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Objective—Endothelial dysfunction is a possible mechanism to explain the association between atherosclerosis and kidney disease. This study evaluated circulating soluble endothelial cell–selective adhesion molecule (sESAM), a marker of endothelial dysfunction, as a risk factor for kidney function decline and albuminuria.

Approach and Results—In the Heart and Soul Study, we measured sESAM from baseline serum samples and defined elevated levels of sESAM by the highest quartile (quartile 4 [Q4]: >65.4 ng/mL). We evaluated the associations of high sESAM with baseline estimated glomerular filtration rate (eGFR) and ratio of urine albumin to creatinine (ACR), and with longitudinal changes in eGFR and ACR. Among 990 participants with sESAM measurements, median sESAM was 54.5 ng/mL (interquartile range, 45.3–65.8). After multivariable adjustment, elevated levels of sESAM were strongly and independently associated with baseline reduced eGFR <60 mL/min per 1.73 m^2 (odds ratio [OR], 11.44; $P<0.0001$) and ACR ≥30 mg/g (OR, 5.23; $P<0.0001$). Associations of sESAM (Q4 versus quartile 1 [Q1]) with change in ACR (β=54.47; $P<0.0001$) were also significant after full adjustment. The association with change in eGFR (1.56%; $P=0.0049$) was not statistically significant after application of the Bonferroni correction for multiple markers. In unadjusted models, sESAM was associated with rapid kidney function loss, defined as 3% annual eGFR decline (OR, 2.28; $P=0.0003$), although this was attenuated by adjustment (OR, 2.11; $P=0.0095$).

Conclusions—sESAM is associated with albuminuria and reduced kidney function in both cross-sectional and longitudinal analyses. These findings implicate endothelial dysfunction as a potential contributor to the elevated kidney disease risk in persons with cardiovascular disease. (***Arterioscler Thromb Vasc Biol*. 2014;34:00-00.**)

Key Words: albuminuria ■ atherosclerosis ■ kidney diseases

Clinical cardiovascular disease (CVD) is independently associated with kidney function decline and development of chronic kidney disease (CKD), defined by estimated glomerular filtration rate (eGFR) <60 mL/min per 1.73 m^2.[1] Mechanisms explaining the higher risk of CKD in this population are incompletely understood.[2] Albuminuria occurs in many individuals with CVD[3] and may be the result of a defect in the endothelial surface monolayer.[4] Endothelial dysfunction is observed in early stages of atherosclerosis and contributes to its progression to more advanced disease.[5,6] Because endothelial dysfunction in atherosclerosis is a systemic process,[7] its effects on renal vasculature may contribute to the development and progression of CKD among patients with atherosclerotic CVD.[7]

Endothelial dysfunction is associated with impaired endothelium-dependent vasodilation and has been observed in persons with proinflammatory or prothrombotic states, including dyslipidemia, coronary artery disease, congestive heart failure, and peripheral artery disease.[5,8] Abnormal endothelial function has also been described in early kidney disease[9,10] and may be responsible for albuminuria,[4] although the role of glomerular endothelial cells in the onset of albuminuria remains controversial.[11] Endothelial dysfunction can be assessed by brachial artery flow–mediated dilation,[12] but this measurement is methodologically challenging.[8] Identification of specific serum markers of endothelial function is an active area of research[13]; one example is endothelial cell-selective adhesion molecule (ESAM), which is expressed in vascular endothelial cells.[14] Increased expression of ESAM is associated with susceptibility to atherosclerosis.[15] In the Dallas Heart Study, a population-based cohort, circulating soluble ESAM (sESAM) was associated with subclinical CVD assessed by coronary artery calcium, abdominal aortic wall thickness, aortic plaque burden, and aortic compliance.[15] sESAM is also expressed at the glomerulus.[16] The association of this selective endothelial function marker with kidney disease in patients with preexisting atherosclerotic heart disease is unknown.

We investigated the associations between sESAM and kidney outcomes among ambulatory individuals with stable coronary heart disease in the Heart and Soul Study cohort. We evaluated a comprehensive array of kidney outcomes, including baseline reduced eGFR and ratio of albumin to creatinine (ACR), change in eGFR, change in ACR, incident

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reduced eGFR <60 mL/min per 1.73 m^2, and rapid kidney function decline. Understanding these associations may help to delineate the role of endothelial dysfunction in kidney disease onset, particularly among patients with established CVD.

