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Associations of HDL metrics with coronary artery calcium score and density among women traversing menopause

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Abstract **The cardioprotective association of high-density lipoprotein cholesterol (HDL-C) may vary by menopause stage or estradiol level. We tested whether associations of comprehensive HDL metrics (HDL subclasses, phospholipid and triglyceride content, and HDL cholesterol efflux capacity [HDL-CEC]) with coronary artery calcium (CAC) score and density vary by menopause stage or estradiol level in women transitioning through menopause. Participants (N = 294; mean age [SD]: 51.3 [2.9]) had data on HDL metrics and CAC measures at one or two time points during the menopause transition. Generalized estimating equations were used for analyses. Effect modifications by menopause stage or estradiol level were tested in multivariable models. In adjusted models, menopause stage modified the associations of specific HDL metrics with CAC measures. Higher small HDL particles (HDL-P) concentrations (p-interaction = 0.008) and smaller HDL size (p-interaction = 0.02) were associated with greater odds of CAC presence in late perimenopause than in pre/early perimenopause stage. Women in the highest estradiol tertile, but not the lower tertiles, showed a protective association of small HDL-P with CAC presence (p-interaction = 0.007). Lower large HDL-P concentrations (p-interaction = 0.03) and smaller HDL size (p-interaction = 0.03) were associated with lower CAC density in late perimenopause than in postmenopause stage. Associations of HDL phospholipid and triglyceride content and HDL-CEC with CAC measures did not vary by menopause stage or estradiol level. We concluded that HDL subclasses may impact the likelihood of CAC presence and the stability of coronary plaque differently over the menopause transition. Endogenous estradiol levels may contribute to this observation.**

Supplementary key words HDL/structure • cardiovascular disease • cholesterol/Efflux • lipoproteins • hormones • calcium score • calcium density • climacteric • menopause • women

Previous studies have shown that high-density lipoprotein cholesterol (HDL-C) levels plateau (1) or decline around menopause (2). However, more recent longitudinal studies have revealed that as women traverse menopause, their HDL-C levels increase (3), and this increase continues even after menopause (4). Despite the strong epidemiological data that higher HDL-C is associated with a lower risk of cardiovascular disease (CVD) (5), the rise in HDL-C in midlife and older women is associated with increased atherosclerosis risk (6–10).

HDL are complex particles that are heterogeneous in size, composition, and function. HDL-C, the cholesterol load of HDL, does not necessarily reflect the overall concentration of HDL particles (HDL-P), the heterogeneous distribution of HDL subclasses, or the content and function of these particles. The limitation of HDL-C as an overall metric of HDL was evident from a recent analysis of longitudinal data from 469 midlife women transitioning through menopause (3). Although HDL-C significantly increased over the menopause transition, adverse changes were reported in other HDL metrics, including declines in large HDL-P concentrations and HDL size, increases in concentrations of small HDL-P and HDL triglyceride content, and declines in the per particle ability to mediate macrophage cholesterol efflux capacity (3), a validated assay of HDL function for human studies. Thus, metrics of HDL subclasses, lipid content, and function may better reflect the clinical utility of HDL in midlife and older women.

Coronary artery calcium (CAC) score, a noninvasive index of the overall burden of coronary

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atherosclerosis, is a strong predictor of CVD risk (11, 12), even among younger populations. Association of HDL-C with this CVD risk indicator has not been consistent in the general population (13, 14) and even reverses in midlife women (8). Higher levels of HDL-C have been linked to a lower odds of CAC in some (13, 14) but not all studies (15, 16). Additionally, in women at midlife, association between HDL-C and CAC varied by menopause stage with higher HDL-C associated with greater risk of left main CAC in women at a later, but not earlier, stage of the menopause transition (8). The dynamic changes in estradiol (E2), one of the main cardinal markers of menopause (17), may contribute to making HDL dysfunctional during the menopause transition. Few studies have assessed associations of comprehensive metrics of HDL with CAC score in midlife women (18, 19), and none has assessed whether these associations vary by menopause stage or level of E2.

Using CAC score, the average calcium density of calcified plaque can be estimated. A growing body of evidence argues that the use of CAC score to predict CVD risk may not be sufficient since higher CAC score could be a result of greater CAC volume, density, or both. Studies suggest that higher calculated calcium density may be protective of CVD (20, 21) independent of CAC volume. A greater CAC density may reflect a stabilizing process in plaque development (22, 23). Associations of CAC density with comprehensive HDL metrics in midlife women have not been characterized, and these associations might also be modified by menopause stage or E2 level.

The Study of Women's Health Across the Nation (SWAN) HDL and Heart ancillary studies provide a great opportunity to enhance our understanding of the usefulness of HDL subclasses, lipid content, and function as biomarkers of CVD risk, beyond HDL-C, in women traversing menopause. The main objective of this research was to test associations of HDL subclasses, phospholipid and triglyceride content, and HDL cholesterol efflux capacity, with CAC score and calculated density, and to assess whether these associations vary by menopause stage or E2l level in midlife women. We hypothesized that associations of HDL subclasses, lipid content, and HDL function with each of the two CAC measures will vary by menopause stage and E2 level with HDL metrics showing protective associations at younger stages of the menopause transition or at higher levels of E2.

MATERIALS AND METHODS

SWAN is an ongoing, multiethnic, multisite, population-based, longitudinal study that aims to characterize the physiological and psychological changes in women as they traverse the menopause transition. The study design of SWAN has been described before (24). Briefly, between 1996 and 1997, 3,302 women between the ages of 42 and 52 years were recruited at seven different sites across the United States: Pittsburgh, PA; Boston, MA; Newark, NJ; Detroit, MI; Chicago, IL; Oakland, CA; and Los Angeles, CA. The eligibility criteria for SWAN recruitment were: (i) having an intact uterus and at least one ovary, (ii) not being pregnant or lactating at the time of recruitment, (iii) having at least one menstrual period within the last 3 months prior to recruitment, (iv) not being on hormone therapy and (v) identifying themselves as White, Black, Hispanic, Chinese, or Japanese.

