Early Midlife Cardiovascular Health Influences Future HDL Metrics in Women: The SWAN HDL Study

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BACKGROUND: Utility of high-density lipoprotein cholesterol (HDL-C) in assessing the antiatherogenic properties of HDL may be limited in midlife women. Novel metrics of HDL function, lipid contents, and subclasses may better reflect the atheroprotective capacities of HDL, supporting the need to evaluate how cardiovascular health affects these metrics in women. We assessed the relationship of early midlife Life’s Simple 7 (LS7) score and its health behavior components with future HDL function (HDL-cholesterol efflux capacity), HDL-phospholipid, HDL-triglyceride, HDL particles (HDL-P) and size, and the relationship between LS7 score and changes in HDL metrics over time.

METHODS AND RESULTS: We analyzed 529 women (baseline age: 46.4 [2.6] years, 57% White) from the SWAN HDL (Study of Women’s Health Across the Nation HDL) study who had baseline LS7 followed by future repeated HDL metrics. Multivariable linear mixed models were used. Higher LS7 score was associated with favorable future HDL profile (higher HDL-phospholipid, total HDL-P and large HDL-P, lower HDL-triglyceride, and larger overall HDL size). Ideal body mass index was associated with higher HDL-cholesterol efflux capacity, HDL-phospholipid, and large HDL-P, lower HDL-triglyceride and small HDL-P, and larger overall HDL size. Ideal physical activity was associated with higher HDL-phospholipid, and total, large, and medium HDL-P. Ideal smoking was associated with less HDL-triglycerides. Diet was not related to HDL metrics. Higher LS7 score and ideal body mass index were associated with slower progression of HDL size over time.

CONCLUSIONS: Novel HDL metrics may better reflect the clinical utility of HDL. Improving lifestyle at midlife, particularly maintaining ideal body mass index, is associated with better future HDL phenotype.

Key Words: cholesterol efflux capacity ■ HDL metrics ■ Life’s Simple 7 ■ lifestyle factors ■ midlife women

The midlife period in women is often accompanied by an increase in risk of cardiovascular disease (CVD) and in cardiometabolic risk factors. Independent of aging, the menopause transition (MT) plays a critical role in accelerating CVD risk. During the MT, changes in lipoproteins, body fat composition, metabolic syndrome, and vascular health contribute to the increased risk of CVD, the leading cause of death in midlife and older women.

Previous studies that have assessed the alterations in the lipoprotein profile around the MT have consistently shown that this period is accompanied by increases in total cholesterol, low-density lipoprotein cholesterol, and apolipoprotein-B (ApoB). However, changes in high-density lipoproteins (HDLs) are more complex. Earlier cross-sectional studies have shown that levels of HDL-cholesterol (HDL-C) do not differ by menopause status or are lower in postmenopausal compared with...
premenopausal women. Nonetheless, more recent longitudinal studies have shown that HDL-C may increase after menopause, and that higher HDL-C after menopause, but not before, is associated with higher risk of CVD. This cluster of observations suggests that HDL may lose its atheroprotective function during the MT. Additionally, lifestyle and pharmacological interventions that raise HDL-C have failed to show a beneficial effect on reducing risk of CVD.

HDLs are complex particles that vary in composition, size, and function. The major anti-atherogenic function of HDL is through its role in the reverse cholesterol transport process, where cholesterol is removed from cells for clearance. Studies have suggested that novel metrics, which directly evaluate the function, lipid contents, and subclasses distribution of the HDL, may provide a better understanding of the cardioprotective qualities of the HDL compared with HDL-C. Despite some inconsistencies in certain studies, findings have suggested that higher cholesterol efflux capacity and more phospholipid content within the HDL, as well as a larger subclass distribution, are associated with a cardioprotective risk profile.

In efforts to measure CVD burden in adults, the Goals and Metrics Committee of the Strategic Planning Task Force of the American Heart Association (introduced a comprehensive new metric in 2011 to monitor the cardiovascular health (CVH) of all Americans through 2020. This CVH metric, termed the Life’s Simple 7 (LS7), encompasses a set of 7 components: 4 health behaviors (body mass index [BMI], physical activity, smoking status, and diet quality) and 3 health factors (blood pressure, total cholesterol, and fasting blood glucose). A higher S7 score is associated with reduced CVD-related morbidity and mortality. However, only 3% of the US population in 2016 had ideal scores on ≥5 components of the LS7, and CVD remains the most common cause of mortality in men and women. This indicates that more action is needed to successfully achieve reductions in CVD morbidity and mortality.

Behavioral modifications and lifestyle changes are linked to increases in HDL-C in different populations. However, the lack of consistent associations of HDL-C with CVD, particularly in midlife women, raises the question whether modifiable health behaviors affect other direct measures of HDL. Thus, in this analysis, we aimed to assess whether a better LS7 score and the modifiable health behavior components of the score (BMI, physical activity, diet, and smoking) early in midlife are associated with a better future HDL metric profile, and whether this early-midlife LS7 score is associated with anti-atherogenic changes in HDL metrics over the MT. We hypothesized that a higher LS7 score and ideal BMI, physical activity, smoking, and diet status at early midlife will be associated with higher HDL cholesterol efflux capacity (HDL-CEC), higher phospholipid and less triglycerides contents, more large and less small HDL subclasses, and a larger overall

**Nonstandard Abbreviations and Acronyms**

- ApoA-I: apolipoprotein A-I
- ApoB: apolipoprotein B
- CVH: cardiovascular health
- HDL-CEC: HDL cholesterol efflux capacity
- HDL-P: HDL-particles
- HDL-PL: HDL-phospholipids
- HDL-Tg: HDL-triglycerides
- LS7: Life’s Simple 7
- MESA: Multi-Ethnic Study of Atherosclerosis
- NMR: nuclear magnetic resonance
- MT: menopause transition
- SWAN: Study of Women’s Health Across the Nation
HDL size in the future as women traverse menopause, and that a better LS7 score and ideal health behavior components will be associated with favorable changes in HDL metrics over time.

