Sex Differences in Circulating Soluble Urokinase-Type Plasminogen Activator Receptor (suPAR) Levels and Adverse Outcomes in Coronary Artery Disease

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Background—Women have higher circulating levels of soluble urokinase-type plasminogen activator receptor (suPAR), and elevated suPAR is associated with cardiovascular risk. The independent association of sex with suPAR and the impact of sex on its association with cardiovascular risk are unknown.

Methods and Results—Plasma suPAR was measured using ELISA in 2 cohorts of 666 asymptomatic individuals (49 years, 65% women) and 4184 patients with coronary artery disease (63 years, 37% women). Independent association of sex with suPAR was studied using linear regression models adjusted for demographics, risk factors, and visceral adiposity in asymptomatic participants. Impact of sex on association of suPAR with all-cause mortality was studied in patients with coronary artery disease using multivariable-adjusted Cox models. Sex-specific suPAR cutoffs for predicting all-cause mortality were calculated. Asymptomatic women had 10% higher suPAR compared with men after adjusting for confounders, and visceral adiposity partly accounted for this association. Over a median follow-up of 5.2 years, 795 deaths were recorded in patients with coronary artery disease. Log$_2$-transformed suPAR was independently associated with mortality (hazard ratio per 1-SD 1.72, 95% CI 1.60–1.85) and an interaction with sex was noted (P=0.005). Association of suPAR with mortality was slightly weaker in women (hazard ratio 1.61, 95% CI 1.41–1.83) compared with men (hazard ratio 1.83, 95% CI 1.67–2.00). However, using sex-specific suPAR cut-offs (4392 pg/mL for women and 3187 pg/mL for men), a similar mortality incidence was observed for both sexes (38.5% and 35.5%, respectively, P=0.3).

Conclusions—Women have 10% higher plasma suPAR levels compared with men. Elevated sex-specific plasma suPAR levels are equally predictive of risk of adverse events in both sexes. (*J Am Heart Assoc*. 2020;9:e015457. DOI: 10.1161/JAHA.119. 015457.)

Key Words: biomarkers • coronary artery disease • outcomes • sex differences • SuPAR

C oronary artery disease (CAD) is the leading cause of mortality worldwide.[1] The existing cardiovascular risk assessment paradigms in the general population and among those with CAD involve ascertainment of high-risk clinical characteristics that are associated with adverse outcomes.[2–4]

These approaches are imperfect and do not capture the effect of subclinical inflammation and immune activation that are integral to the pathobiology of atherosclerosis.[5]

In this context, novel circulating protein-based, inflammatory biomarkers hold a promising role for stratifying

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Clinical Perspective

What Is New?

- Asymptomatic women and those with coronary artery disease have higher plasma soluble urokinase-type plasminogen activator receptor, a marker of systemic immune activation, as compared with men.
- However, the association of elevated plasma soluble urokinase-type plasminogen activator receptor level with adverse cardiovascular outcomes is similar for women and men with coronary artery disease when sex-specific cut-offs are used.

What Are the Clinical Implications?

- Cardiovascular risk assessment tools incorporating suPAR as a risk predictor will likely require sex-specific algorithms for creating prediction models.

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cardiovascular risk.[6] Soluble urokinase-type plasminogen activator receptor (suPAR), a marker of systemic immune activation, inflammation, and thrombogenesis, is one such promising biomarker.[7] SuPAR is typically cleaved off the plasma membrane by the enzymatic processing of the glycosyl-phosphatidylinositol–anchor in podocytes, immature myeloid cells, vascular endothelial cells, and activated T-lymphocytes.[8,9] Both membrane-bound and soluble forms regulate cell adhesion and migration by interacting directly with integrins, and the soluble form's chemotactic properties play a role in recruiting granulocytes, mobilizing hematopoietic stem cells, and in podocyte detachment.[10–14] Elevated circulating suPAR levels are associated with several measures of CAD; it is inversely correlated with coronary flow reserve, is associated with presence of coronary calcium, with CAD severity, and with increased risk of future cardiovascular events and mortality.[15–19] Moreover, unlike other biomarkers, circulating suPAR levels remain stable during acute coronary syndromes and after surgery, making it a possibly more reliable biomarker in these populations.[18,20]

Similar to hsCRP (high-sensitivity C-reactive protein),[21–23] circulating suPAR levels are known to be higher among women compared with men,[24] but the independent association of sex with suPAR is unclear and so are the reasons for these observed sex-based differences. Furthermore, it is unknown whether the association of suPAR with adverse outcomes is influenced by sex. Therefore in this report we have (1) investigated the relationship between suPAR and sex in a cohort of individuals with and without CAD; (2) explored whether the differences in suPAR levels are secondary to sex-based differences in fat mass, fat distribution, or sex hormones; and (3) evaluated the impact of sex on the association of suPAR with adverse outcomes in a cohort of patients with established CAD.

Methods

Study Population

The subjects analyzed in this study were participants of the Emory Center for Health Discovery and Wellbeing (CHDWB) cohort and the Emory Cardiovascular Biobank (EmCAB) cohort. The study designs for CHDWB and EmCAB cohorts have been previously published,[25–27] and the study population is described in Data S1. Our analysis includes 666 participants of CHDWB cohort and 4184 participants of the EmCAB cohort. Both studies were approved by the institutional review board at Emory University and study protocols comply with the Declaration of Helsinki. All participants provided written informed consent at the time of enrollment. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Plasma suPAR Measurement

Plasma levels of suPAR were measured with the suPARnostic ELISA assay (ViroGates, Copenhagen, Denmark), which has a lower limit of detection of 100 pg per milliliter and intra- and interassay variations of 2.75% and 9.17%, respectively.[28]

Adverse Outcomes

Participants of the EmCAB cohort were prospectively followed for the primary outcome of all-cause mortality and the secondary outcome of a composite of cardiovascular death/ nonfatal myocardial infarction (MI). Follow-up data were obtained by annual phone contact, electronic medical record review, and data from the Social Security death index and state records.[27] The cause of death was determined from medical record review or by direct contact with the participants' family member(s). Cardiovascular death and nonfatal MI events were adjudicated by 2 cardiologists blinded to study data. Cardiovascular death was defined as death attributable to an ischemic cardiovascular cause such as fatal MI, stroke, or sudden death secondary to a presumed cardiovascular cause in this high-risk population.[29] Nonfatal MI events were adjudicated using the third universal definition of MI.[30]

Statistical Analysis

Baseline characteristics of participants were stratified by sex in both cohorts and are reported as number (proportion) for categorical variables and means (standard deviation) or

medians [25th percentile–75th percentile] for continuous variables, depending on distribution. Differences between women and men were assessed using χ^2 test for categorical variables and the unpaired t test or Mann–Whitney U test for continuous variables.

