20) | 60,620 Cases and 970,216 controls | European | Including covariates birth year, sex, genotype batch, and PCs 1–4. | Outcome (LDSC regression and MR) |
| HF | Heart Failure Molecular Epidemiology for Therapeutic Targets (HERMES) Consortium (21) | 47,309 Cases and 930,014 controls | European | All studies included age and sex (except for single-sex studies) as covariates in the regression models. PCs were included as covariates for individual studies as appropriate. | Outcome (LDSC regression and MR) |
| CAD | CARDIoGRAMplusC4D and UK Biobank (UKB) (22) | 122,733 Cases and 424,528 controls | European | age, gender, the first 30 PCAs | Outcome (LDSC regression and MR) |
| Stroke | MEGASTROKE Consortium (23) | 67,162 Cases and 454,450 controls, cases including 67,162 AS, 60,341 AIS, 6,688 LAS, 9,006 CES, 11,710 SVS. | European | NA | Outcome (LDSC regression and MR) |
| MI | CARDIoGRAMplusC4D, MIGen and CARDIoGRAM Exome consortia, and ESP EOMI datasets (24) | Interrogated the CARDIoGRAMplusC4D (60,801 cases, 123,504 controls), the MIGen and CARDIoGRAM Exome consortia (42,335 cases, 78,240 controls), and ESP EOMI (4,703 cases, 5,090 controls) datasets | European | NA | Outcome (LDSC regression and MR) |
| Longevity | The previously published GWA studies on longevity (25) | 11,262 cases and 25,483 controls | European | NA | Outcome (LDSC regression and MR) |

AF, atrial fibrillation; HF, heart failure; CAD, coronary artery disease; MI, myocardial infarction.

deviation (SD) difference in serum levels of Ca, VD, and VK. The TwoSampleMR (35) and MRPRESSO (36) packages in R version 4.0.2 was used for all analyses.

## Analyses of pleiotropy and heterogeneity

Analyzing potential pleiotropy is vital given that pleiotropic Ivs have the potential to have an indirect impact on study outcomes, serving as confounders of MR analysis efforts. A range of strategies were utilized herein in an effort to detect possible pleiotropy. Initially, heterogeneity among Ivs when utilizing the fixed-effects IVW approach was detected through Cochrane's $Q$ test. Lower levels of heterogeneity are indicative of the possibility that estimates between Ivs vary based on random chance, which can only occur when pleiotropic effects are not evident. In cases where significant heterogeneity was detected, a multiplicative random-effects IVW model would be implemented. An MR-Egger intercept was additionally performed, with a zero intercept ($p > 0.05$) being indicative of a lack of any pleiotropic bias. The MR-PRESSO method was additionally used for global heterogeneity testing and to detect horizontal pleiotropy (36). To assess the extent to which these

associations were under the influence of any one SNP, a leave-one-out sensitivity analysis was conducted. Moreover, all SNPs included in the GWAS catalog database (37) were searched, with the goal of determining the association between those SNPs and risk factors pertaining to CVD incidence and longevity outcomes. After removing pleiotropic SNPs, causal associations were also analyzed.

### RCS analysis

Restrictive cubic spline analyses entail the use of a piecewise polynomial function capable of examining non-linear relationships between predictors and outcomes in a flexible manner (38). Here, spline models were adjusted for covariates including age, sex, body mass index (BMI), genotype batch, assessment center, and Townsend deprivation index (TDI) (39). Multivariate logistic analyses were used to examine relationships between serum Ca or VD concentrations and CVD incidence at the 25, 50, 75, and 95th centiles. When less than 20% of covariate data was absent, these missing values were accounted for through multiple imputations based upon five replicates and a chained equation method using the R MI procedure. Baseline categorical data were summarized across serum Ca and VD concentrations as percentages, while continuous variables were summarized using means and SDs. A two-sided $p < 0.05$ was the threshold of significance. R version 4.0.2 was used for all analyses.

## Results

### Genetic correlations between serum Ca, vitamin D, vitamin K, and CVDs

When examining genetic correlations pertaining to serum Ca levels, the positive genetic correlation following Bonferroni correction was detected for MI [$r_g$ (SE) = 0.890 (0.012); $p = 1.356E{-}04$] and CAD [$r_g$ (SE) = 0.868 (0.014); $p = 3.601E{-}04$]. Serum Ca levels also exhibited a negative genetic correlation with AF, much as VD levels did with CAD [$r_g$ (SE) = −0.061 (0.029); $p = 0.035$; $p_{adj} = 0.350$] (**Figure 1** and **Supplementary Table 1**). No genetic correlations were detected when examining the relationship between VK (VK1, circulating phylloquinone concentrations) and any CVD subtypes. Results from SNV-based heritability testing suggest that these three tested exposures were unrelated to longevity ($p_{range} = 0.722–0.900$).

### Causal associations between serum Ca, vitamin D, vitamin K, and CVDs

When conducting IVW MR analyses, higher VK1 levels were found to be strongly related to the risk of HF ($p = 0.003$, OR per 1-SD increase: 1.044; CI: 1.015–1.074) and LAS ($p = 0.003$, OR per 1-SD increase: 1.172; CI: 1.054–1.302). Potential associations were also observed between increases in VK1 levels and the risk of AS ($p = 0.031$, OR per 1-SD increase: 1.041; CI: 1.004–1.080). MR analyses also indicated a suggestive association between higher serum Ca concentrations and reduced odds of longevity ($p = 0.014$, OR per 1-SD increase: 0.865; CI: 0.770–0.971), with VD levels being significantly associated with reduced odds of longevity ($p = 4.620E{-}04$, OR per 1-SD increase: 0.777; CI: 0.674–0.895) (**Figure 2** and **Supplementary Table 3**). Fixed-effects IVW estimates failed to reveal any causal associations between VK levels and longevity outcomes. Serum Ca or VD levels were also not found to be causally related to all tested CVDs.

To ensure that the causal inferences drawn from MR analyses are valid, it is critical that it be established that SNV-outcome relationships are the result of a given exposure and not the consequence of horizontal pleiotropy or a similar mechanism. Pleiotropy-resistant sensitivity analyses including Cochrane's $Q$ test, as well as MR-Egger intercept, MR-PRESSO, and leave-one-out sensitivity analyses were thus performed (**Supplementary Figures 2**, **3**). Observed relationships between VK levels and HF/LAS/AS remained robust in these analyses (Cochrane's $Q_{HF} = 13.088$, $p_{HF} = 0.159$; MR-Egger intercept, $p_{HF} = 0.197$; and MR-PRESSO, $p_{HF} = 0.181$; Cochrane's $Q_{LAS} = 3.631$, $p_{LAS} = 0.889$; MR-Egger intercept, $p_{LAS} = 0.010$; and MR-PRESSO, $p_{LAS} = 0.900$; and Cochrane's $Q_{AS} = 4.678$, $p_{AS} = 0.791$; MR-Egger intercept, $p_{AS} = 0.953$; and MR-PRESSO, $p_{AS} = 0.804$) (**Table 2** and **Supplementary Table 4**). Consistent directionality was also evident for the relationships between increases in serum Ca/VD levels and longevity across all of these sensitivity analyses (Cochrane's $Q_{calcium} = 372.154$, $p_{calcium} = 0.983$; MR-Egger intercept, $p_{calcium} = 0.197$; and MR-PRESSO, $p_{calcium} = 0.983$ and Cochrane's $Q_{vitaminD} = 26.940$, $p_{vitaminD} = 0.956$; MR-Egger intercept, $p_{vitaminD} = 0.339$; and MR-PRESSO, $p_{vitaminD} = 0.961$) (**Table 2** and **Supplementary Table 4**).

### Plateau points for serum Ca-associated exposures

In total, serum Ca and VD level data in the UK Biobank were available for 429,863 and 448,777 individuals, respectively. Mean ages and gender distributions for these individuals are summarized in **Supplementary Table 5**. Individual VK data was not available with respect to CVD incidence. Through the use of an RCS regression analysis, curvilinear associations were detected between CVDs and both serum Ca and VD levels. All CVDs (CAD, AF, HF, MI, stroke) at the serum Ca plateau point (2.376 mmol/L, $p < 0.001$) were significantly different, whereas four CVDs (MI, stroke, HF, CAD) differed significantly at the VD plateau point (46.8 nmol/L, $p < 0.001$) (**Figure 3**).

## Discussion

In this study, SNV-based genetic correlations and potential causal relationships between levels of serum Ca-associated exposures and both CVD and longevity were assessed. The resultant data were complementary, indicating that both serum Ca and VD were genetically but not causally related to CVD incidence, whereas these serum Ca and VD levels were causally but not genetically associated with longevity. VK levels were also causally related to CVD incidence, but not related to CVDs or longevity in genetic correlation analyses. Genetic correlation tests heritability and co-heritability between two traits, while MR analysis assesses possible causality between exposures and outcomes, which complement each

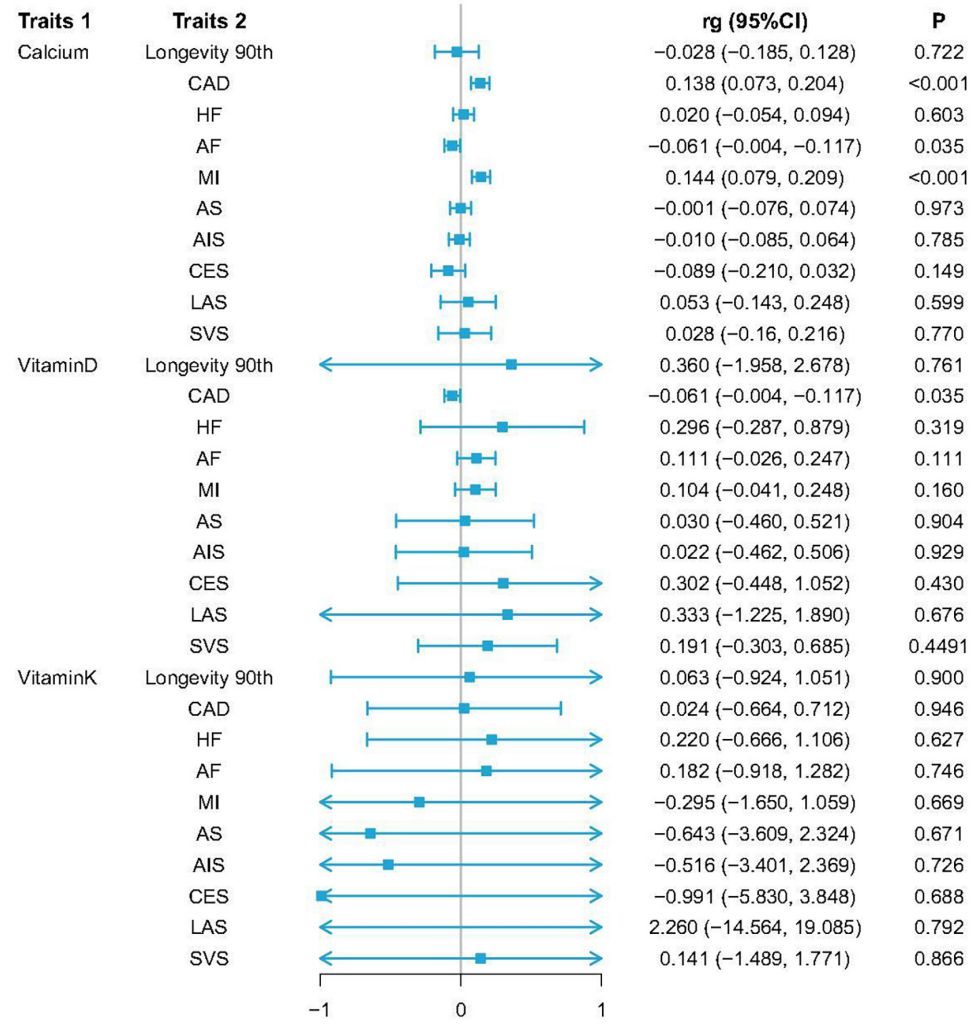


FIGURE 1
Genetic correlation estimates for the associations between serum levels of calcium (Ca), vitamin D (VD), and vitamin K1 (VK1), and cardiovascular diseases (CVDs) as well as longevity. Traits 1 and 2 respectively, correspond to study exposures and outcomes. Error bars denote 95% confidence interval (CIs).

other to indicate a possible relationship between two traits. Plateau points for serum Ca and VD levels associated with reduced CVD risk were also analyzed. Multivariate analyses ultimately revealed that serum Ca and VD levels were non-linearly related to CVD incidence after adjusting for confounding factors ($p < 0.001$) (**Figure 3**). In this European population, the serum Ca plateau point was 2.376 mmol/L, indicating that this concentration was associated with the minimum CVD incidence, in addition to falling within the standard the normal serum Ca clinical reference range (2.2–2.6 mmol/L) (40). Moreover, a VD concentration of 46.8 nmol/L was associated with the lowest risk of CVD incidence.

## Serum Ca and CVD

Calcium is a divalent cation that plays essential roles in diverse physiological processes such as nerve excitation, muscle contraction, the mineralization of the skeleton, and coagulatory function (41, 42). In observational analyses, serum Ca concentrations have been shown to be positively correlated with CVD risk (43, 44). RCT-derived evidence suggests that Ca supplementation, which can lead to acute or persistently elevated serum Ca concentrations (45, 46), can result in a modest increase in the risk of MI and other cardiovascular events (47). An MR analysis of 184,305 participants (including 60,801 CAD cases, of which ∼70% had experienced MI, and 123,504 non-cases) revealed an association between a genetic predisposition toward elevated serum Ca levels and a higher risk of MI and CAD (48). LDSC analyses in this study confirmed a significant positive genetic relationship between serum Ca levels and both CAD ($p = 3.601E{-}04$) and MI ($p = 1.356E{-}04$). Insufficient evidence is currently available regarding the association between serum Ca and AF incidence, in line with the weak negative genetic correlation between these variables observed in this study. The inverse genetic associations between serum Ca and both CAD and MI, and serum Ca and AF may be due to the non-linear relationship between Ca and CVDs itself. Both genetic correlation analysis and MR are based on the assumption of linear relationship between serum Ca and CVDs, which may need to be further explained by observational data. However, our results of RCS just confirmed that there is a U-shaped relationship between them through individual observational data analysis.

Recent epidemiological evidence further suggests that circulating Ca levels are associated with CVD-related mortality and with

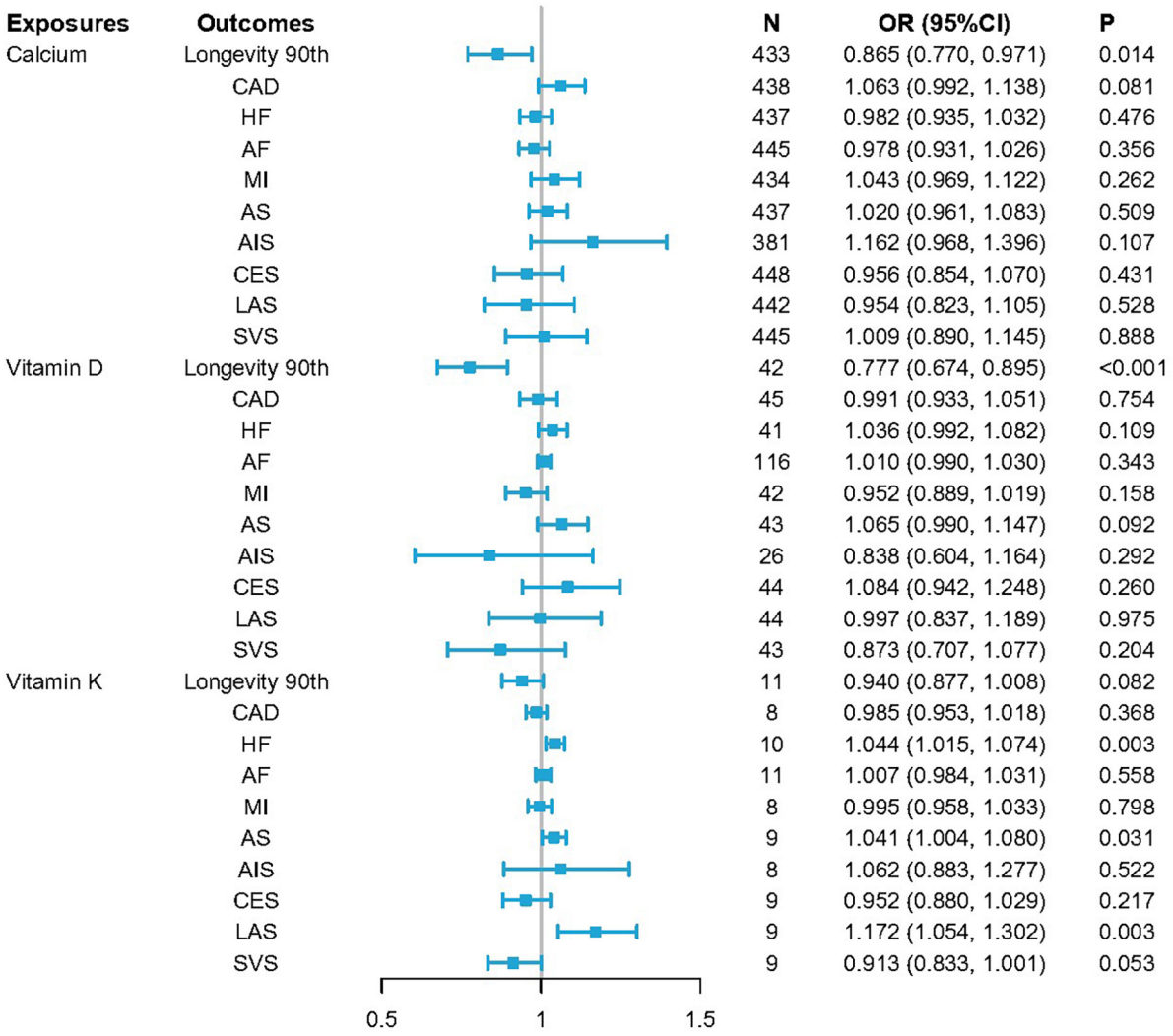


FIGURE 2
$OR_{SD}$ for causal associations between serum levels of calcium (Ca), vitamin D (VD), and vitamin K1 (VK1), and cardiovascular diseases (CVDs) as well as longevity. Odds ratio (OR) estimates for individual single nucleotide variants (SNVs) were made using the inverse-variance-weighted (IVW) method. $OR_{SD}$ = OR for standard deviation (SD) unit increases in risk factors.

longevity (43, 44, 49, 50). Specifically, elevated levels of serum Ca were linked to an increase in the odds of non-fatal CVD (HR = 1.12, 95% CI 1.10–1.14, MI: 1.19, 1.14–1.25) and fatal CVD (HR = 1.41, 95% CI 1.35–1.47; MI: 1.41, 1.31–1.51) (44). The MR analysis conducted herein also revealed a causal relationship between serum Ca levels and longevity ($p$ = 0.014). Higher serum Ca levels were significantly associated with longevity and were negatively correlated with the incidence of AS, SVS, AIS, MI, and CAD, although these latter relationships were not significant. The combination of MR and LDSC results revealed a correlation between serum Ca levels, CVDs, and longevity.