METHODS
SWAN (Study of Women’s Health Across the Nation) is an ongoing, multi-site, multi-ethnic, longitudinal study that aims to characterize the physiologic and psychological changes as women traverse menopause. The design of the SWAN study has been previously described.\textsuperscript{24} In brief, 3302 women aged 42 to 52 years were recruited between 1996 and 1997 at 7 different sites across the United States: Pittsburgh, PA; Detroit, MI; Chicago, IL; Los Angeles, CA; Oakland, CA; Boston, MA; and Newark, NJ. Women were eligible for recruitment to SWAN if they had an intact uterus and at least 1 ovary, were not pregnant or lactating at the time of recruitment, had at least 1 menstrual period within the last 3 months before recruitment, were not on hormone replacement therapy, and self-identified as either White, Black, Hispanic, Chinese, or Japanese.

The SWAN HDL study is an ancillary study to SWAN. This ancillary study aims to characterize the changes of HDL function and composition that accompany ovarian aging, and to understand how these changes influence the atheroprotective associations of HDL in women as they progress through the MT. For SWAN HDL, frozen serum samples from 558 SWAN women (1461 samples over the study period) were used to quantify the function, lipid content, and subclasses of HDL. Women were selected into SWAN HDL if they had participated in at least 1 visit before and 2 visits after the final menstrual period, with available blood samples at the selected visits.\textsuperscript{6}

For this analysis, 29 women who had missing data on the LS7 metrics at SWAN baseline visit were excluded. The final analysis included 529 women who had baseline LS7 score (SWAN visit 0), followed by at least 1 HDL metric at a later visit (coinciding with SWAN follow-up visits 1, 3–9, and/or 12). Out of the 529 women, 13 had HDL metrics measured once, 220 had HDL metrics measured twice, 258 had HDL metrics measured 3 times, 31 women had HDL metrics measured 4 times, and 7 had HDL metrics measured 5 times over midlife. Written informed consent was provided by all participants before enrollment in SWAN, and the study protocols were approved by the institution review board at each study site.

Blood Data Collection
Phlebotomy was performed after a minimum 10-hour overnight fast. The blood draw was scheduled 2 to 5 days after a spontaneous menstrual bleed when possible or randomly within 90 days of the annual SWAN visit when the date of the menstrual cycle could not be determined. Stored samples that have been frozen at \( -80^\circ \text{C} \) and never been thawed before were used for SWAN HDL assays to enhance the validity of results.

HDL-CEC and HDL-Phospholipids and HDL-Triglycerides Contents
HDL-CEC, HDL-phospholipids (HDL-PL), and HDL-triglycerides (HDL-Tg) were measured at a Centers for Disease Control and Prevention–certified lipid laboratory at the University of Pennsylvania. HDL-CEC was measured by the efflux of fluorescence-labeled cholesterol as has been previously described.\textsuperscript{16} In summary, J774 mouse macrophage cells were plated and labeled with 2 \( \mu \text{Ci/mL} \) of \( ^3\text{H} \) cholesterol overnight. The cells were then incubated for 4 hours in the presence of 0.3 mmol 8-(4-chlorophenylthio)-cyclic AMP, an upregulator of ATP-binding cassette transporter-1. To create ApoB-depleted plasma, lipoproteins containing ApoB were removed from plasma by polyethylene glycol precipitation. Cells were then incubated for 2 hours with the equivalent of 1% ApoB-depleted serum or plasma at a 37°C. Cells incubated with media alone were used as the baseline controls. To remove the cell debris and quantify radioactivity by liquid scintillation counting, each medium was collected and passed through a 0.22-\( \mu \text{M} \) filter. Isopropanol extraction was then used to quantify radioactive cellular cholesterol that was incorporated into the cellular lipids. Percent efflux capacity was calculated by the following formula: ([cpm of \(^3\text{H} \) cholesterol in the media–cpm of \(^3\text{H} \) cholesterol in serum-free media]/[cpm of \(^3\text{H} \) cholesterol in the cells+cpm of \(^3\text{H} \) cholesterol in the media]) \times 100. Samples from participants were normalized to a pooled plasma control that was included on each plate. The intra- and interassay coefficients of variation were 3.7% and 10.1%, respectively.

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

HDL Subclasses by Nuclear Magnetic Resonance Spectroscopy
The number of HDL subclasses and overall HDL size were measured by the Nuclear Magnetic Spectroscopy LipoProfile-3 algorithm\textsuperscript{25} using the Vantera Clinical Analyzer, an automated 400-MHz nuclear magnetic resonance (NMR) spectroscopy platform. Lipoprotein...
particle quantification by NMR utilizes composite signal envelopes at 0.6ppm, which contain the signals emitted by the terminal methyl group protons of the HDL contents (phospholipids, triglycerides, cholesteryl ester, and unesterified cholesterol) that are carried in each HDL particle. Signal amplitudes that contribute to the composite plasma signal are produced as a result of the deconvolution of the composite signal. Each lipoprotein subclass produces unique NMR signals, which are specific in frequency and shape. The amplitude of the signal is proportional to the number of particles that are releasing the signal. The line shape of the signal envelope is modeled as a sum of all lipoprotein signals to obtain the amplitude of each subpopulation of subclasses. To quantify the concentration of each subclass, areas of different subpopulations were multiplied by conversion factors and the subclasses were then grouped into small (7.3–8.2 nm), medium (8.2–9.4 nm), or large (9.4–14.0 nm) HDL subclasses. The total HDL particle concentration was calculated as the sum of the concentrations of individual subclasses. The average size of HDL particles was calculated by adding the diameter of each subclass multiplied by its relative mass percentage from NMR signal amplitude. Because of the magnetic property of lipoproteins, which produces signals of different shapes and frequencies for different lipoproteins, NMR spectroscopy does not require the separation of lipoprotein subclasses as is required by electrophoresis or ultracentrifugation. The intra- and interassay coefficients of variation for HDL particles (HDL-P) concentrations and size ranged from 0.6% to 3.7% (intra-assay) and 1.5% to 4.0% (interassay).

