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Lipoprotein Particle Profiles, Standard Lipids, and Peripheral Artery Disease Incidence

Prospective Data From the Women's Health Study

Editorial, see p 2342

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BACKGROUND: Despite strong and consistent prospective associations of elevated low-density lipoprotein (LDL) cholesterol concentration with incident coronary and cerebrovascular disease, data for incident peripheral artery disease (PAD) are less robust. Atherogenic dyslipidemia characterized by increased small LDL particle (LDL-P) concentration, rather than total LDL cholesterol content, along with elevated triglyceride-rich lipoproteins and low high-density lipoprotein (HDL) cholesterol (HDL-C), may be the primary lipid driver of PAD risk.

METHODS: The study population was a prospective cohort study of 27 888 women ≥45 years old free of cardiovascular disease at baseline and followed for a median of 15.1 years. We tested whether standard lipid concentrations, as well as nuclear magnetic resonance spectroscopy–derived lipoprotein measures, were associated with incident symptomatic PAD (n=110) defined as claudication and/or revascularization.

RESULTS: In age-adjusted analyses, while LDL cholesterol was not associated with incident PAD, we found significant associations for increased total and small LDL-P concentrations, triglycerides, and concentrations of very LDL (VLDL) particle (VLDL-P) subclasses, increased total cholesterol (TC):HDL-C, low HDL-C, and low HDL particle (HDL-P) concentration (all *P* for extreme tertile comparisons <0.05). Findings persisted in multivariable-adjusted models comparing extreme tertiles for elevated total LDL-P (adjusted hazard ratio [HR_{adj}] 2.03; 95% CI, 1.14–3.59), small LDL-P (HR_{adj} 2.17; 95% CI, 1.10–4.27), very large VLDL-P (HR_{adj} 1.68; 95% CI, 1.06–2.66), medium VLDL-P (HR_{adj} 1.98; 95% CI, 1.15–3.41), and TC:HDL-C (HR_{adj}, 3.11; 95% CI, 1.67–5.81). HDL was inversely associated with risk; HR_{adj} for extreme tertiles of HDL-C and HDL-P concentration were 0.30 (*P* trend < 0.0001) and 0.29 (*P* trend < 0.0001), respectively. These components of atherogenic dyslipidemia, including small LDL-P, medium and very large VLDL-P, TC:HDL-C, HDL-C, and HDL-P, were more strongly associated with incident PAD than incident coronary and cerebrovascular disease. Finally, the addition of LDL-P and HDL-P concentration to TC:HDL-C measures identified women at heightened PAD risk.

CONCLUSIONS: In this prospective study, nuclear magnetic resonance–derived measures of LDL-P, but not LDL cholesterol, were associated with incident PAD. Other features of atherogenic dyslipidemia, including elevations in TC:HDL-C, elevations in triglyceride-rich lipoproteins, and low standard and nuclear magnetic resonance–derived measures of HDL, were significant risk determinants. These data help clarify prior inconsistencies and may elucidate a unique lipoprotein signature for PAD compared to coronary and cerebrovascular disease.

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Clinical Perspective

What Is New?

- Among women aged 45 years and older without cardiovascular disease at baseline, elevated levels of low-density lipoprotein cholesterol were not associated with future peripheral artery disease (PAD).
- Using both standard lipids and nuclear magnetic resonance-derived lipoprotein measures, we found strong associations of an atherogenic dyslipidemia profile, including small, dense low-density lipoprotein particle concentration, triglyceride-rich lipoproteins, high-density lipoprotein cholesterol and particle concentration, and total cholesterol: high-density lipoprotein cholesterol with incident PAD.
- These same components of atherogenic dyslipidemia were more strongly associated with PAD than with a composite of cardiovascular and cerebrovascular disease, suggesting a unique lipoprotein profile for incident PAD.

What Are the Clinical Implications?

- Focus on low-density lipoprotein cholesterol in terms of atherosclerotic risk prediction underestimates the risk of PAD among middle-aged, low-risk women.
- The addition of nuclear magnetic resonance-derived lipoprotein measures to traditional lipid measures may improve risk assessment for PAD, and importantly may elucidate a novel therapeutic strategy for PAD prevention.
- Ongoing clinical trials are investigating whether treating atherogenic dyslipidemia, rather than elevations in low-density lipoprotein cholesterol alone, is beneficial in preventing PAD.

Atherogenic dyslipidemia, which comprises a triad of increased blood concentrations of small, dense low-density lipoprotein (LDL) particles (LDL-P), decreased high-density lipoprotein (HDL) particles (HDL-P), and increased triglyceride-rich lipoproteins, has been linked to a composite of coronary artery disease and cerebrovascular disease (CCVD).[1] However, the specific lipoprotein components that contribute to peripheral artery disease (PAD) risk are less clear. In contrast to CCVD, the epidemiological data supporting a link between LDL cholesterol (LDL-C) and incident PAD are limited, especially among women.[2,3] Additionally, individuals with heterozygous familial hypercholesterolemia and genetically elevated levels of LDL-C have notably *lower* rates of PAD compared to coronary artery disease (CAD).[4] Instead, studies suggest that dyslipidemia parameters, such as an elevated ratio of total cholesterol (TC):HDL cholesterol (HDL-C), mixed dyslipidemia, and hypertriglyceridemia, may be the strongest lipid risk factors for incident PAD and PAD progression.[2,5–8]

One means of delineating the lipid-related risk in PAD is by using more detailed lipoprotein measures derived from proton nuclear magnetic resonance (NMR) spectroscopy. Standard lipid panels measure the entire plasma cholesterol or triglyceride content in concentration per deciliter of each lipoprotein class. In contrast, NMR spectroscopy quantifies both the number and size of lipoprotein particles.[9] Plasma cholesterol concentration can differ among individuals due to both variations in particle size as well as metabolic processes that regulate the cholesterol and triglyceride content of the lipoprotein particle core, and these differences often lead to discrepant risk estimates based on traditional versus NMR-derived lipoprotein measures.[10] NMR-derived lipoprotein measures are associated with future myocardial infarction (MI),[11–16] stroke,[11,14,16] diabetes,[17,18] and hypertension.[19] To our knowledge, this methodology has not yet been applied to PAD.

Given the lack of robust data showing a link between LDL-C and PAD, we hypothesized that NMR-derived lipoprotein subclass abnormalities associate with incident PAD and would be distinct from those previously described in other cardiovascular disorders.[11] Therefore, in the current study, we evaluated baseline NMR lipoprotein particles and conventional lipid concentrations in a prospective cohort of middle-aged and older American women free of PAD, MI, and stroke at baseline and measured the association of these lipid measures with incident PAD.