The SWAN Heart ancillary study was designed to evaluate subclinical measures of atherosclerosis during the menopause transition. Participants for the Pittsburgh and Chicago sites were recruited in this ancillary study. SWAN Heart assessed CAC at 2 visits (baseline at SWAN visits 4–7 and follow-up at SWAN visits 6–9). The SWAN HDL study, an ancillary study to SWAN, aims to characterize the changes in HDL subclasses, lipid content, and function that accompany ovarian aging and to describe how these changes interact to impact the atheroprotective capacity of HDL in women as they traverse the menopause transition. Five-hundred fifty-eight (n = 558) women from SWAN were selected to be included in SWAN HDL based on having at least one visit before and two visits after menopause onset with available stored blood specimens (a total of 1,461 samples). HDL metrics were measured on stored samples 2–5 times over the menopause transition for each participant (coincident with SWAN visit 1, follow-up visits 3–9, and visit 12).

For the current study, 301 women from SWAN HDL ancillary study had HDL metrics coincident with CAC measurements from the baseline and/or the follow-up visit of SWAN Heart. Women with unknown menopausal status due to hormone therapy use or hysterectomy (n = 7 women with a total of 44 observations) during SWAN follow-up visits were excluded. The final analytical sample included 294 women with CAC scores (236 had CAC measured twice and 58 had CAC measured once for a total of 530 observations). Analysis of calculated CAC density was restricted to those with a nonzero CAC score at any time point, and thus, the final analytical sample for CAC density included 165 women (76 had CAC > 0 twice and 89 had CAC > 0 once for a total of 241 observations with CAC > 0 at any time point).

Written informed consent was provided by all participants prior to enrollment in SWAN. Study protocols were approved by the institution review board at the University of Pittsburgh and the University of Chicago and meet the Declaration of Helsinki principles.

Coronary artery calcium score and density

CAC was quantified at two separate visits by the C-150 Ultrafast CT Scanner (GE Imatron, San Francisco, CA). Briefly, an initial scan was performed to identify anatomic landmarks. A second scan was performed for the evaluation of the coronary arteries, where 30–40 adjacent 3-mm thick images were obtained between the levels of the aortic root to the apex of the heart. Images were obtained during maximal breath holding. Scan data were saved to an optical disk for central scoring using a DICOM workstation and software by Accu-Image, Inc (South San Francisco, CA). This software utilizes the Agatston scoring method (25). CAC was defined as a lesion with an area ≥3 pixels that are hyperattenuated at >130 Hounsfield units (HU). The CAC Agatston unit score was calculated by multiplying the lesion area (mm^2) by a density factor (between 1 and 4) depending on the highest density measurement anywhere in the plaque lesion; the higher the

HU, the higher the density factor. Total volume score was calculated by adding the individual scores of the four major epicardial coronary arteries. The scans were scored by a technologist under the supervision of a cardiologist. This method had high reproducibility when measured in 40 consecutive participants with wide range of calcium. Intraclass correlation for CAC scores was 0.99 (26). CAC score was converted to a binary variable (absent: CAC = 0 or present: CAC > 0) for the analyses.

CAC density was calculated as described by Criqui el al. (20) whenever there was prevalent CAC (Agatston score > 0). Briefly, area score was calculated by dividing the CAC volume score by 3 mm; density was then calculated by dividing the CAC score by the area score. CAC density was log transformed for all analyses.

HDL metrics

At each SWAN visit, phlebotomy was performed after a minimum of a 10-h overnight fast. This was scheduled 2–5 days after a spontaneous menstrual bleed when possible, or randomly within 90 days of the annual SWAN visit when the menstrual cycles had ceased or were less predictable. Stored serum samples that have been frozen at –80°C and never been thawed were used for SWAN HDL assays to enhance the validity of results. For this analysis, samples that were stored for a mean of 17.0 (1.2) years (minimum: 13.5, 19.4) were used.

Cholesterol efflux capacity and HDL contents of phospholipid and triglycerides. Cholesterol efflux capacity (HDL-CEC), HDL-phospholipid (HDL-PL), and HDL triglycerides (HDL-Tg) were all measured at the Rader's laboratory at the University of Pennsylvania. HDL-CEC was evaluated by methods similar to the protocol of Khera *et al.* (27). Briefly, J774 mouse macrophage cells were plated and labeled with 2 μCi/ml of ^{3}H cholesterol overnight. The cells were then incubated for 6 h with 0.3 mM 8-(4-chlorophenylthio)-cyclic AMP (cAMP), an upregulator of the ATP-binding cassette transporter-1 (ABCA1). Proteins containing apolipoprotein B (ApoB) were then removed from plasma by polyethylene glycol precipitation. The cells were then incubated with the equivalent of 1% apolipoprotein B–depleted serum or plasma for 2 h at a 37°C temperature. Each medium was then collected and passed through a 0.22 μM filter to remove cell debris and radioactivity determined by liquid scintillation counting. Media without serum were used as baseline controls. The quantity of radioactive cellular cholesterol was determined after isopropanol extraction. Percent HDL-CEC was calculated as follows: [(cpm of ^{3}H cholesterol in the media − cpm of ^{3}H cholesterol in serum free media)/(cpm of ^{3}H cholesterol in the cells + cpm of ^{3}H cholesterol in the media)] × 100. A pool made from human serum obtained from ProMedDx was used to test the assay reagents and to normalize assays performed on different days during the study. The intra- and interassay coefficients of variation were 3.7% and 10.2%, respectively.

For HDL-PL and HDL-Tg, HDL was isolated from serum by phosphotungstic acid precipitation (FujiFilm Wako Pure Chemical Corporation). HDL-PL and HDL-Tg were then measured according to manufacturer's protocol (Wako: 433-36201 and Roche: 20767107322, respectively) using the Roche Cobas C311 clinical analyzer. The interassay coefficients of variation were 3.5% and 3.9% for HDL-PL and HDL-Tg, respectively.

Nuclear magnetic resonance (NMR) spectroscopy. HDL subclasses and size were quantified at LabCorp (Morrisville, NC) by the NMR Spectroscopy LipoProfile-3 algorithm (28), by the Vantera Clinical Analyzer, an automated 400 MHz NMR spectroscopy platform.