## Vitamin D and CVD

Vitamin D plays an essential role in regulating Ca homeostasis, but the expression of nuclear VD receptor (VDR) by cardiomyocytes and vascular endothelial cells suggests that VD may be directly involved in the development and progression of CVD (51). These data thus prompted a more in-depth analysis of VD in addition to Ca.

In published studies, a 1.41-fold greater risk of CVD mortality (95% CI: 1.18–1.68) for individuals in the lowest plasma VD quintile based on a meta-analysis of prospective cohort studies. Acute VD deficiencies can contribute to inflammation and impaired insulin secretion, thereby increasing the odds of plaque rupture and arterial thrombosis. Chronically insufficient VD levels can contribute to increased arterial stiffness (52). Overall, VD deficiencies are detrimental to cardiovascular or longevity outcomes over any time scale. Observational results suggest that low levels of serum 25-hydroxyVD [25(OH)D], with is the primary form in which VD is stored, are related to an elevated risk of CVD incidence and mortality (53). VD deficiency has also been found to be associated with a more severe cardiovascular risk profile and increased CAD prevalence (54). VD was also shown to suppress NF-κB pathway signaling within cells to inhibit the progression of CAD, highlighting a possible mechanism whereby VD may mitigate vascular inflammation and atherosclerosis (55). The genetic and causal association analyses conducted herein revealed VD levels to be genetically related to CAD ($p$ = 0.035) and causally related to longevity ($p$ = 4.620E–04), confirming the association between VD exposure and these endpoints. An inverse

TABLE 2 Results of potential pleiotropy and heterogeneity assessments.

| Exposures | Outcomes | Cochran's *Q* statistic | *P*-value for Cochran's *Q* | *p*-value for intercept | MR-PRESSO global test |
|---|---|---|---|---|---|
| Calcium | CAD | 665.368 | 1.015E−11 | 0.789 | <1E−04 |
| | MI | 606.473 | 6.667E−08 | 0.758 | <1E−04 |
| | AF | 685.478 | 1.311E−12 | 0.891 | <1E−04 |
| | HF | 505.539 | 0.012 | **0.020** | **0.011** |
| | AIS | 358.083 | 0.784 | 0.865 | 0.787 |
| | AS | 518.219 | 0.004 | 0.821 | **0.004** |
| | CES | 461.504 | 0.308 | 0.912 | 0.317 |
| | LAS | 433.451 | 0.592 | 0.910 | 0.593 |
| | SVS | 486.558 | 0.080 | 0.806 | 0.084 |
| | Longevity 90 | 372.154 | 0.983 | 0.197 | 0.983 |
| Vitamin D | CAD | 31.876 | 0.913 | 0.709 | 0.919 |
| | MI | 25.366 | 0.974 | 0.394 | 0.977 |
| | AF | 102.814 | 0.785 | 0.286 | 0.790 |
| | HF | 42.513 | 0.363 | 0.600 | 0.419 |
| | AIS | 13.268 | 0.973 | 0.885 | 0.973 |
| | AS | 22.313 | 0.995 | **0.030** | 0.995 |
| | CES | 40.566 | 0.577 | 0.582 | 0.599 |
| | LAS | 48.618 | 0.257 | 0.879 | 0.274 |
| | SVS | 69.788 | 0.005 | 0.183 | **0.004** |
| | Longevity 90 | 26.940 | 0.956 | 0.339 | 0.961 |
| Vitamin K1 | CAD | 1.659 | 0.976 | 0.309 | 0.979 |
| | MI | 5.118 | 0.646 | 0.838 | 0.662 |
| | AF | 0.969 | 1.000 | 0.532 | 1.000 |
| | HF | 13.088 | 0.159 | 0.197 | 0.181 |
| | AIS | 14.952 | 0.037 | **0.024** | **0.046** |
| | AS | 4.678 | 0.791 | 0.953 | 0.804 |
| | CES | 7.650 | 0.468 | 0.125 | 0.489 |
| | LAS | 3.631 | 0.889 | **0.010** | 0.900 |
| | SVS | 4.978 | 0.760 | 0.112 | 0.756 |
| | Longevity 90 | 8.363 | 0.593 | 0.421 | 0.617 |

*P*-values below the threshold of 0.05 are displayed in bold.

relationship was observed between VD and CES, AS, HF, and AF incidence, but these relationships did not attain the level of statistical significance.

## Vitamin K and CVD

As a fat-soluble vitamin, VK is required for the activation of certain proteins and has been suggested to play some role in CVD incidence. Through anti-inflammatory activity that has been observed *in vitro* and *in vivo*, VK can potentially protect against vascular calcification, thus lowering the odds of CVD development and all-cause mortality (17). One observational prospective analysis of 601 individuals found lower VD and VK levels to be related to adverse cardiac remodeling and greater all-cause mortality risk (56). Conversely, a meta-analysis of three cohorts in the USA found VK1 levels to be related to all-cause mortality risk but unrelated to CVD (57). Circulating VK1 levels were also found not to be causally associated with CHD in a prior two-sample MR study (RR = 1.00, 95% CI: 0.98–1.04) (58). Here, analyses of different CVD subtypes revealed VK1 levels to be causally associated with HF ($p$ = 0.003), AS ($p$ = 0.031), and LAS ($p$ = 0.003). However, epidemiological data pertaining to correlations between VK1 and various CVD subtypes are lacking at present, underscoring the need for further research focused on this topic and the underlying mechanisms that link VK levels between CVD or other health outcomes.

## Clinical implications

Calcium supplementation is a common practice in the USA, and there is rising clinical interest with respect to the association between these supplements and CVD. Some work suggests that Ca supplements may lower blood pressure and contribute to better

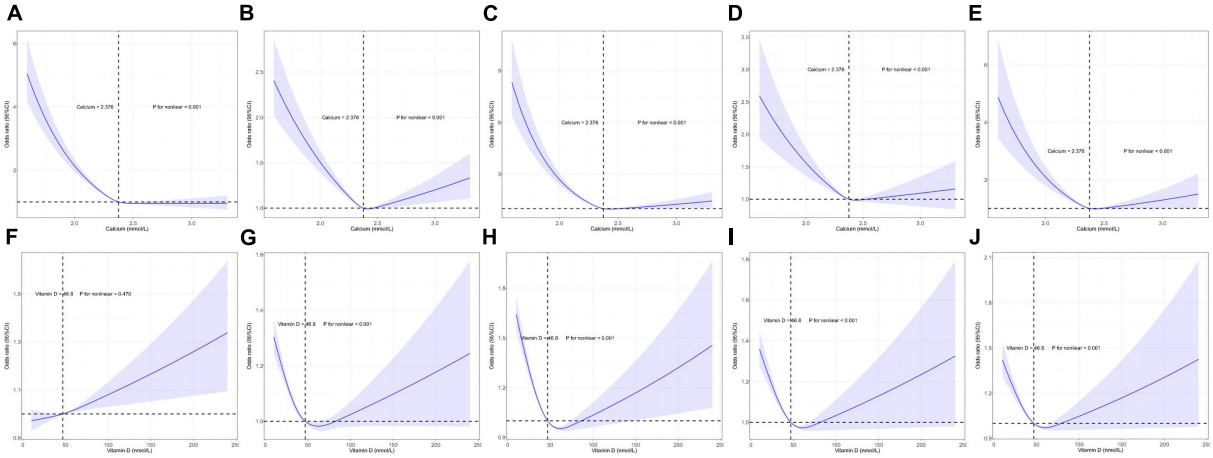


FIGURE 3
Restricted cubic spline model-based analyses of the association between serum calcium (Ca)/vitamin D (VD) levels and the risk of cardiovascular diseases (CVDs). **(A–E)** Curvilinear relationships between serum Ca levels and atrial fibrillation (AF), coronary artery disease (CAD), heart failure (HF), myocardial infarction (MI), and stroke. **(F–J)** Curvilinear relationships between VD concentrations and AF, CAD, HF, MI, and stroke. The *y*-axis represents the log of logistic regression model-derived odds ratios (ORs), while the shaded area denotes the corresponding 95% confidence interval (CIs) for these adjusted ORs. A plateau in CVD risk was evident in the risk function.

serum lipid profiles, yet they also have the potential to increase serum Ca levels, thereby elevating the risk of vascular calcification and concomitant CVD event incidence. Perhaps unsurprisingly, prior research has yielded conflicting results with respect to the relationships between CVD and Ca supplementation (13). This issue is made more complex by the fact that many adults seek to improve their bone health through the combined intake of Ca and VD supplements despite the inconclusive evidence suggesting possible relationship between Ca intake and the risk of CVD (53). Some adverse effects have been reported in individuals utilizing supplemental VD and Ca alone or in combination, with these effects likely being attributable to the dose of supplemental Ca utilized (59). The present results suggest a genetic relationship between serum Ca, VD concentrations, and CVD incidence such that these serum biomarkers may offer value for the selection of appropriate nutritional interventions designed to mitigate CVD-related risk. Importantly, this study enabled the establishment of threshold Ca and VD concentrations in CVD patients and healthy controls, revealing that serum Ca and VD levels of 2.376 mmol/L and 46.8 nmol/L, respectively, were related to the lowest risk of CVD development among individuals of European heritage. When these levels fall too far above or below these levels, they may contribute to CVD development. A study of 441,738 individuals during a median follow-up time of 21 years found that serum Ca concentrations greater than 2.40 nmol/L were associated with increased risk of non-fatal CVD (44). Another study discovered L-shaped associations between VD level and CVD mortality. When VD concentrations were less than 27.70 nmol/L, the risk of death from CVD was increased. When VD concentrations exceeded 54.40 nmol/L, there was no association with all-cause mortality in America (60). Although no studies have yet provided exact epidemiological data on serum Ca and VD concentrations, the range of concentrations given by previous studies supports our results. At the same time, the Ca (2.376 mmol/L) and VD (46.8 nmol/L) plateau points of CVD are within the standard the normal clinical reference range. Accordingly, the intake of Ca or VD from primarily dietary sources may be most appropriate, reserving the minimum necessary Ca/VD supplementation for individuals dealing with Ca/VD intake deficiencies following the exhaustion of other forms of dietary modification (53).

When analyzing supplemental VK1 intake, following adjustment for confounding lifestyle and demographic factors, moderate-to-high VK1 intake levels (87–192 μg/days) were related to a decrease in the odds of all-cause [HR (95% CI): 0.76 (0.72, 0.79)], and CVD-related [HR (95% CI): 0.72 (0.66, 0.79)] (61). These findings confirmed that VK1 levels were related to AS, LAS, and HF. However, individual VK1 data were unavailable such that it was not possible to estimate the threshold levels necessary to minimize the risk of CVD development. As such, further research will be needed to provide specific guidance regarding supplemental VK1 dosing in different populations.

## Strengths and limitations

A major strength of this study is that the analyses of serum Ca-associated exposures for the nine included CVD types and overall longevity were performed using the largest GWAS datasets available. Causal inferences should generally be based upon several study types given that MR analyses are based on three major assumptions that are not always met or fully testable (62, 63). Genetic correlation analyses were thus used herein in an effort to complement MR-related research design limitations. Furthermore, these outcomes included both specific analyses for nine CVD subtypes as well as longevity as a control outcome for co-analyses, thereby strengthening the overall reliability of these findings. An additional strength of this approach is that the genetic instruments employed herein were selected based on a recent European population GWAS dataset for individuals with accessible serum Ca and plasma VD/K1 levels together with summary-level information regarding CVDs and longevity. These results are not likely to have been affected by population stratification bias given that these were GWAS data for individuals who were primarily of European ancestry. Lastly, correlation analyses of the associations between

serum Ca and VD levels and the CVD risk factors enabled the estimation of the serum Ca and VD levels associated with the minimum CVD risk, thus enabling the establishment of recommended threshold levels for these nutrients aimed at mitigating the odds of CVD development.

There are certain limitations to this analysis. For one, the GWAS study used for these analyses was derived from a public database pertaining to a European population, and the results may thus not be applicable to populations of Asian, African, or other ancestries. In addition, individual-level VK data were not available for these European CVD patients, precluding the establishment of optimal concentrations of this vitamin for cardiovascular health.

## Conclusion

Our findings support a genetic correlation between serum Ca and VD and CVD risk, and a causal relationship between VK1 levels and CVD risk. The optimal serum Ca and vitamin plasma D concentrations associated with the minimum risk of CVDs were 2.376 mmol/L and 46.8 nmol/L, respectively. Whether plasma VK1 levels can contribute to improved CVD outcomes and extend lifespan, however, has yet to be established.

## Data availability statement

Publicly available datasets were analyzed in this study. These data can be found here: Summary statistics from the GWAS used in this study are publicly accessible in the published literature and UK Biobank Resource which are shown in **Table 1**.

## Author contributions

XN, LL, YY, CZ, and HS conceived and designed the study, literature search, and wrote the original draft. YY, YL, RL, ZC, and WH did the data collection, formal analysis, and methodology. XN, LL, HS, and QZ did the visualization and methodology. LS, XZ, ZY, HZ, and SZ accessed and verified the data. CH, ZY, and HY did project administration and coordination and reviewed and edited the manuscript. All authors had final responsibility for the decision to submit for publication.

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## Conflict of interest

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

The handling editor declared a shared affiliation with the author, YY at the time of review.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2023.1096662/full#supplementary-material

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# Cognitive decline among older adults with heart diseases before and during the COVID-19 pandemic: A longitudinal cohort study

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**Background:** Little is known about the impact induced by the COVID-19 pandemic on the cognitive function of older adults with heart diseases. This study aimed to examine whether older adults with heart diseases suffered larger cognitive deterioration during the COVID-19 pandemic.

**Methods:** This study leveraged longitudinal data from the Health and Retirement Study (HRS), a nationally representative U.S. aging cohort with objective cognitive assessments measured before and during the pandemic. The interval from HRS waves 13 to 14 (April 2016 to June 2019) was defined as the pre-pandemic period to control the pre-existed cognitive difference between participants with and without heart diseases, and the interval from waves 14 to 15 (June 2019 to June 2021) was defined as the pandemic period. The HRS wave 14 survey was considered the baseline. The heart disease status was defined by a self-reported diagnosis. Linear mixed models were performed to evaluate and compare the cognitive differences during different periods.

**Results:** A total of 9,304 participants (women: 5,655, 60.8%; mean age: 65.8 ± 10.8 years) were included, and 2,119 (22.8%) had heart diseases. During the pre-pandemic period, there was no significant difference (−0.03, 95% CI: −0.22 to 0.15, $P = 0.716$) in the changes in global cognitive scores between participants with and without heart disease. During the pandemic period, a larger decreased change in the global cognitive score was observed in the heart disease group compared with the non-heart disease group (−0.37, 95% CI: −0.55 to −0.19, $P < 0.001$). An enlarged difference in global cognitive score was observed during the pandemic period (−0.33, 95% CI: −0.65 to −0.02, $P = 0.036$).

**Conclusion:** The findings demonstrated that the population with heart diseases suffered more cognitive decline related to the pandemic, underscoring the necessity to provide immediate cognitive monitoring and interventions for the population with heart diseases.

KEYWORDS

**heart diseases, cognitive decline, dementia, COVID-19 pandemic, older adult**

# 1. Introduction

Since 11 March 2020, the World Health Organization has designated the coronavirus disease 2019 (COVID-19) as a global epidemic (1). This pandemic has exerted an unprecedented impact on the multi-dimension of people's lives. Notably, it has intrigued health concerns on non-communicable diseases due to the constraints on healthcare resources and changes in public mental wellbeing and behaviors (2, 3). It is of vital clinical and public health importance to understand the consequence of the pandemic on non-communicable diseases and to better adapt responses to this persistent pandemic crisis.

Heart diseases are the leading cause of morbidity and mortality in older adults (4). During the pandemic, most outpatient visits, elective procedures, cardiac rehabilitation, and telemedicine programs have been canceled or postponed to prioritize the care of patients with COVID-19 (5, 6). The reduced access to healthcare has affected the vulnerable population with heart disease. Moreover, the enforced social isolation during the pandemic has caused a spectrum of mental disorders and unhealthy lifestyles, which are recognized cardiovascular risk factors and contribute to poorer prognosis in the population with heart diseases (7–10). The European Society of Cardiology has issued guidance for the management of cardiovascular diseases during the COVID-19 pandemic to mitigate the deleterious impact of the pandemic (11). However, it is worthwhile that the adverse outcomes of the pandemic on the population with heart diseases might not be limited to cardiac manifestation. Even before the pandemic, accumulated evidence has proven that older adults with heart diseases exhibit elevated risks of cognitive decline and dementia, potentially owing to multiple mechanisms, including atherosclerotic processes, vascular oxidative stress, and inflammation response (12, 13). The latest American Heart Association (AHA) Heart Disease and Stroke Statistics demonstrated that promoting cardiovascular health would help retain cognitive function and achieve healthy aging (14). The exacerbation of cardiovascular health during the pandemic could further exaggerate cognitive decline among older adults with heart diseases. The existing evidence has indicated a significant decline in cognitive function during the pandemic among older adults (15–17). Still, it is important to further identify the most vulnerable population toward the pandemic-induced cognitive decline for service providers and policymakers.