METHODS

Data Availability

The data will not be made available to other researchers for purposes of reproducing the results. However, the methods used in the analysis are available on request.

Study Population

Participants were identified from the WHS (Women's Health Study), a previously completed randomized, double-blind, placebo-controlled trial of low-dose aspirin and vitamin E in the primary prevention of cardiovascular disease.[20] From 1992 to 1995, the study enrolled a total of 39 876 female healthcare professionals in the United States without a history of cancer, MI, stroke, coronary revascularization, or peripheral artery revascularization. At the time of enrollment, women completed questionnaires on baseline demographics, anthropometrics, medical history, and lifestyle factors. Following completion of the trial, willing individuals consented to participate in a longitudinal observational component of the WHS. All participants provided written informed consent, and the study was approved by the institutional review board at Brigham and Women's Hospital.

Before randomization, 28 345 of the participants consented to provide blood samples, and 98.9% (n = 28 024) of these samples underwent NMR lipoprotein profiling. Individuals missing baseline demographic data on body mass

index, as well as history of smoking, hypertension, or hormonal therapy, were excluded from the analysis. In addition, subjects with confirmed prerandomization PAD (n = 30) were excluded from the present analysis. The final study population (n = 27 888) was followed for a median of 15.1 years.

Outcome Ascertainment

Health outcomes of WHS participants were ascertained using annual questionnaires. The primary outcome of interest for the present study was symptomatic lower extremity PAD defined as intermittent claudication and/or peripheral artery revascularization (surgical or percutaneous). To validate reported events, PAD outcomes were initially identified through annual questionnaires, and then confirmed through physician interview and medical records review. For cases of claudication, confirmation was performed using the Edinburgh Claudication Questionnaire, which was administered during telephone interviews conducted by a physician adjudicator. The Edinburgh Claudication Questionnaire is an accepted tool for the detection of PAD that is commonly used in clinical research, and has been validated against in-office physician-diagnosed intermittent claudication with a sensitivity of 91.3% and a specificity of 99.3%.[21] These values for sensitivity and specificity are similar to—and in some cases, higher than—those for reported resting ankle-brachial index.[22] If patients reported lower extremity revascularization on their questionnaire, these events were confirmed by cardiologist review of primary medical records. CCVD was defined as nonfatal MI, percutaneous coronary intervention, coronary artery bypass grafting, nonfatal stroke, or coronary-related death, and these end points were adjudicated as previously described.[23] Utilizing these criteria, we confirmed 130 cases of incident PAD. The most common causes for nonischemic leg pain in disconfirmed cases were venous disease, lower extremity arthritis, lumbar disk disease, and peripheral neuropathy.

Laboratory Analysis

Blood samples were stored in liquid nitrogen (–150°C to –180°C) until analysis. Samples were thawed, aliquoted, and shipped in 200-µL frozen aliquots to LipoScience (now LabCorp, Raleigh, NC) for analysis. The lipoprotein analysis used in the present study is the NMR LipoProfile 4 panel. In this panel, the concentration of each lipoprotein particle subclass is calculated from the NMR signal of terminal methyl groups, and these same NMR signals are used to help calculate weighted-average lipoprotein particle sizes.[10] Particles are classified based on size into the following categories: LDL-P, HDL-P, and very LDL (VLDL-P). Table I in the online-only Data Supplement lists lipoprotein particle diameters.

A core laboratory certified by the National Heart, Lung, and Blood Institute/Centers for Disease Control and Prevention Lipid Standardization Program measured standard lipids and apolipoproteins. LDL-C was measured using a homogeneous direct method with a Hitachi 917 analyzer using reagents from Roche Diagnostics (Indianapolis, IN). HDL-C was measured using a direct enzymatic colorimetric assay, and triglycerides were measured enzymatically with correction for endogenous glycerol. Coefficients of variation were <3% for all standard lipids. Non–HDL-C was calculated by subtracting HDL-C from TC. Apolipoproteins B_{100} and A-1 were measured using immunoturbidometric assays (DiaSorin, Stillwater, MN) with coefficients of variation of 5% and 3%, respectively. High-sensitivity C-reactive protein was measured by a high-sensitivity immunoturbidimetric assay (Denka Seiken, Niigata, Japan).

Statistical Analysis

Continuous data are summarized as either mean±SD or median with interquartile range depending on normality of the distributions. Categorical data are listed as percentages. Between-group differences were assessed by the Wilcoxon rank-sum test for continuous data and the χ^2 test for categorical data. Lipid biomarkers were divided into tertiles. Cox proportional-hazards models were used to estimate the hazard ratio (HR) and 95% CI for each biomarker tertile, and results are presented as top tertile compared to bottom tertile; similar analyses were performed per SD increase of each biomarker. Tests of linear trend across tertiles were performed using the median value from each tertile. We also calculated Spearman rank correlation coefficients to test the relationships between standard and NMR-derived lipoprotein measures.

As preventive therapies instituted at diagnosis of MI or stroke may dramatically alter the subsequent risk of vascular events, we censored women having non-PAD vascular events (e.g., CCVD) at the time of diagnosis. Thus, within these models, follow-up time was censored at the time of the PAD event except in situations in which a CCVD event occurred first—in which case, censoring occurred at the time of the CCVD event. There were a total of 130 confirmed PAD cases, and in 20 of these cases, a CCVD event occurred before the PAD event. Thus, the final population in the current analysis was 110 cases of incident PAD.

Regression models were sequentially adjusted for age followed by smoking pack-years (Model 1). Fully adjusted models (Model 2) were adjusted for age, smoking pack-years, metabolic syndrome, hypertension, postmenopausal hormone therapy, high-sensitivity C-reactive protein, lipid-lowering therapy, and body mass index. Data on smoking pack-years was collected as a categorical variable within WHS, and this variable was divided into the following categories for the purposes of regression modeling: 0, 1 to 10, 11 to 29, and ≥30. All regression results in the text are presented for Model 2 unless otherwise noted. Additionally, all models were adjusted for randomized treatment within the WHS trial. Measures of serum triglycerides were log-transformed for *P* trend analysis due to a right-skewed distribution. Models of LDL particle size were also adjusted for total LDL particle concentration as previously described.[24] Given the inverse correlation of LDL-P subclasses,[24] models assessing each LDL-P subclass were adjusted additionally for the remaining LDL-P subclasses to delineate independent risk associations. The likelihood ratio χ^2 statistic was used to assess model fit. To evaluate the joint effects of LDL-C concentration with LDL-P as well as TC:HDL-C with LDL-P, HDL-P, and VLDL-P, individuals were classified into 4 groups based on the values of each biomarker relative to the population median. Kaplan-Meier survival curves were plotted based on these strata and analyzed using a log-rank test for trend with 3 degrees of freedom. All statistical analyses were performed using SAS statistical software version 9.4 (SAS Institute, Cary, NC). All 95% CIs are 2-tailed, and the *P* value cutoff for all analyses was 0.05.

