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Cholesterol mass efflux capacity and risk of peripheral artery disease: The Multi-Ethnic Study of Atherosclerosis

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Abstract

Background and Aims: We aimed to assess the relationship of HDL (high-density lipoprotein)-mediated cholesterol mass efflux capacity (CMEC) with risk of incident peripheral artery disease (PAD).

Methods: CMEC was measured in 1458 Multi-Ethnic Study of Atherosclerosis participants between 2000 and 2002 as part of a case-control study matched for incident cardiovascular disease and progression of carotid plaque by ultrasound. Incident clinical PAD, adjudicated on the basis of a positive history for the presence of disease-related symptoms or treatment, was ascertained through 2015 in 1419 individuals without clinical PAD at baseline. Subclinical PAD, defined as an ankle-brachial index (ABI) ≤1.0, was assessed among 1255 individuals with a baseline ABI >1.0 and at least one follow-up ABI measurement 3 to 10 years later. Cox proportional hazards and

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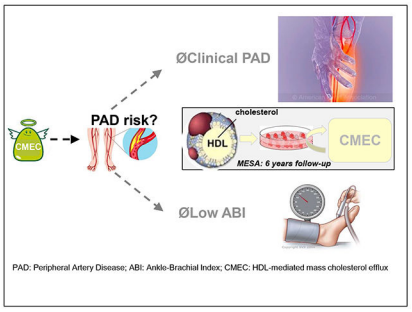
relative risk regression modeling per SD increment of CMEC were used to determine the association of CMEC with clinical and subclinical PAD, respectively.

Results: There were 38 clinical PAD and 213 subclinical PAD events that occurred over a mean follow-up of 6.0 and 6.5 years respectively. After adjustment for age, gender, race, higher CMEC levels were not associated with clinical PAD (hazard ratio 1.25; 95% CI 0.89, 1.75) or subclinical PAD (risk ratio 1.02; 95% CI, 0.94, 1.11).

Conclusions: These findings suggest that HDL-mediated cholesterol efflux is not significantly associated with incident clinical and subclinical PAD.

Graphical Abstract



Introduction

Lower extremity peripheral artery disease (PAD) is a significant public health problem, affecting at least 8.5 million over the age of 40 in the US and 200 million individuals globally.[1, 2] Over 20% of older men and women seen in primary care medical practices have a low ankle–brachial index (ABI) consistent with peripheral arterial disease.[3] PAD is associated with a significant increase in morbidity and mortality.[4]

A low high-density lipoprotein (HDL) cholesterol level has been associated with increased PAD risk.[5, 6] However, a number of RCTs have failed to show an association between pharmacologic improvement of HDL cholesterol levels and a decreased risk of cardiovascular events.[7–9] As a result, the focus has shifted away from actual HDL and HDL cholesterol levels and towards HDL function. The ability to promote reverse cholesterol transport (RCT) from lipid-laden macrophages is a major component of HDL function for atheroprotection.[10] While pharmacologic agents can effectively improve HDL cholesterol levels, they only modestly increase measures of HDL function.[11, 12]

Cholesterol efflux capacity (CEC), which can be measured, is the initial step in RCT and emerged as a biomarker of atherosclerotic cardiovascular disease. While many large cohort studies have found an inverse association between CEC and risk of coronary heart disease, no such relationship has yet been established with non-coronary atherosclerotic disease.[13–16] Additionally, either no relationship or a paradoxical relationship has been reported for CEC with respect to total atherosclerotic burden in the coronary or carotid artery distributions.[14–18] However, we are aware of no studies that have examined the association of CEC with

PAD, defined by the presence of either established clinical disease or a low ABI, an indication of significant subclinical disease.

We determined whether cholesterol mass efflux capacity (CMEC) is associated with the risk of incident PAD in a multi-ethnic cohort. Prior literature has mainly relied upon a radioactive or fluorescent cholesterol tracer to measure efflux of cholesterol from cultured macrophages to HDL. This results in a bidirectional exchange of cholesterol between cells and HDL, and the efflux of labeled cholesterol from cells may be counterbalanced by the uptake of non-labeled cholesterol from HDL. Therefore, quantifying efflux by this method is not an accurate measure of the net movement (efflux minus influx) of cholesterol between cells and HDL. CMEC circumvents this problem by directly measuring the change in cholesterol mass in media[12] and has already been reported to be inversely associated with coronary heart disease in this cohort.[16]