Plasma suPAR levels in both cohorts were highly right-skewed and were $\log_2$-transformed to achieve normality. The independent association of sex with suPAR levels among asymptomatic participants of the CHDWB cohort was determined using linear regression models that were sequentially adjusted for cardiovascular risk factors (Model 1—age, race, diabetes mellitus, current smoking, antihypertensive medication use, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, statin use, estimated glomerular filtration rate [eGFR], and body mass index), hsCRP (Model 2), and visceral fat measures of total body fat and android–gynoid fat ratio (Model 3). A similar analysis was conducted in the EmCAB cohort, following which the independent predictors of suPAR levels were determined separately in women and men of both cohorts using linear regression models.

Cox proportional hazards regression models were used to investigate the relationship of plasma suPAR levels with all-cause mortality and cardiovascular death/MI among participants of the EmCAB cohort. Plasma suPAR level was the independent variable and was analyzed as continuous ($\log_2$-transformed) and categorical (median and quartile level in entire cohort) to study the association with outcomes. Cox models were adjusted for sex, age, race, diabetes mellitus, current smoking, hypertension, body mass index, eGFR, history of CABG, heart failure, peripheral artery disease, acute MI at enrollment, revascularization at enrollment, and cardiovascular medication use (angiotensin-converting enzyme inhibitor or angiotensin-II receptor blocker, aspirin, β-blocker, clopidogrel, and statin). The multiplicative interaction between suPAR levels and sex was examined to test whether the association of suPAR with outcomes depended on sex, and subsequent Cox models were stratified by sex.

The cumulative incidence of all-cause mortality and cardiovascular death/nonfatal MI was plotted across sex-specific deciles of plasma suPAR levels. Lastly, a sex-specific suPAR cutoff for all-cause mortality and cardiovascular death/nonfatal MI was identified based on the maximum likelihood for predicting the respective outcome. To calculate the sex-specific cutoffs, a univariate Cox model was used to calculate the partial likelihood of mortality among women and men. A cutoff that gave the maximum likelihood among all possible cutoffs was then considered to be the optimal sex-specific cut point. Subsequently, suPAR was dichotomized using the candidate cutoff and patients were categorized to either a low- or high-risk category. To minimize the effect of potential data perturbation on the selected optimal cutoff, 500 bootstrap replicates were utilized, and the bootstrap bias

corrected estimate was used as the final optimal sex-specific cutoff. Kaplan–Meier curves were used to visualize the survival of EmCAB participants above and below the sex-specific thresholds.

All analyses were performed using IBM SPSS Statistics Version 25 (Armonk, NY) and R version 3.5.1 (R Foundation for statistical computing, Vienna, Austria). Two-tailed $P<0.05$ were considered statistically significant.

Results

Baseline Characteristics

The baseline characteristics for CHDWB and EmCAB participants are depicted in Table 1 and Table S1, respectively. In the CHDWB cohort, women were younger, more frequently black, had lower blood pressure and triglyceride levels, and higher total cholesterol, high-density lipoprotein cholesterol, eGFR, and hsCRP levels as compared with men (Table 1). Despite having a similar mean body mass index, women had a significantly higher total fat mass and a lower android-to-gynoid fat ratio (Table 1). Notably, plasma suPAR levels in women were 12.6% higher compared with men.

In the larger EmCAB cohort, women were older, more frequently black, had higher body mass index, and hsCRP level, lower eGFR, and lower prevalence of prior CABG, revascularization at enrollment, and cardiovascular medication use as compared with men (Table S1). Plasma suPAR levels in women with CAD were 17.5% higher. Furthermore, median suPAR levels among EmCAB participants (2930 [2275–3929] pg/mL) were significantly higher than in the asymptomatic CHDWB cohort (2543 [2087–3018] pg/mL) ($P<0.001$).

Association of Sex With Plasma suPAR Levels

The impact of sex on suPAR levels in the CHDWB cohort was studied using 4 separate models with stepwise adjustment for covariates (Table 2). After adjustment for cardiovascular risk factors (Model 1) and hsCRP levels (Model 2), suPAR levels were noted to be 16.2% higher ($P<0.001$) in women compared with men (Table 2). To address whether body fat mass or distribution was contributing to this relationship, total body fat and android–gynoid fat ratio were added as covariates in Model 3. The relationship of sex with suPAR was attenuated after controlling for visceral fat measures and women had 10% higher levels ($P=0.005$) as compared with men (Table 2). A similar analysis performed in the EmCAB cohort revealed that sex was an independent predictor of suPAR levels and women with CAD had 11.7% higher levels after adjustment, compared with men (Table S2).