RESULTS

As shown in Table 1, women with incident PAD were more likely to be older, be current smokers, and have higher rates of baseline hypertension. In this population with a low prevalence of baseline diabetes, there was no significant difference in diagnosed diabetes, although individuals with incident PAD were more likely to have a history of metabolic syndrome. Baseline levels of triglycerides, apolipoprotein B_{100}, non–HDL-C, TC:HDL-C, and high-sensitivity C-reactive protein were all higher in individuals who developed PAD. The baseline levels of HDL-C and apolipoprotein A-1 were lower in individuals with incident PAD. There was no statistically significant difference in TC or LDL-C.

Table 2 shows median concentrations of NMR-derived lipoprotein particles according to case status. Total LDL-P and small LDL-P subclass concentrations were

Table 1. Baseline Characteristics of the Study Population

	Women Remaining Free of PAD Events (n=27 778)*	Women Developing PAD Events (n=110)†	*P* Value
Age, mean (SD), y	54.7 (7.1)	59.2 (7.5)	<0.0001
BMI, mean (SD), kg/m^2	25.9 (5.0)	25.7 (4.5)	0.75
Non-Hispanic white, %	95.3	98.2	0.25
Current smoking, %	11.5	47.3	<0.0001
Prior smoking, %	36.6	36.4	1.00
Pack-years, %			
0	52.3	16.5	<0.0001
1–10	15.3	6.4	
11–29	22.6	32.1	
≥30	9.8	45.0	
Diabetes, %	2.5	3.6	0.35
Metabolic syndrome, %	24.6	33.6	0.03
Hypertension, %	25.1	39.1	0.001
Treatment for hypercholesterolemia, %	3.2	5.5	0.17
Family history of premature CAD, %	14.4	18.5	0.22
Exercise ≥1 time/wk, %	43.2	38.2	0.33
Current HT use, %	42.6	36.4	0.21
WHS trial assignment to vitamin E, %	50.1	49.1	0.85
WHS trial assignment to aspirin, %	50.1	50.9	0.92
hsCRP, mg/L	2.0 (0.8–4.4)	2.8 (1.6–6.6)	<0.0001
Standard chemical lipids, mg/dL			
Total cholesterol	208 (184–235)	214 (185–246)	0.13
LDL cholesterol	121 (101–144)	130 (103–153)	0.05
HDL cholesterol	52 (43–62)	44 (37–55)	<0.0001
Triglycerides	118 (84–175)	146 (104–218)	0.0001
Apolipoproteins, mg/dL			
Apolipoprotein B_{100}	100 (84–121)	115 (91–133)	<0.0001
Apolipoprotein A-1	149 (132–168)	138 (124–152)	<0.0001
Non–HDL cholesterol, mg/dL	154 (129–182)	172 (137–200)	0.0007
Total cholesterol:HDL cholesterol	3.97 (3.23–4.92)	4.66 (3.87–6.02)	<0.0001

Values are median (25th to 75th percentile) unless otherwise indicated. *P* values for continuous variables were obtained from the Wilcoxon rank-sum test. *P* values for categorical variables were obtained using the chi-square test. BMI indicates body mass index; CAD, coronary artery disease; hsCRP, high-sensitivity C-reactive protein; HDL, high-density lipoprotein; HT, hormonal therapy; LDL, low-density lipoprotein; PAD, peripheral artery disease; and WHS, Women's Health Study.

*Number missing: 234 for race; 227 for pack-years; 15 for diabetes, 49 for metabolic syndrome; 20 for treatment for hypercholesterolemia; 462 for family history of premature CAD; 10 for exercise; 86 for hsCRP; 87 each for total cholesterol and HDL cholesterol; 86 each for LDL cholesterol and triglycerides; 224 for apolipoprotein B_{100}; 220 for apolipoprotein A-1; and 88 each for non-HDL cholesterol and total cholesterol: HDL cholesterol.

†Number missing: 1 for race; 1 for pack-years; 2 for family history of premature CAD; and 4 each for apolipoprotein B_{100} and apolipoprotein A-1.

Table 2. Baseline NMR Lipoprotein Profile

	Women Remaining Free of PAD Events (n=27 778)*	Women Developing PAD Events (n=110)†	*P* Value
NMR lipoprotein particle concentrations, nmol/L			
LDL particles			
Total	1567 (1329–1839)	1723 (1495–1989)	<0.0001
Large	306 (160–467)	233 (69–474)	0.02
Medium	156 (0–347)	128 (0–285)	0.03
Small	953 (685–1336)	1208 (875–1599)	<0.0001
VLDL particles			
Total	166.5 (130.3–208.4)	180.1 (145.3–221.9)	0.005
Very large	0.1 (0.1–0.2)	0.2 (0.1–0.5)	0.003
Large	1.6 (0.3–4.3)	2.9 (0.6–5.5)	0.004
Medium	15.8 (8.5–25.5)	20.8 (13.2–31.9)	0.0009
Small	55.4 (34.0–81.8)	62.2 (36.3–85.2)	0.22
Very small	84.3 (58.9–114.8)	87.8 (64.9–118.9)	0.18
HDL particles			
Total	24 400 (22 000–27 000)	23 150 (20 950–25 250)	0.0009
Large	2100 (1300–3300)	1600 (1050–2500)	0.0002
Medium	5300 (3700–7200)	3850 (2600–6200)	<0.0001
Small	16 300 (14 100–18 700)	16 750 (15 150–18 650)	0.11
NMR average particle size, nm			
LDL particles	20.9 (20.6–21.2)	20.7 (20.4–21.1)	0.003
VLDL particles	42.5 (38.6–47.9)	44.0 (39.1–50.8)	0.04
HDL particles	8.9 (8.7–9.2)	8.7 (8.6–9.1)	0.0001

Values are median (25th to 75th percentile). *P* values were obtained from the Wilcoxon rank-sum test. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; NMR, nuclear magnetic resonance; PAD refers to peripheral artery disease; and VLDL, very low-density lipoprotein.

*Number missing: 581.

†Number missing: 6.

higher in women with PAD, whereas large LDL-P, medium LDL-P, and total HDL-P were significantly lower. Among HDL-P subclasses, all but small HDL-P were lower in individuals with PAD. Total very LDL (VLDL) particles (VLDL-P), very large VLDL-P, large VLDL-P, and medium VLDL-P concentrations were higher in those with PAD. Differences in small and very small VLDL-P concentrations did not reach statistical significance. Consistent with data for particle subclass concentrations, women developing PAD had smaller average LDL-P and HDL-P size and a larger average VLDL-P size.