We, therefore, aimed to examine whether older adults with heart diseases suffered larger cognitive deterioration during the COVID-19 pandemic. The present study was designed in the framework of a well-established U.S. aging cohort. We took advantage of the available objective cognitive assessments measured before and during the pandemic, to account for the existing difference in cognitive function between people with and without heart diseases preceding the pandemic, and thus accurately detecting the impact directly related to the pandemic. We hypothesized that the pandemic would induce an enlarged gap in cognitive function between people with and without heart disease.

# 2. Materials and methods

## 2.1. Study population

The Health and Retirement Study (HRS) is a nationally representative longitudinal cohort study of U.S. community dwellers aged 50 years and older, which has been conducted biennially since 1992. Detailed conception and methods of this study have been well documented elsewhere (18). The HRS was approved by the Institutional Reviewing Board at the University of Michigan and the National Institute on Aging (HUM00061128), and all participants have provided written informed consent.

The timeline of the present study is exhibited in **Figure 1**. The interval between HRS wave 13 (April 2016 to April 2018) and wave 14 (April 2018 to June 2019) was considered the control period. The interval between wave 14 and wave 15 (March 2020 to June 2021) was considered the pandemic period. The first confirmed COVID-19 case in the United States was reported on 20 January 2020 (19), and the number of cumulative-confirmed cases during HRS wave 15 from 1 March 2020 to 30 June 2021 elevated from 32 to 33.78 million.

HRS wave 14 was considered the baseline. As shown in **Supplementary Figure 1**, among a total of 17,146 participants who attended the wave 14 survey, we excluded 7,295 participants without complete data on cognitive assessment at any one wave from wave 13 to 15 surveys and 547 participants with existing dementia before the pandemic. Finally, 9,304 participants were included in the present study. **Supplementary Table 1** shows the differences in characteristics between included and excluded participants. The excluded participants were significantly older and less healthy.

## 2.2. Heart diseases ascertainment

We identified the heart disease status by the following question in HRS wave 14: "Has a doctor told you that you have had a heart attack, coronary heart disease, angina, congestive heart failure, or other heart problems?" Participants who reported "Yes" were defined as having heart diseases; otherwise, they were regarded as without heart diseases.

## 2.3. Cognitive assessments

The HRS evaluated cognitive function *via* an adapted version of the Telephone Interview for Cognitive Status (20), which is a sensitive screening tool fit for large-scale population-based surveys. The validity and consistency of the HRS cognitive assessments have been well documented (21, 22).

The HRS assessed memory (the immediate and delayed word recall test ranged from 0 to 20 points) and executive function (one was the serial 7's subtraction test, which ranged from 0 to 5 points to evaluate working memory, and another was the counting backward test, which ranged from 0 to 2 points to evaluate processing speed and attention) on all respondents. The global cognitive score was the summary of two component scores (ranging from 0 to 27 points), and the higher score manifested better cognitive performance. As shown in previous studies, participants with a global cognitive score

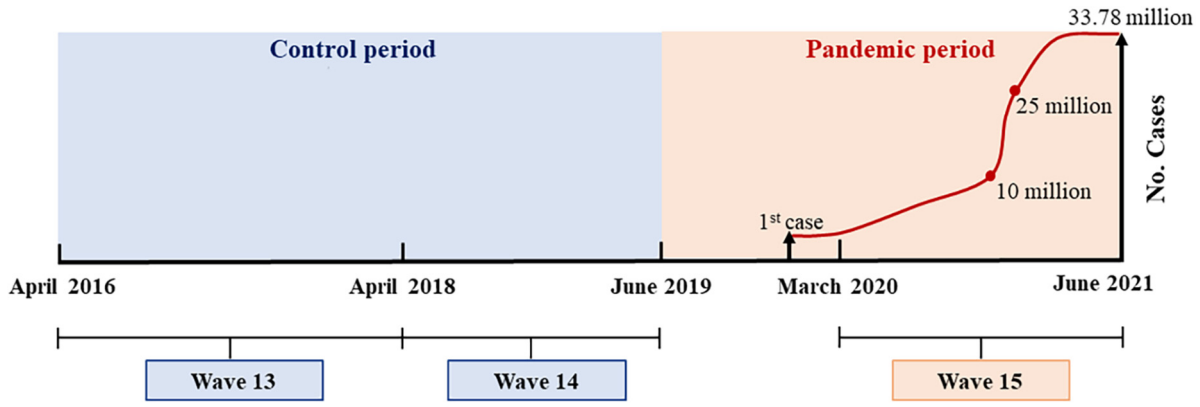


FIGURE 1
Timeline of Health and Retirement Study (HRS) surveys and cumulative-confirmed coronavirus disease 2019 (COVID-19) cases in the United States.

of fewer than 7 points were regarded as having dementia (21, 23). The definition of dementia in the present study was a self-reported diagnosis of dementia with a global cognitive score of fewer than 7 points.

## 2.4. Covariates

Potential confounders commonly associated with heart diseases and cognitive decline were selected as *a priori* based on the previous literature (12, 24, 25). These included age at baseline (years), sex, race, educational level, cohabitation status, current lifestyle including smoking, drinking, and physical activity, depressive symptoms, and status of chronic diseases including hypertension, diabetes, stroke, cancer, and chronic lung diseases. Race was divided as white ethnicity or not. A high educational level referred to those who received an education of 12 years or above. The cohabitation status was categorized as living alone at present or not. Participants were categorized into current drinkers (no less than once a week) and non-drinkers (including ex-drinkers), as well as current smokers and non-smokers (including ex-smokers). Physical activity was defined as engaging in weekly moderate or vigorous physical activities at least once. Depressive symptoms were evaluated by using 8-item Center for Epidemiologic Studies Depression Scale (CES-D, the total score ranged from 0 to 8 points), consistent with prior studies (26, 27), and participants who scored 4 or above were regarded as having depressive symptoms. Hypertension was defined as systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mmHg, or self-reported diagnosis of hypertension or use of anti-hypertension drugs. Diabetes was defined as an $HbA_{1c}$ of ≥6.5% or self-reported diagnosis of diabetes or the use of anti-diabetic therapy. Other identifications of chronic diseases were based on self-reported diagnoses.

## 2.5. Statistical analysis

The results were presented as the percentage for categorical variables, as well as means ± standard deviations (SD) for continuous variables. Baseline characteristic differences between different heart diseases status were compared by the *t*-test or chi-square test for continuous and categorical variables, respectively.

Linear mixed models were employed to compare the differences in the changes of global cognitive scores by heart disease status during the pre-pandemic period and pandemic period. We adjusted all the covariates mentioned earlier in the linear mixed model. Heart disease status and time were included as classified variables in the model. Time = 0, 2, and 4 were referred to as waves 13, 14, and 15 of the HRS, respectively. At first, least square means (LSMs) and 95% confidence intervals (CIs) after multivariable adjustment of global cognitive scores by heart disease status and time were derived from models. Then, LSM differences in global cognitive scores between heart diseases status at each wave were calculated, and thus, the differences between heart diseases status in the changes of global cognitive scores during the pre-pandemic period and the pandemic period could be estimated, respectively. Finally, we considered the pre-pandemic period as the reference and determined whether the difference between heart disease status in the changes in global cognitive scores during the COVID-19 pandemic period was larger.

In addition, we also repeated the analysis on every single cognitive domain. In sensitivity analysis, we explored potential modified effects of covariates and COVID-19 infection which was defined as the participant self, or his relatives or friends had an infection of COVID-19, on the differences in global cognitive scores between people with and without heart diseases during the pandemic period compared with those during the pre-pandemic period. *Z*-test was applied to examine interaction effects between different subgroups (28).

All analyses were conducted by SAS 9.4 software (SAS Institute Inc., Cary, NC, USA), and a two-sided α value of 0.05 was considered as statistical significance.

# 3. Results

## 3.1. Baseline characteristics

A total of 9,304 participants (women: 5,655, 60.8%; mean age: 65.8 ± 10.8 years) who attended the HRS wave 13–15 surveys were included in the present analysis. All of them have completed cognitive assessments in each of the three waves. There were 2,119 participants with heart disease (22.8%) and 7,185 participants without heart disease (77.2%). The distribution of baseline characteristics by

TABLE 1 Baseline characteristics of included participants, by heart diseases status.

| Characteristics | Heart disease group ($n = 2119$) | Non-heart disease group ($n = 7185$) | $P$* |
|---|---|---|---|
| Age (years) | 70.3 ± 10.8 | 64.4 ± 10.4 | <0.001 |
| Female (%) | 1172 (55.3) | 4483 (62.4) | <0.001 |
| White (%) | 1453 (68.6) | 4372 (60.8) | <0.001 |
| High educational level (%) | 1706 (80.5) | 5800 (80.7) | 0.826 |
| Living alone (%) | 880 (41.5) | 2641 (36.8) | <0.001 |
| Current smoking (%) | 249 (11.8) | 1010 (14.1) | 0.006 |
| Current drinking (%) | 696 (32.8) | 2916 (40.6) | <0.001 |
| Physical active (%) | 1329 (62.7) | 5233 (72.8) | <0.001 |
| Depressive symptoms (%) | 393 (18.5) | 904 (12.6) | |
| Chronic diseases status | | | |
| Hypertension (%) | 1718 (81.1) | 4550 (63.3) | <0.001 |
| Diabetes (%) | 881 (41.6) | 2062 (28.7) | <0.001 |
| Stroke (%) | 346 (16.3) | 355 (4.9) | <0.001 |
| Cancer (%) | 408 (19.3) | 884 (12.3) | <0.001 |
| Chronic lung diseases (%) | 405 (19.1) | 566 (7.9) | <0.001 |
| Cognitive scores | | | |
| Global cognitive score | 15.3 ± 3.8 | 16.0 ± 4.0 | <0.001 |
| Memory score | 9.9 ± 3.1 | 10.6 ± 3.2 | <0.001 |
| Executive function score | 5.4 ± 1.7 | 5.4 ± 1.7 | 0.638 |

Data are presented as mean ± SD or n (%).
*The differences between heart disease participants and non-heart disease participants were tested using the *t*-test or chi-square test.

heart disease status is shown in Table 1. Overall, participants with heart diseases were older and had a larger proportion of white ethnicity, with a lower percentage of women, drinking, smoking, and physical activity, while a higher percentage of those having depressive symptoms and chronic diseases exhibited lower global cognitive scores and memory scores.

## 3.2. Differences in cognitive changes before and during the pandemic

As shown in Table 2, after adjusting for multiple covariates, in the heart disease group, the LSM of global cognitive scores in wave 13, wave 14, and wave 15 was 15.70 (95% CI: 15.54 to 15.85), 15.91 (95% CI: 15.76 to 16.07), and 15.32 (95% CI: 15.14 to 15.49), respectively. In the non-heart disease group, the LSM in each wave was 15.60 (95% CI: 15.51 to 15.68), 15.85 (95% CI: 15.77 to 15.93), and 15.62 (95% CI: 15.53 to 15.72), respectively. There were no significant differences in global cognitive scores between people with and without heart diseases before the pandemic (wave 13 and wave 14, respectively), while the global cognitive score in the heart disease group was significantly lower than that in the non-heart disease group at wave 15. During the pre-pandemic period, significantly increased changes in global cognitive scores were observed both in the heart disease group and non-heart disease group, respectively. However, no significant difference in the changes in global cognitive scores between people with and without heart diseases was detected (LSM difference: −0.03, 95% CI: −0.22 to 0.15, $P = 0.716$).

During the pandemic period, significant decreases in global cognitive scores from wave 14 to wave 15 were observed in both the heart disease group and the non-heart disease group, respectively. A larger decreased change in global cognitive score was observed in the heart disease group compared with the non-heart disease group (−0.37, 95% CI: −0.55 to −0.19, $P < 0.001$). Compared with the change in the global cognitive score during the pre-pandemic period, people with different heart diseases status exhibited disproportionate cognitive decline during the pandemic: −0.81 (95% CI: −1.09 to −0.54, $P < 0.001$) in the heart disease group and −0.48 (95% CI: −0.63 to −0.33, $P < 0.001$) in the non-heart disease group, respectively. Furthermore, using the cognitive difference between people with and without heart diseases during the pre-pandemic period as the reference, we found that the extent of global cognitive difference among groups was significantly larger during the pandemic period (−0.33, 95% CI: −0.65 to −0.02, $P = 0.036$).

In addition, generally consistent results were yielded in specific cognitive domains. As shown in Tables 3, 4, in the heart disease group, the LSM of memory scores in each wave was 10.15 (95% CI: 10.02 to 10.28), 10.45 (95% CI: 10.32 to 10.57), and 9.97 (95% CI: 9.82 to 10.11), and the LSM of executive function scores was 5.55 (95% CI: 5.48 to 5.62), 5.47 (95% CI: 5.40 to 5.54), and 5.36 (95% CI: 5.28 to 5.43), respectively. In the non-heart disease group, the LSM of memory scores in each wave was 10.14 (95% CI: 10.07 to 10.21), 10.46 (95% CI: 10.39 to 10.52), and 10.26 (95% CI: 10.18 to 10.36), and the LSM of executive function scores was 5.46 (95% CI: 5.42 to 5.49), 5.39 (95% CI: 5.36 to 5.43), and 5.36 (95% CI: 5.32 to 5.40), respectively. There were no significant differences in the changes in memory scores or executive function scores between people with and without heart diseases during the pre-pandemic period. Significantly larger decreased changes were observed in the heart disease group compared with the non-heart disease group in memory scores (−0.28, 95% CI: −0.44 to −0.12, $P < 0.001$), as well as in executive function scores during the pandemic period (−0.09, 95% CI: −0.16 to −0.01, $P = 0.020$). After accounting for the cognitive difference during the pre-pandemic period, people with heart diseases experienced −0.26 (95% CI: −0.54 to 0.02, $P = 0.068$) points of decline in memory scores, as well as −0.07 (95% CI: −0.20 to 0.05, $P = 0.254$) points of decline in executive function scores during the pandemic period.

## 3.3. Sensitivity analyses

Subgroup analyses were conducted to explore potential modified effects. We observed that the difference in global cognitive scores between people with and without heart diseases during the pandemic period compared with that during the pre-pandemic period was −0.59 (95% CI: −1.01 to −0.17, $P = 0.006$) among female participants, significantly larger than male participants (0.03, 95% CI: −0.42 to 0.49, $P = 0.893$), and the $P$-value for interaction was 0.047. We also found that the pandemic-related difference in global cognitive scores was −0.58 (95% CI: −0.96 to −0.19, $P = 0.003$) among physical active participants, significantly larger than those physical inactive participants (0.13, 95% CI: −0.39 to 0.65, $P = 0.616$), with a $P$-value for the interaction of 0.030. Neither other covariate

TABLE 2 Differences in the changes of global cognitive scores before and during the pandemic period, by heart disease status.

| Global cognitive scores, LSM (95% CI)* | | | | |
|---|---|---|---|---|
| | Heart diseases group ($n = 2119$) | Non-heart diseases group ($n = 7185$) | LSM differences between groups* | *P* for differences between groups* |
| **Before pandemic** | | | | |
| Wave 13 (2016) | 15.70 (15.54, 15.85) | 15.60 (15.51, 15.68) | 0.10 (−0.09, 0.28) | 0.301 |
| Wave 14 (2018) | 15.91 (15.76, 16.07) | 15.85 (15.77, 15.93) | 0.06 (−0.12, 0.24) | 0.491 |
| LSM differences between waves* | 0.22 (0.06, 0.38) | 0.25 (0.16, 0.34) | −0.03 (−0.22, 0.15) | 0.716 |
| *P* for differences between waves* | 0.008 | <0.001 | 0.716 | / |
| **During pandemic** | | | | |
| Wave 14 (2018) | 15.91 (15.76, 16.07) | 15.85 (15.77, 15.93) | 0.06 (−0.12, 0.24) | 0.491 |
| Wave 15 (2020) | 15.32 (15.14, 15.49) | 15.62 (15.53, 15.72) | −0.31 (−0.51, −0.11) | 0.003 |
| LSM differences between waves* | −0.60 (−0.76, −0.44) | −0.23 (−0.31, −0.14) | −0.37 (−0.55, −0.19) | <0.001 |
| *P* for differences between waves* | <0.001 | <0.001 | <0.001 | / |
| **During pandemic vs. Before pandemic** | | | | |
| Differences in LSM differences between two periods* | −0.81 (−1.09, −0.54) | −0.48 (−0.63, −0.33) | −0.33 (−0.65, −0.02) | 0.036 |
| *P* for differences in LSM differences between two periods* | <0.001 | <0.001 | 0.036 | / |

*Differences were calculated by linear mixed model, after adjusting for age, sex, race, education, cohabitation status, current smoking, current drinking, physical active, depressive symptoms, status of hypertension, diabetes, stroke, cancer, and chronic lung diseases.

TABLE 3 Differences in the changes of memory scores before and during the pandemic period, by heart disease status.

| Memory scores, LSM (95% CI)* | | | | |
|---|---|---|---|---|
| | Heart diseases group ($n = 2119$) | Non-heart diseases group ($n = 7185$) | LSM differences between groups* | *P* for differences between groups* |
| **Before pandemic** | | | | |
| Wave 13 (2016) | 10.15 (10.02, 10.28) | 10.14 (10.07, 10.21) | 0.01 (−0.14, 0.16) | 0.901 |
| Wave 14 (2018) | 10.45 (10.32, 10.57) | 10.46 (10.39, 10.52) | −0.01 (−0.16, 0.13) | 0.881 |
| LSM differences between waves* | 0.30 (0.15, 0.44) | 0.32 (0.24, 0.39) | −0.02 (−0.18, 0.14) | 0.805 |
| *P* for differences between waves* | <0.001 | <0.001 | 0.805 | / |
| **During pandemic** | | | | |
| Wave 14 (2018) | 10.45 (10.32, 10.57) | 10.46 (10.39, 10.52) | −0.01 (−0.16, 0.13) | 0.881 |
| Wave 15 (2020) | 9.97 (9.82, 10.11) | 10.26 (10.18, 10.36) | −0.29 (−0.46, −0.13) | <0.001 |
| LSM differences between waves* | −0.48 (−0.62, −0.34) | −0.20 (−0.27, −0.12) | −0.28 (−0.44, −0.12) | 0.001 |
| *P* for differences between waves* | <0.001 | <0.001 | 0.001 | / |
| **During pandemic vs. Before pandemic** | | | | |
| Differences in LSM differences between two periods* | −0.77 (−1.02, −0.53) | −0.51 (−0.65, −0.38) | −0.26 (−0.54, 0.02) | 0.068 |
| *P* for differences in LSM differences between two periods* | <0.001 | <0.001 | 0.068 | / |

*Differences were calculated by the linear mixed model, after adjusting for age, sex, race, education, cohabitation status, current smoking, current drinking, physical active, depressive symptoms, status of hypertension, diabetes, stroke, cancer, and chronic lung diseases.

nor COVID-19 infection was observed to play a modified role (**Supplementary Figure 2**).