Table 1. Baseline Characteristics of CHDWB Participants Overall and Stratified by Sex

Participant Characteristics	Overall (n=666)	Women (n=436)	Men (n=230)	P Value
Age, y (SD)	48.7 (10.9)	48.0 (10.2)	50.0 (12.0)	0.014
Black race (%)	153 (23.0)	133 (30.5)	20 (8.7)	<0.001
Diabetes mellitus (%)	75 (11.3)	53 (12.2)	22 (9.6)	0.367
Antihypertensive use (%)	152 (22.8)	99 (22.7)	53 (23.0)	0.923
Systolic blood pressure, mm Hg (SD)	120.8 (15.9)	119.8 (16.7)	122.9 (14.0)	0.002
Diastolic blood pressure, mm Hg (SD)	76.3 (10.9)	74.7 (10.7)	79.1 (10.8)	<0.001
Current smoking (%)	39 (5.9)	20 (4.6)	19 (8.3)	0.081
Total cholesterol, mg/dL	192.0 [169.0, 218.0]	195.0 [172.3, 219.0]	189.5 [164.8, 213.3]	0.008
High-density lipoprotein cholesterol, mg/dL	61.0 [50.0, 75.0]	67.0 [54.0, 81.0]	51.0 [44.0, 61.0]	<0.001
Triglycerides, mg/dL	86.0 [65.0, 121.0]	80.0 [62.3, 108.0]	100.0 [74.0, 147.0]	<0.001
Low-density lipoprotein cholesterol, mg/dL (SD)	110.5 (31.6)	109.8 (32.5)	111.9 (29.8)	0.233
eGFR, mL/min per 1.73 m^2 (SD)	96.0 (15.8)	97.2 (16.4)	93.7 (14.3)	0.002
Body mass index, kg/m^2 (SD)	27.9 (6.4)	28.2 (7.4)	27.3 (4.0)	0.434
Body fat, lb*	59.6 [45.1, 78.4]	62.4 [46.2, 84.1]	53.4 [43.4, 68.1]	<0.001
Android-to-gynoid fat ratio (SD)*	0.47 [0.35, 0.62]	0.40 [0.31, 0.50]	0.64 [0.53, 0.74]	<0.001
Statin use (%)	107 (16.1)	39 (8.9)	68 (29.6)	<0.001
hsCRP, mg/L*	1.5 [0.5, 3.6]	1.8 [0.5, 4.2]	1.0 [0.5, 1.9]	<0.001
suPAR, pg/mL*	2543 [2087–3018]	2619 [2193–3089]	2378 [1937–2743]	<0.001

Continuous variables are presented as mean (SD) or median [25–75th percentile] and categorical variables are presented as count (proportion). CHDWB indicates Emory Center for Health Discovery and Wellbeing; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; suPAR, soluble urokinase-type plasminogen activator receptor.
*Visceral adiposity measures and biomarkers reported as medians with interquartile ranges. Visceral adiposity measured in 623 participants (407 women, 216 men) and high-sensitivity CRP in 596 participants (393 women, 203 men).

We stratified both cohorts by sex and evaluated the sex-specific independent predictors of plasma suPAR levels (Tables S3 and S4). Among female participants in the CHDWB cohort, diabetes mellitus and body fat correlated positively with suPAR levels, while increasing eGFR, high-density lipoprotein, and android-gynoid fat ratio were inversely correlated (Table S3). Age was a predictor in men, while statin use correlated negatively with suPAR levels (Table S3). Importantly, estradiol levels in women and total testosterone levels in men were not associated with suPAR (Table S3). Among participants of the EmCAB cohort, diabetes mellitus, current smoking, heart failure, peripheral artery disease, and hsCRP levels correlated positively with suPAR levels in both men and women, with eGFR and statin use exhibiting a negative correlation (Table S3).

Association of suPAR With Adverse Outcomes and the Impact of Sex

Participants in the EmCAB cohort were followed for a median duration of 5.2 [2.1–6.9] years, during which 795 all-cause deaths (301 in women and 494 in men) and 604 cardiovascular death/MI (226 in women and 378 in men) events were

recorded. Plasma suPAR was stratified by median (2930 pg/mL) and quartile (2275, 2930, and 3929 pg/mL) levels in the overall EmCAB cohort. The association of continuous and categorical suPAR levels with all-cause mortality and cardiovascular death/MI was assessed using multivariable-adjusted Cox models, and the hazard ratios for these associations are depicted in Table 3. High suPAR (1-SD increase in log$_2$-transformed level) in the overall cohort was independently associated with a nearly 1.7-fold increased risk of adverse outcomes. High suPAR (1-SD increase in log2-transformed level) in the overall cohort was independently associated with a nearly 1.7-fold increased risk of adverse outcomes. This association was not attenuated after further adjustment for hsCRP. Both high suPAR (hazard ratio 1.81 [95% CI 1.66–1.98] and 1.58 [95% CI 1.43–1.76] for all-cause mortality and cardiovascular death/nonfatal MI, respectively) and high hsCRP (1-SD increase in log2-transformed level) were independently associated with adverse outcomes (hazard ratio 1.19 [95% CI 1.09–1.30] and 1.20 [95% CI 1.08–1.33] for all-cause mortality and cardiovascular death/nonfatal MI, respectively).

Circulating suPAR level above the median level in the overall cohort was independently associated with a 2- to

Table 2. Independent Association of Female Sex With Plasma suPAR Levels Among CHDWB Participants

Model	Estimate (95% CI)	P Value
Unadjusted	12.6% (7.7%, 17.7%)	<0.001
Model 1*	16.0% (10.2%, 22.1%)	<0.001
Model 2†	16.2% (10.2%, 22.4%)	<0.001
Model 3‡	10.4% (3.0%, 18.3%)	0.005

CHDWB indicates Emory Center for Health Discovery and Wellbeing; suPAR, soluble urokinase-type plasminogen activator receptor.
*Adjusted for covariates including age, race, diabetes mellitus, smoking, antihypertensive use, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, statin use, estimated glomerular filtration rate, and body mass index. Total cholesterol, high-density lipoprotein, and body mass index were log-transformed.
†Model 2 adjusted for covariates included in Model 1 and log-transformed high-sensitivity C-reactive protein.
‡Model 3 adjusted for the covariates included in Model 2 as well as log-transformed body fat mass and android-to-gynoid fat ratio.

3-fold increase in the risk of adverse outcomes, and a level in the highest quartile was associated with a 3- to 4-fold increase in risk compared with those in the lowest quartile (Table 3). Interestingly, we observed a strong multiplicative interaction between sex and both suPAR categories for all-cause mortality risk (P-interactions=0.005, 0.007, and 0.001 for $\log_2$-transformed, median, and quartile analyses, respectively). In sex-stratified analyses, the strength of the association between suPAR levels and outcomes was consistently higher among men as compared with women (Table 3).