Table II in the online-only Data Supplement shows Spearman correlation coefficients for NMR lipoproteins with standard lipid and apolipoprotein measures in the total sample. Total LDL-P concentration correlated strongly with LDL-C (r=0.71), as well as apolipoprotein B-100 (r=0.86), non–HDL-C (r=0.78), and TC:HDL-C (r=0.66). Large and small LDL-P concentration correlated modestly with LDL-C (r=0.27 and 0.25, respectively). Large HDL-P correlated strongly with HDL-C (r=0.75), but total HDL-P (r=0.52) and the medium HDL-P subclass (r=0.50) showed more modest correlations. Table III in the online-only Data Supplement lists Spearman correlation coefficients for NMR lipoproteins with themselves. Total LDL-P strongly correlated with small LDL-P (r=0.63), and total VLDL-P most strongly correlated with very small VLDL-P (r=0.69). HDL-P had similar positive correlations with large, medium, and small HDL-P subclasses (r=0.35, 0.49, and 0.51, respectively).

Figure 1 and Table IV in the online-only Data Supplement show the results from Cox regression analyses adjusted for both age and nonlipid risk factors in women classified based on *standard* lipid and apolipoprotein tertiles. The strongest positive risk association was with TC:HDL-C (multivariable-adjusted HR, 3.11; 95% CI, 1.67 to 5.81; *P* trend=0.0005). Serum triglyceride concentration was strongly associated with incident PAD in age-adjusted, but not multivariable-adjusted, models (adjusted HR, 1.46; 95% CI, 0.82 to 2.61; *P* trend=0.22). Significant findings were also seen for both apolipoprotein B_{100} and non–HDL-C in age-adjusted but not multivariable-adjusted models. Importantly, no significant associations were seen for TC or LDL-C. In both age-adjusted and multivariable-adjusted mod-

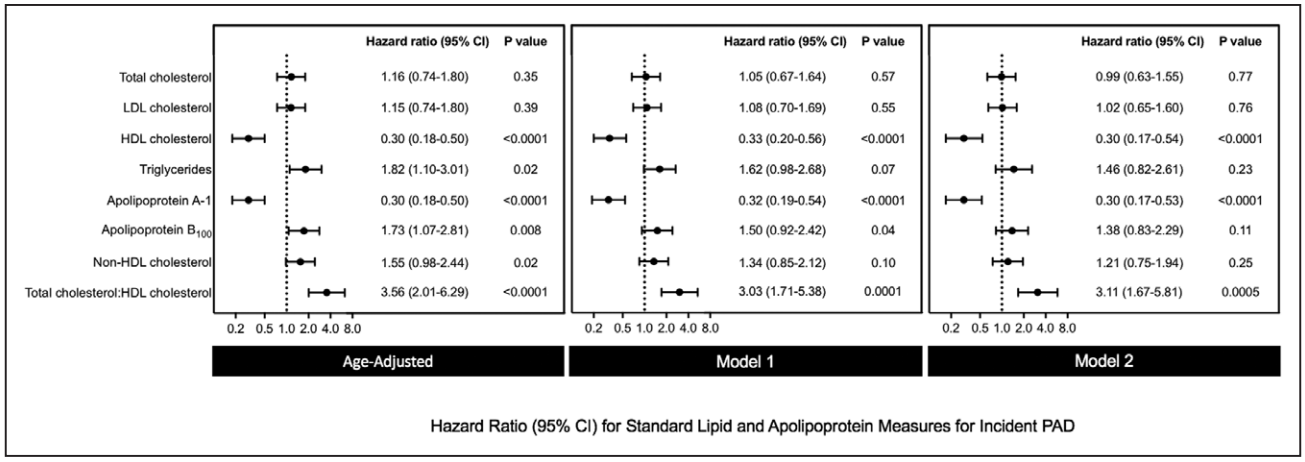

Figure 1. Risk associations between standard lipid and apolipoprotein measures and incident peripheral artery disease.
Hazard ratios and 95% CIs for the top versus bottom tertile of standard lipid and apolipoprotein measures. Model 1 adjusted for age and smoking pack-years. Model 2 adjusted for age, smoking pack-years, metabolic syndrome, hypertension, hormonal therapy, high-sensitivity C-reactive protein, lipid-lowering therapy, randomized treatment assignment, and body mass index. HDL indicates high-density lipoprotein; and LDL, low-density lipoprotein.

els, HDL-C concentration was inversely associated with PAD with a 70% lower relative risk for the lowest tertile versus the highest (adjusted HR, 0.30; 95% CI, 0.17 to 0.54; *P* trend<0.0001). There was a similar strong inverse association seen with apolipoprotein A-1 in fully adjusted models (HR, 0.30; 95% CI, 0.17 to 0.53; *P* trend<0.0001). Overall, similar results were seen for incident PAD per SD increase of each biomarker (Table V in the online-only Data Supplement), although the association for apolipoprotein B_{100} reached statistical significance in the fully adjusted model (HR, 1.23; 95% CI, 1.02 to 1.49; *P* trend=0.03).

Similarly, age-adjusted and multivariable-adjusted analyses for *total NMR-derived* lipoprotein particle concentrations are displayed in Figure 2 and Table VI in the online-only Data Supplement. In contrast to the null association of LDL-C with PAD, total LDL-P was the strongest positive lipoprotein risk factor (adjusted extreme tertile HR, 2.03; 95% CI, 1.14 to 3.59; *P* trend=0.02). Both LDL-P size (adjusted extreme tertile HR, 0.60; 95% CI, 0.36 to 1.02; *P* trend=0.02) and HDL-P size (adjusted extreme tertile HR, 0.39; 95% CI, 0.21 to 0.70; *P* trend=0.002) were inversely associated with incident PAD. No significant association was seen for total VLDL-P concentration or VLDL-P size. Total HDL-P was inversely associated with PAD (adjusted HR, 0.29; 95% CI, 0.16 to 0.52; *P* trend<0.0001). The associations between each biomarker analyzed per SD and incident PAD are displayed in Table VII in the online-only Data Supplement.