## 4. Discussion

Leveraging longitudinal data from a nationally representative aging cohort in the United States, we observed that older adults with heart diseases exhibited a greater cognitive decline compared with those without heart diseases during the COVID-19 pandemic, while no significant difference in the change of cognitive function was detected during the pre-pandemic period. After accounting for the existing cognitive difference during the pre-pandemic period, we demonstrated that the magnitude of cognitive difference between people with and without heart diseases was significantly enlarged during the pandemic period.

To our current knowledge, this is one of the largest studies to demonstrate the deterioration in cognitive function during the pandemic among the general older population and, more importantly, the first one to identify an enlarged gap in cognitive function related to the pandemic between people with and without heart diseases. A few studies have suggested that older adults experienced a cognitive decline during the pandemic, although these studies were limited in small sample sizes, convenience

TABLE 4 Differences in the changes of executive function scores before and during the pandemic period, by heart disease status.

| Executive function scores, LSM (95% CI)* | | | | |
|---|---|---|---|---|
| | Heart diseases group ($n = 2119$) | Non-heart diseases group ($n = 7185$) | LSM differences between groups* | *P* for differences between groups* |
| **Before pandemic** | | | | |
| Wave 13 (2016) | 5.55 (5.48, 5.62) | 5.46 (5.42, 5.49) | 0.09 (0.01, 0.17) | 0.023 |
| Wave 14 (2018) | 5.47 (5.40, 5.54) | 5.39 (5.36, 5.43) | 0.08 (0.00, 0.16) | 0.050 |
| LSM differences between waves* | −0.08 (−0.14, −0.01) | −0.07 (−0.10, −0.03) | −0.01 (−0.09, 0.06) | 0.720 |
| *P* for differences between waves* | 0.017 | <0.001 | 0.720 | / |
| **During pandemic** | | | | |
| Wave 14 (2018) | 5.47 (5.40, 5.54) | 5.39 (5.36, 5.43) | 0.08 (0.00, 0.16) | 0.050 |
| Wave 15 (2020) | 5.36 (5.28, 5.43) | 5.36 (5.32, 5.40) | −0.01 (−0.09, 0.08) | 0.875 |
| LSM differences between waves* | −0.12 (−0.18, −0.05) | −0.03 (−0.06, 0.00) | −0.09 (−0.16, −0.01) | 0.020 |
| *P* for differences between waves* | <0.001 | 0.092 | 0.020 | / |
| **During pandemic vs. Before pandemic** | | | | |
| Differences in LSM differences between two periods* | −0.04 (−0.15, 0.07) | 0.04 (−0.03, 0.10) | −0.07 (−0.20, 0.05) | 0.254 |
| *P* for differences in LSM differences between two periods* | 0.499 | 0.253 | 0.254 | / |

*Differences were calculated by the linear mixed model, after adjusting for age, sex, race, education, cohabitation status, current smoking, current drinking, physical active, depressive symptoms, status of hypertension, diabetes, stroke, cancer, and chronic lung diseases.

samples, or lacking objective cognitive assessments measured before and during the pandemic. French PA-COVID study observed an accelerated cognitive decline during the pandemic, compared with 15 years of cognitive trajectory preceding the pandemic among 263 older adults (15). A Japanese survey of 955 older people reported that social isolation was associated with self-reported cognitive impairment during the pandemic (16), while an online survey of 640 Belgium older adults found only those with depressive symptoms exhibited self-perceived cognitive decline during the pandemic, and this study sample mainly focused on individuals with high socioeconomic status (17). Data collected by online surveys inclined to rule out disadvantaged people who do not possess Internet access (29). Our study observed pandemic-related cognitive decline in the both heart disease group and the non-heart disease group, together with these previous findings, emphasizing that increased attention should be paid to cognitive decline among older adults during the pandemic. Moreover, our results showed that the cognitive function gap between people with and without heart diseases significantly grow further during the pandemic. Identifying this vulnerable group is of pivotal importance to provide targeted cognitive monitoring and training as the pandemic progressed.

Our subgroup analyses identified that sex might play a potential modified role in the pandemic-related cognitive difference between people with and without heart diseases, and a larger cognitive difference was presented among female participants. Similarly, previous findings have shown that women were especially susceptible to mental disorders during the pandemic (7, 30, 31). This evidence indicated the sex disparities related to the COVID-19 pandemic and underscored the importance to support vulnerable women. In addition, we also observed that a significantly smaller cognitive difference was exhibited among physical inactive participants, probably because these participants had a much lower cognitive function at the baseline due to their poor health status, with a global cognitive score of 15.04 points at wave 14 in the physical inactive group while 16.21 points in the physical active group (data not shown). Therefore, physical inactive participants were likely to have less room to decline on the cognitive test (32). A more sophisticated cognitive assessment in the future study might help clarify this question.

The atherosclerotic process and induced hypoxic–ischemic brain injury have been well documented to link heart diseases and cognitive decline (33). In addition, the shared vascular factors could also contribute to cognitive decline through multiple biological pathways, such as oxidative stress and inflammation responses (34, 35). It is plausible that the enlarged cognitive gaps between people with and without heart diseases during the pandemic might be attributed to COVID-19 infection (36). The presence of heart disease was associated with a more severe course and higher mortality of COVID-19. The infection could in turn lead to cardiac complications such as myocarditis, arrhythmia, and heart failure, as well as lasting cognitive deficits (37, 38), whereas the proportion of patients with COVID-19 in the present study was too small (2.6%, data not shown) to detect the cognitive decline directly due to COVID-19 infection. Therefore, the deleterious impact of the pandemic on health service access and lifestyle changes was more likely to account for our findings. The nationally representative data from the UK showed that the incident use of cardiovascular disease medicines has drastically decreased compared with the pre-pandemic level, and such missed treatment was estimated to result in more than 13,000 additional cardiovascular disease events (39). In addition, several studies have indicated a decrease in hospitalization rate in patients with heart failure during the pandemic compared with 2019, and the admitted patients exhibited significantly more severe symptoms and higher mortality (40, 41). The diminished access to healthcare might be partly because most outpatient visits and cardiac activities have been deferred or canceled to guarantee the capacity for the care of patients with COVID-19 (5, 42) and partly because people avoided seeking medical care for fear of getting infected (43, 44). The deterioration of prognosis compounded by

stress and anxiety during the pandemic made older adults with heart diseases more vulnerable to cognitive decline. Moreover, the social isolation caused by quarantine has exacerbated cardiovascular risk factors such as physical inactivity, obesity, and unhealthy food habits (45). For example, Beydoun et al. found that among the HRS participants, the onset of the COVID-19 pandemic was associated with increased BMI, elevated numbers of cardiometabolic risk factors, and chronic morbidities (10). Taken together, all these repercussions of the COVID-19 pandemic on cardiovascular health could further exacerbate cognitive decline among older adults with heart diseases.

The present study draws strength from the nationally representative longitudinal cohort to provide a comprehensive picture of the COVID-19 pandemic as a determinant of aging issues regarding cognitive decline. By employing objective assessments of cognitive function measured during a similar period preceding the pandemic as control, we were able to unpack and compare the pandemic-related cognitive decline between participants with and without heart diseases.

## 5. Limitations

Nevertheless, our findings should be interpreted with caution given the following limitations. First, the ascertainment of heart diseases was based on self-reported doctor diagnoses, which might lead to a misclassification of heart disease cases and bias our findings to a null. In addition, due to the relatively low response rates of questions on specific heart disease types in the HRS, we were not able to further explore whether the observed associations differed by heart disease types. Second, the cognitive assessment was less elaborate given the large-scale population-based setting. Third, although multiple important covariates have been adjusted, other unmeasured and unavailable determinants such as genetic susceptibility and dietary intake were likely to confound our results. Fourth, 7,842 participants from the HRS wave 14 survey were excluded due to incomplete cognitive data or pre-existed dementia, non-response analyses showed significant differences in characteristics between individuals included and excluded, selection bias could not be ruled out, and the generalizability of our findings might be compromised.

## 6. Future directions

Further investigations with more comprehensive measurements on the diagnosis of heart diseases might yield more accurate estimations and provide more information. In addition, using a more sophisticated neuropsychological assessment might provide insights into other cognitive domains and have a higher capacity to detect more subtle cognitive decline. Furthermore, future studies conducted in non-U.S. populations, with a longer follow-up time during the pandemic, are warranted to verify our findings. Moreover, future policy and guidance should be in place for the immediate provision of cognitive monitoring and interventions for the vulnerable population with heart diseases to mitigate the adverse impact of the pandemic.

## 7. Conclusion

In conclusion, this study illustrated the deteriorated cognitive status among older adults and an enlarged gap in cognitive function between people with and without heart diseases related to the COVID-19 pandemic. The findings underscore the necessity to provide immediate cognitive monitoring and interventions for the population with heart diseases.

## Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://hrsonline.isr.umich.edu/.

## Ethics statement

The Health and Retirement Study was approved by the Institutional Reviewing Board at the University of Michigan and the National Institute on Aging (HUM00061128). The participants provided their written informed consent to participate in this study.

## Author contributions

FZ and WX conceived and designed the study, obtained the funding, had full access to all of the data in the study, and took responsibility for the integrity of the data and the accuracy of the data analysis. RH, CL, DG, and WX performed the statistical analysis. RH, CL, WX, and FZ drafted and revised the manuscript. All authors contributed to the data interpretation and final approval of the manuscript.

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## Conflict of interest

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

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.1077800/full#supplementary-material

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# Pregnancy loss and risk of incident CVD within 5 years: Findings from the Women's Health Initiative

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**Background:** Previous studies have demonstrated an increased risk of cardiovascular disease (CVD) in women with a history of pregnancy loss. Less is known about whether pregnancy loss is associated with age at the onset of CVD, but this is a question of interest, as a demonstrated association of pregnancy loss with early-onset CVD may provide clues to the biological basis of the association, as well as having implications for clinical care. We conducted an age-stratified analysis of pregnancy loss history and incident CVD in a large cohort of postmenopausal women aged 50–79 years old.

**Methods:** Associations between a history of pregnancy loss and incident CVD were examined among participants in the Women's Health Initiative Observational Study. Exposures were any history of pregnancy loss (miscarriage and/or stillbirth), recurrent (2+) loss, and a history of stillbirth. Logistic regression analyses were used to examine associations between pregnancy loss and incident CVD within 5 years of study entry in three age strata (50–59, 69–69, and 70–79). Outcomes of interest were total CVD, coronary heart disease (CHD), congestive heart failure, and stroke. To assess the risk of early onset CVD, Cox proportional hazard regression was used to examine incident CVD before the age of 60 in a subset of subjects aged 50–59 at study entry.

**Results:** After adjustment for cardiovascular risk factors, a history of stillbirth was associated with an elevated risk of all cardiovascular outcomes in the study cohort within 5 years of study entry. Interactions between age and pregnancy loss exposures were not significant for any cardiovascular outcome; however, age-stratified analyses demonstrated an association between a history of stillbirth and risk of incident CVD within 5 years in all age groups, with the highest point estimate seen in women aged 50–59 (OR 1.99; 95% CI, 1.16–3.43). Additionally, stillbirth was associated with incident CHD among women aged 50–59 (OR 3.12; 95% CI, 1.33–7.29) and 60–69 (OR 2.06; 95% CI, 1.24–3.43) and with incident heart failure and stroke among women aged 70–79. Among women aged 50–59 with a history of stillbirth, a non-significantly elevated hazard ratio was observed for heart failure before the age of 60 (HR 2.93, 95% CI, 0.96–6.64).

**Conclusions:** History of stillbirth was strongly associated with a risk of cardiovascular outcomes within 5 years of baseline in a cohort of postmenopausal women aged 50–79. History of pregnancy loss, and of stillbirth in particular, might be a clinically useful marker of cardiovascular disease risk in women.

KEYWORDS

**cardiovascular disease, pregnancy loss, miscarriage, stillbirth, epidemiology**

# Introduction

Approximately one out of four clinically recognized pregnancies ends in pregnancy loss; rates of subclinical pregnancy loss are far higher (1). Among women with a history of pregnancy loss, an increased risk of cardiovascular disease (CVD) has been observed (2–6); rates are even higher among women with a history of recurrent pregnancy loss (RPL) and stillbirth (2, 5–9). In a meta-analysis conducted by Oliver-Williams et al., a history of pregnancy loss was associated with 45% greater odds of developing coronary heart disease (CHD), while RPL was associated with nearly twofold greater odds (10). More recently, Parker et al. found odds ratios of 1.19 (95% CI 1.08–1.32), 1.18 (95% 1.04–1.34), and 1.27(95% CI 1.07–1.51) for CHD among women with a history of one miscarriage, two or more miscarriages, and any history of stillbirth, respectively, among participants in the Women's Health Initiative (WHI), a large-scale prospective study of postmenopausal women (5).

The association between pregnancy loss and age at CVD onset is less well understood. Previous research has shown a stronger association between pregnancy loss and heart disease in very young women (<age 35) than older women (9); however, less is known about the relative strength of the association among women in midlife (aged 50–59) compared with older age. Whether a history of pregnancy loss is associated with an increased risk of early-onset CVD (before the age of 60) is a question of interest and might provide clues about the biological basis for the association. Although all factors underlying the association between pregnancy loss and CVD risk are not understood, a genetic basis has been suggested (11). As the contribution of genetic factors to disease risk appears to be particularly strong for early-onset disease (12), a demonstrated association between pregnancy loss and early-onset CVD might be indirect evidence in support of the postulated genetic basis.

Additionally, such findings might inform the clinical management of women with a history of pregnancy loss. It has been proposed that, in addition to conventional cardiovascular risk factors, such as dyslipidemia, diabetes, and hypertension, the inclusion of reproductive factors into cardiovascular risk profiles might aid clinicians in identifying patients who would benefit from monitoring and control of cardiovascular disease risk factors (13). In particular, the addition of reproductive factors into cardiovascular risk profiles might be most beneficial for predicting the risk of heart disease in younger women, prior to the onset of conventional cardiovascular risk factors (14).

In the current study, we sought to expand upon the work of Parker et al. (5) by assessing whether the association between a history of pregnancy loss and CVD risk in postmenopausal women differs across age strata; in particular, whether the association is strongest among women under the age of 60. We conducted all-ages and age-stratified analyses of the associations between pregnancy loss and risk of total CVD and three major types of CVD (CHD, congestive heart failure, and stroke) within 5 years of baseline among WHI participants.

# Methods

## Study setting and study population

The WHI cohort has previously been described in detail (15). Briefly, WHI is a large-scale prospective study of postmenopausal women, aged 50–79 at baseline, who were enrolled at 40 clinical centers throughout the United States between 1993 and 1998. The main WHI study concluded in 2005; follow-up of surviving participants is ongoing in WHI Extension Studies (16). The WHI study involves both an observational study (OS) arm and three overlapping randomized trials. The latter comprise a hormone replacement therapy clinical trial (CT) for the prevention of CHD, and two studies of non-hormone treatment: dietary modification for the prevention of breast and colorectal cancer, and calcium/vitamin D supplementation for hip fracture prevention (17).

Participants who were screened for the CT but were either ineligible or unwilling to undergo randomization were invited to participate in the OS (18), a prospective longitudinal study comprising a periodic collection of data on participant demographic and lifestyle factors and health outcomes. The OS focuses on identifying novel risk factors and biomarkers of disease; primary outcomes of interest are CHD, stroke, breast and colorectal cancer, fracture, and mortality (17). In total, 161,808 participants enrolled in the WHI; the OS cohort comprised 93,676 participants (18, 19). At baseline, 31.7, 44.0, and 24.3% of the cohort were aged 50–59, 60–69, and 70–79, respectively (17).

The current analysis was limited to OS participants to exclude the potential effects of CT interventions on CVD outcomes. Participants eligible for inclusion were those who had ever been pregnant, for whom complete reproductive history information was available and who were free of cardiovascular disease at baseline. Of the 93,676 OS participants, 73,805 (78.8%) met the inclusion criteria (Figure 1).

## Exposure assessment

Reproductive history data were collected at the second WHI screening visit by questionnaire (14). Pregnancy history data included self-reported gravidity, parity, number of live births, spontaneous miscarriages, and stillbirth following a pregnancy lasting at least 6 months. Exposures considered in the current analysis were (1) any history of pregnancy loss (defined as at least one miscarriage and/or stillbirth), (2) history of RPL (defined as a history of two or more miscarriages and/or stillbirths), (20) and (3) any history of stillbirth.

## Outcomes assessment

The ascertainment and adjudication of primary and secondary outcomes for WHI have been described in detail previously (21). In brief, OS study participants were contacted by mail annually to collect self-reported outcomes, as well as updated exposure data (15). The adjudication of outcomes for all OS participants continued through August 2009, allowing for an average duration of follow-up for OS participants of 12 years (17). The initial adjudication of outcomes was performed by a physician adjudicator at a local clinical center and consisted of a physician review of hospital discharge summaries, relevant diagnostic tests, and death certificates. Primary and safety outcomes were subsequently confirmed by central adjudication; a review of primary cardiovascular outcomes was performed by the WHI Cardiovascular Central Adjudication Committee (21).