**HDL-C and Apolipoprotein A-I**

Lipid fractions were determined in EDTA-treated plasma. From SWAN baseline visit until follow-up visit 7, fasting HDL-C was separated with heparin-2M manganese chloride, whereas apolipoprotein (ApoA-I) was measured using the immunonephelometry using Behring reagents on the Behring Nephelometer II at the Medical Research Laboratory (Lexington, KY). For SWAN follow-up visits 9 and 12, fasting HDL-C was separated with heparin-2M manganese chloride at the University of Michigan Pathology laboratory (Ann Arbor, MI), and ApoA-I was measured by using reagents from Beckman Coulter (Brea, CA) at the University of Pittsburgh Heinz laboratory. The results of HDL-C and ApoA-I from University of Michigan Pathology and the University of Pittsburgh Heinz laboratories were calibrated by converting them to equivalent Medical Research Laboratory values.

**American Heart Association’s Life’s Simple 7**

The LS7 was evaluated at the SWAN baseline visit. Each component of the LS7 (BMI, physical activity, smoking, diet, total cholesterol, fasting blood glucose, and blood pressure) was categorized into either ideal (score=2), intermediate (score=1), or poor (score=0), based on AHA guidelines and as described in Table 1. A total LS7 score was calculated by summing the score of each of the 7 components. In the SWAN study, the LS7 components were assessed as follows:

**Body Mass Index**

BMI was calculated as measured weight (kg)/height (m)^2.

**Physical Activity**

The physical activity component was assessed by the Kaiser Physical Activity Survey, which is an adaptation of the Baecke physical activity questionnaire. Participants were asked about the frequency, duration, and intensity of sports. Women were classified based on the minutes per week of activity for ≥9 months/y.

**Smoking Status**

Smoking status was derived from self-reported questionnaires where women were asked about their current smoking status, whether they had stopped smoking, and the month and year when they last smoked.

**Diet Quality**

The diet component was assessed by the food frequency questionnaire. Five components contributed to the diet quality score: fruits and vegetables, fiber-rich whole grains, fish consumption, sugar-sweetened beverages, and daily sodium intake. In the context of a healthy dietary pattern that is consistent with a Dietary Approaches to Stop Hypertension–type, consuming ≥4.5 cups/d of fruits and vegetables, ≥2 servings/wk of fish, ≥3 servings/d of whole grains, ≤36 oz/wk of sugar-sweetened beverages, and ≤1500 mg/d of sodium was considered within target. Diet was classified into ideal, intermediate, or poor based on the number of components met.

**Blood Pressure**

Systolic and diastolic blood pressure were measured according to a standardized protocol, with readings taken on the right arm, while the subject was seated with feet flat on the floor for at least 5 minutes before measurement. An average of 2 sequential blood pressures was reported. Blood pressure treatment was determined from self-administered questionnaires.

**Total Cholesterol**

Total cholesterol was quantified at Medical Research Laboratory at the SWAN baseline visit. The Hitachi 747–200
clinical analyzer was used to measure total cholesterol by an automated cholesterol oxidase assay. Lipid treatment was determined by self-administered questionnaires.

**Fasting Blood Glucose**

Fasting blood glucose was quantified at the Medical Research Laboratory at the SWAN baseline visit. The Hitachi 747 to 200 clinical analyzer was used to measure fasting blood glucose by the hexokinase reaction. Fasting blood glucose levels at the SWAN baseline visit were calibrated to increase comparability with later SWAN glucose measures, which were measured at other laboratories. Antidiabetic medication use was determined by self-administered questionnaires.

**Study Covariates**

Study covariates were assessed at the SWAN baseline visit, coinciding with the time of LS7 assessment. Race, education status, economic hardship (defined as difficulty paying for basics), and alcohol use were self-reported. Race was categorized as White, Black, or Other (Hispanics, Chinese, or Japanese). Age was calculated as the difference between the visit date and the date of birth. Time between HDL measures and the LS7 measure was calculated as the difference between the dates of the 2 visits. Menopause status was self-reported based on bleeding patterns over the past 12 months and was categorized as either premenopausal (no changes in menstrual bleeding patterns over the previous 12 months) or early perimenopausal (at least 1 bleed within the last 3 months with some perceived changes in menstrual cycle intervals). CRP (C-reactive protein) was measured at the Medical Research Laboratory on serum or plasma collected at SWAN baseline visit by immunonephelometry using Behring reagents on the Behring Nephelometer II. Medical Research Laboratory results were calibrated to the hs-CRP (high-sensitivity CRP) ELISA assay. A random sample from SWAN women was selected and the distribution of CRP within different menopausal status groups, race/ethnicity groups, and study visits were checked to confirm a representative sample of the SWAN cohort, and calibration equations were applied.

**Statistical Analysis**

Descriptive statistics including mean (SD), median (Q1, Q3), or frequencies (%) were used to summarize participants’ characteristics at the SWAN baseline visit, as appropriate. Distribution of continuous variables was assessed, and skewed variables (HDL-Tg and hs-CRP) were log-transformed to reduce skewness. Because of the small number of women in the intermediate smoking and physical activity categories, poor and intermediate women for these components were combined in regression analysis.