In multivariable models that evaluated *lipoprotein particle subclass concentrations*, small LDL-P remained significantly associated with incident PAD (adjusted HR, 2.17; 95% CI, 1.10 to 4.27; *P* trend=0.02) (Figure 3; Table VIII in the online-only Data Supplement). No residual association was seen for large or medium LDL-P. Both large and medium HDL-P were associated with protection

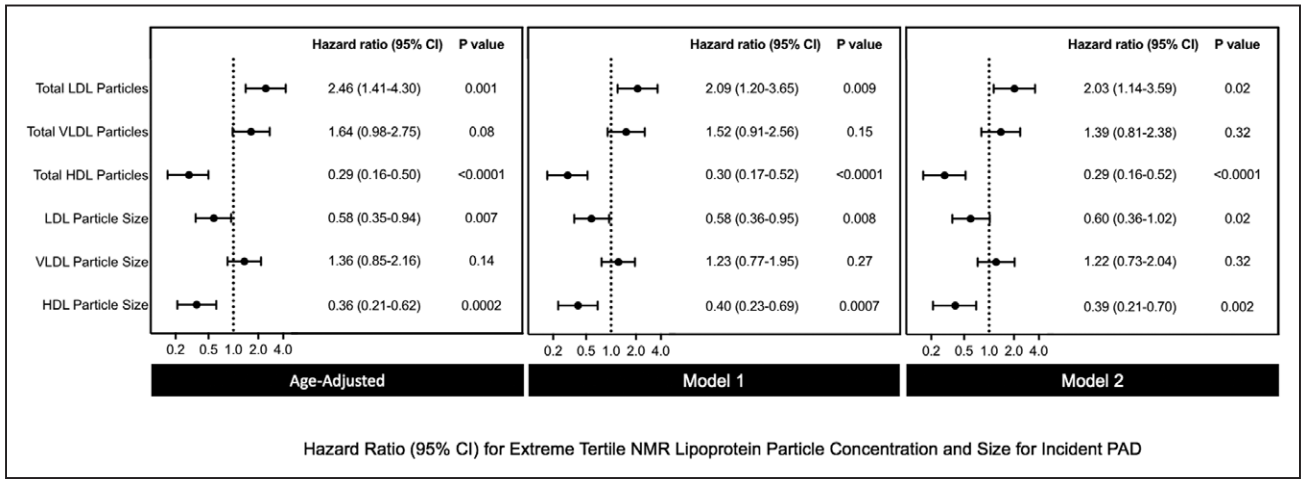

Figure 2. Risk associations between nuclear magnetic resonance lipoprotein particle concentrations and size and incident peripheral artery disease.
Hazard ratios and 95% CIs for the top versus bottom tertile of nuclear magnetic resonance lipoprotein particle concentrations and sizes. Model 1 adjusted for age and smoking pack-years. Model 2 adjusted for age, smoking pack-years, metabolic syndrome, hypertension, hormonal therapy, high-sensitivity C-reactive protein, lipid-lowering therapy, randomized treatment assignment, and body mass index. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; and VLDL, very LDL.

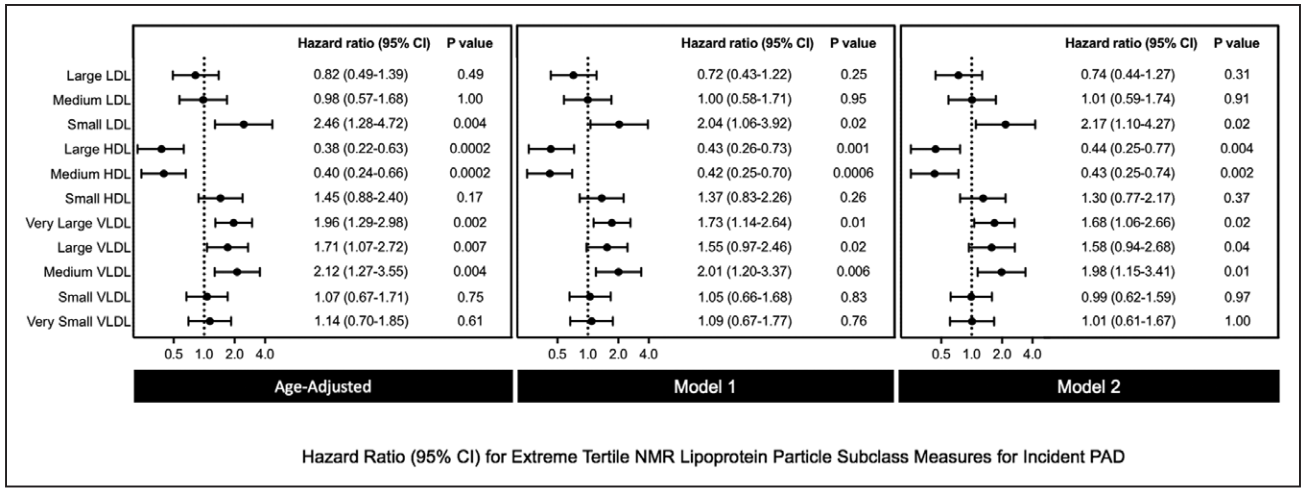

Figure 3. Risk associations between nuclear magnetic resonance lipoprotein particle subclasses and incident peripheral artery disease.
Hazard ratios and 95% CIs for the top versus bottom tertile of nuclear magnetic resonance lipoprotein particle subclasses. Model 1 adjusted for age and smoking pack-years. Model 2 adjusted for age, smoking pack-years, metabolic syndrome, hypertension, hormonal therapy, high-sensitivity C-reactive protein, lipid-lowering therapy, randomized treatment assignment, and body mass index. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; and VLDL, very LDL.

against PAD (adjusted HR, 0.44; 95% CI, 0.25 to 0.77; *P* trend=0.004; and adjusted HR, 0.43; 95% CI, 0.25 to 0.74; *P* trend=0.002, respectively). Of VLDL-P subclasses, very large (size range: 90 to 240 nm), large (size range: 50 to 89 nm), and medium VLDL-P (size range: 37 to 49 nm) were significantly associated with incident PAD (adjusted HR, 1.68; 95% CI, 1.06 to 2.66; *P* trend=0.02; adjusted HR, 1.58; 95% CI, 0.94 to 2.68; *P* trend=0.04; and adjusted HR, 1.98; 95% CI, 1.15 to 3.41; *P* trend=0.01, respectively). Table IX in the online-only Data Supplement shows the association per SD increase in each biomarker.