In order to identify sex-specific optimal cut-offs for the association of suPAR levels with adverse outcomes, women and men were stratified by sex-specific suPAR deciles. The cumulative incidence of all-cause mortality and cardiovascular death/MI across sex-specific suPAR deciles is depicted in Figure 1A and 1B. Overall, the incidence of both adverse outcomes increased across sex-specific suPAR deciles, but the progressive increase in adverse events in women occurred among those above the fifth decile (>3059 pg/mL), whereas in men, the increase in risk began at levels above the sixth decile (>2918 pg/mL) (Figure 1A and 1B). The incidence of adverse events among both men and women was similar at the highest sex-specific suPAR levels (deciles 9 and 10), suggesting the potential utility of creating sex-specific suPAR cutoffs for predicting outcomes.

The sex-specific suPAR cutoffs for all-cause mortality were 4392 pg/mL for women (76th percentile) and 3187 pg/mL for men (64th percentile). The corresponding cutoffs for cardiovascular death/MI were 3888 pg/mL for women (67th percentile) and 2941 pg/mL for men (56th

Table 3. Association of Plasma suPAR With Adverse Outcomes in EmCAB Participants

	Overall HR (95% CI)	P Value	Women HR (95% CI)	P Value	Men HR (95% CI)	P Value
All-cause mortality*						
$\log_2$-transformed suPAR (per 1-SD)	1.72 (1.60–1.85)	<0.001	1.61 (1.41–1.83)	<0.001	1.83 (1.67–2.00)	<0.011
Median suPAR	2.63 (2.20, 3.16)	<0.001	1.91 (1.41, 2.59)	<0.001	3.07 (2.45, 3.84)	<0.001
suPAR quartile I	Referent		Referent		Referent	
suPAR quartile II	1.20 (0.89, 1.62)	0.242	0.67 (0.40, 1.12)	0.129	1.49 (1.03, 2.18)	0.037
suPAR quartile III	2.37 (1.80, 3.12)	<0.001	1.24 (0.79, 1.96)	0.348	3.07 (2.17, 4.34)	<0.001
suPAR quartile IV	3.87 (2.93, 5.12)	<0.001	1.76 (1.13, 2.74)	0.013	5.64 (3.96, 8.03)	<0.001
Cardiovascular death/MI†						
$\log_2$-transformed suPAR (per 1-SD)	1.57 (1.44–1.71)	<0.001	1.59 (1.38–1.85)	<0.01	1.59 (1.43–1.77)	<0.001
Median suPAR	2.23 (1.82, 2.73)	<0.001	1.84 (1.30, 2.61)	0.001	2.43 (1.89, 3.12)	<0.001
suPAR quartile I	Referent		Referent		Referent	
suPAR quartile II	1.09 (0.78, 1.52)	0.597	0.93 (0.52, 1.69)	0.822	1.11 (0.75, 1.65)	0.601
suPAR quartile III	1.97 (1.46, 2.65)	<0.001	1.37 (0.79, 2.38)	0.266	2.23 (1.56, 3.19)	<0.001
suPAR quartile IV	2.97 (2.19, 4.04)	<0.001	2.18 (1.27, 3.73)	0.005	3.25 (2.24, 4.73)	<0.001

Plasma suPAR level stratified by median (2930 pg/mL) and quartile (2275, 2930, and 3929 pg/mL) levels in the overall EmCAB cohort. Cox proportional hazards regression models adjusted for sex, age, race, diabetes mellitus, current smoking, hypertension, body mass index, estimated glomerular filtration rate, history of coronary artery bypass graft, heart failure, peripheral artery disease, acute MI at enrollment, revascularization at enrollment, and cardiovascular medication (angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker, aspirin, β-blocker, clopidogrel, and statin) use. EmCAB indicates Emory Cardiovascular Biobank; HR, hazard ratio; MI, myocardial infarction; suPAR, soluble urokinase-type plasminogen activator receptor.
*The multiplicative interaction of sex with log-transformed suPAR (P=0.005), median suPAR (P=0.007) and suPAR quartiles (P=0.001) was significant for all-cause mortality in the overall cohort.
†The multiplicative interaction of sex with log-transformed suPAR (P=0.037) was significant, with median suPAR (P=0.061) was nominal; and with suPAR quartiles (P=0.182) was not significant for cardiovascular death/MI in the overall cohort.
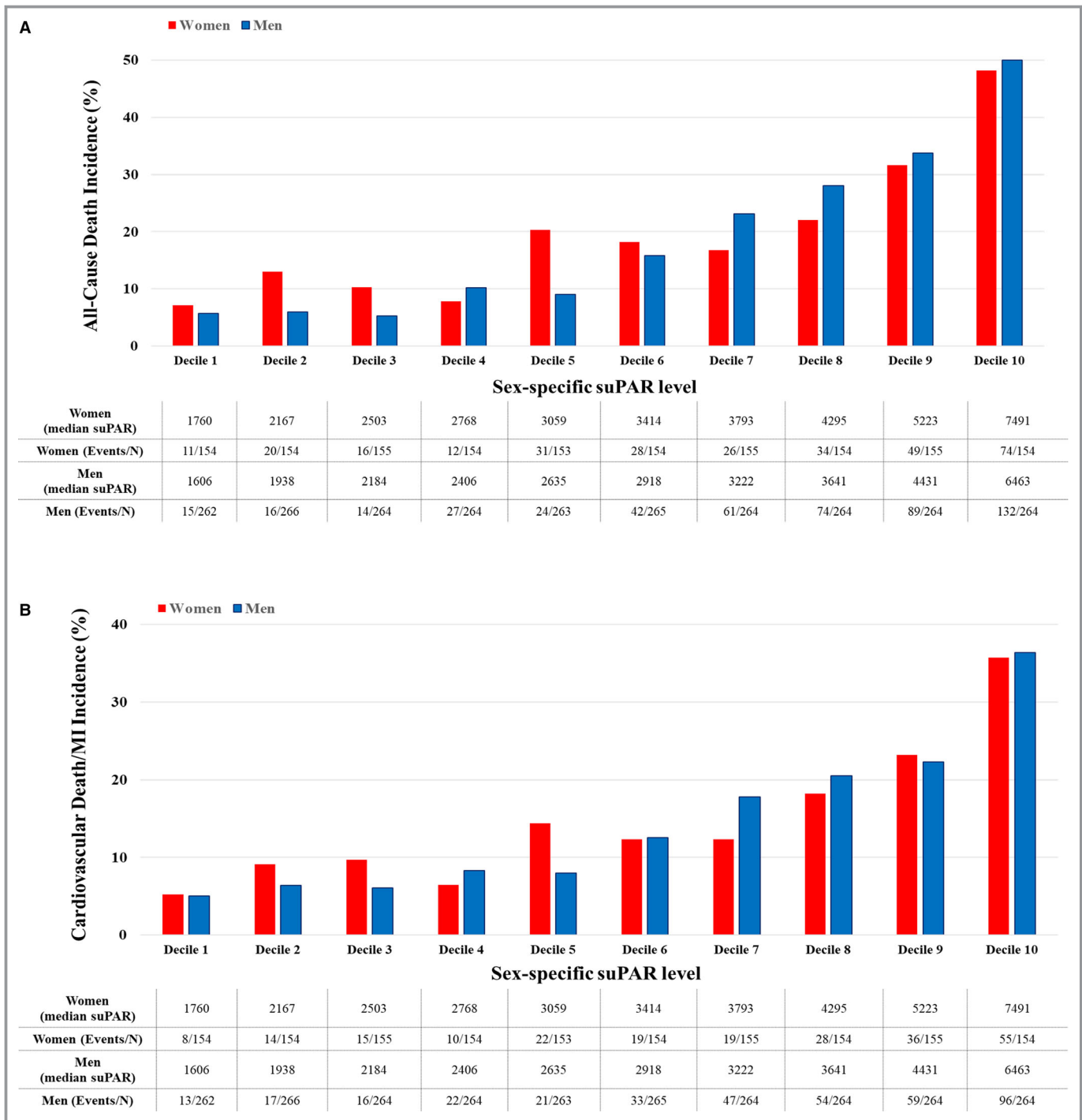
Figure 1. Cumulative incidence of adverse outcomes across sex-specific suPAR deciles. Sex-specific cumulative incidence of all-cause mortality (**A**) and cardiovascular death/nonfatal MI events (**B**) across deciles of plasma suPAR levels. The cumulative incidence of adverse outcomes across increased across sex-specific suPAR deciles, but the progressive increase in women occurred in those above the fifth decile, whereas in men, the increase in risk began at levels above the sixth decile (>2918 pg/mL). The incidence of adverse outcomes among both men and women was similar at the highest sex-specific suPAR levels (deciles 9 and 10). MI indicates myocardial infarction; suPAR, soluble urokinase–type plasminogen activator receptor.