Lipoprotein particle quantification by NMR utilizes composite signal envelopes at 0.8 ppm that contain the signals emitted by terminal methyl group protons of the unesterified cholesterol, cholesterol ester, phospholipids, and triglycerides, which are carried within each HDL particle. Signal amplitudes that contribute to the composite plasma signal are produced as a result of the deconvolution of the composite signal. Different NMR signals that are unique in frequency and shape are produced by each lipoprotein subclass. The amplitude of the signal is proportional to the number of particles that are releasing the signal. The line shape of the signal envelope was modeled as a sum of all lipoprotein signals in order to obtain the amplitude of each subclass subpopulation. Areas of different subpopulations were multiplied by conversion factors to quantify the concentrations, which were then grouped into small (7.3–8.2 nm), medium (8.2–9.4 nm), or large (9.4–14 nm) HDL subclasses. Total HDL particle (HDL-P) concentration was obtained by adding up the concentrations of all subclasses. Overall average size of HDL-P was calculated by adding the diameter of each subclass multiplied by its relative mass percentage from NMR signal amplitude. NMR spectroscopy does not require the separation of lipoprotein subclasses as required by electrophoresis or ultracentrifugation due to the magnetic property of lipoproteins that produce signals of different shapes and frequencies for different lipoproteins. The intra- and interassay coefficients of variation for HDL-P concentrations and size ranged from 0.6% to 3.7% (intraassay) and 1.5%–4.0% (interassay).

HDL-cholesterol and apolipoprotein A-I (ApoA-I). Lipid fractions were determined in EDTA-treated plasma. Fasting HDL-C (29, 30) and ApoA-I were measured at the Medical Research Laboratory (MRL), Lexington, KY, for SWAN baseline visit until follow-up visit 7 or at the University of Michigan Pathology (UM), Ann Arbor, MI, at SWAN follow-up visit 9. HDL-C was separated with heparin-2M manganese chloride and measured by an automated cholesterol oxidase assay on a Hitachi 747-200 clinical analyze at MRL or by the ADVIA Direct-HDL Cholesterol (D-HDL) method at UM. ApoA-I was measured by immunonephelometry (BN1A-100; Behring Diagnostics, Westwood, MA) at MRL or by Beckman-Coulter (Brea, Ca) at UM. UM results were calibrated so that results are comparable to those produced by the MRL.

Study covariates

Race/ethnicity was self-reported at the baseline SWAN visit. Age, menopause status, body mass index (BMI), physical activity, systolic blood pressure, alcohol use, smoking status, the homeostasis model assessment of insulin resistance index (HOMA-IR), triglycerides, large density lipoprotein cholesterol (LDL-C), E2 levels, cycle day of blood draw, complement protein C3, diabetes status, and medication use were collected at every visit.

Age was calculated as the difference between visit date and birth date. BMI (kg/m^2) was calculated as the measured weight (in kg) divided by measured height^2 (in m). Physical activity score was ascertained by the modified Kaiser Permanente Health Plan Activity Survey (31). Systolic blood pressure was averaged over two measures. HOMA-IR was

calculated as [fasting insulin (mU/L) × fasting glucose (mmoles/L)]/22.5.

Fasting triglyceride levels were evaluated by the Hitachi 747-200 clinical analyzer at MRL (SWAN baseline visit − visit 7) or by the ADVIA assay at UM (SWAN visit 9). UM results were calibrated to match MRL results. LDL-C was calculated by the Friedewald equation when triglycerides were <400 mg/dl (32). Complement C3 was performed on the Alfa-Wasserman ACE analyzer using the K-ASSAY (Kamiya Biomedical Company) complement C3 reagents. E2 was performed on the Bayer Diagnostics Automated Chemiluminesence System:180 instruments. E2 was measured in duplicates and the average of the two measures was reported. The lower limit of detection for E2 ranged between 2 and 4 pg/ml; any value below the LLD was randomly assigned a number between 0 and the LLD. The interassay and intra-assay coefficients of variance for E2 were 10.6% and 6.4% respectively. In this analysis, no observation had E2 levels below the LLD. Cycle day of blood draw was categorized into two groups: either within 2–5 days of the menstrual cycle or not.

Menopause status was determined in SWAN at each visit based on the frequency and pattern of the menstrual bleeding and hormone therapy use within the past 12 months prior to every visit. In this analysis, menopausal status was categorized as either premenopause/early perimenopause (no changes in menstrual bleeding within the last three months or at least one menstrual bleed within the last three months with some perceived changes in cycle intervals), late perimenopause (no menstrual bleed within the last 3 months but at least one cycle within the last 12 months), or postmenopause (no menstrual cycle within the last 12 months, either due to natural menopause or surgical menopause by bilateral salpingo-oophorectomy). Frequency of alcohol use was acquired from self-administered questionnaires and categorized into either less than once/month or ≥ once/month. Smoking status was self-reported and categorized as never smoker, past smoker, or current smoker. Diabetes status was classified based the use of antidiabetic medication or maximum glucose ≥ 126 mg/dl. Use of any cardiovascular medication was defined as the self-reported use of antidiabetics, antihypertensive, and/or antilipids (statins or nonstatins).

Statistical analysis

At each time point, CAC score was categorized into either absent (CAC score = 0) or present (CAC score > 0), and CAC density was calculated when CAC was present. CAC density and volume score were skewed, thus log-transformed. Characteristics of participants included in the CAC presence as well as CAC density analysis were summarized at the first time point. Additionally, we compared the characteristics participants at the first time point by CAC presence (supplemental Table S1) as well as by menopause status (supplemental Table S2). Intercorrelations between different CAC measures and the HDL metrics at the first visit were assessed by Pearson's or Spearman's correlations as appropriate.

Associations between each HDL metric and presence of CAC and CAC density were tested using generalized estimating equation modeling for binary and continuous outcomes, respectively. Repeated measures for each of the CAC outcomes were modeled as a function of repeated measures of each HDL metric at concurrent visits. Generalized estimating equation models empirically provide robust standard errors to adjust for errors from miss-specifying the correlation matrices. Models were adjusted for study site, race/ethnicity, age, menopausal status, BMI, alcohol consumption, physical activity, log-transformed triglycerides, LDL-C, E2, cycle day of blood draw, and C3. Additionally, HDL-C and ApoA-I models were adjusted for total HDL-P levels, whereas HDL metric models were adjusted for HDL-C. Convergence of the models and the consistency of standard errors were checked to ensure the robustness of the models despite the small sample sizes. CAC density models were additionally adjusted for log-transformed volume score. Since HDL-Tg and CAC density were both log-transformed, those associations were presented per percent increase in HDL-Tg for more interpretable results (https://kenbenoit.net/assets/courses/ME104/logmodels2.pdf).