Materials and methods

Cohort

MESA is a National Heart, Lung, and Blood Institute–funded multicenter community-based study. The study recruited 6814 adults aged 45–84 years and free of clinically recognized cardiovascular disease from six field centers to undergo baseline examination between 2000 and 2002.[19] The study participants self-identified with one of four race/ethnic groups: non-Hispanic white (38%), African-American (28%), Hispanic (22%), and Chinese (12%). Follow-up visits 2, 3, 4, and 5 were conducted in 2002–2004, 2004–2005, 2005–2007, and 2010–2012, respectively. Follow-up at 10 years (MESA exam 5) was 76% (n=4655) of those alive. Institutional review boards at each site approved the study, and all participants gave informed consent.

HDL-mediated cholesterol mass efflux measurement

CMEC was determined from stored samples obtained at exam 1 in a nested case-control study. MESA participants were matched for incident CVD over a mean of 10.2 years of follow-up (n=465 cases and 465 age- and sex-matched controls) and progression of carotid plaque by ultrasound (n=407 cases and 407 age- and sex-matched controls). Carotid plaque score (range 0-12) was defined as the number of carotid plaques in the internal, bifurcation, and common segments of both carotid arteries. Progression was defined as any increase in the carotid plaque score from Exam 1 to Exam 5. Controls for incident CVD were required to have at least as much event-free follow-up time as the incident CVD cases. For carotid plaque progression, controls had to have been measured at exam 5 and found to have no progression in carotid plaque. Age matching used 5-year intervals.

Plasma HDL preparation——Blood samples were collected from all subjects after 12-hour fasting. ApoB-containing particles was precipitated from plasma by adding 100μl of plasma to 40μl of 20% polyethyleneglycol (PEG, Sigma P-2139 in 200mM glycine, pH10) solution. This mixture was incubated at room temperature for 15 min. After this incubation, the solution was centrifuged at 4,000rpm for 20 min. The supernatant, containing HDL

fractions, was removed and used for experiments as previously described.[20] Independently, HDL-2 fraction was isolated from plasma by ultracentrifugation as previously described.[21]

Human THP-1 macrophages——Cholesterol efflux measurements were performed at Columbia University in a completely blinded fashion and data transmitted to the University of Washington for unblinding. THP-1 monocytes were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) at 37°C in 5% CO2. Cells were treated with 100nM PMA (Phorbol myristate acetate) for 24 h to facilitate differentiation into macrophages. Then, adherent macrophages were incubated with 50μg/mL acetyl-LDL and 3μM LXR agonist (TO901317) for 24 hours before cholesterol efflux studies.

***Isotopic cholesterol efflux* assay——**Bone-marrow-derived macrophages were cultured for 24h in 10% FBS in DMEM containing 50μg/mL acetyl-LDL and 2μCi/mL of [3H]-cholesterol. Cholesterol efflux was performed for 3h in 0.2% BSA DMEM containing different concentrations or volumes of HDL as acceptors. The cholesterol efflux was expressed as the percentage of the radioactivity in cells plus medium.

Cholesterol mass analysis——CMEC was analyzed in DMEM containing 0.2% BSA in the presence of polyethylene glycol-HDL matched by volume (ratio 7:1). After 6 hours incubation with HDL, the mass of total cholesterol was determined from the collected media by colorimetric assay. The HDL mediated cholesterol efflux was calculated by subtraction of cholesterol mass of the medium cultured with or without cells. This allows the determination of the net cholesterol efflux driven by HDL particles reflecting the ability of HDL to remove cellular cholesterol.[12] The assay was run in triplicate; the intra-assay coefficient of variation was 4.6%.

Western blot analysis——Aliquots of 20μg of HDL-2 were boiled at 95°C for 10 minutes in SDS buffer (6.25.10-3 M Tris-Hcl pH6.8, 2%SDS, 5% 2-mercaptoethanol, 10% sucrose and 0.002% Coomassie blue). Then, HDL proteins were resolved by 10% SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. Primary antibodies for LCAT (400-107A2), PAFAH (160603), ApoE (ab1906) or ApoA-I (ab17278) were purchased from Novus Biologicals (Littleton, CO), Cayman Chemical (Ann Arbor, MI) and Abcam (Santa Cruz, CA). Anti-mouse and rabbit secondary antibodies were obtained from GE Healthcare. Specific protein signals were revealed using the ECL detection system (Amersham Biosciences).