Linear mixed models with random intercepts and autoregressive covariance structure were used to evaluate the independent associations between baseline LS7 score and repeated measures of each HDL metric later in life. Potential confounders were added to the models and the parsimonious model with the best fit was chosen based on likelihood ratio tests. Final models were adjusted for time between LS7 score and HDL metrics measures, race, education status, and the following covariates at baseline: age, economic hardship, menopause status, alcohol use, and log-transformed hs-CRP.
To investigate the associations between each health behavior component at baseline and HDL metrics later in life, linear mixed models with random intercepts were used to model baseline BMI, physical activity, smoking and diet quality categories, separately, in relation to repeated measures of HDL metrics in final models. In these analyses, HDL metrics were modeled as z-scores to increase the comparability of the metrics between the different components. Z-scores were calculated for each HDL metric from the mean and SD.

To assess whether baseline baseline health behavior components are associated with changes in future HDL metrics, random-intercept linear mixed-effect models with repeated measures of each HDL metric were fitted as a function of each health behavior component, time since first HDL measure, and their interaction. Time since first HDL measure was calculated as the time difference between each repeated HDL metric and the first available HDL metric. The beta estimate of the interaction represents the yearly change in each HDL metric per 1 SD increase in LS7 score. To assess whether baseline health behavior components are associated with changes in future HDL metrics, random-intercept linear mixed-effect models of repeated measure of each HDL metric were fitted as function of each health behavior component, time since first HDL measure, and their interaction. The beta estimate for the interaction represents the yearly change in the z-score of the HDL metric per group (ideal, intermediate or poor).

All analyses were run using SAS v9.4 (SAS Institute, Cary, NC).

RESULTS

Characteristics of Women Included in This Analysis at the SWAN Baseline Visit

The baseline characteristics of women included in this analysis are presented in Table 2. Women, on average, were 46.4 (SD: 2.6) years old, mostly White (56.5%) or Black (26.7%), and were all premenopausal or early perimenopausal at time of LS7 assessment. The average LS7 score was 8.5 (SD: 2.0), which is ranked as intermediate, and ranged between 2 and 13. Time between the LS7 assessment and the first HDL metric measure was 3.9 (SD: 1.4) years. The women had up to 5 repeated HDL metrics. The time elapsed between the first and second HDL metrics assessment was 2.7 (SD: 1.3) years, that between the first and the third HDL metrics assessment was 8.5 (SD: 2.6) years, that between the first and the fourth HDL metrics assessment was 8.9 (SD: 2.8) years, and that between the first and the fifth HDL assessment was 9.3 (SD: 2.0) years.

Thirty-six percent of SWAN women had an ideal LS7 score (between 10 and 14), 48% had an ideal BMI, and 88% had an ideal smoking status (Figure 1).

### Table 2. Characteristics of Women Included in This Analysis

| Characteristic                          | N=529 |
|-----------------------------------------|-------|
| Age, y, mean (SD)                       | 46.4 (2.6) |
| Race, n (%)                             |       |
| White                                   | 299 (56.5%) |
| Black                                   | 141 (26.7%) |
| Other*                                  | 89 (16.8%) |
| Menopause status, n (%)                 |       |
| Premenopause                            | 328 (62.4%) |
| Early perimenopause                     | 198 (37.6%) |
| Education level, n (%)                  |       |
| College degree                          | 154 (29.2%) |
| Some college/college degree             | 281 (53.3%) |
| <High school degree                     | 92 (17.5%) |
| Economic hardship, n (%)                |       |
| No                                      | 364 (68.9%) |
| Yes                                     | 164 (31.1%) |
| Alcohol use, n (%)                      |       |
| <1 drink/mo                             | 253 (47.8%) |
| ≥1 drink/mo                            | 276 (52.2%) |
| Lipid medication users, n (%)           |       |
| No                                      | 527 (99.6%) |
| Yes                                     | 2 (0.4%) |
| BMI, kg/m², median (Q1, Q3)             | 25.5 (22.5, 30.6) |
| Systolic blood pressure, mmHg, median (Q1, Q3) | 112.0 (104.0, 124.0) |
| Estradiol, pg/dL, median (Q1, Q3)       | 58.1 (34.5, 85.5) |
| Follicle-stimulating hormone, mL.U/mL, median (Q1, Q3) | 16.3 (11.5, 24.2) |
| Life’s Simple 7 Score, mean (SD)        | 8.5 (2.0) |
| hs-CRP, mg/dL, median (Q1, Q3)          | 1.5 (0.5, 4.0) |
| HDL metrics at first available visit    |       |
| HDL-CEC, % (mean, SD)                   | 3.96 (0.7) |
| HDL-Tg, mg/dL, median (Q1, Q3)          | 17 (14, 21) |
| HDL-PL, mg/dL, mean (SD)                | 54.8 (10.6) |
| Total HDL-P, µmol/L, mean (SD)          | 35.1 (6.3) |
| Large HDL-P, µmol/L, mean (SD)          | 8.4 (3.6) |
| Medium HDL-P, µmol/L, mean (SD)         | 11.3 (6.2) |
| Small HDL-P, µmol/L, mean (SD)          | 15.4 (7.1) |
| HDL size, nm, mean (SD)                 | 9.5 (0.5) |
| HDL-C, mg/dL, mean (SD)                 | 59.5 (14.3) |
| ApoA-I, mg/dL, mean (SD)                | 165.6 (27.1) |

Hs-CRP: 1 mg/dL=10 mg/L; HDL-C: 1 mg/dL=0.026 mmol/L; ApoA-I: 1 mg/dL=0.01 g/L. ApoA-I indicates apolipoprotein A-I; BMI, body mass index; HDL, high-density lipoprotein; HDL-CEC, HDL cholesterol efflux capacity; HDL-P, HDL particles; HDL-PL, HDL-phospholipids; HDL-Tg, HDL triglycerides; hs-CRP, high-sensitivity C-reactive protein; and SWAN, Study of Women’s Health Across the Nation.