Outcomes for the current analysis were adjudicated total CVD (fatal and non-fatal) and three major types of CVD: CHD, heart failure, and stroke, occurring within 5 years of baseline. These

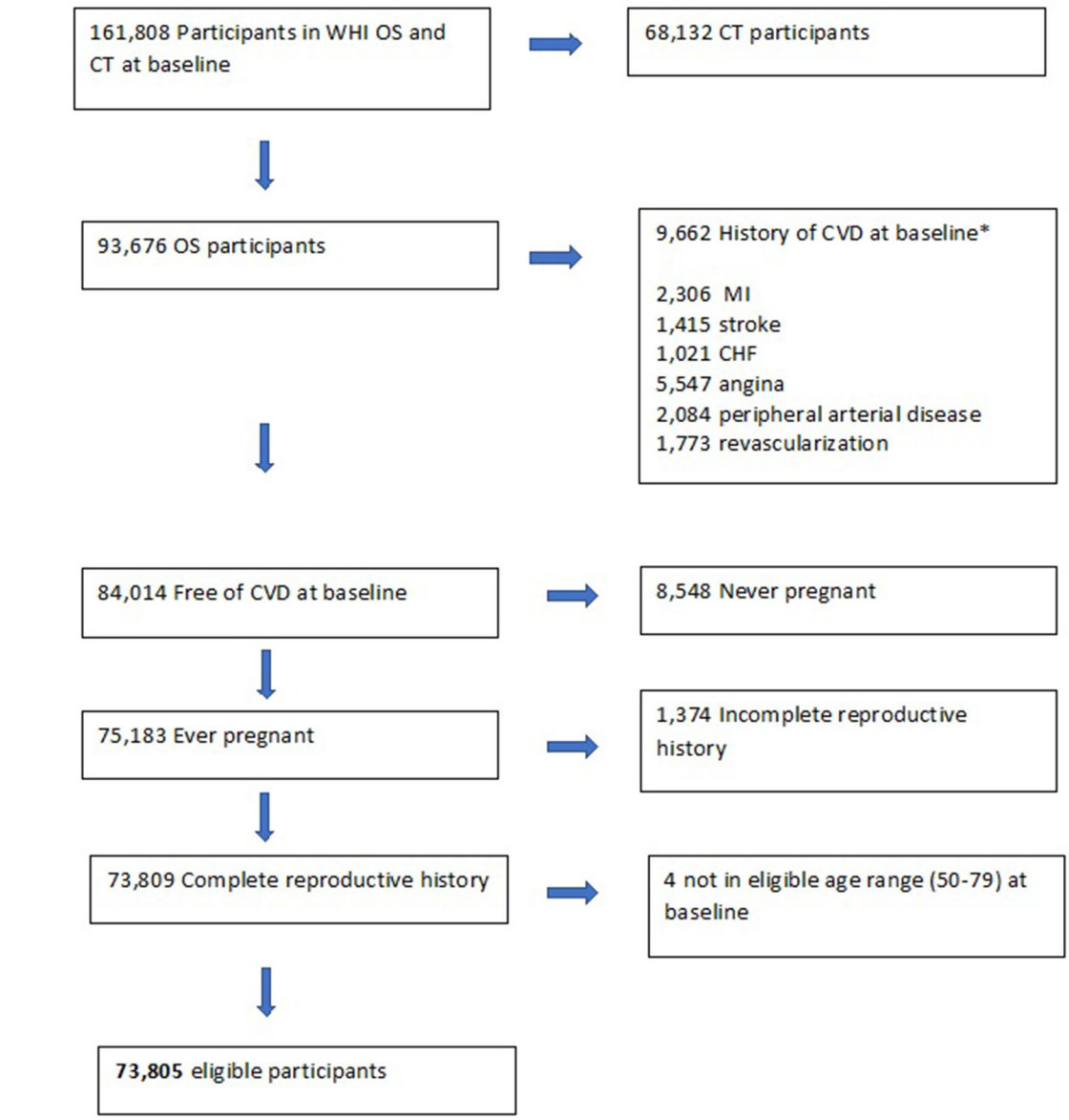


FIGURE 1
Identification of WHI participants eligible for inclusion in the current study.

comprised primary (CHD) or secondary (CVD, heart failure, and stroke) cardiovascular outcomes in the WHI CT and were also ascertained among OS participants; (21) methods for ascertainment of these outcomes were therefore well documented and consistent across local clinical centers.

Cardiovascular outcomes were defined as in the WHI OS. Non-fatal CVD outcomes were defined as CHD, stroke, heart failure, peripheral vascular disease, angina, coronary artery bypass graft (CABG), coronary revascularization, and pulmonary embolism (21). Fatal CVD outcomes were defined as death due to cerebrovascular, definite CHD, possible CHD, pulmonary embolism, other cardiovascular, or unknown cardiovascular causes.

The outcome of CHD in WHI OS participants was defined as hospitalized myocardial infarction (MI) (definite or probable) or coronary death (21). Definite and probable MI events were identified by an algorithm comprising medical history data, electrocardiogram readings, and cardiac enzyme/troponin levels, as available (22). Silent MI events were not ascertained in OS participants; therefore, silent MI was not considered as an outcome in this analysis. Fatal coronary outcomes comprised out-of-hospital as well as hospitalized deaths: coronary death was identified based on a physician review of medical records and death certificate data and was defined as death consistent with an underlying cause of death of CHD (21).

Outcome of heart failure was defined as signs and symptoms of heart failure together with one of the following: pulmonary edema on X-ray; ventricular dilation/poor ventricular function; or physician diagnosis and treatment for heart failure. Stroke was defined as rupture or obstruction of the brain arterial system, resulting in rapid neurological deficit persisting for 24 h or more. Stroke outcome comprised stroke, hemorrhagic stroke, or cause of death reported as stroke. Heart failure and stroke not resulting in hospitalization were not considered as WHI outcomes (21).

## CVD risk factor assessment

Physical measurements, blood specimens, and an inventory of current medication/supplement use were collected from OS participants during a baseline clinic visit. Participants also completed questionnaires covering medical history, family history, reproductive history, lifestyle, and behavioral factors (15).

## Confounding variables

Multivariable models were adjusted for socioeconomic factors, CVD risk factors, and other covariates identified *a priori* as potential confounders. Socioeconomic factors included in the models were education level (<high school education, high school graduate, some college/associate's degree, college graduate) and neighborhood socioeconomic status (NSES) quartile; the latter is a composite measure based on census tract-level neighborhood variables (23). Other covariates included were number of pregnancies (continuous), smoking status (never, former, or current smoker), race/ethnicity (American Indian/Alaskan Native, Asian/Pacific Islander, Black/African American, Hispanic/Latino, non-Hispanic White, or Other), aspirin use (yes/no), and body mass index (BMI) at baseline (continuous).

## Data analyses

Demographic and reproductive history were compared for three exposure categories: any history of pregnancy loss, a history of RPL, and any history of stillbirth. Multivariable logistic regression analyses were performed to assess associations between pregnancy loss and incident outcome events within 5 years of study entry. To finely adjust for age, analyses were conducted for each 1-year interval of age at study entry. The results of logistic regression analyses for each 1-year age interval were in turn used to assess associations in the entire study sample and to conduct an age-stratified analysis across three age strata: 50–59, 60–69, and 70–79 at baseline.

Multivariable logistic regression models were adjusted for socioeconomic factors, CVD risk factors, and other covariates identified *a priori* as potential confounders, as described above. Odds ratios for calculated outcomes were calculated for each of the three age strata. As the outcome of total CVD comprises the individual outcomes of CHD, heart failure, and stroke, the analyses were considered to involve three rather than four separate outcomes; thus, adjustment for multiple comparisons was not performed.

To assess the significance of associations between age and pregnancy loss exposures, regression analyses including a term for the interaction between age at baseline and history of pregnancy loss were performed for the full study sample for each outcome. A likelihood ratio test was used to compare models including a term for the interaction between age and pregnancy loss exposure to models with no interaction term.

To examine associations between a history of pregnancy loss and early-onset CVD, Cox regression analyses were used to assess incident CVD, CHD, heart failure, and stroke occurring before the age of 60 in the subset of study participants aged 50–59 at baseline. Cox regression analyses were adjusted for the same set of *a priori* established confounders included in the logistic regression analyses.

All analyses were performed using Stata 16.0 (24).

# Results

## Study sample

Table 1 shows descriptive statistics for the study sample across the three exposure categories (any history of pregnancy loss [miscarriage and/or stillbirth], a history of RPL [two or more miscarriages and/or stillbirths], and a history of stillbirth). A history of any pregnancy loss was reported by 33.8% of study subjects; histories of RPL and stillbirth were reported by 11.9 and 4.1% of subjects, respectively.

Several demographic and lifestyle factors differed across the categories of pregnancy loss. Compared with women with no history of pregnancy loss, a higher percentage of women with any history of pregnancy loss were aged 60–69 or 70–79 at baseline, identified as Black/African American or Hispanic/Latino, had either less than a high school education or had completed some college or an associate's degree, were in the lowest quartile of NSES, were obese, and were former or current smokers. Additionally, significant differences in reproductive histories were reported: women with any history of pregnancy loss were more likely than those with no history of loss to report five or more total pregnancies and either no live births or several (five or more) live births.

Differences between women with and without a history of RPL and stillbirth were nearly identical to those observed between those with and without any history of pregnancy loss. A higher percentage of women with a history of either RPL or stillbirth were aged 60–69 at baseline compared with women with no history of those exposures; the percentage of women with a history of stillbirth who were aged 70–79 at baseline was also higher than those with no history of stillbirth. Additionally, women with a history of either RPL or stillbirth were more likely to identify as Black/African American or Hispanic/Latino, have less than a high school education or have completed some college or an associate's degree, be in the lowest NSES quartile, be obese, and be current smokers; women with a history of RPL were also more likely to be former smokers than those with no history of RPL. Women reporting a history of either RPL or stillbirth were more likely than women without the respective exposures to report five or more total pregnancies and either no live births or several live births (five or more).

## Logistic regression analysis, all age groups

Within 5 years of baseline, 2,735 (3.71%) study subjects experienced CVD events; incident CHD, heart failure, and stroke occurred in 756 (1.02%), 641 (0.87%), and 724 (0.98%) subjects,

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TABLE 1 Baseline demographic characteristics, cardiovascular disease risk factors, and reproductive history in selected participants from the Women's Health Initiative Observational Study (WHI OS).

| | No history of pregnancy loss $N = 44,840$ | Any history of pregnancy loss (miscarriage or stillbirth) $N = 24,965$ | No history of recurrent (2+) pregnancy loss $N = 65,009$ | History of recurrent pregnancy loss $N = 8,796$ | No history of stillbirth $N = 70,751$ | History of stillbirth $N = 3,054$ |
|---|---|---|---|---|---|---|
| Demographics | *n* (%) | *n* (%) | *n* (%) | *n* (%) | *n* (%) | *n* (%) |
| Age group | | | | | | |
| 50–59 | 16,794 (34.4) | 7,671 (30.7)[a] | 2,986 (33.8) | 2,479 (28.2)[a] | 23,615 (33.4) | 850 (27.8)[a] |
| 60–69 | 21,392 (43.8) | 11,476 (46.0)[a] | 28,681 (44.1) | 4,187 (47.6)[a] | 31,440 (44.4) | 1,428 (48.6)[c] |
| 70–79 | 10,654 (21.8) | 5,818 (23.3)[a] | 14,342 (22.1) | 2,130 (24.2) | 15,696 (22.2) | 776 (25.4)[a] |
| Race/ethnicity | | | | | | |
| American Indian/ Alaskan Native | 187 (0.4) | 121 (0.5)[c] | 266 (0.4) | 42 (0.5) | 289 (0.4) | 19 (0.6) |
| Asian/Pacific Islander | 1,501 (3.1) | 633 (2.5)[a] | 1.954 (3.0) | 180 (2.1)[a] | 2,051 (2.9) | 83 (2.7) |
| Black/African American | 3,290 (6.8) | 2,282 (9.2)[a] | 4,606 (7.1) | 966 (11.0)[a] | 5,149 (7.3) | 423 (13.9)[a] |
| Hispanic/Latino | 1,678 (3.5) | 1,056 (4.2)[a] | 2,269 (3.5) | 465 (5.3)[a] | 2,492 (3.5) | 242 (8.0)[a] |
| White, non-Hispanic | 41,501 (85.2) | 20,522 (82.4)[a] | 55,036 (84.9) | 6,996 (79.8)[a] | 59,802 (84.8) | 2,230 (73.3)[a] |
| Other/unknown | 541 (1.1) | 280 (1.1) | 705 (1.1) | 116 (1.3)[c] | 776 (1.1) | 45 (1.5) |
| Education | | | | | | |
| <HS diploma | 2,180 (4.5) | 1,310 (5.3)[a] | 2,920 (4.5) | 570 (6.5)[a] | 3,206 (4.6) | 284 (9.4)[a] |
| High school | 8,163 (16.84) | 3,970 (16.0)[b] | 10,769 (16.7) | 1,364 (15.6)[c] | 11,630 (16.6) | 503 (16.6) |
| Some college/ associate's degree | 17,563 (36.2) | 9,542 (38.5)[a] | 23,589 (36.6) | 3,516 (40.3)[a] | 25,910 (36.9) | 1,195 (39.5)[b] |
| College graduate | 20,558 (42.4) | 9,938 (40.1)[a] | 27,220 (42.2) | 3,276 (37.5)[a] | 29,452 (42.0) | 1,044 (34.5)[a] |
| Neighborhood socioeconomic status (NSES) quartile | | | | | | |
| 1 (lowest) | 10,598 (24.2) | 5,944 (26.6)[a] | 14,251 (24.4) | 2,291 (29.2)[a] | 15,616 (24.6) | 926 (33.8)[a] |
| 2 | 10.942 (25.0) | 5,599 (25.0) | 14,613 (25.1) | 1,928 (24.6) | 15,872 (25.0) | 669 (24.4) |
| 3 | 11,110 (25.4) | 5,438 (24.3)[b] | 14,663 (25.1) | 1,885 (24.0)[c] | 15,950 (25.2) | 598 (21.8)[a] |
| 4 (highest) | 11,141 (25.4) | 5,394 (24.1)[a] | 14,796 (25.4) | 1.739 (22.2)[a] | 15,990 (25.2) | 545 (19.9)[a] |
| CVD risk factors | | | | | | |
| Body mass index category | | | | | | |
| Underweight (<18.5) | 545 (1.1) | 263 (1.1) | 723 (1.1) | 85 (1.0) | 754 (1.1) | 30 (1.0) |
| Normal (18.5–24.9) | 20,054 (41.6) | 9,532 (38.6)[a] | 26,467 (41.2) | 3,119 (36.0)[a] | 28,376 (40.4) | 973 (31.5)[a] |
| Overweight (25.0–29.9) | 16,520 (34.2) | 8,421 (34.1) | 21,977 (34.2) | 2,964 (34.2) | 23,890 (34.2) | 1,051 (34.9) |
| Obese (>=30) | 11,138 (23.1) | 6,467 (26.2)[a] | 15,097 (23.5) | 2,508 (28.9)[a] | 16,653 (23.8) | 952 (31.6)[a] |
| Aspirin use | | | | | | |
| Yes | 8,629 (17.7) | 4,498 (18.0) | 11,546 (17.8) | 1,581 (18.0) | 12,605 (17.8) | 522 (17.1) |
| No | 40,211 (82.3) | 20,467 (82.0) | 52,463 (82.2) | 7,215 (82.0) | 58,146 (82.2) | 2,532 (82.9) |
| Smoking status | | | | | | |
| Never smoked | 25,086 (52.0) | 12,238 (49.6)[a] | 33.099 (51.5) | 4,225 (48.7) | 35,784 (51.2) | 1,540 (51.2) |
| Former smoker | 20,441 (42.4) | 10,731 (43.5)[b] | 27,344 (42.6) | 3,828 (44.2)[b] | 29,922 (42.8) | 1,250 (41.6) |
| Current smoker | 2,733 (5.7) | 1,698 (6.9)[a] | 3,807 (5.9) | 615 (7.1)[a] | 4,204 (6.0) | 218 (7.2)[b] |

*(Continued)*

TABLE 1 (Continued)

| | No history of pregnancy loss $N = 44,840$ | Any history of pregnancy loss (miscarriage or stillbirth) $N = 24,965$ | No history of recurrent (2+) pregnancy loss $N = 65,009$ | History of recurrent pregnancy loss $N = 8,796$ | No history of stillbirth $N = 70,751$ | History of stillbirth $N = 3,054$ |
|---|---|---|---|---|---|---|
| Reproductive history | | | | | | |
| Number of pregnancies | | | | | | |
| 1 | 5,469 (11.2) | 621 (2.5)[a] | 6,077 (9.4) | 0 (0.0)[a] | 6,027 (8.5) | 63 (2.1)[a] |
| 2 | 15,530 (31.8) | 1,667 (6.7)[a] | 16,912 (26.0) | 285 (3.2)[a] | 16,969 (24.0) | 228 (7.5)[a] |
| 3 to 4 | 21,781 (44.6) | 10,809 (43.3)[b] | 30,399 (46.8) | 2,191 (24.9)[a] | 31,443 (44.4) | 1,147 (37.6)[a] |
| 5 or more | 6,060 (12.4) | 11,868 (47.5)[a] | 11,621 (17.9) | 6,307 (71.7)[a] | 16,312 (23.1) | 1,616 (52.9)[a] |
| Number of live births | | | | | | |
| 0 | 1,195 (2.5) | 1,168 (4.7)[a] | 1,907 (2.9) | 456 (5.2)[a] | 2,240 (3.2) | 123 (4.0)[b] |
| 1 | 5,478 (11.2) | 2,259 (9.1)[a] | 6,891 (10.6) | 846 (9.6)[b] | 7,397 (10.5) | 340 (11.1) |
| 2 | 15,984 (32.7) | 6,393 (25.6)[a] | 20,398 (31.4) | 1,979 (22.5)[a] | 21,640 (30.6) | 737 (24.1)[a] |
| 3 to 4 | 20,852 (42.7) | 10,740 (43.1) | 27,964 (43.0) | 3,638 (41.4)[b] | 30,311 (42.8) | 1,291 (42.3) |
| 5 or more | 5,331 (10.9) | 4,395 (17.6)[a] | 7,849 (12.1) | 1,877 (21.3)[a] | 9,163 (13.0) | 563 (18.4)[a] |

[a] $p < 0.001$ for exposed vs. unexposed subjects.
[b] $p < 0.01$ for exposed vs. unexposed subjects.
[c] $p < 0.05$ for exposed vs. unexposed subjects.