Peripheral artery disease – Clinical PAD and low ABI

During follow-up, clinical PAD was identified by self-report of a hospitalized PAD diagnosis by the participant at (1) MESA clinic visits, (2) follow-up phone call, or (3) participant notification. A PAD diagnosis was also found during review of medical records for other events. Follow-up for this analysis extended through 2015. Two physician members of the MESA mortality and morbidity review committee independently classified events. The full committee made final classifications if there were disagreements. “Definite” PAD required more than a physician diagnosis as follows.

PAD was defined as symptomatic disease including intermittent claudication, ischemic ulcers, or gangrene. The disease had to be symptomatic and have a diagnostic procedure or require therapeutic intervention. Physician adjudicators recorded the diagnosis one or more of (1) lower extremity claudication, (2) atherosclerosis of arteries of the lower extremities, (3) arterial embolism and/or thrombosis of the lower extremities, (4) abdominal aortic aneurysm. In addition to symptoms, participants had to have one or more of the following: a) Ultrasonographically- or angiographically-demonstrated obstruction or ulcerated plaque (≥50% of the diameter or ≥75 of the cross-sectional area) demonstrated on ultrasound or angiogram of the iliac arteries or below, b) Absence of pulse by Doppler in any major vessel of the lower extremities, c) Exercise test positive for lower extremity claudication, d) Surgery, angioplasty, or thrombolysis for peripheral vascular disease, e) Amputation of one or more toes or part of the lower extremity because of ischemia or gangrene, f) Exertional leg pain relieved by rest in combination with either physician-diagnosed claudication diagnosed or an ankle-arm blood pressure ratio ≤0.8.

As for subclinical PAD, the ABI was performed at the baseline examination, as well as clinic exam 3 and clinic exam 5. To obtain the measurements used to calculate the ABI, participants rested supine for 5 minutes, and then systolic blood pressures were measured in both arms and legs with the appropriate-sized cuffs. For each leg, the systolic blood pressure in each posterior tibial and dorsalis pedis artery was measured using a continuous-wave Doppler ultrasound 5-mHz probe. The leg-specific ABI was calculated as the higher systolic blood pressure in the posterior tibial or dorsalis pedis divided by the average of the left and right brachial pressures. In the event that left and right brachial pressures differed by 10 mmHg or more, the higher of the brachial pressures was chosen, since subclavian stenosis could be present. The lower of the two leg-specific ABIs was considered the index ABI and used for analysis.

Subclinical PAD was defined as an ABI ≤1.0. Although this is traditionally defined by an ABI of less than 0.90, a large meta-analysis indicated that ABI values between 0.91 and 1.0 were associated with increased cardiovascular disease (CVD) mortality compared to ABI >1.0.[22] Within the Multi-Ethnic Study of Atherosclerosis (MESA), an ABI ≤1.0 was significantly associated with incident CVD and with prevalence of subclinical atherosclerosis.[23, 24]

Individuals with an ABI ≤1.0 at the baseline visit were excluded from the incident clinical PAD and low ABI analyses respectively. Participants with evidence of non-compressible vessels (ABI>1.4) at baseline were also excluded from all ABI analyses (n=7). Incident clinical PAD required a physician-adjudicated diagnosis of “definite” PAD as defined above. Incident low ABI was defined as a decline in ABI to ≤1.0 in either leg. If only one follow-up ABI was available, then that was used for the analysis. If both follow-up ABIs were available, then exam 5 was used unless the participant already met criteria for ABI decline at Exam 3.

Measurement of covariates

Standardized questionnaires were used at baseline to obtain age, sex, race/ethnicity, physical activity, alcohol consumption, smoking history, and medication usage, including

antihypertensive, lipid-lowering, and antidiabetic drug use. Body mass index (BMI) was calculated as body mass (kilograms) divided by the square of the body height (meters). Three separate systolic and diastolic resting blood pressure measurements were taken in seated participants, with the last two measurements being averaged for analysis. Physical activity was recorded as participant-reported number of intentional exercise metabolic equivalent (MET)-minutes per week. Cigarette smoking was calculated in pack-years and also defined as current, former, or never. Hypertension was defined as a self-report of physician diagnosis and use of an anti-hypertensive medication, or systolic blood pressure ≥140, or diastolic blood pressure ≥90 mmHg. Glucose and total cholesterol were measured from fasting blood samples. Diabetes was defined as a fasting glucose >125 mg/dl or use of anti-diabetic medications. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equations.[25]

Statistical methods

Descriptive characteristics were provided according to presence or absence of incident subclinical PAD. Comparisons were performed using the $\chi 2$ test for categorical variables and two sample t-tests for continuous variables. We used multivariable regression splines to explore the linearity assumption for CEC. We found no evidence of non-linearity and variables were used continuously to maximize power and information. For all analyses, we accounted for the sampling scheme used to identify the nested case-control participants by reweighting participants according to the inverse of the estimated probability that they were included in the sample.