*Other Race category included 1 Hispanic, 47 Chinese, and 41 Japanese women.
However, no woman achieved ideal diet quality, and 79% had poor physical activity status.

**Associations Between Baseline LS7 Score With HDL Metrics Later in Life**

Associations between baseline LS7 score and future metrics of function and contents, HDL-C, and ApoA-I are presented in Table 3. In the univariate analysis (Model 1), a higher LS7 score was associated with higher HDL-CEC, HDL-PL, HDL-C, and ApoA-I, and lower HDL-Tg. After adjusting for potential confounders (Model 2), the results for HDL-CEC became insignificant, but the other adjusted associations remained statistically significant. The Akaike Information Criterion showed an improvement in model fit in fully adjusted compared with unadjusted models.

Associations between baseline LS7 score and future HDL subclasses and size are presented in Table 4. In the univariate analysis (Model 1), a higher LS7 score was associated with higher concentrations of total HDL-P, large HDL-P, and medium HDL-P, lower small HDL-P, and larger overall HDL size. Adjusting for potential confounders (Model 2), the results for medium and small HDL-P became insignificant, but the other associations remained statistically significant. The Akaike Information Criterion showed an improvement in model fit in fully adjusted compared with unadjusted models.

**Association of Baseline LS7 Score and Individual Components of Health Behaviors With Changes in HDL Metrics Since First HDL Measure**

In final models, higher baseline LS7 score was associated with a slower increase in HDL size as time progressed ($P_{interaction}=0.005$; Table S2). Compared with women with ideal BMI, women in the poor BMI group had larger increases in HDL size ($P_{interaction}=0.006$, Figure 4A) over time. Medium HDL-P did not significantly change with time within each physical

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**Figure 1. Distribution of LS7 components among study participants at SWAN baseline visit.**

AHA LS7 Categories Classification: Poor: LS7 score 0–4; Intermediate: LS7 score 5–9; Ideal: LS7 score 10–14. AHA indicates American Heart Association; BMI, body mass index; LS7, Life’s Simple 7; and SWAN, Study of Women’s Health Across the Nation.
activity group, but the interaction with time was significant between women with ideal compared with poor/intermediate physical activity ($P_{interaction}=0.021$, Figure 4B), indicating that the latter group had a larger increase in medium HDL-P compared with the former.

**DISCUSSION**

In this cohort of midlife women, better CVH is associated with a favorable profile of HDL function, lipid content, and subclasses distribution. Healthier modifiable lifestyle risk factors during early midlife are linked to higher future cholesterol efflux capacity and phospholipid contents, lower triglyceride contents, and a shift of the subclasses distribution towards larger HDL particles. In particular, poorer BMI status appears be associated with lower anti-atherogenic cholesterol efflux capacity of the HDL, lower phospholipid contents, and smaller size of HDL subclasses. A sedentary lifestyle with lack of sufficient physical activity is associated with lower phospholipid contents, whereas smoking is associated with higher triglyceride contents of the HDL. In light of the current uncertainty of the clinical utility of HDL-C in midlife women, the current findings provide critical information on modifiable risk factors that can improve other metrics of HDL that could be more relevant to midlife women.

Despite the perception that HDL-C is associated with reduced CVD morbidity and mortality, most recent clinical guidelines have discarded HDL-C as a target for primary and secondary prevention.\(^{30,31}\) Whether novel metrics of HDL could be potential targets to guide novel CVD therapies and preventive strategies is still unclear. This is of particular importance in midlife women when a loss in the atheroprotective functions of the HDL, which is not captured by HDL-C, may occur. We have previously shown that the MT is accompanied by a decline in large HDL-P and overall HDL size, an increase in HDL-Tg and small HDL-P, and a decline in HDL function close to the final menstrual period.\(^{6,32}\) This HDL profile has been linked to worse CVD risk in various cohorts,\(^{16-19}\) including midlife women.\(^{33}\) Moreover, in SWAN HDL, higher estradiol in midlife was linked to higher HDL-CEC, larger HDL subclass distribution, and higher HDL-Tg; however, the positive association between higher estradiol and HDL-Tg was strongest after 2 years from the final menstrual period.\(^{34}\) These results indicate a potential loss of the protective effects of estradiol on HDL as women transition into menopause. This has been corroborated in SWAN, where the presumably protective association between HDL-C on arterial calcification was only apparent with high estradiol levels but tended to be the opposite at lower levels of estradiol.\(^{10}\) It is important to understand how other interventions, including behavioral modifications, may impact other metrics of HDL found to be more relevant to midlife women.