percentile). The Kaplan–Meier survival curves with the respective sex-specific cutoffs for all-cause mortality and cardiovascular death/MI are depicted in Figure 2A and 2B, respectively. Women and men with plasma suPAR levels above the respective sex-specific cutoffs had a similar incidence of all-cause mortality (38.5% for women and 35.5%
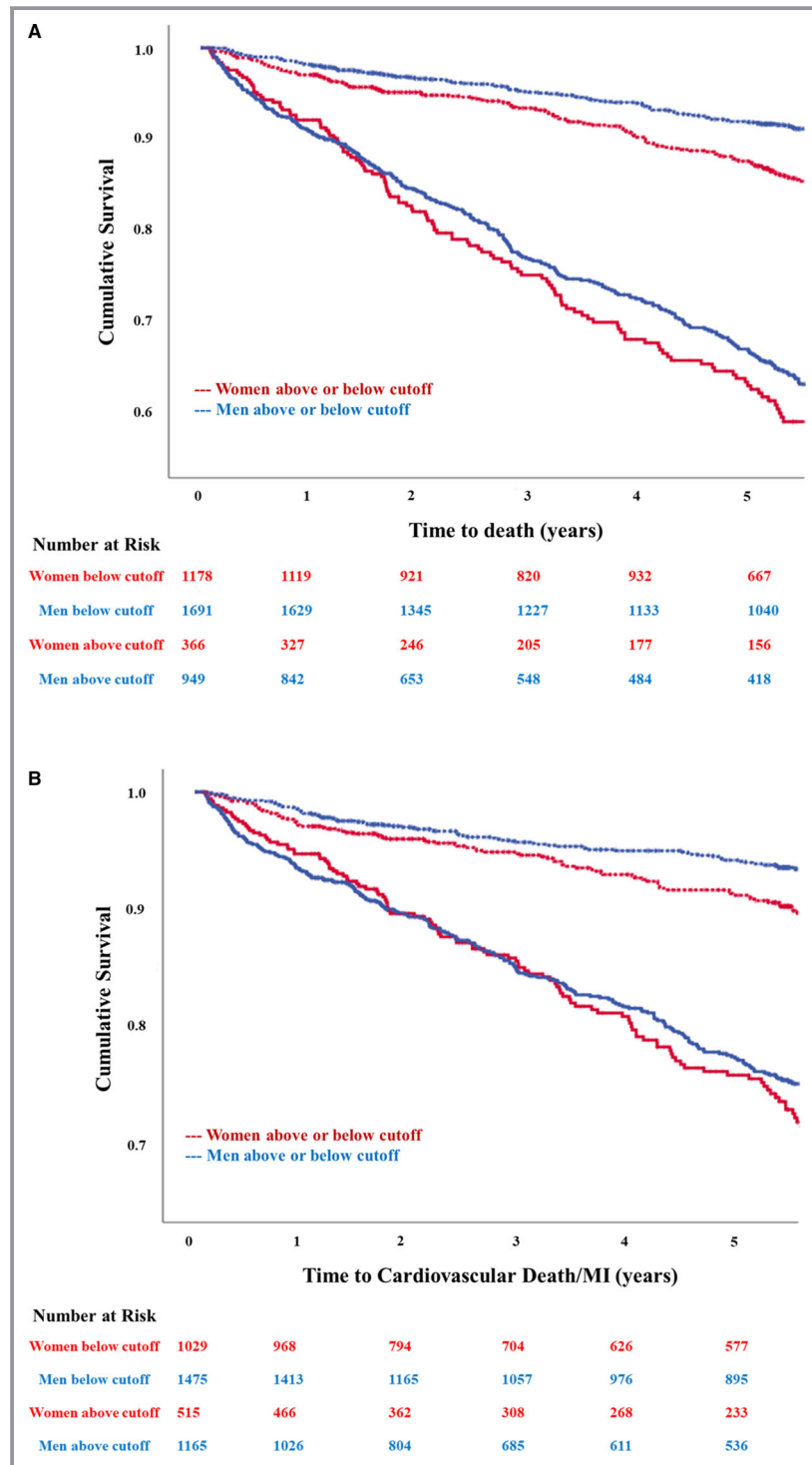
Figure 2. Kaplan–Meier survival among men and women above or below sex-specific suPAR cutoffs. Kaplan–Meier curves for survival from all-cause mortality (**A**) and cardiovascular death/nonfatal MI events (**B**) among men and women above or below the respective sex-specific suPAR cutoffs. The sex-specific suPAR cutoffs for all-cause mortality were 4392 pg/mL for women (76th percentile) and 3187 pg/mL for men (64th percentile). The corresponding cutoffs for cardiovascular death/MI events were 3888 pg/mL for women (67th percentile) and 2941 pg/mL for men (56th percentile). MI indicates myocardial infarction; suPAR, soluble urokinase-type plasminogen activator receptor.