In exploratory analyses, effect modification of menopause stage and E2 levels on the relation between each of the HDL metrics and CAC measures were evaluated in the multivariable models by including interaction terms in these models. All analyses were run using SAS v9.4 (SAS Institute, Cary, NC).

RESULTS

Table 1 presents the characteristics for women included in the CAC presence analysis and the subset of women in the CAC density analysis with available data at the first time point. The mean age of the women included in the analysis was 51.1 (2.9) years, 65.8% were White, and 60.2% were pre/early perimenopausal. More than 50% of the women had a CAC score > 0 at the first visit and were included in the CAC density analysis.

At the first time point, women who had CAC score > 0 were older, had higher BMI, systolic blood pressure, C3, LDL-C, triglycerides, and HDL-Tg levels, and lower E2, HDL-C, ApoA-I, large HDL-P, and HDL-PL, with smaller HDL size and lower physical activity scores compared with women with no CAC (supplemental Table S1). Women with CAC score > 0 also reported more frequent use of CVD medications. When women were compared by menopause status (supplemental Table S2), late perimenopausal and postmenopausal women were older than pre/early perimenopausal women. Postmenopausal women had higher levels of C3, triglycerides, ApoA-I, total HDL-P, and HOMA-IR and were more frequently diabetics compared with pre/early perimenopausal women. Postmenopausal women also had lower E2 levels compared with both groups. When characteristics of women at the first time point were compared by E2 tertiles (data not shown), women in the highest tertile (T3) were younger, more likely to be pre-/early perimenopausal, had lower BMI, C3, and HOMA-IR, and more likely to have CAC = 0 compared with women in the lowest tertile group.

Correlations among the different CAC metrics are presented in supplemental Table S3. CAC score was negatively correlated with volume score and area score, but not with CAC density. Volume score and area score were positively correlated with each other, but negatively with CAC density. Correlations among the

TABLE 1. Characteristics for participants included in either CAC presence or CAC density analysis at first visit

Characteristics	Cohort Used for CAC Presence Analysis (n = 266)[a]	Cohort Used for CAC Density Analysis (n = 126)[a]
Age, years, mean (SD)	51.1 (2.9)	51.5 (2.8)
Race, n (%)		
White	175 (65.8%)	70 (55.6%)
Black	91 (34.2%)	56 (44.4%)
Menopausal status, n (%)		
Pre-/early perimenopausal	160 (60.2%)	74 (58.7%)
Late perimenopausal	24 (9.0%)	14 (11.1%)
Postmenopausal (BSO/Natural)	82 (30.8%)	38 (30.2%)
BMI, kg/m^2, median (Q1, Q3)	27.8 (24.6, 33.1)	32.9 (28.4, 37.1)
Physical activity score, mean (SD)	7.9 (1.7)	7.52 (1.7)
C3, mg/dl mean (SD)	137.0 (30.3)	149.5 (28.8)
Estradiol, pg/ml, median (Q1, Q3)	31.6 (16.3, 77.4)	26.1 (15.4, 51.4)
Alcohol consumption, n (%)		
None- ≤1 drink/month	108 (42.2%)	57 (46.3%)
>1 drink/month	148 (57.8%)	66 (53.7%)
Systolic blood pressure (mmHg), mean (SD)	117.6 (16.4)	122.7 (16.8)
HOMA-IR, median (Q1, Q3)	1.96 (1.44, 3.12)	2.72 (1.77, 4.55)
Diabetes, n (%)		
No	256 (96.2%)	120 (95.2%)
Yes	10 (3.8%)	6 (4.8%)
Smoking, n (%)		
Never smoker	157 (59.3%)	75 (59.5%)
Past smoker	77 (29.1%)	38 (30.2%)
Current smoker	31 (11.7%)	13 (10.3%)
Any use of CVD medication,[b] n (%)		
No	209 (78.6%)	91 (72.2%)
Yes	57 (21.4%)	35 (27.8%)
LDL-C, mg/dl, mean (SD)	120.6 (34.1)	126.6 (35.7)
Triglycerides, mg/dl, median (Q1, Q3)	104.0 (76.0, 141.0)	118.5 (87.0, 165.0)
HDL-C, mg/dl, mean (SD)	56.5 (13.7)	52.8 (11.6)
ApoA-I, mg/dl, mean (SD)	163.0 (26.8)	158.4 (24.8)
Total HDL-P umol/L, mean (SD)	34.8 (6.8)	34.0 (6.9)
Large HDL-P, umol/L, mean (SD)	7.6 (3.5)	6.6 (3.1)
Medium HDL-P, umol/L, median (Q1, Q3)	10.0 (5.7, 13.8)	9.8 (5.7, 14.0)
Small HDL-P, umol/L, mean (SD)	16.6 (7.0)	16.9 (6.8)
HDL size, nm, mean (SD)	9.4 (0.6)	9.3 (0.5)
HDL-PL, mg/dl, mean (SD)	53.2 (10.9)	51.5 (9.8)
HDL-Tg, mg/dl, median (Q1, Q3)	18 (15, 21)	19 (16, 23)
HDL-CEC, %, mean (SD)	3.84 (0.64)	3.73 (0.57)
CAC score, median (Q1, Q3)	0 (0, 6.52)	8.76 (3.09, 24.03)
CAC density score, median (Q1, Q3)	-	3.45 (2.86, 4.36)
CAC area score, median (Q1, Q3)	-	2.54 (0.80, 7.52)
CAC volume score, cm^3, median (Q1, Q3)	-	7.62 (2.40, 22.57)
CAC categories, n (%)		
CAC = 0	140 (52.6%)	0 (0%)
CAC > 0	126 (47.4%)	126 (100%)

BMI, body mass index; CAC, coronary artery calcification; C3, complement protein 3; HDL-C, High-density lipoprotein cholesterol; HDL-CEC, HDL cholesterol efflux capacity; HDL-P, HDL particle; HDL-PL, HDL-phospholipids; HDL-Tg, HDL-triglycerides; LDL-C, low-density lipoprotein cholesterol.