Generalized linear models with log link and Gaussian error structure were used to calculate adjusted prevalence ratios (PRs) and 95% confidence intervals (CIs) (using robust standard errors) for the association of baseline CEC with prevalent subclinical PAD. Models were initially adjusted for age, sex, and race/ethnicity (Model 1). Additional adjustments included BMI, cigarette smoking, diabetes, eGFR, HDL-cholesterol and total cholesterol, hypertension, physical activity, and lipid-lowering therapy (Model 2).

For the analysis of incident subclinical PAD, we again used generalized linear models with log link, but with offset to accommodate differential time to exposure. Estimates of the rate ratios (RR) of incident low ABI were obtained with 95% confidence intervals based on robust standard errors. Models were similar as above except for the additional adjustment of baseline ABI in model 2. We repeated the incident subclinical PAD analysis using a traditional ABI cut-off of ≤0.9. Cox hazard models were used to estimate the hazard ratio (HR) of incident clinical PAD associated with CMEC. Due to the low number of incident clinical PAD events, only model 1 variables were included for this analysis.

Finally, we performed a sensitivity analysis defining incident low ABI as a decline in ABI of at least 0.15 and to 1.0 or less in either leg. This approach for ABI decline has been advocated to limit the impact of regression to the mean and measurement error and avoid small clinically insignificant changes being included in the incident low ABI definition.[26]

Results

Of the 1458 participants included for this study analysis, 203 had subclinical PAD at baseline. Adjusting for age, sex, race, BMI, cigarette smoking, diabetes, eGFR, HDL-cholesterol, total cholesterol, hypertension, physical activity, and lipid-lowering therapy, CMEC was not associated with a low baseline ABI at baseline (prevalence ratio (PR) per SD increment: 1.01; 95% confidence interval [CI]: 0.98, 1.04).

After excluding individuals with a baseline ABI ≤1.0, with evidence of non-compressible vessels, or without a follow-up ABI, 1255 participants were included in the prospective incident subclinical PAD analysis (mean age=64.1 years; 44.4% female; 38.5% white, 24.5% African-American, 24.2% Hispanic, and 12.7% Chinese) and 213 developed a low ABI (mean follow-up=6.5 years). Table 1 shows that participants who developed a low ABI were older, less likely to be male, and more likely to be African-American, have a higher BMI, have a higher SBP, be a current smoker, have diabetes, and report aspirin and anti-hypertensive medication use. Mean CMEC levels did not differ between the two groups.

In fully adjusted analyses, higher CMEC was not associated with a lower risk of developing a low ABI (relative risk (RR) 1.03; 95% CI 0.97, 1.10) (Table 2). Results were similar when additionally requiring an ABI decline of at least 0.15 (RR 1.13; 95% CI 0.77, 1.66 (104 events)). When using a traditional ABI cut-off of ≤0.9 instead, higher CMEC was also not associated with a lower risk of an incident low ABI (RR 1.05; 95% CI 0.90, 1.23) amongst 1301 eligible participants (70 events).

There were 38 incident clinical PAD events amongst 1419 participants followed for this outcome (mean follow-up=6.0 years). Higher CMEC was not associated with developing clinical PAD (hazard ratio 1.25; 95% CI 0.89, 1.75) (Table 2).

Discussion

In this multi-ethnic cohort we found no association between higher CMEC and risk of developing either a low ABI or clinical PAD. Prospective relationships of CMEC with lower extremity PAD have not been previously investigated and our findings are the first to examine the relationship between HDL CMEC and risk of PAD.