for men, *P*=0.3) and cardiovascular death/MI (24.9% for women and 23.5% for men, *P*=0.6).

Discussion

We investigated the determinants and implications of sex-based differences in plasma suPAR levels and the impact of sex on the prognostic value of suPAR in patients with CAD. First, both asymptomatic women and those with CAD have 10% to 12% higher circulating suPAR levels compared with men after adjusting for potential confounders. Second, higher body fat and an increasing visceral fat distribution in women are at least partly responsible for the higher suPAR levels. Third, elevated suPAR levels have a similar association with adverse cardiovascular outcomes in both women and men with CAD when sex-specific suPAR cutoff values are utilized.

Sex and suPAR Levels

It is well established that women have higher levels of inflammatory markers including hsCRP, IL-6, serum amyloid A, D-dimer, and lipoprotein phospholipase A2,[21–23,31,32] some of which are attributed, at least partly, to visceral adiposity in women.[23] Herein, we report that levels of plasma suPAR, the circulating form of uPAR, a measure of systemic inflammation and immune activation,[7] are also higher in women, even after adjusting for demographics, risk factors, medication use, and systemic inflammation measured as circulating hsCRP levels. Previous studies have shown that visceral adiposity is associated with higher suPAR levels,[33,34] and we observed that the association of female sex with suPAR was slightly attenuated but remained significant after adjusting for visceral adiposity measures.

Impact of Sex on the Association of suPAR With Adverse Outcomes

Elevated plasma suPAR levels are associated with risk of adverse cardiovascular and renal outcomes in the general population and among patients with established cardiovascular disease.[19,35–37] Prior work from our group has additionally shown that the association of suPAR with outcomes is independent of other biomarkers including fibrin degradation products, heat shock protein-70, and very importantly, hsCRP levels.[38]

Our observations regarding the impact of sex on the association of suPAR with adverse outcomes are similar to healthy Danish participants in the MONICA (Monitoring trends and determinants of cardiovascular disease) study where the hazard ratio of the top tertile was 1.7 in women compared with 2.1 for men using the same cut-off value.[19] Herein we demonstrate, using sex-specific suPAR deciles, that the incidence of cardiovascular events was similarly elevated in both men and women at the highest levels. Lastly, we observed significant overlap between survival curves for women and men above or below the derived sex-specific cutoff values. Overall, these findings suggest that elevated plasma suPAR levels have a similar association with adverse cardiovascular events among both men and women when sex-specific levels are utilized.

Clinical Implications

Our findings regarding the association of sex with suPAR levels and the impact of sex on the association of suPAR with adverse outcomes have important implications for future research focused on leveraging biomarkers to improve cardiovascular risk assessment. The Reynold's risk score exemplifies this potential clinical application and incorporates hsCRP into the primary prevention risk assessment algorithm. Since sex is a predictor of hsCRP levels and impacts the association of hsCRP with outcomes, sex-specific Reynold's risk score equations have been created.[39,40] As the evidence base for the clinical applicability of suPAR grows, similar sex-specific algorithms will be necessary for using suPAR.

Strengths and Limitations

Strengths of our study include analysis of 2 large, clinically and ethnically diverse cohorts. The primary prevention cohort underwent extensive phenotyping, including visceral fat distribution studies, and provided important mechanistic insights regarding sex differences. Patients with CAD validated the observed sex differences in suPAR levels, and these patients were followed for adjudicated outcomes in order to develop sex-specific suPAR cutoff values for secondary risk assessment. However, the suPAR cutoff values determined in our study cannot necessarily be extrapolated to the general population. We have not evaluated the impact of change in cardioprotective medications over time in this study, although our data suggest that suPAR values were lower in patients with CAD treated with statins. Lastly, we cannot exclude the possibility of residual confounding explaining the relationship between sex, suPAR levels, and adverse outcomes in CAD, given the observational nature of this study.

Conclusions

Women with and without CAD have 10% to 12% higher plasma suPAR levels compared with men. An elevated suPAR level is equally predictive of an increased risk of adverse

cardiovascular events in women and men, when sex-specific levels are utilized.

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Disclosures

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SUPPLEMENTAL MATERIAL

Data S1.

Study Population

The Emory Center for Health Discovery and Wellbeing (CHDWB) was established in 2008 as an initiative aiming towards the prevention of the chronic diseases through promotion of a healthy lifestyle in employees of Emory University and Georgia Institute of Technology, Atlanta, Georgia, USA.(1) Our analysis includes 666 unique participants without known CAD that had plasma suPAR measured at the time of enrollment. Subjects with an acute illness, recent hospitalization within the year prior to enrollment, pregnant women, and individuals with poorly controlled medical comorbidities were excluded.