[a]266 women had nonmissing CAC score and HDL metrics at the first visit of whom 126 women had nonmissing CAC density and HDL metrics at the first visit.

[b]Defined as the self-reported use of antidiabetics, antihypertensive, and/or antilipids.

different HDL metrics were presented in supplemental Table S4.

Univariate associations between CAC measures and study covariates

Black race, older age, and higher BMI, C3, LDL-C, and log-triglycerides levels were associated with higher odds of CAC presence. Older age was associated with higher CAC density (Table 2).

Longitudinal associations of HDL metrics with CAC measures

Table 3 presents the associations of different HDL metrics with odds of CAC presence or CAC density. In unadjusted models (Model 1), higher concentrations of HDL-C, ApoA-I, large HDL-P, HDL-PL, HDL-CEC %, and larger overall HDL size were associated with lower odds of CAC presence, whereas higher HDL-Tg was associated with higher odds of CAC presence. These associations were attenuated in multivariable models (Model 2), except for medium HDL-P, which was associated with higher odds of CAC presence. In the unadjusted models (Model 1), only higher small HDL-P was associated with higher CAC density; however, this was no longer significant in adjusted models. Adding systolic blood pressure, log-HOMA-IR, and cigarette smoking to final models did not change these results (data not shown).

TABLE 2. Univariate associations of covariates with CAC > 0 and log-transformed CAC density score[a]

Covariates	CAC > 0		CAC density[b,c]	
	Or (95% CI)	*P*	β (SE)	*P*
Age, years	1.25 (1.03, 1.52)	0.02	0.11 (0.03)	<0.0001
Race		0.001		0.38
White	——	——	——	——
Black	2.09 (1.34, 3.26)	0.001	−0.05 (0.06)	0.38
Menopausal status		0.93		0.02
Pre-/early perimenopausal	——	——	——	——
Late perimenopausal	1.05 (0.74, 1.50)	0.78	0.13 (0.08)	0.12
Postmenopausal (Natural/bilateral oophorectomy)	1.05 (0.68, 1.72)	0.75	0.17 (0.06)	0.005
BMI, kg/m^2	4.48 (3.15, 6.37)	<0.0001	0.02 (0.03)	0.45
Physical activity score	0.79 (0.65, 0.96)	0.02	0.02 (0.03)	0.52
C3, mg/dL	2.03 (1.60, 2.57)	<0.0001	−0.02 (0.03)	0.58
Estradiol[c]	0.87 (0.73, 1.03)	0.11	−0.04 (0.03)	0.21
Alcohol consumption, n (%)		0.18		0.60
None- ≤1 drink/month	——	——	——	——
>1 drink/month	0.75 (0.49, 1.14)	0.18	0.03 (0.06)	0.60
LDL-C, mg/dL	1.23 (1.01, 1.49)	0.04	0.06 (0.03)	0.05
Triglycerides[c]	1.58 (1.28, 1.96)	<0.0001	0.03 (0.02)	0.27
Systolic blood pressure, mmHg	1.94 (1.56, 2.43)	<0.0001	0.02 (0.03)	0.43
HOMA-IR[c]	2.67 (2.02, 3.51)	<0.0001	0.06 (0.02)	0.02
Diabetes status		0.06		0.007
No	——	——	——	——
Yes	1.99 (0.96, 4.13)	0.06	0.22 (0.08)	0.007
Smoking		0.91		0.74
Never smoker	——	——	——	——
Past smoker	1.10 (0.69, 1.73)	0.69	0.04 (0.06)	0.53
Current smoker	1.01 (0.56, 1.81)	0.98	−0.03 (0.10)	0.79
Any use of CVD medication		0.02		0.28
No	——	——	——	——
Yes	1.66 (1.09, 2.52)	0.02	0.07 (0.06)	0.28

BMI, body mass index; C3, complement protein 3; LDL-C, low-density lipoprotein cholesterol.
[a]For continuous variables, data presented per 1-SD increase.
[b]Adjusted for log-transformed volume score.
[c]Log-transformed.

Effect modification of menopause stage on the associations of HDL metrics with CAC measures

In multivariable models, menopause stage independently modified the associations of specific HDL metrics with CAC measures (Table 4: CAC score; Table 5: CAC density). Higher concentrations of small HDL-P and smaller HDL size were associated with greater odds of CAC presence in late perimenopause

TABLE 3. Longitudinal associations of HDL metrics with CAC > 0 and CAC density

HDL Metrics	CAC > 0[b]				CAC density[a,c]			
	Model 1		Model 2		Model 1		Model 2	
	Or (95% CI)	*P*	Or (95% CI)	*P*	% (95% CI)	*P*	% (95% CI)	*P*
HDL-C, mg/dL	0.60 (0.47, 0.75)	<0.0001	0.78 (0.51, 1.19)	0.24	1.27 (−4.27, 7.14)	0.67	−1.48 (−10.76, 8.88)	0.77
ApoA-I, mg/dL	0.79 (0.64, 0.96)	0.02	0.82 (0.56, 1.21)	0.32	1.00 (−4.86, 7.22)	0.73	−3.18 (−10.37, 4.58)	0.40
Total HDL-P, umol/L	0.86 (0.71, 1.05)	0.14	1.13 (0.79, 1.60)	0.51	2.81 (−3.44, 9.47)	0.38	−0.20 (−8.17, 8.53)	0.97
Large HDL-P, umol/L	0.62 (0.50, 0.77)	<0.0001	1.08 (0.64, 1.84)	0.77	−1.73 (−7.23, 4.11)	0.56	−4.63 (−14.92, 6.89)	0.42
Medium HDL-P, umol/L	1.03 (0.86, 1.23)	0.73	1.46 (1.12, 1.90)	0.006	−3.25 (−9.32, 3.16)	0.31	−5.36 (−11.47, 1.24)	0.11
Small HDL-P, umol/L	1.03 (0.86, 1.24)	0.72	0.76 (0.58, 1.01)	0.05	6.99 (0.81, 13.46)	0.03	5.98 (−0.74, 13.24)	0.08
HDL size, nm	0.66 (0.54, 0.82)	0.0002	1.04 (0.70, 1.55)	0.85	−2.56 (−8.04, 3.26)	0.38	−1.61 (−10.14, 7.74)	0.73
HDL-PL, mg/dL	0.76 (0.61, 0.94)	0.01	1.39 (0.82, 2.35)	0.22	1.34 (−4.46, 7.49)	0.67	−4.10 (−14.93, 8.01)	0.49
HDL-Tg, %	1.34 (1.12, 1.60)	0.0001	1.03 (0.75, 1.41)	0.87	−3.68 (−8.74, 1.67)	0.17	−7.12 (−14.69, 1.12)	0.09
HDL-CEC, %	0.79 (0.64, 0.97)	0.03	0.89 (0.60, 1.30)	0.55	−0.63 (−6.16, 5.23)	0.83	−4.99 (−13.76, 4.67)	0.30