TABLE 2 Odds of incident cardiovascular disease (CVD) within 5 years of baseline in WHI participants aged 50–79 with and without a history of pregnancy loss, recurrent pregnancy loss, and stillbirth.

| Exposure | Adjusted odds ratio (95% CI)* | | | |
|---|---|---|---|---|
| | Total CVD | CHD | Heart failure | Stroke |
| History of pregnancy loss | 1.09 (0.99, 1.20) | **1.29 (1.08, 1.54)** | 1.10 (0.90, 1.35) | 1.03 (0.86, 1.25) |
| History of recurrent (2+) pregnancy loss | **1.17 (1.03, 1.34)** | 1.16 (0.90, 1.49) | **1.35 (1.03, 1.76)** | 1.15 (0.90, 1.49) |
| History of stillbirth | **1.47 (1.22, 1.75)** | **1.81 (1.31, 2.50)** | **1.76 (1.26, 2.45)** | **1.53 (1.08, 2.16)** |

*Adjusted for age (meta-analysis of 1-year age intervals), education, NSES, number of pregnancies, smoking status, race/ethnicity, aspirin use, and BMI. CIs are not corrected for multiple comparisons. Bold values indicate a statistically significant association.

respectively. After adjustment for confounders, a history of any pregnancy loss was significantly associated with incident CHD (OR 1.29 [1.08, 1.54]) 5 years post-baseline, while a history of RPL was associated with both incident CVD (OR 1.17 [1.03, 1.34]) and heart failure (OR 1.35 [1.03, 1.76]). A history of stillbirth was significantly associated with all CVD outcomes, with adjusted ORs of 1.47 (1.22, 1.75), 1.81 (1.31, 2.50), 1.76 (1.26, 2.45), and 1.53 (1.08, 2.16) for incident CVD, CHD, heart failure, and stroke, respectively (Table 2).

## Logistic regression analysis, age-stratified

Interaction terms between age and pregnancy loss exposures were not significant for any cardiovascular outcome (Supplemental Table 1). Therefore, results of the age-stratified analysis are provided simply to augment the all-ages analysis (Table 3).

After adjustment for confounders, a history of any pregnancy loss was not associated with greater odds of incident CVD 5 years post-baseline in any age group, while RPL was associated with increased odds of CVD among women aged 60–69 at baseline. A history of stillbirth was associated with incident CVD within 5 years among all age groups, with adjusted ORs of 1.99 (1.16, 3.43), 1.46 (1.11, 1.92), and 1.37 (1.05, 1.78) among women aged 50–59, 60–69, and 70–79 at baseline, respectively.

A history of any pregnancy loss was associated with incident CHD among women aged 70–79 at baseline (OR 1.34 [1.03, 1.73]). Stillbirth was associated with incident CHD among women aged 50–59 and 60–69 at baseline, with adjusted ORs of 3.12 (1.33, 7.29) and 2.06 (1.24, 3.43), respectively.

A history of RPL was associated with heart failure among women aged 50–59 (OR 2.18 [1.001, 4.76]) and 60–69 (OR 1.54 [1.03, 2.31]) at baseline. A history of stillbirth was associated with heart failure among women aged 70–79 at baseline (OR 1.69 [1.04, 2.76]); marginally insignificant adjusted ORs of 2.45 (0.96, 6.22) and 1.65 (0.97. 2.79) were observed among women aged 50–59 and 60–69, respectively.

A history of RPL was associated with stroke among women aged 50–59 at baseline (OR 2.60 [1.10, 6.16]). A history of stillbirth was associated with stroke among women aged 70–79 at baseline (OR 1.77 [1.11, 2.80]).

## Survival analysis, early-onset cardiovascular outcomes

In the subset of 24,465 study participants aged 50–59 at baseline, the rate of incident CVD before the age of 60 was 1.95 events per 1,000 person-years; rates of incident CHD, heart failure, and stroke before the age of 60 were 0.64, 0.49, and 0.20 per 1,000

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TABLE 3 Odds of incident cardiovascular disease (CVD) within 5 years in women with and without a history of pregnancy loss, recurrent pregnancy loss, and stillbirth, by age at study baseline.

| Exposure | Age group | Adjusted odds ratio (95% CI)* | | | |
|---|---|---|---|---|---|
| | | Total CVD | CHD | Heart failure | Stroke |
| Any pregnancy loss | 50–59 | 1.19 (0.89, 1.59) | 1.45 (0.85, 2.46) | 1.18 (0.63, 2.22) | 0.86 (0.44, 1.69) |
| | 60–69 | 1.08 (0.94, 1.25) | 1.20 (0.90, 1.59) | 1.10 (0.81, 1.49) | 1.18 (0.88, 1.58) |
| | 70–79 | 1.07 (0.93, 1.24) | **1.34 (1.03, 1.73)** | 1.09 (0.81, 1.46) | 0.95 (0.73, 1.24) |
| Recurrent (2+) loss | 50–59 | 1.25 (0.82, 1.89) | 0.75 (0.32, 1.77) | **2.18 (1.001, 4.76)** | **2.60 (1.10, 6.16)** |
| | 60–69 | **1.33 (1.10, 1.62)** | 1.16 (0.78, 1.71) | **1.54 (1.03, 2.31)** | 1.12 (0.75, 1.68) |
| | 70–79 | 1.00 (0.82, 1.23) | 1.25 (0.88, 1.78) | 1.04 (0.70, 1.55) | 0.90 (0.62, 1.30) |
| Stillbirth | 50–59 | **1.99 (1.16, 3.43)** | **3.12 (1.33, 7.29)** | 2.45 (0.96, 6.22) | 0.97 (0.23, 4.06) |
| | 60–69 | **1.46 (1.11, 1.92)** | **2.06 (1.24, 3.43)** | 1.65 (0.97, 2.79) | 1.32 (0.75, 2.31) |
| | 70–79 | **1.37 (1.05, 1.78)** | 1.35 (0.84, 2.19) | **1.69 (1.04, 2.76)** | **1.77 (1.11, 2.80)** |

*Adjusted for age (meta-analysis of 1-year age intervals), education, NSES, number of pregnancies, smoking status, race/ethnicity, aspirin use, and BMI. CIs are not corrected for multiple comparisons. Bold values indicate a statistically significant association.

person-years, respectively. (Supplemental Table 2). After adjustment for confounders, Cox proportional hazard regression analyses did not demonstrate significantly increased hazard ratios for cardiovascular outcomes before the age of 60 (Supplemental Table 3), although a marginally non-significantly elevated hazard ratio for heart failure (2.53 [0.96, 6.65]) was observed among women with a history of stillbirth.

## Discussion

In a large cohort of postmenopausal women aged 50–79, after adjustment for cardiovascular risk factors, a history of stillbirth was found to be associated with all cardiovascular outcomes within 5 years of study entry. Although we did not observe an interaction between age and pregnancy loss exposures for cardiovascular outcomes, we conducted an age-stratified analysis to determine whether meaningful patterns emerged. In the age-stratified analysis, the strongest association between a history of stillbirth and total incident CVD was observed among women aged 50–59, with smaller but still significant associations observed among women aged 60–69 and 70–79, although overlapping confidence intervals were observed for all age groups. Additionally, stillbirth was associated with incident CHD within 5 years among women aged 50–59 and 60–69. Among women aged 50–59 with a history of stillbirth, the risk estimate for heart failure was elevated but marginally insignificant after adjustment for confounders ($p = 0.06$), possibly due to the relatively small number of cases of heart failure occurring within 5 years of baseline in this age group ($n = 70$).

In a subset of study participants aged 50–59 at baseline, the proportion of study subjects experiencing cardiovascular outcomes before the age of 60 was small, and pregnancy loss exposures were not associated with significant increases in hazard ratios for any cardiovascular outcome. However, as with the age-stratified analysis, the hazard ratio for heart failure before the age of 60 was non-significantly ($p = 0.06$) elevated among women with a history of stillbirth.

The results of our analysis contribute to the existing literature demonstrating an increased risk of CVD among women with a history of pregnancy loss (2–9), and in particular a history of stillbirth (5, 9). Further, although we did not observe a significant interaction between age and pregnancy loss exposures in our analyses, our findings of strong associations between a history of stillbirth and incident CVD and CHD in women aged 50–59 augment the existing literature. The question of whether a history of pregnancy loss is more predictive of CVD risk in young women has been previously considered; however, previous work has examined CVD risk in very young women (under the age of 35) compared with older women. In a population-based study comprising more than 1 million women, Ranthe et al. found that in women under the age of 35, the rates of MI, cerebral infarction, and renovascular hypertension increased by 35–55% with each documented miscarriage. In women aged 35 and over, rates of the above outcomes increased by 6–7% per additional miscarriage (9). As the analysis of Ranthe et al. used dichotomized age groups of <35 and 35 and older, their study did not address the question of whether the association between pregnancy loss and risk of heart disease is stronger for women in midlife compared with older age.

In our all-ages analysis, our findings of higher odds of CVD, CHD, and heart failure among women with a history of stillbirth compared with any history of pregnancy loss (miscarriage or stillbirth) are consistent with previous research (5, 9, 25) and demonstrate biological plausibility. While numerous factors—including maternal diabetes, hypertensive disorders, other chronic diseases, maternal infection, and fetal genetic abnormalities—are known to increase the risk of pregnancy loss throughout gestation (26), the etiology of pregnancy loss also differs markedly by gestational age. Chromosomal abnormalities are likely to lead to losses early in pregnancy, while factors associated with loss in mid-to-late pregnancy include antiphospholipid syndrome, cervical weakness, anomalies of the uterus, infection, and placental insufficiency (27, 28). As stillbirth is more likely to occur because of maternal health factors than miscarriage, particularly miscarriage occurring in early pregnancy, stillbirth may also be more reflective of women's cardiovascular risk.

The stronger association observed between stillbirth and CVD may also provide clues about the biological basis for this association. In particular, the role that underlying vascular pathology and

endothelial dysfunction (the failure of the epithelial cells to regulate homeostasis in the vascular system) (29) may play in the observed association between pregnancy loss and CVD risk is of interest. Endothelial dysfunction has been shown to be associated with adverse pregnancy outcomes (30), is an early marker of atherosclerosis (31), and is thought to play a role in the pathogenesis of heart failure (32). It has been postulated that endothelial dysfunction resulting in poor placentation during a woman's reproductive years and the development of CVD later in life might underlie the observed associations between adverse pregnancy outcomes and CVD risk (28).

As a larger proportion of stillbirths than miscarriages are attributable to placental factors, such a mechanism would be consistent with the results of our analysis. Furthermore, unlike miscarriages occurring because of fetal chromosomal abnormalities, which are primarily due to *de novo* errors of meiosis and are unlikely to recur (27), vascular pathology is likely to lead to recurrent loss (9). This would be consistent with the findings of previous research in this area showing that the risk of CVD increases with the number of previous pregnancy losses, (5, 9) and also with the increased odds of CVD and heart failure observed in the current study among women with a history of recurrent loss.

The results of our analysis complement previous research suggesting that the inclusion of a history of pregnancy loss—in particular, a history of stillbirth—into cardiovascular risk profiles might improve risk prediction. In their study of CVD among WHI participants with a history of pregnancy loss, Parker et al. noted that a history of pregnancy loss might prove to be a clinically useful marker of future CVD risk in women (5). Even in studies finding a high prevalence of the conventional risk factors of smoking, hypertension, diabetes, and dyslipidemia among patients with CVD, approximately 15% of women experiencing CVD events had none of these factors (33). Further, the positive predictive value of conventional factors has been called into question, as a high percentage of patients who do not experience CVD events also demonstrate one or more of these factors (34).

The 2011 revision of the American Heart Association's (AHA) 'Evidence-Based Guidelines for Cardiovascular Disease Prevention in Women' called for complications of pregnancy to be considered in evaluating a woman's lifetime risk of CVD (35). Noting that complications of pregnancy may be seen as a "failed stress test" (35), whereby pre-existing vascular or metabolic disease or endothelial dysfunction are revealed (13), the guidelines recommend postpartum follow-up to monitor and control cardiovascular risk factors for women experiencing pregnancy-related complications. However, the 2011 AHA guidelines cite only a history of pre-eclampsia, gestational diabetes, or pregnancy-induced hypertension as major cardiovascular risk factors; no mention is made of pregnancy loss.

Similarly, the 2018 "Guideline on the Management of Blood Cholesterol" from the American College of Cardiology (ACC) states that, in order to assess atherosclerotic CVD risk in women, clinicians should obtain "a thorough pregnancy-related history"0.3 (36) Examples given of pregnancy-associated disorders associated with increased risk of atherosclerotic CVD include hypertension in pregnancy, pre-eclampsia, gestational diabetes, and delivery of a low-birthweight or preterm infant; as in the 2011 AHA guidelines, pregnancy loss is not explicitly mentioned.

A 2017 editorial on pregnancy-related events and CVD risk assessment recommended that clinicians include the following in women's cardiovascular risk profiles: a history of preterm delivery, pre-eclampsia, gestational hypertension, gestational diabetes, and infant size (13). While the editorial does note recent research showing an association between pregnancy loss and CVD in later life, the authors state only that "the mechanism remains uncertain" and stop short of explicitly calling for the consideration of pregnancy loss to be regarded as a risk factor for CVD. More recently, a position paper from the European Society of Cardiology explicitly mentioned the apparent association between recurrent pregnancy loss and CVD risk, in support of a statement that pregnancy history is integral to cardiovascular risk assessment in women (37).

In a recent analysis using data from the WHI OS, Parikh et al. considered the contribution of reproductive risk factors to CHD risk prediction. In a model adjusted for conventional CHD risk factors, a history of miscarriage and stillbirth were associated with a risk of incident CHD. The inclusion of reproductive factors resulted in a modest improvement in model discrimination, although net reclassification of risk category (<5%, 5–10%, or >10% 10-year risk of CHD) for women with CHD events was not significantly improved ($p = 0.18$) (14). The authors postulated that the inclusion of reproductive factors into risk profiles might be most efficacious in predicting CHD risk in younger women, prior to the onset of the conventional risk factors of dyslipidemia, diabetes, and hypertension.

The current analysis, like that of Parikh et al., comprised postmenopausal women; thus, we cannot draw from our results any conclusions about the strength of the association between pregnancy loss and cardiovascular outcomes in premenopausal women, or consider the question of whether a significant interaction between age and pregnancy loss history might be observed in a younger population. Nonetheless, our findings of a strong association between a history of stillbirth and risk of incident CVD within 5 years in women aged 50–59 may support the suggestion that pregnancy loss history could be efficacious as a clinical predictor of CVD risk in younger women.

Strengths of our analysis include the large sample size, lengthy follow-up, and consistent well-documented methods for ascertainment of cardiovascular outcomes in the WHI OS. Additionally, our study has certain limitations. As noted in Methods, potential confounders of the association between pregnancy loss and CVD risk were identified *a priori* and included as covariates in our models. As these were assessed at entry into the WHI, when participants were past reproductive age, it is possible that some of the factors may in fact be mediators rather than confounders of the association between pregnancy loss and risk of CVD. Thus, adjusting for these covariates may have attenuated our ability to detect an association between exposure and outcome.

As history of pregnancy loss in WHI participants was based upon self-reported reproductive history data, exposure misclassification is a possible limitation of our analysis. However, analysis of self-reported pregnancy losses has demonstrated good agreement with medical records data (38), and maternal recall of pregnancy-related events has shown good reproducibility and validity, even for events that occurred several decades previously (39). Further, the degree of misclassification of pregnancy loss in our study sample is unlikely to differ among women who did and did not

experience cardiovascular outcomes, and any such non-differential exposure misclassification would be expected to lead to more conservative results.

Potential misclassification of fatal coronary outcomes was an acknowledged limitation of the WHI outcome ascertainment (21). Additionally, as non-hospitalized heart failure and stroke were excluded from the WHI outcomes, we could not consider such events in our analysis. However, as with exposure misclassification, the effect of such outcome misclassification in our analysis would be unlikely to explain the observed associations, as there is no reason to suspect that misclassification of fatal outcomes or non-hospitalized events would occur differentially between women with and without a history of stillbirth.

The decision to exclude WHI participants with a self-reported history of CVD is a potential source of bias in our analysis. As our study sample by definition had to survive CVD-free until entry into the WHI cohort, we cannot exclude the possibility that our sample is biased in favor of event-free survival, particularly for subjects in the oldest age stratum. However, limiting the study sample to participants free of CVD at baseline enabled us to assess the risk of CVD in a previously healthy population, using the well-documented methods of ascertainment of CVD outcomes in the WHI cohort. Additionally, our methodology is consistent with that of a previous analysis of pregnancy loss and CVD in the WHI cohort (5).

Finally, although our analyses of cardiovascular outcomes did not demonstrate significant interactions between age and history of pregnancy loss, we lacked data on two factors that deserve further consideration: gestational age and maternal age at pregnancy loss. Recent studies of spontaneous late-second trimester pregnancy losses suggest that placental pathology plays an important role and that the etiology of later-term pregnancy loss is similar to that of stillbirth (40). Compared with losses in early pregnancy, late-term (after 12 weeks gestation) miscarriage and stillbirth both demonstrate stronger associations with the development of such clinical CVD risk factors as hypertension and type II diabetes; (41) correspondingly, late-term miscarriages are also likely to be more strongly associated with maternal future risk of CVD. As the WHI reproductive history questionnaire did not address gestational age at miscarriage, we were not able to examine this question in our analysis.

Similarly, while maternal age is strongly associated with the risk of pregnancy loss (42, 43), this is primarily due to an increased incidence of chromosomal abnormalities with increasing maternal age (27). As maternal factors account for a higher proportion of pregnancy losses in young women than in those of advanced maternal age, it is reasonable to speculate that losses experienced by young women may be more predictive of future CVD risk. As with gestational age, data on maternal age at pregnancy loss were not collected by the WHI; thus, we were unable to consider the potential importance of maternal age at loss in our analysis.