The Emory Cardiovascular Biobank (EmCAB) is an ongoing prospective registry of patients undergoing cardiac catheterization for evaluation of CAD at three Emory Healthcare affiliated hospitals.(2) Our study includes participants enrolled between 2003 and 2015. Within the EmCAB cohort, there were 4,184 unique participants who underwent plasma suPAR measurement at enrollment and were followed for adverse outcomes. We excluded patients with active cancer, organ transplantation, severe valvular heart disease, and missing follow-up.

Cardiovascular risk factors

Participants in both cohorts were interviewed to obtain information about demographic characteristics, medical history, medication use, and behavioral habits. In the CHDWB cohort, physical measurements included vital signs, height, weight, and body mass index (BMI) calculated as weight (in kilograms) divided by height (in meters)-squared. Hypertension, hypercholesterolemia, and diabetes mellitus were defined according to the Joint National Committee, Adult Treatment Panel III, and American Diabetes Association criteria, respectively,

and smoking habits were recorded and classified as nonsmoker or ever smoker if there was a lifetime history of smoking at least 100 cigarettes. Fasting blood samples were collected for a lipid profile, metabolic panel, and hsCRP measurement (Quest Diagnostics, Madison, NJ, USA). Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation.(3) Lastly, body fat composition was measured as fat mass and android-to-gynoid fat ratio using dual-energy x-ray absorptiometry (iDXA, GE Lunar Densitometry, General Electric Company, Boston, MA, USA), which is considered a gold standard measure for the identification of whole-body fat mass within a 2% coefficient of variation.(4) The android region included an area from the top of the iliac crest to 20% of the distance from the iliac crest to the bottom of the subject's head.(5) The gynoid region extended from the top of the greater trochanter down a distance twice the height of the android region.(5) Overall, 10.5% and 6.5% participants had data missing for hsCRP and visceral adiposity measures in the cohort. This data was assumed to be missing at random and was imputed using the Visualization and Imputation of Missing values (VIM) R package by utilizing the k-nearest neighbors approach.(6)

In the EmCAB cohort, the prevalence of hypertension, hyperlipidemia, and diabetes was determined by physician diagnosis and/or treatment. Medical records and International Classification of Diseases-9 (ICD-9) codes were reviewed to confirm participant-reported medical history. Previous history of coronary artery bypass grafting (CABG), heart failure, peripheral artery disease, and the presence of an acute myocardial infarction and revascularization of any coronary artery at time of enrollment were recorded. The use of angiotensin converting enzyme inhibitor (ACEi)/angiotensin-II receptor blocker (ARB), aspirin, beta blocker, clopidogrel, and statin was recorded as well.(2)

Table S1. Baseline characteristics of EmCAB participants overall and stratified by sex.

Participant Characteristics	Overall (n=4,184)	Women (n=1,544)	Men (n=2,640)	p-value
Age, years (SD)	63.1 (12.2)	63.9 (12.7)	62.7 (11.8)	0.003
Black race (%)	924 (22.1)	454 (29.4)	470 (17.8)	< 0.001
Diabetes (%)	1,411 (33.9)	512 (33.3)	899 (34.2)	0.564
Hypertension (%)	3,233 (77.6)	1219 (79.4)	2014 (76.6)	0.051
Current smoking (%)	347 (8.3)	120 (7.8)	227 (8.6)	0.384
eGFR, ml/min/1.73 m^2 (SD)	73.0 (24.6)	71.9 (25.9)	73.7 (23.8)	0.030
Body mass index, kg/m^2 (SD)	29.9 (6.4)	30.4 (7.6)	29.6 (5.6)	< 0.001
History of CABG (%)	977 (23.4)	231 (15.0)	746 (28.3)	< 0.001
History of Heart Failure (%)	1,314 (31.4)	479 (31.0)	835 (31.6)	0.704
History of Peripheral Artery Disease (%)	683 (16.3)	239 (15.5)	444 (16.8)	0.260
Acute MI at enrollment (%)	361 (8.6)	123 (8.0)	238 (9.0)	0.254
Revascularization at enrollment (%)	2,161 (51.6)	629 (40.7)	1532 (58.0)	< 0.001
Aspirin use (%)	3,180 (76.0)	1084 (70.2)	2096 (79.4)	< 0.001
Clopidogrel use (%)	1,862 (44.5)	568 (36.8)	1294 (49.0)	< 0.001
ACEi/ARB* use (%)	2,371 (56.7)	813 (52.7)	1558 (59.0)	< 0.001
Beta blocker use (%)	2,833 (67.7)	1001 (64.8)	1832 (69.4)	0.003
Statin use (%)	2,972 (71.0)	1005 (65.1)	1967 (74.5)	< 0.001
hsCRP, mg/L	2.8 [1.1, 7.3]	3.7 [1.5, 9.0]	2.4 [1.0, 6.2]	< 0.001
suPAR, pg/ml	2,930 [2,275-3,929]	3,245 [2,503-4,295]	2,761 [2,183-3,641]	< 0.001
Death (%)	795 (19.0)	301 (19.5)	494 (18.7)	0.540
Cardiovascular death/nonfatal MI (%)	604 (14.4)	226 (14.6)	378 (14.3)	0.785

EmCAB, Emory Cardiovascular Biobank; eGFR, estimated glomerular filtration rate; CABG, coronary artery bypass graft; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; hsCRP, high-sensitivity C-reactive protein; suPAR, soluble urokinase-type plasminogen activating receptor; MI, myocardial infarction. Continuous variables are presented as mean (SD) or median [25-75th percentile] and categorical variables are presented as count (proportion). hsCRP measured in 3,645 participants (1,336 women and 2,309 men).

Table S2. Independent association of female sex with plasma suPAR levels among EmCAB participants.