Although our findings contrast with published literature demonstrating an association between higher CMEC and a reduced risk of coronary heart disease, they are consistent with studies reporting no associations of CMEC with atherosclerotic burden and risk of cerebrovascular disease.[16–18] Similarly, in a recent MESA analysis, no relationship was observed between CMEC and ultrasound-measured carotid plaque progression.[16] This study also found an inverse (protective) relationship between CMEC and CHD but no relationship between CMEC and non-hemorrhagic stroke.[16] Of note, mean CMEC levels were significantly lower in individuals with CVD compared to those without (2.9 mg/dL vs. 3.1 mg/dL) whereas those with carotid plaque progression had higher mean CMEC levels verse those without (3.1 mg/dL vs. 2.8 mg/dL). While CMEC was inversely associated with stroke in the Dallas Heart Study in a secondary analysis, there were only 30 stroke events and stroke subtype was not distinguished.[15]

HDL confers atheroprotective benefit through its ability to act as both the acceptor of cholesterol from cells and as a cholesterol carrier in the subsequent RCT pathway, defined as the process by which cholesterol moves out of arterial foam cells in peripheral tissues, enters the circulation, and is eventually excreted in the feces.[27] Atherosclerosis development is, however, not uniform throughout the vascular tree and PAD is a distinct form of atherosclerosis. In the Reduction of Atherothrombosis for Continued Health registry, 40% of people with PAD had no concomitant coronary or cerebrovascular disease.[28] Although traditional cardiovascular risk factors are common to all types of atherosclerotic disease, significant differences in the strength of these associations have been demonstrated depending on the form of disease. Cigarette smoking and diabetes mellitus have particularly strong associations with the development of PAD, carrying an over 3-fold increased risk each, compared with hypertension and dyslipidemia, which have more modest effects.[29] Cigarette smoking is 2 to 3 times more likely to cause PAD compared with coronary artery disease.[30]

Numerous other factors have also been suggested to influence regionally distinct atherosclerotic lesion development including differences in hemodynamics and the underlying wall structure. Varied hemodynamics across arterial beds produce differences in shear stress patterns and in the relative residence times of the lipoproteins, blood borne molecules and inflammatory cells that come in contact with the endothelial cells.[31, 32] Hemodynamic patterns may also alter the gene expression profile of endothelial cells in subtle different ways so that these cells respond differently to cardiovascular risk factors.[33] Finally, site-specific arterial wall structure can also differentially impact lesion development based on its degree of elastic versus muscular composition.

Important differences found in peripheral arterial plaque composition compared to other vascular beds, in particular, may help to explain the lack of an inverse association between CMEC and incident PAD. In a post-mortem study analyzing the coronary, carotid, and superficial femoral arteries from 100 individuals, distinct artery-dependent patterns of atherosclerosis were found.[34] The femoral arteries were least affected by atherosclerosis—foam cells plaques were least commonly found in this artery and foam cell lesions were rare.[34] The development of advanced atherosclerosis in the femoral arteries was strongly age-dependent and dominated by fibrous plaques.[34] Similar findings were seen *in vivo* based on an intravascular ultrasound imaging of coronary and peripheral artery lesions.[35] Compared with coronary arteries, fibroatheromatous plaque, including both thin cap and thick cap subtypes, were relatively infrequently observed in renal and iliac arteries whereas the more stable plaque phenotypes characterized by fibrocalcification and pathological intimal thickening were more often observed.[35]

These differences are important in gauging the potential impact of RCT. Impaired cholesterol efflux is known to be associated with the prevalence of both thin-cap fibroatheroma and noncalcified plaques, phenotypes that most reliably predict risk of plaque rupture and acute coronary syndrome, based on studies in individuals with increased cardiovascular risk or undergoing clinically indicated coronary angiography.[36–38] In a study of 100 patients with psoriasis, CEC inversely correlated with non-calcified atherosclerotic burden as assessed by coronary computed angiography.[37] Similarly, amongst 85 patients

with stable coronary artery disease and treated with intensive statin therapy, improvements in CEC were associated with an increase in fibrous cap thickness as seen on optical coherence tomography.[36] Development of a low ABI or symptomatic PAD may be more reflective of atherosclerosis progression rather than unstable plaque rupture, which is more commonly seen in CHD. Therefore, the impact of RCT may be less important in the setting of PAD.