In summary, the results of the current analysis contribute to the literature on pregnancy loss and incident CVD and support the suggestion that the inclusion of history of pregnancy loss—and stillbirth in particular—in cardiovascular risk profiles may be clinically useful. Future research should strive to elucidate the common mechanisms underlying pregnancy loss and cardiovascular disease risk; additionally, the question of whether data on gestational age and maternal age at pregnancy loss might be useful clinical markers of CVD risk in women should be considered.

## Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: Women's Health Initiative datasets are made available to researchers upon approval of a paper proposal by WHI. Requests to access these datasets should be directed to helpdesk@whi.org.

## Ethics statement

The current analysis was reviewed and approved by Human Subjects Division, Office of Research, University of Washington. The WHI project was reviewed and approved by the Fred Hutchinson Cancer Research Center (Fred Hutch) IRB in accordance with the U.S. Department of Health and Human Services regulations at 45 CFR 46 (approval number: IR# 3467-EXT). WHI participants provided written informed consent for use of deidentified data for secondary analyses.

## Author contributions

CW, MK, DE, SP, and IP were responsible for the study design, data analysis and interpretation, and manuscript preparation. CK, RW, KP, and SS-J contributed to data analysis and interpretation and to manuscript revision. All authors have approved the final version of the manuscript for publication.

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## Conflict of interest

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

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2023.1108286/full#supplementary-material

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# Cardiovascular health and potential cardiovascular risk factors in young athletes

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**Introduction:** Cardiovascular disease remains the most common cause of death worldwide, and early manifestations are increasingly identified in childhood and adolescence. With physical inactivity being the most prevalent modifiable risk factor, the risk for cardiovascular disease is deemed low in people engaging in regular physical exercise. The aim of this study was to investigate early markers and drivers of cardiovascular disease in young athletes pursuing a career in competitive sports.

**Methods:** One hundred and five athletes (65 males, mean age 15.7 ± 3.7 years) were characterized by measurement of body impedance to estimate body fat, blood pressure (BP), carotid femoral pulse wave velocity (PWV) to evaluate arterial elasticity, ergometry to assess peak power output, echocardiography to calculate left ventricular mass, and blood tests.

**Results:** Systolic BP was elevated in 12.6% and thereby more than twice as high as expected for the normal population. Similarly, structural vascular and cardiac changes represented by elevated PWV and left ventricular mass were found in 9.5% and 10.3%. Higher PWV was independently associated with higher systolic BP ($\beta = 0.0186$, $p < 0.0001$), which in turn was closely correlated to hemoglobin levels ($\beta = 0.1252$, $p = 0.0435$). In this population, increased left ventricular mass was associated with lower resting heart rate ($\beta = -0.5187$, $p = 0.0052$), higher metabolic equivalent hours ($\beta = 0.1303$, $p = 0.0002$), sport disciplines with high dynamic component ($\beta = 17.45$, $p = 0.0009$), and also higher systolic BP ($\beta = 0.4715$, $p = 0.0354$).

**Conclusion:** Despite regular physical exercise and in the absence of obesity, we found an unexpected high rate of cardiovascular risk factors. The association of PWV, systolic BP, and hemoglobin suggested a possible link between training-induced raised hemoglobin levels and altered vascular properties. Our results point toward the need for thorough medical examinations in this seemingly healthy cohort of children and young adults. Long-term follow-up of individuals who started excessive physical exercise at a young age seems warranted to further explore the potential adverse effects on vascular health.

KEYWORDS

child, adolescent, pulse wave velocity, left ventricular mass, blood pressure

## Introduction

Cardiovascular (CV) disease is a leading cause of death and disability worldwide (1). The World Health Organization (WHO) accounts 32% of all deaths (17.9 million per year) mainly to myocardial infarction and stroke (https://www.who.int/health-topics/cardiovascular-disease). Physical inactivity is the most prevalent modifiable risk factor in this regard (2) prompting the WHO to devise a global action plan (3).

While clinical manifest CV disease usually presents in adulthood, the underling arteriosclerotic process begins much earlier (4). Starting already in childhood, the fractured elastin lamellae in the aorta and elastic arteries cause continuous remodeling, which leads to vascular stiffening. The lost elasticity raises pulse pressure and the aortic pulse wave velocity (PWV), which can later contribute to the development of left ventricular hypertrophy, cardiac failure, and microvascular disease in highly perfused organs such as the brain and kidneys (4). Although these developments are physiological to a certain extent, the concept of early vascular aging describes an accelerated remodeling process driven by the interaction of genetic predisposition with certain risk factors like arterial hypertension, insulin resistance, microinflammation, and dyslipidemia (5). Especially cardiorespiratory fitness and adiposity are connected to stiffer blood vessels even in young children (6). Symptomatic endpoints such as chronic heart failure, myocardial infarction, and stroke are rare in young individuals. Yet, subclinical changes can be noninvasively assessed by measuring PWV or left ventricular mass (LVM) in children and young adults (7, 8). Both parameters are well-defined surrogate markers and are associated with cardiovascular disease later in life (9, 10).

In this context, it is generally assumed that athletes are in good cardiovascular health; after all the amount of physical activity performed in this group, it must be considered sufficient to comply with the recommendations by the WHO (11). Reports have shown normal to reduced PWV in adult elite athletes performing swimming (12), endurance sports (13), and a diverse set of other sport disciplines (14). However, there is also evidence for strength and resistance training elevating PWV in older adults (15). Indeed, a recent study showed that even in young adults engaging in different sports, there were markedly raised PWV values (16). Increases in LVM, also called *athletes' heart*, are frequently seen in athletes and are greater in endurance sports (17). This has also been observed in active children and adolescents (18) albeit being not as pronounced (19). Younger athletes appear to have primary chamber dilation and less hypertrophy (20) causing a more eccentric remodeling (21). This hypertrophy is often regarded as a physiological adaption to exercise. Yet in some cases, there is a morphological overlap with primary cardiomyopathies, posing a potential threat to athletes (22). Similar to chances in LVM, there is also mixed evidence for the effect of competitive sports on blood pressure (BP). Up to a third of a large contemporary cohort of young athletes formally fulfilled the criteria of arterial hypertension (23), while others saw a decrease of diastolic BP (24). Of note, young non-endurance athletes in the latter study presented with increased systolic BP. When looking at other known risk factors such as classical blood biomarkers, young athletes tend to have lower levels of low-density lipoprotein and higher levels of high-density lipoprotein (25).

The aim of our study was to investigate the CV risk in young athletes pursuing a career in competitive sports. We comprehensively assessed vascular and cardiac surrogate markers indicative of subclinical CV damage and several blood parameters known to reflect CV risk.

## Methods

This prospective cross-sectional study recruited young athletes from the Olympic Training Centre Lower Saxony in Hannover, Germany, over a period of 4 months (October, November, and December 2016 and September 2017). One hundred and five out of 340 children and young adults between 7.9 and 28.6 years of age, who engaged in their annual sport medical examination, consented to be enrolled. The participants were active in different competitive sports disciplines: tennis ($n = 7$), cycling ($n = 8$), gliding ($n = 4$), basketball ($n = 19$), judo ($n = 10$), karate ($n = 3$), swimming ($n = 8$), field hockey ($n = 5$), rugby ($n = 7$), decathlon ($n = 1$), handball ($n = 1$), sailing ($n = 12$), water ski ($n = 6$), boxing ($n = 7$), and running ($n = 7$). We grouped sport disciplines based on their dynamic or static components, respectively, into high, moderate, and low according to the Mitchell classification (26). The study was approved by the local institutional review board (file number 3339–2016) and, if applicable, the consent from the respective parents was acquired prior to participation.

All examinations were carried out in the morning. The athletes were allowed to have a light breakfast, of their choice, mostly containing long carbohydrates. Examinations always followed the same order: blood samples were taken first, followed by anthropometric measurements, bioimpedance analysis, BP and resting heart rate (HR), ECG, echocardiography, measurement of PWV, and ergometer tests. Bioimpedance analysis was carried out using the InBody720 device (InBody Europe B.V., Eschborn, Germany). Standardized measurement of BP and resting HR were performed in a seated position with a validated oscillometric device (Dinamap, Carescape V100; GE Healthcare, Chicago, IL, United States) after 5 min of rest as described previously (27). Carotid femoral PWV was evaluated using the oscillometric Vicorder device (Skidmore Medical Limited, Bristol, United Kingdom; Software Version 4) according to the recommendations of the Task Force III on clinical applications of arterial stiffness (28).

We calculated $z$-scores for BMI, height, weight (29), body fat percentage (30), BP (31), resting HR (32), and PWV (7). Obesity was defined as BMI $z$-score $\geq$1.645 (reflecting values above the 95th percentile). Similarly, elevated BP values were defined as either systolic or diastolic BP $z$-scores $\geq$1.645. In case participants exceeded the upper age range of the respective reference cohort, we calculated their $z$-scores based on the highest age available for reference as the cut-off values generally used in adulthood correspond very well to $z$-scores reflecting the 90th–95th percentiles in adolescents of 16–18 years of age. This

approach was taken for BMI, BP, resting HR, as well as body fat and was only used to allow for better visualization of the results.

Transthoracic echocardiography was performed using the ultrasound GE Vivid I, equipped with a 1–5 MHz transducer, according to the recommendations of the American Society of Echocardiography (33). All examinations and measurements were carried out by one experienced consultant specialized in cardiology following a standardized protocol. Left ventricular end-diastolic wall thickness and end-diastolic dimensions were obtained from the parasternal long-axis view at the level of the papillary muscles using M-Mode. Measurements were made only if image quality was excellent and allowed for unequivocal identification of all relevant structures. Relative wall thickness was calculated and a score greater than 0.42 cm was considered abnormal (34). We calculated LVM (35), LVM index (36), and LVM *z*-scores (8).

The athletes' cardiorespiratory fitness was examined through a slightly modified bicycle ergometry (Viasprint 150P, Ergoline, Bitz, Germany) test (37). In brief, all participants started with a load of 50 W and were increased by 17W every minute (10 W for athletes with a bodyweight below 40 kg). HR was measured continuously; BP and blood lactate concentration (Ebio 6666, Eppendorf, Hamburg) were measured at rest, 1 min after the start of exercise, and every 3 min during the exercise. Termination criterion of the ergometry was the physical exhaustion of the athletes, determined by subjective exhaustion, or if cadence could not be maintained above 60 revelations/min. The maximum wattage was related to body weight and lean body mass.

Questionnaires adapted from the German Health Interview and Examination Survey for Children and Adolescents (38) were used to capture participants' activities (hours per week spent on training within their respective sport's discipline, other physical activities, and years of involvement in competitive sports). In addition, we retrieved information on pre-existing illnesses of participants. An individual metabolic equivalent (MET) value was assigned to each of the indicated sport disciplines depending on their respective intensity (39). MET hours were then calculated by multiplying the number of training hours (reported by the athletes) with the respective sport-specific MET score.

Blood samples were analyzed in one central laboratory (Klinikum Region Hannover, Hannover, Germany) and included small blood count, electrolytes, creatinine, urea, total bilirubin, lactate dehydrogenase, aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma gamma-glutamyltransferase, creatine kinase, total cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, C-reactive protein, and ferritin. The estimated glomerular filtration rate was calculated by the Schwartz bedside formula for athletes who were under 18 years of age (40) and by the CKD-Epi equation for those who were 18 years or older (41). Hemoglobin *z*-scores adjusted for age were calculated using data from the German Health Interview Examination Survey for Children and Adolescents (42).

Statistical analysis was performed using SAS 9.4M6 (Statistical Analysis Software, Cary, NC, United States). Continuous variables are given as mean ± SD. A *p*-value of <0.05 was considered statistically significant. Two-sided *t*-tests were used for the comparison of continuous variables. Data were further evaluated by the use of multivariable regression models. For the outcomes, LVM and PWV, we started with setting up a basic model corrected for the covariates sex, age, and height and using raw data (not *z*-scores) as outcome. Potential predictors were chosen based on prior knowledge: systolic BP (7), resting HR (38), sport discipline (26), MET hours (39), and hemoglobin (40). Hemoglobin was also chosen as hemoglobin values were significantly raised in our study group. Each of these predictors was added separately as an independent variable to the basic models. For the full models, only predictors showing a *p*-value <0.05 were selected.

TABLE 1 Basic characteristics of all participants and according to sex.

| | Total ($n = 105$) | Boys ($n = 65$) | Girls ($n = 40$) | |
|---|---|---|---|---|
| Body composition | M ± SD | M ± SD | M ± SD | *p* |
| Age, years | 15.7 ± 3.7 | 16 ± 3.8 | 15.1 ± 3.4 | 0.2203 |
| Height, cm | 170.2 ± 12.2 | 172.8 ± 12.2 | 166 ± 10.9 | **0.0045** |
| Weight, kg | 59.4 ± 14.6 | 61.7 ± 16.3 | 55.8 ± 10.6 | **0.0441** |
| BMI, kg/m² | 20.2 ± 3.1 | 20.3 ± 3.4 | 20.1 ± 2.4 | 0.7337 |
| Body fat, % | 12.3 ± 6.5 | 9.7 ± 5 | 16.5 ± 6.4 | **<.0001** |
| Muscle mass, % | 48.8 ± 3.9 | 50.4 ± 3.1 | 46.1 ± 3.7 | **0.0004** |
| Lean body mass, kg | 45.0 ± 12.4 | 48.5 ± 13.6 | 38.9 ± 6.8 | **0.0002** |
| Training | M ± SD | M ± SD | M ± SD | |
| Experience, years of involvement | 6.5 ± 3.3 | 6.1 ± 3.4 | 7.1 ± 3.1 | 0.1573 |
| Current exposure, hours of training/week | 9.4 ± 5.4 | 9.3 ± 5 | 9.5 ± 6.1 | 0.8558 |
| Intensity, MET hours | 96.3 ± 71.3 | 94 ± 67.3 | 99.8 ± 77.3 | 0.6943 |
| Max. power output, W | 229.2 ± 59.1 | 243.5 ± 62.8 | 206 ± 44.3 | **0.0015** |
| Max. power output, watt/kg bodyweight | 3.9 ± 0.5 | 4.0 ± 0.5 | 3.7 ± 0.5 | **0.0079** |
| Max. power output, watt/kg lean body mass | 4.4 ± 0.5 | 4.4 ± 0.5 | 4.5 ± 0.6 | 0.7375 |

*p*-value of <0.05 in bold.
M, mean; SD, standard derivation; BMI, body mass index; MET, metabolic equivalent of task; *p*, *p*-value.

## Results

We enrolled a total of 105 young athletes (65 males; 62%) at the Olympic Training Centre Lower Saxony in Hannover. Table 1 shows the basic characteristics of all participants, also categorized by sex. The mean age was 15.7 ± 3.7 (range 7–28) years. As reflected by the calculated *z*-scores (Figure 1), athletes were significantly taller and heavier compared to the WHO reference cohort ($p < 0.0001$ for height and weight), while their BMI was close to average. Athletes' body composition showed a significantly lower proportion of fat ($p < 0.0001$ compared to the underlying reference cohort; Figure 1). The athletes participated in competitive sports for an average of 6.5 ± 3.3 years and were currently attending training sessions for 9.4 ± 5.4 h per week resulting in an average of 96.3 ± 71.3 MET hours. The overall relative maximum power output was 3.9 ± 0.5 W/kg. Compared

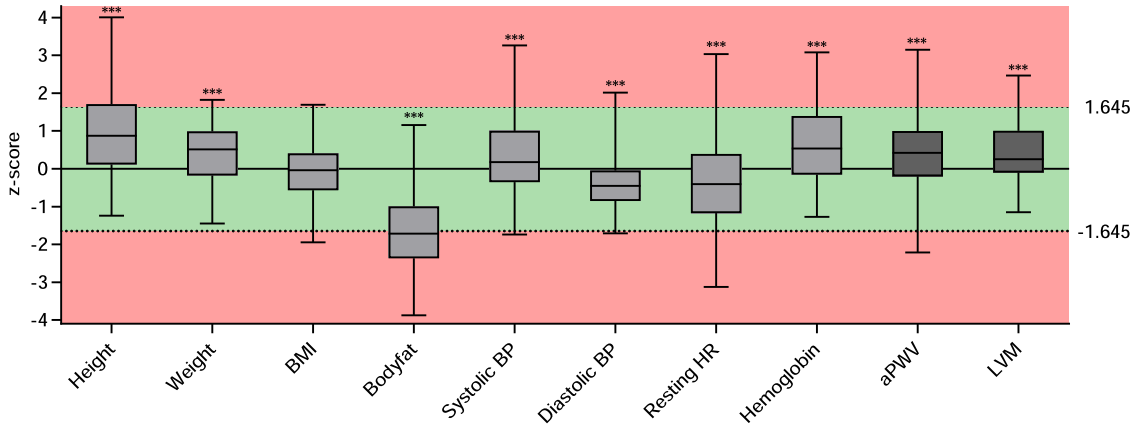


FIGURE 1
*z*-scores for parameters resembling cardiovascular risk or being indicative for cardiovascular health. Data are presented as boxplots of the available *z*-scores. The box includes the interquartile range with the median denoted in the middle. Minimum and maximum are depicted as whiskers. *** indicates a significance of $p < 0.001$ in a one sample *t*-test compared to "0." The red background represents *z*-scores smaller than −1.645 or greater than 1.645 corresponding to the 5th and 95th percentile, respectively. BMI, body mass index; BP, blood pressure; HR, heart rate; PWV, pulse wave velocity; LVM, left ventricular mass.

to a reference cohort consisting of healthy children, the participants of our study were in the upper average range (43). None of the participants reported smoking.