Figure 4 (Figure I and Table X in the online-only Data Supplement) displays multivariable-adjusted risk associations for standard lipid, apolipoprotein, and NMR lipoprotein measures for both incident PAD and CCVD ranked by magnitude of the risk estimate. Results are

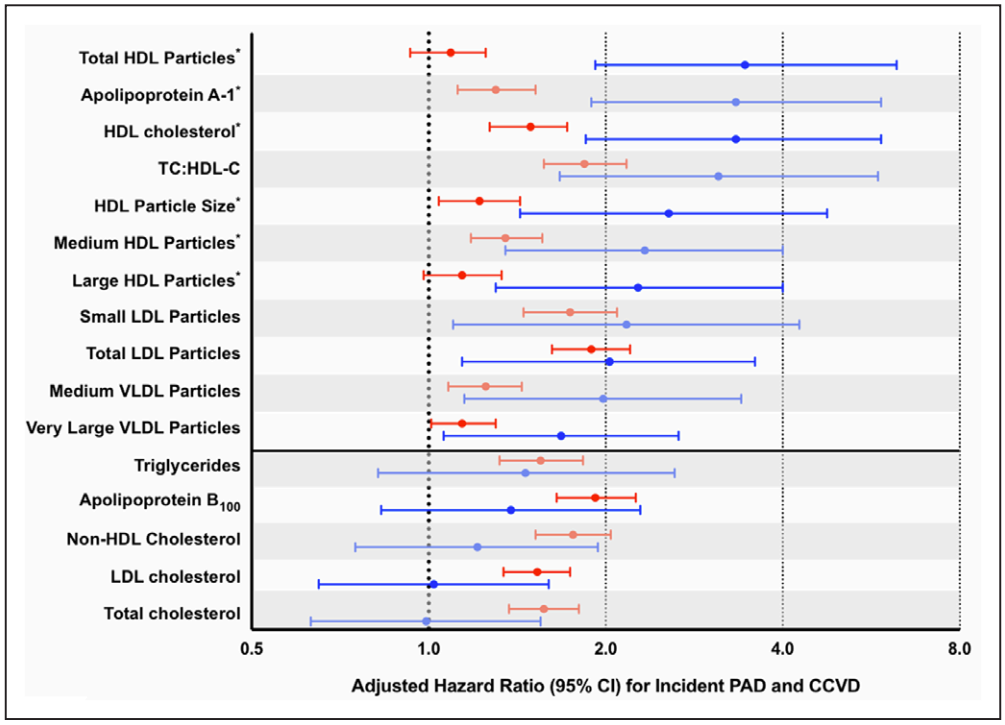

Figure 4. Risk associations between nuclear magnetic resonance lipoprotein and standard lipid measures with incident peripheral artery disease (PAD) versus incident coronary and cerebrovascular disease (CCVD).
Hazard ratios and 95% CIs for the top versus bottom tertile of incident PAD (blue) and CCVD (red), adjusted for age, smoking pack-years, metabolic syndrome, hypertension, hormonal therapy, high-sensitivity C-reactive protein, lipid-lowering therapy, randomized treatment assignment, and body mass index. Measures displayed include all standard lipid and apolipoprotein assays, as well as nuclear magnetic resonance–derived measures with a statistically significant association for incident PAD. Horizontal line separates markers of atherogenic dyslipidemia from other measures without statistical significance for incident PAD. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; TC:HDL-C, total cholesterol:HDL cholesterol; and VLDL, very LDL.

displayed comparing highest versus lowest tertile, except for biomarkers associated with protection against incident disease, in which case results are presented as lowest versus highest tertile to facilitate comparisons. Of all measures analyzed, HDL-P, apolipoprotein A-1, HDL-C, and TC:HDL-C had the largest HRs for incident PAD (Figure 4). Statistically significant associations were also seen for HDL-P size, medium and large HDL-P, small and total LDL-P, and medium, large, and very large VLDL-P.

Although TC and LDL-C were not associated with incident PAD, both were associated with incident CCVD (adjusted HR, 1.57; 95% CI, 1.37 to 1.80; *P* trend<0.0001; and adjusted HR, 1.53; 95% CI, 1.34 to 1.74; *P* trend<0.0001, respectively [Figure 4; Figure II and Table X in the online-only Data Supplement]). Among standard lipid and apolipoprotein measures, adjusted HRs for apolipoprotein B_{100} and non–HDL-C were nominally larger and statistically significant only for incident CCVD, while HDL-C, apolipoprotein A-1, and TC:HDL-C were more strongly associated with incident PAD. Of the NMR-derived lipoprotein measures, total LDL-P, small LDL-P, large and medium subclasses of VLDL-P, and total HDL-P appeared more strongly associated with incident PAD than incident CCVD.

Women were categorized based on both LDL-C and total LDL-P concentration (above or below median) to evaluate the joint role of these biomarkers in PAD risk prediction (Figure 5A). Overall, women with total LDL-P values above the population median were at highest risk of incident PAD, irrespective of their LDL-C measure. Additionally, we reclassified women based on total LDL-P, HDL-P, and VLDL-P concentrations and TC:HDL-C (above or below median; Figure 5B through 5D). Even among those with elevated TC:HDL-C, the addition of total LDL-P or total HDL-P concentration further differentiated individuals based on their risk of incident PAD. VLDL-P levels above the population median did not increase the incidence of PAD beyond the risk of an elevated TC:HDL-C.

DISCUSSION

In this prospective evaluation comparing standard lipid and NMR-derived lipoprotein measures, we found that TC:HDL-C, as well as total and small LDL-P concentration, had strong positive associations for incident PAD, particularly in comparison with LDL-C, non–HDL-C, and $apoB_{100}$, which had no significant associations in multivariable models. Medium, large, and very large VLDL-P were also significantly associated with PAD, while plasma triglycerides were a significant risk predictor in age-adjusted models only. In aggregate, these findings provide evidence that the atherogenic dyslipidemia profile is an important determinant of PAD risk in women, and this profile appears more strongly linked to incident PAD than to CCVD. Although small, retrospective stud-