Materials and Methods

Materials and Methods section is available in the online-only Supplement.

Results

Participant Characteristics

Among 990 individuals with ischemic heart disease and serum measurements of sESAM at baseline, mean age was 66.7 (±11) years, 81% were men, 60% were white, 70% had hypertension, and 26% had diabetes mellitus. Mean $eGFR_{creatinine\text{-}cystatin\ C\ (cr\text{-}cys)}$ at baseline was 70.6 (±22.2) mL/min per 1.73 m^2, and the prevalence of $eGFR_{cr\text{-}cys}$ <60 mL/min per 1.73 m^2 at baseline was 31%. Individuals in the highest quartile of sESAM were more likely to be older, men, and white, and to have much lower baseline eGFR and higher baseline ACR and modestly lower high-density lipoprotein and low-density lipoprotein levels (Table 1).

Associations of sESAM With Baseline Reduced eGFR and Albuminuria

sESAM was strongly associated with eGFR <60 mL/min per 1.73 m^2 at baseline. After full adjustment, the highest quartile had 11-fold increased odds of eGFR <60 mL/min per 1.73 m^2 (Table 2). Similarly, the highest quartile had 5-fold increased odds of baseline urine ACR ≥30 mg/g (Table 2). These associations remained strong after additional adjustment for ACR and were attenuated by adjustment for eGFR. These findings were especially strong among black individuals (adjusted odds ratio [OR] for eGFR <60: 70; P<0.0001; adjusted OR for ACR ≥30: 16; P=0.0001).

Associations With Change in Urine ACR

Compared with the lowest quartile, adjusted mean increase in ACR from baseline to year 5 was 54 mg/g higher among patients in the highest sESAM quartile (Table 3). The adjusted mean increase in ACR was similar among individuals with preserved and reduced eGFR. Our sensitivity analysis using proportional odds and ordinal categories of ACR change gave similar results (OR, 2.55; P=0.0171).

Associations With Changes in Kidney Function

The highest quartile of sESAM compared with the lowest was associated with a loss in $eGFR_{cr\text{-}cys}$ of 1.56% per year after full adjustment (P=0.0049; Figure). This association was minimally affected by additional adjustment for ACR (1.36%; P=0.0166). These results were not statistically significant at the experiment-wide P=0.0017.

Table 1. Baseline Characteristics by Quartile of Soluble Endothelial Cell–Selective Adhesion Molecule

	Q1 (0.4–45.1 ng/mL)	Q2 (45.1–53.8 ng/mL)	Q3 (53.8–65.3 ng/mL)	Q4 (65.4–130 ng/mL)	*P* Value
Age, y*	62 (10)	66 (10)	67 (11)	72 (10)	0.0001
Men*	73%	80%	86%	86%	0.0001
Race					
White	44%	60%	67%	69%	0.0001
Latino	9%	11%	8%	7%	
Asian	14%	12%	11%	9%	
Black	28%	15%	12%	11%	
Other	4%	3%	2%	4%	
Tobacco, pack-years*	22 (22)	21.1 (22.5)	20.9 (21.7)	21.3 (23)	0.8596
HDL, mg/dL*	46 (14)	46 (13)	44 (15)	46 (14)	0.0374
LDL, mg/dL*	110 (35)	105 (31)	102 (35)	101 (34)	0.0012
C-reactive protein, mg/L	4.4 (6.8)	4.5 (10.3)	3.9 (5.3)	5.5 (9.5)	0.2219
Hypertension	70.5%	64.8%	70.2%	75.6%	0.075
Type 2 diabetes mellitus	23%	24%	29%	29%	0.276
ACEI/ARB	45.5%	50%	56.6%	52.8%	0.092
Statins	59.5%	70%	65%	63%	0.133
eGFR, mL/min per 1.73 m^2					
cr*	76 (19)	68 (15)	65 (18)	52 (20)	0.0001
cys*	85 (21)	77 (17)	70 (18)	54 (23)	0.0001
cr-cys*	86 (19)	77 (17)	70 (18)	52 (19)	0.0001
cr-cys <60	14.8%	16.0%	27.1%	58.1%	0.0001
ACR, mg/g†	8.0 (4.4–13.8)	7.8 (4.1–13.9)	8.3 (4.9–17.1)	11.8 (6.9–54)	0.0001
ACR ≥30 mg/g	10.4%	9.5%	12.3%	31.6%	0.0001

Values reported as *mean (SD) or †median (IQR). ACEI indicates angiotensin-converting enzyme inhibitor; ACR, albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; cr, creatinine; cys, cystatin C; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; Q, quartile; and SD, standard deviation.