Table 2 gives an overview of additional parameters assessed to determine cardiovascular health and blood parameters resembling classical and nonclassical cardiovascular risk factors. Mean systolic BP was 118 ± 13 mmHg and mean diastolic BP was 62 ± 7 mmHg. Athletes displayed higher mean systolic BP (*z*-score 0.4 ± 1) and lower mean diastolic BP value (*z*-score −0.4 ± 0.6) compared to reference values ($p = 0.0006$ and $p < 0.0001$, respectively, Figure 1). Twelve athletes (12.6%) had elevated systolic BP levels; only one athlete (1.05%) displayed an elevated diastolic BP. Mean resting HR was 71.2 ± 13.7 and was significantly lower than reference (*z*-score −0.4 ± 1.4; $p = 0.00015$, Figure 1).

Laboratory parameters demonstrated normal kidney and liver function, no overt dyslipidemia, and no sign of myolysis or anemia. The athletes had higher levels of hemoglobin with a *z*-score of 0.6 ± 1.1 compared to the KIGGS-reference cohort ($p < 0.0001$, Figure 1). Of note, boys demonstrated a lower hemoglobin *z*-score when compared to girls (see Supplementary Material Table S1 and Figure S1).

In addition to risk factors, we assessed structural vascular and cardiac changes indicative for cardiovascular end organ damage (Table 2). Mean PWV was 5.6 ± 0.6 m/s. The average *z*-score of 0.4 ± 0.9 was elevated and significantly higher than in the underlying reference cohort ($p < 0.0001$, Figure 1). Nine athletes (9.45%) displayed elevated PWV values. Regression models showed that greater age and height were independent determinants of higher PWV [(a) in Table 3]. When added individually to the basic model for PWV, higher systolic BP ($p < 0.0001$), more MET hours ($p = 0.0182$), and higher hemoglobin ($p = 0.0435$) had a significant positive effect on PWV, while resting HR and sport discipline did not independently influence

TABLE 2 Parameters either resembling classical and nonclassical cardiovascular risk factors or being indicative of cardiovascular health.

| | Total ($n = 105$) | Boys ($n = 65$) | Girls ($n = 40$) | |
|---|---|---|---|---|
| Cardiovascular parameters | M ± SD | M ± SD | M ± SD | *p* |
| Systolic BP, mmHg | 117.6 ± 12.6 | 119.5 ± 12.8 | 114.5 ± 11.9 | **0.0479** |
| Diastolic BP, mmHg | 61.6 ± 7.1 | 61.4 ± 7.4 | 62 ± 6.8 | 0.7110 |
| Resting heart rate, bpm | 71.2 ± 13.7 | 70.5 ± 13.8 | 72.2 ± 13.7 | 0.5514 |
| PWV, m/s | 5.6 ± 0.6 | 5.6 ± 0.6 | 5.4 ± 0.6 | 0.0840 |
| Left ventricular mass, g | 132.4 ± 40 | 146.4 ± 40.5 | 113.4 ± 30.6 | **<.0001** |
| Left ventricular mass index, g/m$^{2.16}$ | 40.1 ± 8.8 | 42.7 ± 8.9 | 36.7 ± 7.5 | **0.0013** |
| Left ventricle relative wall thickness, cm | 0.3 ± 0.03 | 0.3 ± 0.03 | 0.3 ± 0.03 | 0.9247 |
| Laboratory tests | M ± SD | M ± SD | M ± SD | |
| Hemoglobin, g/dl | 14.3 ± 1 | 14.6 ± 1.1 | 13.9 ± 0.8 | **0.0003** |
| Cholesterol, mg/dl | 158.5 ± 30.5 | 155.4 ± 32.6 | 163.6 ± 26.3 | 0.2128 |
| LDL, mg/L | 96.8 ± 22.5 | 95.9 ± 24.5 | 98.1 ± 19 | 0.6481 |
| HDL, mg/dl | 54.9 ± 11.9 | 53.1 ± 11.1 | 58.1 ± 12.7 | **0.0486** |
| Creatinine, mg/dl | 0.75 ± 0.15 | 0.8 ± 0.2 | 0.7 ± 0.1 | 0.1174 |
| Estimated GFR, ml/min/1.73 m$^2$ | 102.3 ± 15.1 | 104.4 ± 15.9 | 98.7 ± 13 | 0.0680 |
| Bilirubin, mg/dl | 0.7 ± 0.4 | 0.6 ± 0.3 | 0.7 ± 0.4 | 0.7565 |
| AST, U/L | 26.4 ± 9.2 | 28.1 ± 10.5 | 23.7 ± 5.7 | **0.0283** |
| ALT, U/L | 18.7 ± 7.7 | 20.7 ± 8.5 | 15.3 ± 4.8 | **0.0009** |
| Alkaline phosphatase, U/L | 204.4 ± 134.6 | 231.2 ± 152.3 | 160 ± 82.8 | **0.0126** |
| LDH, U/L | 182.3 ± 36.7 | 186.8 ± 37.3 | 174.9 ± 35.1 | 0.1311 |
| CK, U/L | 182.2 ± 158.7 | 202.1 ± 179.4 | 148.1 ± 109.3 | 0.1006 |

*p*-value. *p*-value of <0.05 in bold.
M, mean; SD, standard derivation; BP, blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; GFR, glomerular filtration rate; AST, aspartate-aminotransferase; ALT, alanine-aminotransferase; LDH, lactate dehydrogenase; CK, creatine kinase; PWV, pulse wave velocity; *p*.

Table 3 Basic models for (a) PWV and (b) LVM corrected for sex, age, and height.

| Variables | (a) PWV ($R^2 = 0.3226$) | | | (b) LVM ($R^2 = 0.6549$) | | |
|---|---|---|---|---|---|---|
| | β | SE | p | β | SE | p |
| Intercept | 1.4932 | 0.8336 | **0.0766** | −181.76 | 40.428 | **<.0001** |
| Female (ref: male) | −0.0174 | 0.1166 | 0.8818 | −13.368 | 5.5158 | **0.0175** |
| Age | 0.0403 | 0.0167 | **0.0181** | 3.9496 | 0.8414 | **<.0001** |
| Height | 0.0202 | 0.0054 | **0.0003** | 1.5034 | 0.2661 | **<.0001** |

*p*-value of <0.05 in bold.
PWV, pulse wave velocity; LVM, left ventricular mass; β, regression coefficient; SE, standard error; $R^2$, explained variance.

PWV [(a) in Table 4]. In the full model [(b) in Table 4], systolic BP and MET hours were significantly positive related to PWV.

Mean LVM was 132.4 ± 40 g and LVM *z*-score was higher in our cohort than in the reference cohort (8) (*z*-score 0.4 ± 1, $p < 0.0001$; Figure 1) with elevated LVM in nine athletes (10.3%). When the different sport disciplines were classified based on their dynamic component, athletes engaging in sports with a high dynamic component displayed significantly higher LVM (Supplementary Material Table S2). There was no difference in LVM according to the static component (Supplementary Material Table S3). Regression models showed greater age, height, and male sex were significantly associated with higher LVM [(b) in Table 3]. Adding systolic BP ($p = 0.0354$), MET hours ($p = 0.0002$), and high dynamic component of sports discipline ($p = 0.0009$) individually to the basic model for LVM turned out to have a significant positive effect on LVM, while resting HR demonstrated an inverse effect ($p = 0.0052$). Hemoglobin did not independently influence LVM (Table 5). In the full model, systolic BP, resting heart rate, and high dynamic component of sports discipline retained their significant independent effect on LVM [(b) in Table 5].

Table 4 Advanced models for PWV: (a) basic model including only one additional independent variable at a time; (b) full model with all variables.

| Variables | a: Basic model + 1 | | | b: Full model ($R^2 = 0.4256$) | | |
|---|---|---|---|---|---|---|
| | β | SE | p | β | SE | P |
| Intercept | *For details please refer to supplement table S4a–S9a* | | | 0.7735 | 1.0107 | 0.4463 |
| Female (ref: male) | | | | 0.0154 | 0.1125 | 0.8917 |
| Age | | | | 0.0115 | 0.0173 | 0.5063 |
| Height | | | | 0.0111 | 0.0055 | **0.0455** |
| Systolic BP mmHg | 0.0186 | 0.0043 | **<.0001** | 0.0160 | 0.0045 | **0.0005** |
| Resting HR bpm | −0.0018 | 0.0041 | 0.6572 | | – | |
| MET hours | 0.0019 | 0.0008 | **0.0182** | 0.0016 | 0.0008 | **0.0394** |
| Hemoglobin, g/dl | 0.1252 | 0.0611 | **0.0435** | 0.0457 | 0.0587 | 0.4393 |
| High dynamic component (ref.: low and moderate) | 0.0921 | 0.1158 | 0.4285 | | – | |

*p*-value of <0.05 in bold.
PWV, pulse wave velocity; β, regression coefficient; SE, standard error; BP, blood pressure, HR, heart rate; MET, metabolic equivalent of task; $R^2$, explained variance.

Table 5 Advanced models for LVM: (a) basic model including only one additional independent variable at a time; (b) full model with all variables.

| Variables | a: Basic model + 1 | | | b: Full model ($R^2 = 0.7677$) | | |
|---|---|---|---|---|---|---|
| | β | SE | p | β | SE | P |
| Intercept | *For details please refer to supplement table S4b–S9b* | | | −148.04 | 41.483 | **0.0006** |
| Female (ref: male) | | | | −12.282 | 4.6259 | **0.0097** |
| Age | | | | 3.7895 | 0.7845 | **<.0001** |
| Height | | | | 1.0903 | 0.2450 | **<.0001** |
| Systolic BP, mmHg | 0.4715 | 0.2204 | **0.0354** | 0.5402 | 0.1911 | **0.0061** |
| Resting HR, bpm | −0.5187 | 0.1805 | **0.0052** | −0.5191 | 0.1648 | **0.0024** |
| MET hours | 0.1303 | 0.0339 | **0.0002** | 0.0536 | 0.0374 | 0.1560 |
| Hemoglobin, g/dl | −1.9466 | 3.0329 | 0.5228 | | – | |
| High dynamic component (ref.: low and moderate) | 17.450 | 5.0435 | **0.0009** | 12.315 | 5.3338 | **0.0237** |

*p*-value of <0.05 in bold.
LVM, left ventricular mass; β, regression coefficient; SE, standard error; BP, blood pressure, HR, heart rate; MET, metabolic equivalent of task; $R^2$, explained variance.

## Discussion

This observational study characterized a cohort of young athletes pursuing a career in competitive sports. In this seemingly healthy population on regular exercise and without obesity, we found a high rate of potential cardiovascular risk factors, illustrating the importance of regular follow-up by sports medicine. Systolic BP elevation in more than 10% of the participants reflects a rate twice as high as to what one expects in the normal population. Similarly, structural vascular and cardiac changes represented by elevated PWV and LVM were found twice more often. Higher PWV was independently associated with higher systolic BP, which in turn was closely correlated with higher hemoglobin levels. This suggested a possible link between training-induced increased hemoglobin levels and vascular stiffness in a small subgroup of this population. Increased left ventricular mass, often discussed as a physiological reaction to physical exercise and higher fitness level, was clearly associated with lower resting HR and high dynamic component of sport discipline, but also higher systolic BP in our cohort.

Our study extends previous findings, which showed elevated PWV levels in young athletes (16), by providing additional data that advance our understanding of potential drivers of accelerated PWV. The full multivariable model confirms that BP significantly enhances PWV, which has already been demonstrated in healthy populations (7) and several patient groups (44). The effect of systolic BP superseded other independent variables, like hemoglobin, which we had found of importance when added to the basic model. Interestingly, Chen et al. observed a correlation of higher hemoglobin level with BP in 3,776 healthy children (45). They hypothesized that hemoglobin in higher concentrations acts as a nitric oxide (NO) scavenger and leads to less free NO causing vasoconstriction. This vasoconstriction could then cause increased systolic BP and over a longer time period may also explain our observation of faster PWV. Thereby, BP and hemoglobin would be interdependent parts in a common chain of effects; hence, after adding both into one model, hemoglobin is no longer independently associated with PWV. Along those lines, a positive

correlation between hemoglobin and increased PWV was previously described by others in middle-aged adults (46) and elderly women (47). These findings have to be seen in context with the markedly increased hemoglobin *z*-scores in our cohort. However, physical exercise has been widely described to enhance NO release and thereby to have a positive effect on vascular function in athletes (48).

Of note, 12% of the characterized athletes had an elevated systolic along with low-normal diastolic BP. Whether this condition reflects an innocent, "spurious" phenotype or whether it is a true form of hypertension requiring follow-up and even treatment is heavily debated (49). While a large study found middle-aged adults with isolated systolic hypertension to have a higher risk for cardiovascular morbidity and mortality during their 31-year follow up period (50), elevated systolic BP values in young physically active men have long been deemed normal (51). The underlying pathomechanisms differ by age: stiffer vessels due to vascular aging cause both elevated central and brachial BP, whereas in young healthy individuals, central systolic BP might get augmented due to lower HR and higher stroke volume resulting in increased brachial BP (52). The same study found about 20% of young individuals with isolated systolic hypertension, normal stroke volume, and increased PWV, which matches our observations. Isolated systolic hypertension of the young seems to be a heterogeneous condition, and at least in some individuals, it might be associated with premature stiffening of the vasculature and therefore constitutes a true cardiovascular risk. This has recently been highlighted in a study comparing adolescents with isolated systolic hypertension and peers diagnosed with white coat hypertension. The former group demonstrated significantly higher aortic PWV, LVMI, and a higher incidence of left ventricular hypertrophy (53).

Ten percent of the examined athletes demonstrated elevated LVM, for which we demonstrated systolic BP as an associated risk factor on the one hand. On the other hand, we confirmed observations made by others indicating an independent association with both increased MET hours (i.e., increasing intensity and duration of the performed sport) and lower resting HR (54). Moreover, sport disciplines with a high dynamic component were associated with higher LVM. This is in line with observations from a recent large sample of adult athletes (55). As in our full model MET hours did not have an independent significant influence on LVM, the effect of the training intensity might be carried by the highly dynamic component in our cohort. Interestingly, diastolic BP did not independently influence LVM, which supports our finding of elevated systolic blood pressure driving an increase in LVM. As none of the athletes demonstrated an increased relative wall thickness above 0.42 cm, left ventricular hypertrophy can be classified as eccentric (34), which is suggestive for a physiological adaptation to intense exercise. To which extent the described elevation of LVM resembled truly a benign "athletes' heart" and how it is best differentiated from hypertensive heart disease is subject to ongoing research, in both junior and senior athletes (56, 57). Recently, *z*-scores for LVM adapted to child and adolescent athletes were proposed (58), raising the question whether elevating cut-off levels to have fewer pathological findings is the correct approach in light of the presented data and without any long-term follow-up in these young individuals.

Hemoglobin *z*-score was significantly higher in girls than in boys. A possible explanation for this phenomenon could be amenorrhea in female participants due to a high training load. Amenorrhea is part of "The Female Athletes Triad" (59) as well as osteoporosis and disordered eating, which was not the focus of our study. Still, our data highlight an additional adverse effect on the long-term health of professional female athletes.

Despite our cohort reflecting a broad age range and various sport disciplines, we were able to not only confirm known findings but also discover potential drivers for cardiovascular alterations using multivariable modeling. Like others (16), we sought to categorize sports disciplines into endurance and strength in order to evaluate whether they affect the cardiovascular system differently (60). However, such classification was difficult to apply, since most athletes either engage in disciplines that already feature strength and endurance components like boxing and basketball or follow training schedules that incorporate both components. In addition, our study had a cross-sectional design. These limitations emphasize the need for further investigations with homogenous sport groups or even an interventional and probably longitudinal design. Another limitation is that we did not perform central BP measurements, which would have helped to further assess the young athletes with elevated systolic BP values. We also did not engage in extensive electrophysiological investigations like Holter monitoring. The latter would have uncovered arrhythmias, which are among the leading causes for sudden cardiac death besides congenital heart defects and hypertrophic cardiomyopathies in young athletes (61).

In our cohort of young athletes, we found a high rate of potential cardiovascular risk factors and described an association between increased PWV and systolic BP alongside raised hemoglobin levels. We, like others, assumed an etiological relationship between those parameters linked by NO and therefore identifying hemoglobin as a potential risk factor. This observation is of particular importance since in the context of competitive sports, high normal hemoglobin levels are seen as a marker of good physical capacity because of the strong relationship between total hemoglobin mass and maximal aerobic capacity (62). Taken together, regular sports and exercise prevent risk factors like obesity, dyslipidemia, or hyperglycemia and, therefore, have an overall positive effect on health. Our results point toward the need of regular medical examinations in this seemingly healthy cohort of children and young adults. Long-term follow-up of individuals who started excessive physical exercise at a young age seems warranted to further explore potential adverse effects on vascular health.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, upon reasonable request.

## Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Hannover Medical School (file number 3339-2016). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

## Author contributions

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

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## Conflict of interest

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

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2023.1081675/full#supplementary-material

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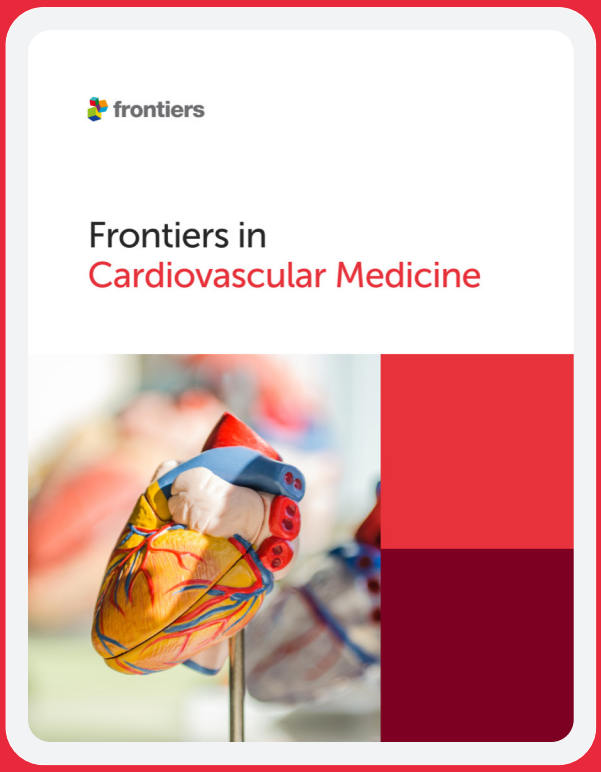


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