**Table 3. Unadjusted and Multivariable-Adjusted Associations Between Baseline LS7 Score and Future HDL Function, Lipid Contents, and ApoA-I**

| HDL function, contents, and ApoA-I | AIC | $\beta$ (SE) | $P$ value |
|----------------------------------|-----|--------------|-----------|
| Model 1 | 2157.3 | 0.11 (0.03) | <0.001 |
| Model 2 | 2074.9 | 0.02 (0.03) | 0.603 |

Data presented as increase or decrease in HDL metric per 1-SD increase in LS7 score. Model 1: Unadjusted. Model 2: Adjusted for time between LS7 score and HDL metrics measures, race, education status, and baseline age, economic hardship, menopause status, alcohol use, and log-hs-CRP. AIC indicates Akaike Information Criterion; ApoA-I, apolipoprotein A-I; HDL, high-density lipoproteins; HDL-CEC, HDL cholesterol efflux capacity; HDL-PL, HDL phospholipids; HDL-Tg, HDL triglycerides; hs-CRP, high-sensitivity C-reactive protein; and LS7, Life’s Simple 7.
Our results propose that lifestyle factors during midlife are linked to the functionality and composition of HDL. Table S3 summarizes potential pathways linking BMI, physical activity, and smoking with HDL metrics. In particular, a healthy midlife BMI appears to be strongly associated with better cholesterol efflux capacity, higher phospholipids, and more large HDL-Ps. These results are consistent with previous findings on the relation between weight and HDL metrics in different cohorts. In the INTERLIPID and Insulin Resistance Atherosclerosis Study cohorts, higher BMI was associated with more small and medium HDL-P, smaller overall HDL size, and less concentrations of large HDL-P.35,36 However, in the MESA (Multi-Ethnic Study of Atherosclerosis) cohort, HDL-C mass efflux did not differ by BMI classes.37 The differences in findings may be because of the cross-sectional design of the analysis, the fact that groups were compared by unadjusted ANOVA, or the variation in BMI categorization compared with our study. Moreover, this study included both men and women, and did not assess whether this relation differed by sex.

An increase in activity of enzymes linked to HDL metabolism, such as cholesterol ester transfer proteins and hepatic lipase, may be observed in states of obesity.38 Cholesterol ester transfer proteins mediate the transfer of cholesteryl esters from HDL-P to triglyceride-rich proteins, which are then taken up by the liver, in exchange for triglycerides.39 Triglyceride-rich HDLs produce smaller HDL particles.40 Smaller HDL-P, elevated triglyceride content, and diminished phospholipids in the particle, in turn, could lead to detrimental effects on the cholesterol efflux capacity and function of the HDL.41,42 This is of particular relevance to midlife women, because women gain significant weight as they transition through midlife.43 It is important to note that BMI was identified as a health behavior in the original LS7 score,20 but BMI is a biological variable that is a consequence of genetic factors, energy intake, and energy expenditure, among others.

Furthermore, in our study, higher physical activity was associated with more phospholipid and less triglycerides content and higher concentrations of total, large, and medium HDL-Ps. Physical activity, whether aerobic or resistance, has been linked to lower CVD risk in the general population. In the WOMAN (Women On the Move through Activity and Nutrition) study, higher leisure physical activity was associated with higher total HDL-P and larger HDL size.44 Exercising has been linked to reductions in cholesterol ester transfer protein concentrations and increases in nitric oxide bioavailability45 and to increases in lipoprotein lipase activity and alterations in immune and endothelial function.

Our study showed that in women, smoking was associated with higher triglyceride contents in the HDL but not associated with other HDL metrics. In healthy

| Total HDL-P (μmol/L) | Large HDL-P (μmol/L) | Medium HDL-P (μmol/L) | Small HDL-P (μmol/L) | HDL size (nm) |
|---------------------|----------------------|-----------------------|---------------------|--------------|
| AIC | β (SE) | P value | AIC | β (SE) | P value | AIC | β (SE) | P value | AIC | β (SE) | P value |
| Model 1 | 8497.5 | 1.06 | <0.001 | 8654.0 | 0.51 | <0.001 | 8664.4 | 0.46 | <0.001 | 1359.8 | 0.12 | <0.001 |
| Model 2 | 8272.6 | 0.62 | <0.001 | 8671.9 | 0.56 | <0.001 | 8543.2 | 0.10 | <0.001 | 1324.7 | 0.10 | <0.001 |

Data presented as increase or decrease in HDL metric per 1-SD increase in LS7 score. Model 1: Unadjusted. Model 2: Adjusted for time between LS7 score and HDL metrics measures, race, education status, and baseline age, economic hardship, menopause status, alcohol use, and log-hs-CRP. AIC indicates Akaike Information Criterion; HDL, high-density lipoproteins; HDL-P, HDL particles; hs-CRP, high-sensitivity C-reactive protein; and LS7, Life’s Simple 7.
participants from the MESA study, Shea et al reported that HDL cholesterol mass efflux capacity did not differ by smoking status. However, in women from the INTERLIPID study, smoking more cigarettes was associated with less total and large HDL-P by NMR, despite no differences in HDL-C. Smoking increases the activity of cholesterol ester transfer proteins and hepatic lipase, and could inhibit the activity and concentration of lecithin–cholesterol acyltransferase, impairing HDL maturation, and potentially reducing levels of larger HDL-P. The lack of associations between smoking and other metrics of HDL, particularly HDL subclasses, may be because of the small number of smokers in this analysis, resulting in lower power to detect between-group differences for other HDL metrics.

We did not find any significant associations between diet and HDL metrics in women. It is important to note that in our study population, no women achieved ideal diet, which could explain the lack of observed associations with HDL metrics between groups. This pattern has been reported previously in SWAN women. Despite the fact that we did not observe an association between diet and HDL metrics, this finding of low frequency of healthy diet in midlife women calls attention to the need for more awareness about diet in US women. Moreover, investigating the associations between HDL metrics and dietary patterns is not straightforward because of the complex nature of dietary habits, because food and nutrients are not consumed as single components or in isolation. Furthermore, we used the food frequency questionnaire to categorize diet based on Dietary Approaches to Stop Hypertension patterns. Other approaches to diet assessment may result in different findings.

