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# Soluble P-selectin predicts lower extremity peripheral artery disease incidence and change in the ankle brachial index: The Multi-Ethnic Study of Atherosclerosis (MESA)

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ABSTRACT

*Objective:* To determine the association of circulating P-selectin with prevalent and incident peripheral artery disease (PAD), the ankle brachial index (ABI), and change in the ABI.
*Methods:* The Multi-Ethnic Study of Atherosclerosis (MESA) is a prospective population-based cohort study including 6814 European descent, African American, Hispanic and Chinese men and women aged 45–84 at baseline. Four clinical exams took place after the baseline exam. After excluding those with ABI>1.4, prevalent and incident PAD were defined as an ABI≤0.90. ABI progression was defined as progression from a normal ABI (0.91–1.4) to abnormal (≤0.90 or >1.4) at a later exam.
*Results:* In adjusted models, each SD (13 ng/mL) higher P-selectin was significantly associated with 0.007 lower ABI (95% CI ((−0.011, −0.004)), $p < 0.001$), and an average change in the ABI of −0.006 ((−0.010, −0.003, $p < 0.001$). P-selectin was significantly associated with a 1.17-fold greater odds of prevalent PAD ((1.02, 1.33), $p = 0.03$), and a 30% greater risk of incident PAD ((1.11, 1.53), $p = 0.001$), as well as progression from a normal ABI to an ABI≤ 0.90 ($p = 0.003$), but not to an ABI>1.4 ($p = 0.96$). Addition of P-selectin to models containing traditional PAD risk factors and markers of inflammation/coagulation significantly improved the net reclassification for ABI progression ($p = 0.03$), but was only marginally significant for incident PAD ($p = 0.06$).
*Conclusions:* P-selectin is significantly associated with the development of PAD. However, further research is needed in population-based studies to confirm prospective associations of P-selectin with incident PAD and change in the ABI, as well as its potential predictive ability.


## 1. Introduction

Between 2000 and 2010, the global burden of peripheral artery disease (PAD) increased by almost 29% in low and middle income countries and 13% in high income countries [1]. PAD is associated with increased morbidity and mortality [2–5], as well as decreased functional status and quality of life [6–9]. Given the burden and comorbid conditions associated with PAD, there is a continuing need for a thorough study of biomarkers related to obstructive lower extremity atherosclerosis that could possibly lead to therapeutic targets to prevent or treat PAD.

The role of P-selectin in the atherosclerotic process involves the activation, rolling and attachment of leukocytes, as well as bonding of endothelial cells via ligand interaction [10–12]. P-selectin levels correlate with the severity of PAD [13], and there is some evidence for the specificity of P-selectin for PAD [14–16]. For example,

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among those with PAD, treatment with anti-platelet agents such as clopidogrel, aspirin, and cilostazol [17], as well as atorvastatin [18], appears to reduce levels of P-selectin effectively.

Only two population-based cohorts have examined the association of P-selectin with lower extremity PAD [19,20]. In these studies, P-selectin was not significantly associated with prevalent PAD, intermittent claudication, or ABI categories (<0.9, 0.9–1.0, >1.0–1.4) [19,20]. However, associations of soluble P-selectin with the ABI and PAD, especially in a larger multi-ethnic cohort, are not well characterized. Furthermore, to our knowledge no diverse population-based cohort has examined the prospective association of P-selectin with incident PAD or change in the ABI. Thus, using data from the Multi-Ethnic Study of Atherosclerosis (MESA), we examined the association of P-selectin with prevalent and incident PAD, levels of and change in the ABI, as well as progression from a normal to an abnormal ABI. We examined the interactions of race/ethnicity, sex, and diabetes with P-selectin for each of these outcomes. Additionally, we sought to determine whether P-selectin contributed to the prediction of PAD above and beyond traditional risk factors, as well as beyond additional markers of inflammation and coagulation.

## 2. Methods

### 2.1. Study participants

MESA participants were recruited from six field sites in the United States—Forsyth County, NC (Wake Forest), Northern Manhattan/Bronx, NY (Columbia), Baltimore/Baltimore County, MD (Johns Hopkins), St. Paul, MN (University of Minnesota), Chicago, IL (Northwestern), and Los Angeles County, CA (UCLA). Details of recruitment have been previously published [21]. MESA complies with the Declaration of Helsinki, and the Institutional Review Boards at each field site, as well as the Coordinating Center (University of Washington, Seattle), approved the study. Briefly, MESA recruited 6814 men and women ages 45–84 years free of cardiovascular disease, and the cohort is 53% women with a racial/ethnic composition of approximately 38% non-Hispanic white, 28% African American, 23% Hispanic and 11% Asian, primarily of Chinese descent. The baseline exam (Exam 1) occurred from 2000 to 2002, with Exam 2 from 2002 to 2004, Exam 3 from 2004 to 2005, Exam 4 from 2005 to 2007, and Exam 5 from 2010 to 2012. The current study includes MESA participants with both P-selectin and ABI measurements, and includes data from Exams 2, 3, and 5.