Model	Estimate (95% CI)	p-value
Unadjusted	13.6% (11.4%, 15.9%)	< 0.001
Model 1[†]	12.0% (10.0%, 16.1%)	< 0.001
Model 2[‡]	11.7% (9.5%, 13.9%)	< 0.001

suPAR, soluble urokinase-type plasminogen receptor activator; EmCAB, Emory Cardiovascular Biobank. [†]Adjusted for covariates including age, race, diabetes, current smoking, hypertension, body mass index, estimated glomerular filtration rate, history of coronary artery bypass graft, heart failure, peripheral artery disease, acute MI at enrollment, revascularization at enrollment, and cardiovascular medication (Angiotensin-converting enzyme inhibitor/Angiotensin II receptor blocker, aspirin, beta blocker, clopidogrel, and statin) use. [‡]Model 2 adjusted for covariates included in Model 1 and log-transformed high-sensitivity C-reactive protein. High-sensitivity C-reactive protein measured in 3,645 participants (1,336 women, 2,309 men).

Table S3. Sex-specific independent predictors of plasma suPAR levels among CHDWB participants.

Characteristic	Women		Men	
	Beta-estimate (95% CI)	p-value	Beta-estimate (95% CI)	p-value
Age (per year)	0.000 (-0.005, 0.005)	0.966	0.012 (0.006, 0.018)	< 0.001
Black race	-0.002 (-0.092, 0.087)	0.957	-0.035 (-0.229, 0.160)	0.726
Diabetes	0.123 (0.006, 0.239)	0.039	0.129 (-0.061, 0.318)	0.184
Antihypertensive use	0.075 (-0.014, 0.165)	0.098	0.036 (-0.098, 0.170)	0.602
Systolic blood pressure (per mmHg)	0.002 (-0.001, 0.004)	0.238	0.000 (-0.004, 0.004)	0.862
Current smoking	0.111 (-0.060, 0.282)	0.203	0.068 (-0.119, 0.255)	0.477
Total cholesterol[†]	0.096 (-0.118, 0.310)	0.379	-0.189 (-0.497, 0.119)	0.228
HDL-cholesterol[*†]	-0.234 (-0.394, -0.074)	0.004	-0.096 (-0.340, 0.148)	0.439
eGFR[*] (per 10 ml/min/1.73 m^2)	-0.004 (-0.007, -0.001)	0.004	-0.001 (-0.005, 0.004)	0.802
Body mass index[†]	0.010 (-0.354, 0.374)	0.957	-0.020 (-0.746, 0.707)	0.958
Body fat mass[†]	0.325 (0.119, 0.531)	0.002	0.004 (-0.279, 0.287)	0.976
Android-to-gynoid fat ratio[†]	-0.175 (-0.329, -0.021)	0.026	0.052 (-0.298, 0.195)	0.682
Statin use	-0.038 (-0.180, 0.104)	0.599	-0.176 (-0.313, -0.039)	0.012
hsCRP[*†]	-0.026 (-0.066, 0.014)	0.202	0.040 (-0.026, 0.105)	0.239
Estradiol[‡]	0.028 (-0.016, 0.071)	0.210	-	-
Total testosterone[‡]	-	-	-0.041 (-0.172, 0.091)	0.542

suPAR, soluble urokinase-type plasminogen activator receptor; CHDWB, Emory Center for Health Discovery and Wellbeing; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein. [†]Values were log-

transformed before analysis. ‡Estradiol and testosterone levels available in 383 women and 197 men, respectively, and log-transformed before analysis.

Table S4. Sex-specific independent predictors of plasma suPAR levels among EmCAB participants.

Characteristic	Women		Men	
	Beta-estimate (95% CI)	p-value	Beta-estimate (95% CI)	p-value
Age	-0.001 (-0.003, 0.002)	0.657	0.000 (-0.002, 0.002)	0.780
Black race	0.007 (-0.055, 0.070)	0.821	0.013 (-0.068, 0.219)	0.640
Diabetes mellitus	0.185 (0.124, 0.245)	< 0.001	0.152 (0.108, 0.197)	< 0.001
Hypertension	0.024 (-0.048, 0.096)	0.513	0.000 (-0.049, 0.050)	0.986
Current smoking	0.112 (0.010, 0.215)	0.032	0.224 (0.151, 0.298)	< 0.001
History of CABG	0.082 (0.003, 0.160)	0.041	0.022 (-0.026, 0.069)	0.373
History of Heart Failure	0.097 (0.035, 0.158)	0.002	0.107 (0.062, 0.152)	< 0.001
History of Peripheral Artery Disease	0.104 (0.025, 0.183)	0.010	0.130 (0.070, 0.186)	< 0.001
Acute MI at enrollment	-0.008 (-0.111, 0.094)	0.872	-0.008 (-0.065, 0.081)	0.827
Revascularization at enrollment	0.019 (-0.052, 0.090)	0.596	-0.037 (-0.085, 0.011)	0.128
Body mass index (per kg/m^2)	0.006 (0.002, 0.010)	0.002	0.002 (-0.002, 0.005)	0.431
eGFR[‡] (per 10 ml/min/1.73 m^2)	-0.011 (-0.013, -0.010)	< 0.001	-0.012 (-0.013, -0.011)	< 0.001
ACEi/ARB use	0.016 (-0.043, 0.076)	0.592	0.004 (-0.041, 0.048)	0.878
Aspirin use	-0.037 (-0.107, 0.034)	0.311	-0.066 (-0.126, -0.007)	0.028
Beta blocker use	0.016 (-0.049, 0.080)	0.635	0.058 (0.009, 0.108)	0.020
Clopidogrel use	0.058 (-0.011, 0.127)	0.102	-0.013 (-0.063, 0.037)	0.618
Statin use	-0.128 (-0.195, -0.061)	< 0.001	-0.059 (-0.114, -0.003)	0.039
hsCRP[†]	0.046 (0.029, 0.063)	< 0.001	0.042 (0.029, 0.054)	< 0.001

suPAR, soluble urokinase-type plasminogen activator receptor; EmCAB, Emory Cardiovascular Biobank; CABG, coronary artery bypass graft; MI, myocardial infarction; eGFR, estimated glomerular filtration rate; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; hsCRP, high-sensitivity C-reactive protein. †High-sensitivity CRP measured in 3,645 participants (1,336 women and 2,309 men) and log-transformed before analysis.

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