Additionally, we found that lower BMI and increased physical activity were associated with a smaller increase in HDL size and a larger increase in medium HDL-P, respectively. This is in an unexpected direction to what we hypothesized. However, in the SWAN HDL ancillary study, we have reported that the changes in HDL metrics in midlife women are not linear over the MT; thus a linear association may not reflect the actual changes in HDL metrics over time. Moreover, SWAN has previously reported that large HDL-P increases after menopause and that greater levels of large HDL-P and a larger HDL size could be associated with less HDL-CEC close to menopause, suggesting a decline in the efficiency of the larger HDLs to promote efflux capacity and indicating a potential dysfunctionality in larger HDLs close to menopause.
Similar findings were also observed in women from the MESA study. The results of this analysis should be interpreted with care because some potential limitations exist. Except for BMI, the SWAN HDL study had a small number of participants in the intermediate CVH groups; thus we had to combine the poor and intermediate groups. Given the likely larger differences expected to exist between the poor and ideal groups, this may have biased the results towards the null. Moreover, smoking status, diet, and physical activity were assessed by self-reported questionnaires. This may have introduced recall bias. The food frequency questionnaire may have different validities across different racial/ethnic groups, where previous studies have shown that the food frequency questionnaire may be limited in its ability to assess nutrient intake and may underestimate racial differences in eating patterns in Black compared with White women. We also included one measure of HDL function; however, lifestyle factors may affect other functions of the HDL such as the anti-oxidative and anti-inflammatory capacities, which were not measured in this study. Moreover, HDL subclasses were measured using NMR spectroscopy; however, multiple methods have been developed to assess HDL subclasses and size, such as ion mobility, ultracentrifugation, and gel electrophoresis, which is the criterion standard for HDL-P assessment. Further studies should utilize other methods to aim to replicate our findings, which would increase the validity of our results. The results may not be generalizable to all racial/ethnic groups, particularly Hispanics, because of the small number of Hispanics included in this analysis. The major strengths of this analysis are the inclusion of a comprehensive profile of HDL properties, which was measured repeatedly over the MT. CVH was measured by the American Heart Association’s LS7 score, which has been frequently investigated in relation to future CVD. This measure is simple, easy, and could be readily computed in research and clinical settings. Moreover, this score provides a simultaneous evaluation of several lifestyle factors that are often interdependent.

In summary, achieving better CVH during early midlife may be tied to a better future HDL metric profile in women. This indicates that following a healthy lifestyle during early midlife may reverse or limit the dysfunction that occurs in the HDL as women transition through menopause. Even though women, in general, are advised to follow a healthy lifestyle including maintaining a healthy weight, being physically active, eating a healthy diet, and avoiding smoking, only 20% of adult American women have an ideal score on more than 5 CVH metrics. Future studies, and particularly interventional studies, should assess whether modifying lifestyle factors would impact HDL metrics and, potentially, reduce burden of future CVD. Because behavioral modifications and lifestyle changes are the initial targets for CVD prevention and risk reduction, assessing whether these lifestyle factors contribute to the prevention of the HDL dysfunction around menopause would
be compelling. This could provide a novel method to predict and assess CVD risk in the clinical setting and would allow the understanding of how improving lifestyle behaviors in midlife women could prevent CVD through its impact on HDL metrics.

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**Supplemental Material**

Tables S1–S3

Figures S1

**ARTICLE INFORMATION**

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**Figure 4.** Associations between baseline LS7 components and change in HDL metrics.

A, Associations between BMI categories and changes in HDL size; B, Associations between physical activity categories and changes in medium HDL-P. Data presented as the yearly change in z-score of each HDL metric per group. Models adjusted for time between LS7 score and HDL metrics, race, and baseline age, education level, economic hardship, menopause status, alcohol use, and log-hs-CRP. BMI indicates body mass index; HDL, high-density lipoproteins; HDL-P, HDL particles; hs-CRP, high-sensitivity C-reactive protein; and LS7, Life’s Simple 7. * Significant differences between groups.
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Table S1. Adjusted means for the associations between LS7 components and future HDL metrics.