### 2.2. P-selectin measurement

Soluble P-selectin was measured at Exam 2 in plasma with the human soluble P-selectin/CD62P Immunoassay kit (R&D Systems, Minneapolis, MN). The minimum detection limit was 0.5 ng/mL, and the inter-assay coefficient of variation was 6.7% at a mean concentration of 182 ng/mL.

### 2.3. Ankle brachial index and peripheral artery disease

For the ABI, which includes data from Exams 3 and 5 for the current study, systolic blood pressure was measured in both the left and right brachial, dorsalis pedis, and posterior tibial arteries using a hand-held Doppler instrument with a 5-mHz probe. The ABI was calculated for both the left and right sides as maximum systolic blood pressure in the posterior tibial artery and dorsalis pedis, divided by the average of the left and right brachial pressures. As previous studies have shown a strong association between PAD and subclavian stenosis [22], in the event that left and right brachial pressures differed by 10 mmHg or more, the higher of the brachial pressures was used. If a pulse was detected when the cuff was inflated to 300 mmHg, the ABI was classified as “incompressible”. For these analyses, minimum of the left and right leg ABI was used, and then those with ABI>1.4 were excluded. However, we also examined associations with the ABI defined by first excluding those with ABI>1.4 on either side, then using the minimum of the left and right side ABI.

Prevalent PAD at Exam 3 was defined as ABI≤0.90, excluding those with ABI>1.4. Seven participants had a lower extremity revascularization or angioplasty prior to Exam 3 and also attended Exam 3; these participants were considered to have prevalent PAD regardless of ABI values at Exam 3 and were excluded from ABI analysis. Incident PAD was defined as ABI≤0.90 at Exam 5, also excluding those with ABI>1.4, as well as prevalent PAD. Participants with no evidence of PAD at Exam 3 were eligible for examination of PAD incidence at Exam 5; ABI≤0.90 at Exam 5 defined incident PAD. ABI progression was defined only for participants with a normal ABI value at Exam 3 (0.91–1.4) as progression to significant lower extremity disease, i.e. an ABI value of ≤0.90 indicating PAD or >1.4 indicating arterial stiffening, at Exam 5.

### 2.4. Covariate measurement

Information on age, sex, race/ethnicity, and smoking was obtained via interview and questionnaires. Height was measured by a stadiometer to the nearest 0.1 of a centimeter. Weight was measured to the nearest pound using a platform balance scale. Body mass index (BMI) was calculated as weight in kilograms per height in meters squared. Medication use was obtained via medication inventory. Systolic and diastolic blood pressures were determined by averaging the last two of three measurements taken with the Dinamap automated blood pressure device (GE Healthcare).

Plasma HDL-cholesterol, fasting triglycerides, fasting plasma glucose, C-reactive protein, IL-6, D-dimer and fibrinogen were measured at a central laboratory after a requested 12 h fast. LDL-cholesterol was calculated using the Friedewald formula in those with triglycerides<400 mg/dL. HDL-cholesterol was measured using the cholesterol oxidase cholesterol method (Roche Diagnostics) after precipitation of non-HDL-cholesterol with magnesium/dextran, and has a laboratory CV of 2.9%. Triglycerides were measured using Triglyceride GB reagent (Roche Diagnostics, Indianapolis, IN 46250) on the Roche COBAS FARA centrifugal analyzer, and have a laboratory CV of 4.0%. Serum fasting glucose was measured by rate reflectance spectrophotometry using thin film adaptation of the glucose oxidase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY 14650) with a laboratory CV of 1.1%. Hemoglobin A1c (HbA1c) was analyzed on the Tosoh $A_1c$ 2.2 Plus Glycohemoglobin Analyzer (Tosoh Medics, Inc., San Francisco, CA 94080) using an automated high performance liquid chromatography method, with a laboratory CV range of 1.4–1.9%. Serum creatinine was measured by rate reflectance spectrophotometry using thin film adaptation of the creatine amidinohydrolase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY), with a laboratory CV of 2.2%. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equations [23]. CRP was measured using the BNII nephelometer (N High Sensitivity CRP; Dade Behring Inc., Deerfield, IL) with an intra-assay CVs range of 2.3–4.4% and inter-assay CVs range of 2.1–5.7%. IL-6 was measured by ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN) with a laboratory CV of 6.3%. Fibrin fragment D-dimer was measured using an immuno-turbidimetric assay (Liatest D-DI; Diagnostica Stago, Parsippany, NJ) on the Sta-R analyzer