Model 1: Unadjusted.
Model 2: Adjusted for study site, race/ethnicity, time-varying age, menopausal stage, BMI, physical activity, alcohol use, log-triglycerides, LDL-C, C3, log-E2, cycle day of blood draw, and total HDL-P for HDL-C and ApoA-I models or HDL-C for HDL subclasses, content, and function measures.
For HDL-Tg, which was log transformed in the model, data are presented as % increase in CAC density (95% CI) per 25.7% increase in HDL-Tg (calculated as; a = log ([100 + 25.7]/100) and $e^{\beta \times a}$); 25.7% represents the % change to the median in HDL-Tg.
[a]CAC density model 2 additionally adjusted for volume score.
[b]OR (95% CI) presented per 1-SD increase in HDL metric (or per 1-SD increase in log-HDL-Tg).
[c]For CAC density score, data presented as % increase in CAC density (95% CI) per 1-SD increase in an HDL metric (calculated from log-CAC score by the following formula: $[e^{\beta \times SD} - 1] \times 100$).

TABLE 4. Effect modification of menopausal stage on associations of HDL metrics with CAC presence in multivariable adjusted models[a]

HDL Metrics	CAC Presence: Pre-/early Peri- OR (95% CI)[b]	CAC Presence: Late Peri- OR (95% CI)[b]	CAC Presence: Postmenopausal OR (95% CI)[b]	*P* Value for Effect Modification of Menopausal Stage
HDL-C, mg/dL	0.85 (0.53, 1.38)	0.67 (0.37, 1.23)	0.72 (0.41, 1.28)	0.73
ApoA-I, mg/dL	0.88 (0.54, 1.44)	0.81 (0.40, 1.66)	0.76 (0.49, 1.19)	0.86
Total HDL-P, umol/L	0.98 (0.59, 1.64)	1.84 (0.91, 3.75)	1.09 (0.72, 1.65)	0.33
Large HDL-P, umol/L	1.23 (0.71, 2.11)	0.66 (0.32, 1.38)	0.93 (0.48, 1.81)	0.17
Medium HDL-P, umol/L	1.58 (1.08, 2.32)	0.93 (0.42, 2.04)	1.49 (1.07, 2.08)	0.44
Small HDL-P, umol/L	0.55 (0.37, 0.84)	2.37 (1.12, 5.02)[c,d]	0.76 (0.54, 1.07)	0.008
HDL size, nm	1.33 (0.85, 2.10)	0.51 (0.29, 0.90)[c]	0.94 (0.55, 1.61)	0.02
HDL-PL, mg/dL	1.64 (0.92, 2.91)	1.06 (0.45, 2.51)	1.29 (0.74, 2.25)	0.45
HDL-Tg[e]	0.96 (0.65, 1.44)	0.84 (0.39, 1.81)	1.14 (0.77, 1.71)	0.67
HDL-CEC, %	0.82 (0.49, 1.38)	1.21 (0.63, 2.34)	0.86 (0.55, 1.34)	0.59

[a]Model adjusted for study site, race/ethnicity, time varying age, body mass index, physical activity, alcohol use, log-triglycerides, LDL-C, C3, log-E2, cycle day of blood draw, and total HDL-P for HDL-C and ApoA-I models or HDL-C for HDL subclasses, contents, and function.
[b]ORs are per 1-SD increase in an HDL metric (or per 1-SD increase in log-HDL-Tg).
[c]Differs significantly from pre-/early perimenopausal.
[d]Differs significantly from postmenopausal.
[e]Log-transformed.

stage than pre/early perimenopause stage. Lower concentrations of large HDL-P and smaller HDL size were associated with lower CAC density in late perimenopause than postmenopause stage. Adding systolic blood pressure, log-HOMA-IR, and cigarette smoking to final models did not impact these findings (data not shown).

Effect modification of E2 on the associations of HDL metrics with CAC measures

In multivariable models, we observed modest effect modification by E2; higher concentrations of small HDL-P were associated with significantly lower odds of CAC presence only in women in the highest E2 tertiles Table 6. E2 did not modify the associations between CAC density and HDL metrics (data not shown).

DISCUSSION

In this sample of midlife women, neither HDL-C nor metrics of HDL subclasses (with the exception of medium HDL-P), lipid content, and function were independently associated with CAC score or density. However, associations between specific HDL metrics and CAC measures varied by menopause stage and/or estradiol level as hypothesized. In particular, higher small HDL-P concentrations and smaller HDL size were associated with greater odds of CAC presence in women when they were in the late perimenopause stage compared with when they were in the pre/early perimenopause stage. Additionally, among women with E2 values in the highest tertile, we observed a protective association of small HDL-P with CAC presence, regardless of menopausal stage. Lower large HDL-P

TABLE 5. Effect modification of menopausal stage on associations of HDL metrics with CAC density in multivariable adjusted models