Our study has limitations. Although the diagnosis of clinical PAD involved a comprehensive adjudication process, the number of overall events was low (<2%) and this may have affected the power for this analysis. CMEC was measured in a select group of MESA participants and in a case-control fashion for the development of CVD and carotid plaque but not PAD. Low HDL cholesterol is an established risk factor for PAD and HDL function has been previously shown to correlate with HDL cholesterol levels.[15] We cannot exclude the possibility that findings may have been different in a more representative cohort where baseline HDL cholesterol levels were significantly lower in those who developed PAD. Although our results did adjust for lipid lowering therapy and anti-hypertensive use, the effects of medications including statins and angiotensin converting enzyme inhibitors on CMEC are unknown. ABI measurements did not include a post-exercise value and, therefore, may not have detected PAD in some individuals.

In conclusion, we found no significant association between CMEC and risk of either clinical or incident subclinical PAD. Our findings suggest that CMEC and potentially RCT may differentially impact atherosclerotic development across various vascular beds that are likely due to plaque-related differences. Further study, however, is needed in larger cohorts to confirm our findings and better elucidate underlying mechanisms.

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Highlights

- No studies that have examined the association of cholesterol-efflux capacity (CEC) with PAD
- We determined whether cholesterol mass efflux capacity (CMEC), a measure that quantifies the net movement of cholesterol between cells and HDL, is associated with the risk of incident PAD in a multi-ethnic cohort.
- After adjustment for age, gender, race, higher CMEC levels were not associated with development of clinical PAD or a low ABI
- Our findings suggest that CMEC may differentially impact atherosclerotic development across various vascular beds

Table 1.

Baseline characteristics of MESA participants according to the presence or absence of incident subclinical PAD[a]

Characteristic	No PAD (n=1042)	PAD (n=213)	*p*-value[b]
Age	63.7 ± 9.7	66.9 ± 9.2	<0.001
Male, %	616 (59%)	83 (39%)	<0.001
Race, %			
White	413 (40%)	72 (34%)	ref
Chinese	141 (13%)	19 (9%)	0.190
African-American	220 (21%)	87 (41%)	<0.001
Hispanic	268 (26%)	35 (16%)	0.223
Body mass index, kg/m^2	28.0 ± 4.9	28.9 ± 6.0	0.041
Systolic blood pressure, mmHg	126.3 ± 20.4	134.1 ± 20.9	<0.001
Diastolic blood pressure, mmHg	72.7 ± 10.3	72.0 ± 9.9	0.605
Total cholesterol, mg/dL	193.5 ± 33.4	191.9 ± 32.9	0.458
LDL cholesterol, mg/dL	117.8 ± 29.5	115.6 ± 29.9	0.391
HDL cholesterol, mg/dL	49.9 ± 14.8	50.5 ± 14.5	0.842
Diabetes, %	118 (11%)	43 (20%)	<0.001
Smoking status, %			
Never	545 (52%)	99 (46%)	Ref
Former	391 (37%)	82 (38%)	0.192
Current	106 (10%)	32 (15%)	0.013
eGFR, ml/min/1.73 m^2	73.4 ± 14.0	71.4 ± 17.0	0.078
Anti-hypertensive use, %	397 (38%)	112 (53%)	<0.001
Intentional exercise, MET-min/week	1510 ±2114	1482 ±1698	0.732
Lipid-lowering therapy, %	161 (15.5%)	37 (17.5%)	0.234
Efflux mass, mg cholesterol/dL serum	3.0 ± 1.9	3.1 ± 1.9	0.412

HDL=high-density lipoprotein; eGFR=estimated glomerular filtration rate

[a]Continuous variables expressed as mean (SD). Categorical variables are N (percent).

[b]Comparisons made between no PAD and PAD. Fisher's exact used to make statistical comparison.

Table 2.

Associations of efflux mass with incident PAD[a]

	Low ABI					Clinical PAD		
	Events/# at risk	**Model 1[b] RR (95% CI)**	***p*-value**	**Model 2[c] RR (95% CI)**	***p*-value**	**Events/# at risk**	**Model 1 HR (95% CI)**	***p*-value**
Continuous								
Per SD unit	213/1255	1.02 (0.94, 1.11)	0.623	1.03 (0.97, 1.10)	0.314	38/ 1419	1.25 (0.89, 1.75)	0.203

PAD=peripheral arterial disease, ABI=ankle-brachial index

[a]Results of multivariable Cox Proportional Hazards Models (clinical PAD) and relative risk regression models (low ABI)

[b]Model 1 adjusted for age, sex, and race/ethnicity

[c]Model 2 adjusted for Model 1 + BMI, cigarette smoking, diabetes, eGFR, HDL-cholesterol and total cholesterol, hypertension, physical activity, lipid-lowering therapy, and baseline ABI