|                  | HDL-CEC (%) | HDL-Tg (log) | HDL-PL (mg/dL) | Total HDL-P (µmol/L) | Large HDL-P (µmol/L) | Medium HDL-P (µmol/L) | Small HDL-P (µmol/L) | HDL size (nm) | HDL-C (mg/dL) | ApoA-I (mg/dL) |
|------------------|-------------|--------------|----------------|----------------------|----------------------|-----------------------|----------------------|---------------|--------------|---------------|
| **AHA BMI**      |             |              |                |                      |                      |                       |                      |               |              |               |
| Poor             | β (SE)      | β (SE)       | β (SE)         | β (SE)               | β (SE)               | β (SE)                 | β (SE)               |               |              |               |
| 3.82 (0.06)*     | 2.94 (0.02)*| 51.97 (0.97)*| 34.77 (0.55)   | 7.56 (0.33)*         | 10.59 (0.52)         | 16.65 (0.61)           | 9.35 (0.05)          | 55.42 (1.33)* | 157.72 (2.52)*|
| Intermediate     | 3.97 (0.05) | 2.90 (0.02)* | 54.15 (0.88)*  | 35.95 (0.50)         | 7.86 (0.31)*         | 10.60 (0.47)           | 17.48 (0.56)*        | 9.37 (0.05)*  | 58.90 (1.21)* | 164.37 (2.32)|
| Ideal            | 4.06 (0.05) | 2.84 (0.02)  | 57.05 (0.73)   | 36.18 (0.42)         | 9.44 (0.25)          | 11.67 (0.39)           | 15.08 (0.47)         | 9.63 (0.04)   | 64.17 (1.01)  | 170.53 (1.92)|
| **p-trend**      | <0.001      | <0.001       | <0.001         | 0.053                | <0.001               | 0.073                  | 0.018                | <0.001        | <0.001        | <0.001        |
| **AHA Diet**     |             |              |                |                      |                      |                       |                      |               |              |               |
| Poor             | 3.98 (0.04) | 2.88 (0.01)  | 54.81 (0.59)   | 35.69 (0.33)         | 8.54 (0.21)          | 11.09 (0.31)           | 16.05 (0.37)         | 9.49 (0.03)   | 60.36 (0.82)  | 165.0 (1.54)|
| Intermediate     | 3.95 (0.06) | 2.88 (0.03)  | 55.56 (1.05)   | 35.94 (0.59)         | 8.53 (0.37)          | 11.22 (0.55)           | 16.25 (0.66)         | 9.51 (0.05)   | 61.11 (1.46)  | 167.4 (2.75)|
| **p-trend**      | 0.755       | 0.896        | 0.514          | 0.689                | 0.9651               | 0.836                  | 0.781                | 0.704         | 0.633         | 0.435         |
| **AHA Physical Activity** |          |              |                |                      |                      |                       |                      |               |              |               |
| Poor/Intermediate| 3.96 (0.03) | 2.89 (0.01)  | 54.54 (0.56)   | 35.32 (0.31)         | 8.43 (0.20)          | 10.91 (0.30)           | 16.00 (0.36)         | 9.48 (0.03)   | 59.87 (0.78)  | 164.1 (1.46)|
| Ideal            | 4.01 (0.07) | 2.85 (0.03)  | 57.48 (1.12)   | 38.21 (0.62)         | 9.23 (0.39)          | 12.33 (0.59)           | 16.65 (0.71)         | 9.56 (0.06)   | 64.30 (1.55)  | 173.88 (2.92)|
| p-trend | 0.497 | 0.244 | 0.011 | <0.001 | 0.044 | 0.020 | 0.367 | 0.187 | 0.006 | 0.001 |
|---------|-------|-------|-------|--------|-------|-------|-------|-------|-------|-------|
| AHA Smoking |       |       |       |        |       |       |       |       |       |       |
| Poor/Intermediate | 4.00 (0.08) | 2.97 (0.03) | 54.79 (1.25) | 35.12 (0.70) | 8.28 (0.43) | 10.97 (0.66) | 15.90 (0.79) | 9.48 (0.06) | 58.42 (1.73) | 164.65 (3.26) |
| Ideal | 3.97 (0.03) | 2.87 (0.01) | 55.00 (0.57) | 35.83 (0.32) | 8.58 (0.20) | 11.14 (0.30) | 16.12 (0.36) | 9.49 (0.03) | 60.83 (0.78) | 165.65 (1.48) |
| p-trend | 0.646 | 0.001 | 0.871 | 0.319 | 0.507 | 0.810 | 0.784 | 0.778 | 0.177 | 0.767 |

Models adjusted for time between Life’s Simple 7 score and HDL metrics, race, and baseline age, education level, economic hardship, menopause status, alcohol use and log-hs-CRP. Bonferroni’s adjustment used for multiple group comparisons when appropriate.

* Significantly different from the ideal group.
Table S2. Associations between baseline LS7 score with changes in HDL metrics.

|                  | HDL Function and Contents |                  |                  |                  |                  |                  |
|------------------|---------------------------|------------------|------------------|------------------|------------------|------------------|
|                  | HDL-CEC                   | HDL-Tg*          | HDL-PL           | HDL-C            | ApoA-I           |
|                  | β (SE)                    | p-value          | β (SE)           | p-value          | β (SE)           | p-value          | β (SE)           | p-value          |
| LS7 Score        | 0.002 (0.003)             | 0.403            | -0.0002 (0.002)  | 0.908            | 0.03 (0.05)      | 0.525            | -0.04 (0.06)     | 0.520            | 0.17 (0.15)      | 0.280            |
|                  |                           |                  |                  |                  |                  |                  |                  |                  |                  |
|                  | HDL Subclasses and Size   |                  |                  |                  |                  |                  |
|                  | Total HDL-P               | Large HDL-P      | Medium HDL-P     | Small HDL-P      | HDL Size         |
|                  | β (SE)                    | p-value          | β (SE)           | p-value          | β (SE)           | p-value          | β (SE)           | p-value          |
| LS7 Score        | -0.0001 (0.03)            | 0.998            | -0.01 (0.02)     | 0.336            | -0.05 (0.03)     | 0.154            | 0.06 (0.04)      | 0.070            | -0.006 (0.002)   | 0.005            |

The beta estimate represents the yearly change in each HDL metric per 1 standard deviation increase in LS7 score. Models adjusted for time between LS7 assessment and HDL metrics, race, baseline age, education status, economic hardship, alcohol use and log-hs-CRP. * Log-transform
Table S3. Summary of Potential Impact of Smoking, BMI and Physical Activity on HDL Metabolism.

|                               | Ideal Smoking Status | Ideal BMI Status | Ideal Physical Activity |
|-------------------------------|----------------------|------------------|-------------------------|
| CETP                          | ↓                    | ↓                | ↓                       |
| Hepatic Lipase                | ↓                    | ↓                | -                       |
| PLTP                          | ↓                    | -                | -                       |
| LCAT                          | ↑                    | -                | -                       |
| Lipoprotein Lipase            | -                    | -                | ↑                       |
| Oxidation                     | ↓                    | -                | ↓                       |

CETP: Cholesteryl ester transfer protein; PLTP: Phospholipid Transfer Protein; LCAT: lecithin-cholesterol acetyl transferase
Figure S1. Associations between baseline BMI and Physical Activity with traditional HDL-C and ApoA-I. 

Models adjusted for time between Life’s Simple 7 score and HDL metrics, race, and baseline age, education level, economic hardship, menopause status, alcohol use and log-hs-CRP. 

* Significant difference between groups