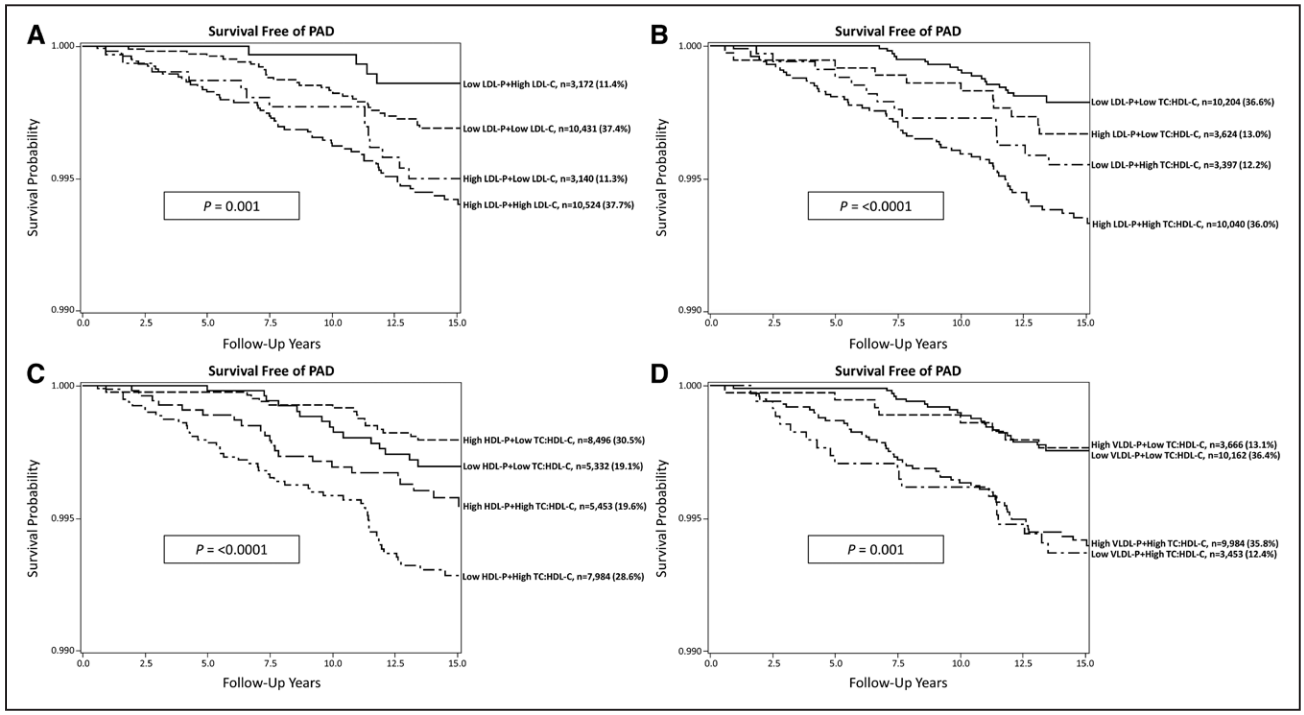

Figure 5. Joint effects of nuclear magnetic resonance lipoprotein and standard lipid measures with incident peripheral artery disease (PAD). **A**, PAD survival curve according to LDL-C and LDL-P particle concentration (above or below population median). **B**, PAD survival curve according to LDL-P particle concentration and TC:HDL-C (above or below population median). **C**, PAD survival curve according to HDL-P particle concentration and TC:HDL-C (above or below population median). **D**, PAD survival curve according to VLDL-P particle concentration and TC:HDL-C (above or below population median). HDL-C indicates high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle concentration; LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particle concentration; TC:HDL-C, total cholesterol:HDL-C; and VLDL-P, very low-density lipoprotein particle concentration.

ies have shown an association between atherogenic dyslipidemia and PAD,[25,26] the current data provide a more robust evaluation of this important issue, including lipid subclassification, which may explain prior inconsistencies. Our data also show a joint association of total LDL-P concentration with measures of both LDL-C and TC:HDL-C, such that women with elevations of total LDL-P were at higher risk of PAD irrespective of these traditional lipid measures alone. Thus, if confirmed in other cohorts, our data suggest that LDL particle number may provide important prognostic information for PAD incidence in women, among whom few prospective data currently exist.

In contrast to well established associations for CCVD, the link between LDL-C and PAD is not robust. Few published studies have demonstrated that elevations of LDL-C are associated with incident PAD.[2,3] In the Physicians' Health Study, which was restricted to men, elevated LDL-C was a risk factor for developing PAD, but had no added value beyond the association with TC:HDL-C in models adjusting for both.[2] Data from the Cardiovascular Health Study showed that LDL-C measures within the highest quartile were associated with incident PAD in both men and women.[3] Of note, this cohort comprised older individuals (aged >65 years, mean baseline age ≈74 years) and included both subjects with prior cardiovascular disease, as well as men. Risk associations were not provided separately for women. Indeed, in contrast to CAD, published data on the link between LDL-C and PAD are notably absent from several large prospective cohorts having measured lipid levels and follow-up for PAD, including the Framingham Offspring Study,[27] the MESA study (Multi-Ethnic Study of Atherosclerosis),[5] and the Edinburgh Artery Study.[28]

Studies of patients with familial hypercholesterolemia and, thus, hereditary elevations in LDL-C, may also be informative. In these studies, prevalence of clinical coronary and cerebrovascular disease is higher than clinical PAD. In a large cohort of 2752 individuals with molecularly confirmed heterozygous familial hypercholesterolemia, 0.5% had a history of peripheral revascularization, while 9.0% had undergone coronary revascularization.[4] Other cohorts have also noted a lower prevalence of PAD compared to CAD in individuals with heterozygous familial hypercholesterolemia.[29,30] LDL particle concentrations were not reported in any of these investigations.

In addition to a risk association with TC:HDL-C, the present study found that both total LDL-P and small LDL-P concentrations were linked to PAD in women, and LDL particle size was inversely associated with incident PAD. Even among women with TC:HDL-C measures above the median, the addition of total LDL-P concentration values identified women at even greater risk of PAD. There are several potential explanations for these findings. Importantly, with even modestly elevated levels of serum triglycerides, triglyceride-rich lipoproteins (such as VLDL) exchange triglyceride molecules with cholesterol from large LDL particles with cholesterol-rich cores.[10] As a result, large LDL particles become enriched for triglycerides and undergo subsequent hydrolysis and conversion to small LDL. Individuals with smaller LDL particles also tend to have greater concentrations of LDL particles, which may further explain the risk association seen in our analysis.[24] Although prospective data for PAD are sparse, it is interesting to note that metabolic syndrome has been linked to a heightened risk of PAD,[31] and the predominant dyslipidemia pattern in these individuals is elevated small LDL-P and relatively normal LDL-C.[32]

Our findings pertaining to triglyceride-rich lipoproteins, such as VLDL, are of particular interest. These lipoproteins can cause increased inflammation, monocyte activation, and endothelial dysfunction.[33] In addition, the size of triglyceride-rich lipoproteins may be important. As previously discussed, large VLDL particles serve as a reservoir for triglyceride exchange with cholesterol-rich large LDL particles, thus facilitating their transition from large to highly atherogenic small LDL particles.[10] As a potential second mechanism, partially hydrolyzed VLDL particles in the ≤70 nm range (which includes large and medium VLDL particles in the current analysis) are small enough to traverse the endothelial barrier.[34] These cholesterol ester-enriched VLDL remnants may bind to and be retained by the connective tissue matrix, where uptake by arterial macrophages leads to foam cell formation. Previous studies have also shown triglyceride levels to be associated with PAD risk,[7,8] and trial data suggest both triglyceride lowering and raising of HDL-C with fibrate therapy may reduce claudication severity.[35] We found stronger risk associations for triglyceride-rich very large (90 to 240 nm), large (50 to 89 nm), and medium (37 to 49 nm) VLDL particles than for triglyceride level alone or even non–HDL-C concentration, suggesting that packaging in these triglyceride-rich lipoprotein particles may be the more potent driver of PAD risk.