HDL Metrics	CAC Density[a]: Pre-/early Peri- % (95% CI)[b]	CAC Density[a]: Late Peri- % (95% CI)[b]	CAC Density[a]: Postmenopausal % (95% CI)[b]	*P* Value for Effect Modification of Menopausal stage[a]
HDL-C, mg/dL	0.69 (−11.47, 14.53)	17.19 (0.92, 36.09)[d]	−6.88 (−16.13, 3.39)	0.03
ApoA-I, mg/dL	−0.74 (−11.92, 11.57)	18.74 (−1.48, 42.75)[d]	−6.73 (−14.09, 1.00)	0.08
Total HDL-P, umol/L	0.26 (−9.91, 11.67)	7.47 (−7.26, 24.53)	−2.29 (−12.20, 8.75)	0.50
Large HDL-P, umol/L	−4.81 (−15.12, 6.77)	6.00 (−10.84, 26.01)[d]	−17.06 (−30.40, −1.16)	0.03
Medium HDL-P, umol/L	−4.56 (−13.34, 5.11)	−0.84 (−14.07, 14.42)	−7.12 (−15.39, −7.12)	0.72
Small HDL-P, umol/L	4.28 (−4.94, 14.31)	2.67 (−11.57, 19.28)	9.25 (−0.81, 20.42)	0.69
HDL size, nm	−0.51 (−9.85, 9.80)	11.34 (−3.07, 27.90)[d]	−10.67 (−20.37, 0.21)	0.03
HDL-PL, mg/dL	−2.07 (−14.60, 12.19)	5.57 (−9.85, 23.50)[d]	−11.80 (−24.24, 2.70)	0.06
HDL-Tg, %	−2.21 (−12.46, 9.24)	−17.35 (−29.17, −3.55)	−9.86 (−18.65, −0.13)	0.18
HDL-CEC, %	−8.29 (−19.84, 4.91)	8.88 (−4.07, 23.60)[c,d]	−7.30 (−16.70, 3.15)	0.08

For HDL-Tg, which was log transformed in the model, data are presented as % increase in CAC density (95% CI) per 25.7% increase in HDL-Tg (calculated as; $a = \log([100+25.7]/100)$ and $e^{\beta \times a}$); 25.7% represents the % change to the median in HDL-Tg.
[a]Model adjusted for study site, race/ethnicity, time varying age, volume score, body mass index, physical activity, alcohol use, log-triglycerides, LDL-C, C3, log-E2, cycle day of blood draw, and total HDL-P for HDL-C and ApoA-I models or HDL-C for HDL subclasses, contents, and function.
[b]Data presented as % increase in CAC density (95% CI) per 1-SD increase in an HDL metric (calculated from log-CAC score by the following formula: $[e^{\beta \times SD} - 1] \times 100$).
[c]Differs significantly from pre-/early perimenopausal.
[d]Differs significantly from postmenopausal.

TABLE 6. Effect modification of E2 on associations of HDL metrics with CAC presence in multivariable adjusted models

HDL Metrics	CAC > 0[a]			*P* Value for Effect Modification of E2[a]
	Tertile 0[f]	Tertile 1[f]	Tertile 2[f]	
	OR (95% CI)[b]	OR (95% CI)[b]	OR (95% CI)[b]	
HDL-C, mg/dL	0.65 (0.36, 1.17)	0.96 (0.62, 1.50)	0.62 (0.32, 1.18)	0.22
ApoA-I, mg/dL	0.82 (0.53, 1.26)	1.10 (0.67, 1.81)	0.59 (0.35, 0.996)[d]	0.11
Total HDL-P, umol/L	1.19 (0.78, 1.81)	1.41 (0.90, 2.21)	0.78 (0.47, 1.29)[d]	0.15
Large HDL-P, umol/L	0.82 (0.41, 1.64)	1.05 (0.58, 1.91)	1.20 (0.70, 2.04)	0.46
Medium HDL-P, umol/L	1.37 (0.89, 2.12)	1.41 (0.91, 2.17)	1.61 (1.09, 2.39)	0.81
Small HDL-P, umol/L	0.92 (0.66, 1.29)	1.03 (0.63. 1.68)	0.45 (0.28, 0.73)[c,d]	0.007
HDL size, nm	0.80 (0.49, 1.32)	0.97 (0.55, 1.72)	1.55 (0.95, 2.53)[c]	0.08
HDL-PL, mg/dL	1.49 (0.79, 2.82)	1.23 (0.70, 2.15)	1.59 (0.86, 2.96)	0.60
HDL-Tg[e]	1.24 (0.81, 1.90)	1.27 (0.75, 2.15)	0.75 (0.49, 1.16)	0.10
HDL-CEC, %	0.85 (0.49, 1.49)	1.21 (0.79, 1.87)	0.64 (0.36, 1.11)[d]	0.11

[a]Model adjusted for study site, race/ethnicity, time varying age, body mass index, menopausal status, physical activity, alcohol use, log-triglycerides, LDL-C, and C3 and Total HDL-P (for HDL-C or ApoA-I) or HDL-C (for HDL subclasses, content, and function) and cycle day of blood draw.
[b]ORs are per 1-SD increase in an HDL metric (or per 1-SD increase in log-HDL-Tg).
[c]Differs significantly from Tertile 0.
[d]Differs significantly from Tertile 1.
[e]Log-transformed.
[f]E2 tertiles: 0 < T0 ≤ 17.5 pg/ml; 17.75 pg/ml < T1 ≤ 43 pg/ml; T2 > 43 pg/mL.

concentrations and smaller HDL size were associated with lower CAC density in late perimenopause stage than postmenopause stage. The current findings suggest that the cardioprotective features of HDL subclasses may change over the menopause transition and that these changes may be driven by the E2 fluctuation accompanying this stage of women's lives.

Very limited data exist on associations of HDL subclasses with CAC score in midlife or older women. In postmenopausal women not using hormone therapy from the Healthy Women Study, associations between NMR HDL subclasses and CAC score were not significant except for large HDL size, which was significantly associated with lower odds of CAC presence (18). In another analysis from the same cohort, a higher concentration of large HDL-P was associated with lower CAC risk; however, this was not independent of HDL-C (19). Our finding of no relationship between HDL metrics and CAC score after adjusting for traditional risk factors is in agreement with these two studies. Because both of these two studies were conducted only in postmenopausal women, effect modification by menopause stage could not be assessed. We are not aware of other studies that have assessed associations of HDL subclasses, lipid content, and function with CAC density in a similar population as in our study. The null reported associations between HDL metrics and CAC measures among all women in the current study could be related to the fact that less dense CAC plaque occurs in younger women (<55 years of age) who hypothetically are more protected by their preserved E2 level (33). Interestingly, associations between CAC measures and HDL metrics were only evident once menopausal stage or E2 level was taken into consideration, supporting the aforementioned hypothesis.