Our findings with regard to HDL-C are not new, and several previous studies have found a negative correlation between HDL-C concentration and PAD.[36,37] Indeed, given the null risk association between TC and incident PAD, HDL-C (along with triglyceride-rich VLDL) is the primary driver of risk for TC:HDL-C in our study. However, we also note that concentrations of apolipoprotein A-1, HDL-C, and total HDL-P concentration, as well as HDL-P size were inversely associated with PAD. Additionally, low levels of HDL-C identified women at heightened risk for PAD beyond that of TC:HDL-C. HDL particle subclasses vary in terms of both cholesterol and apolipoprotein A-1 composition,[38] and some data suggest that large HDL particles are protective against CAD.[39] Increased concentration of small HDL-P is associated with prediabetes,[40] insulin-resistance,[41] and ab-

dominal obesity,[42] again suggesting a potential mechanistic link between atherogenic dyslipidemia and PAD.

These findings have several important clinical implications. First, they add to the growing body of evidence that the atherogenic dyslipidemia phenotype is a precursor to PAD. Second, our findings suggest that focus on LDL-C as a clinical risk factor for PAD, at least in women, may be insufficient, and that further characterization of LDL and VLDL particle concentrations may identify women at heightened risk of PAD who would otherwise remain undetected. Our data also suggest there may be important differences in the development of atherosclerosis and thrombosis in different arterial beds. Indeed, in clinical practice, many patients develop severe manifestations of PAD, but never exhibit overt evidence of CCVD, such as angina or MI. This was also seen in our analysis, in which 95 of 110 total individuals with incident PAD never suffered a CCVD event (data not shown). Although LDL-C may be an important risk factor for subclinical atherosclerosis in PAD as it is in CAD, our findings suggest that a lipoprotein profile of elevated triglyceride-rich lipoproteins, increased LDL particle (in particular small particle) concentration, and low HDL particle concentration may be more important in the pathogenesis of symptomatic PAD.

Our findings may be relevant for therapeutic trials in PAD patients. Observational studies have suggested a benefit of statin therapy on limb outcomes.[43,44] Arya et al. found that among 155 647 patients with incident PAD in the Veterans Affairs health system, statin utilization within the first year was associated with large and significant reductions in lower extremity amputation compared to individuals prescribed antiplatelet therapy alone.[44] However, because of statin pleiotropy,[45–47] it remains unclear whether this benefit was due to LDL-C reduction, inflammation reduction, or improvement in atherogenic dyslipidemia. Finally, it remains possible that individuals receiving statins in this observational study were also benefitting from more guideline-directed therapy overall. Data from the FOURIER (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Patients With Elevated Risk) randomized clinical trial of evolocumab, a PCSK9 inhibitor with potent LDL-C–lowering effects, and modest improvements in triglyceride and HDL-C levels, but no substantive high-sensitivity C-reactive protein reduction, add some clarity in this regard.[48] A 42% reduction in major adverse limb events was observed.[49] However, treatment effects on LDL particle number and other components of atherogenic dyslipidemia are currently unavailable and may yet explain these findings. Whether treatment of atherogenic dyslipidemia per se improves limb-related vascular outcomes will be assessed in the recently initiated PROMINENT trial (Pemafibrate to Reduce Cardiovascular Outcomes by Reducing Triglycerides In Patients With Diabetes) (NCT03071692) of pemafibrate to reduce cardiovascular events in patients with elevated triglycerides and low HDL-C.

Strengths of the present study include the prospective design, large sample size, long-term follow-up, and homogeneity of our study population, which may reduce confounding. However, several potential limitations should be considered. First, the WHS has no male enrollees, and the majority of participants were white and healthy at baseline. Thus, our conclusions may not be generalizable to other groups. It is unclear from our data whether NMR lipoprotein profiling would be beneficial in high-risk individuals or in those receiving lipid-lowering therapy, since the study population was comprised of relatively healthy women enrolled in 1992 to 1995. Second, because our study is observational, residual unmeasured confounding may be present. However, data collected on a broad range of established cardiovascular risk factors were available for multivariable adjustment. Third, the use of symptomatic PAD as the primary end point by definition excludes subclinical disease that might otherwise have been detected with the use of ankle-brachial index or abnormal pulse examination; however, we believe that our data are not only mechanistically relevant but also clinically important because claudication and ischemia requiring limb revascularization are the principal clinical manifestations of PAD. Importantly, each case included in this analysis was confirmed through rigorous methods with the use of a validated claudication questionnaire, cardiovascular physician interview, and medical record review. In addition, women enrolled in the WHS are female health professionals and are therefore less likely to encounter barriers to medical care, which may otherwise have led to underdiagnosis. Furthermore, although potential misclassification resulting from atypical or occult disease may have occurred, this, if anything, would have biased our results toward the null by inclusion of potentially misclassified cases in the event-free group. In terms of the traditional lipid measures used in the present study, more refined methods of calculating LDL-C have been developed.[50] However, the performance of the calculated LDL-C variable in this study was likely adequate given that LDL-C was associated with incident CCVD, as expected. Finally, given our relatively small sample size, the study may be underpowered to detect risk associations for some biomarkers, although numerous statistically significant associations were identified.

In summary, our data show that both standard lipid as well as NMR-derived lipoprotein measures indicative of atherogenic dyslipidemia are associated with PAD in women, whereas LDL-C, non–HDL-C, and apolipoprotein B_{100} were not. Measures of total and small LDL-P concentration further identified women at heightened risk of PAD beyond standard lipid measures. Importantly, our data also indicate that this lipoprotein signature may be unique to PAD in comparison to coronary

or cerebrovascular atherosclerosis. Our results require confirmation; the biological construct is not only plausible, but raises the intriguing possibility that therapeutic modulation of these lipoprotein abnormalities may have clinical benefits in patients at risk for PAD for whom few medical treatment options currently exist.

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Disclosures

Dr Ridker is listed as a coinventor on patents held by the Brigham and Women's Hospital that relate to the use of inflammatory biomarkers in cardiovascular disease, which have been licensed to AstraZeneca and Siemens; has received investigator research support from Kowa Research Institute, Novartis, Pfizer, and Astra-Zeneca; has served as a consultant to Jannsen, Novartis, and Sanofi-Regenerson; and serves as Co-Principal Investigator of the PROMINENT trial (NCT03071692). Dr Mora receives research grant support from Atherotech Diagnostics for research outside the current work; served as a consultant to Amgen, Lilly, Pfizer, and Quest Diagnostics; and is coinventor on a patent on the use of nuclear magnetic resonance–measured GlycA for predicting risk of colorectal cancer. Dr Pradhan receives investigator-initiated research support from Kowa Research Institute and serves as Co-Principal Investigator of the PROMINENT trial (NCT03071692). The other authors report no conflicts.

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