The mechanism linking HDL with CAC formation is biologically plausible. HDL may have a direct anticalcifying effect by triggering osteoprotegerin production, known to inhibit calcium formation, and reducing mRNA expression of tumor necrosis factor alpha (TNF-α), known to enhance the calcification process (34). Moreover, the content of calcium in plaque atheroma seems to be related to specific HDL subclasses. Higher levels of smaller, immature HDL particles are associated with a worse plaque phenotype (greater noncalcified plaque, higher plaque volume, and enlarged necrotic core). On the other hand, the larger mature HDL subclasses are associated with a lower risk phenotype (less low-density noncalcified plaque, lower plaque volume, and less necrotic core (35)). Because the menopause transition has been linked to changes in the distribution of HDL subclasses (3), it is plausible that the associations between HDL subclasses and CAC measures vary by menopause stage. During the perimenopause stage, women experience an increase in small HDL-P and a decrease in large HDL-P (3). In the current study, we found that increases in small HDL-P and decreases in large HDL-P were associated with a greater odds of CAC presence or a lower CAC density, respectively, during the late perimenopause than in the postmenopause stage.

An interesting finding in this study is that the direction of the association between small HDL-P and odds of CAC presence varied by menopause stage. We found that higher concentrations of small HDL-P were associated with a lower odds of CAC presence in the pre/early perimenopause stage, but with greater risk in late perimenopause stage. The protective effect of small HDL-P on CAC presence was only evident at the highest E2 tertile. The reported findings suggest changes in the atherogenic features of the small HDL-P over the menopause transition that may be driven by the variability of E2 level at midlife. The lipidome of

small HDL-P may be modified as women traverse menopause, potentially explaining this finding. Change in HDL lipidome could play a key role in HDL atheroprotective functionality. In normolipidemic subjects, small dense high-density lipoprotein particles are enriched with negatively charged phospholipids such as phosphatidylserine, which positively correlate with multiple cardioprotective features (36) of HDL (cholesterol efflux capacity, antioxidative activity, antithrombotic activity, cell-free anti-inflammatory activity, and antiapoptotic activity). Moreover, the types of lipids could impact the fluidity of surface lipids, a critical determinant of the ability of HDL to accept cellular cholesterol (37) that can significantly impact both antioxidative (38) and anti-inflammatory effects of HDL (39). High sphingomyelin and free cholesterol content reduce the fluidity of surrounding lipids and cellular cholesterol efflux (40, 41). A reduction in the phosphatidylcholine–sphingomyelin ratio and in the phospholipidic layer membrane fluidity ws reported in elderly, supporting the notion of potential changes in lipidome that could contribute to how the subclasses interact with CVD risk overtime.

The current study has some limitations, results on CAC density were necessarily limited to those with prevalent CAC, limiting the generalizability to individuals with calcified disease. CAC density was estimated using four-point scale rather than a continuous HU scale, which may limit our ability to identify all potential associations. We did not adjust for multiple testing in the interaction analyses since these analyses were exploratory; however, the consistency of the findings, such as the association between smaller HDL size and small HDL-P with a worse CAC profile particularly in the late-perimenopause period and in women with lower E2 tertiles, increases our confidence that the results are not due to chance. Moreover, the sample size in this study is small, particularly for the late-perimenopausal group. Despite this small sample size, we found a significant difference when comparing this group to other menopause status groups. However, future studies with larger sample sizes should aim to replicate these findings. The samples used for assessment of HDL metrics were stored for a mean of 17.0 (1.2) years. The long duration of storage could have impacted the quantified levels of HDL metrics; however, since all samples have been stored under the same conditions in the SWAN study, we do not expect this to bias the observed associations, as any impact will affect all samples in a similar direction. We only measured cholesterol efflux capacity as a measure of HDL function; however, other functions such as antioxidative activity, antithrombotic activity, and antiapoptotic activity could be related to CAC; future studies should aim to investigate a more comprehensive HDL function profile in relation to CAC around menopause. The HDL proteome has been previously linked to states of chronic inflammation, and changes in the proteome have been linked to changes in HDL function (42). However, the impact of menopause transition on HDL proteome has not been described before. Whether the HDL proteome impacts CAC should be assessed in future studies. Major strength of this study is being one of the first studies assessing CVD risk prediction of multiple metrics of HDL beyond HDL-C in midlife women, a population among whom the clinical utility of HDL-C has been questioned recently.

In summary, HDL subclasses may impact the likelihood of CAC development and CAC density differently over the menopause transition. This may be driven by the variability of endogenous estradiol levels during midlife. Future studies should investigate pathways through which certain HDL subclasses may become dysfunctional in midlife women. Such studies allow the potential to identify clinically relevant, atheroprotective HDL components that could replace HDL-C as a factor used in risk prediction equation (43) in midlife women.

Data availability

The authors declare that all supporting data are available within the article (and its supplemental data). SWAN provides access to public use datasets that include data from SWAN screening, baseline, and follow-up visits (https://agingresearchbiobank.nia.nih.gov/ and http://www.swanstudy.org/swan-research/data-access/). To preserve participant confidentiality, some, but not all, of the data are contained in the public use datasets. Investigators who require assistance accessing the public use dataset may contact the SWAN Coordinating Center (swanaccess@edc.pitt.edu).

Supplemental data

This article contains supplemental data.

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Author contributions

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Conflict of interest

D. J. R. is the founder of VascularStrategies. All other authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations

C3, complement potein C3; CAC, coronary artery calcium; E2, estradiol; HDL-CEC, HDL cholesterol efflux capacity; HDL-P, HDL-particles; HDL-PL, HDL phospholipids; HDL-Tg, HDL triglycerides; HOMA-IR, homeostasis model assessment of insulin resistance index; HU, Hounsfield units; MRL, Medical Research Laboratory; UM, University of Michigan.

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