Table 2. Associations of Quartiles of Soluble Endothelial Cell–Selective Adhesion Molecule With Baseline CKD Assessed by eGFR and ACR

	Q1	Q2	Q3	Q4	*P* Overall
eGFR <60 mL/min per 1.73 m^2					
Unadjusted OR	Ref	1.41	3.22	16.0	<0.0001
99.83% CI		0.59–3.36	1.46–7.11	7.43–34.41	
P value		0.2212	<0.0001	<0.0001	
Adjusted* OR	Ref	1.12	2.56	11.44	<0.0001
99.83% CI		0.43–2.89	1.07–6.12	4.89–26.76	
P value		0.7454	0.0016	<0.0001	
Adjusted* plus ACR	Ref	1.12	2.53	9.58	<0.0001
99.83% CI		0.42–3.03	1.02–6.3	3.87–23.73	
P value		0.7177	0.0014	<0.0001	
ACR ≥30 mg/g					
Unadjusted OR	Ref	0.75	1.12	3.52	<0.0001
99.83% CI		0.28–2.02	0.45–2.75	1.61–7.69	
P value		0.3557	0.7044	<0.0001	
Adjusted* OR	Ref	0.95	1.36	5.23	<0.0001
99.83% CI		0.31–2.89	0.47–3.93	1.96–13.91	
P value		0.8788	0.3598	<0.0001	
Adjusted* plus eGFR	Ref	0.82	1.05	2.83	0.0006
99.83% CI		0.27–2.52	0.35–3.11	0.93–8.6	
P value		0.5888	0.8965	0.00334	

Threshold *P* value for significance=0.0017. *P* overall gives results of test of heterogeneity. ACR indicates albumin-to-creatinine ratio; CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; OR, odds ratio; Q, quartile; and Ref, reference.

*Adjusted for age, race, sex, hypertension, pack-years, type 2 diabetes mellitus, high-density lipoprotein, low-density lipoprotein, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, statins, and high-sensitivity C-reactive protein.

In unadjusted models, when compared with the lower 3 quartiles, the highest quartile of sESAM was associated with rapid loss in kidney function, defined as a loss of $eGFR_{cr\text{-}cys}$ of >3% per year (Table 4). Adjusted models were not significant after applying the 29-marker Bonferroni correction to the *P* value. sESAM was not associated with incident reduced eGFR <60 mL/min per 1.73 m^2 (adjusted OR, 1.32; *P*=0.56).

Associations of Other Biomarkers With Longitudinal Outcomes

As described in the Materials and Methods section (in the online-only Supplement), sESAM was measured as a panel with 29 total biomarkers. Markers meeting the threshold *P* value for significance of 0.0017 for the longitudinal ACR outcome included ESAM, whey acidic protein 4C, and lymphotoxin β-receptor. Markers significant for percent change in eGFR included tumor necrosis factor receptor-1a and whey acidic protein 4C. None of the markers was significant for rapid kidney function loss (Table I in the online-only Data Supplement).

Discussion

In a cohort of individuals with known ischemic heart disease, we found higher levels of sESAM to be strongly associated with baseline reduced eGFR and higher ACR. sESAM was significantly associated with worsening ACR after full multivariable adjustment and with rapid kidney function loss in unadjusted models only. sESAM was not significantly associated with annual eGFR loss after application of Bonferroni correction for multiple markers tested on the same platform on which sESAM was measured. Higher sESAM may indicate early endothelial dysfunction that precedes kidney function decline among individuals with established CVD.

This is the first study to our knowledge to examine the relationship between sESAM and changes in kidney function in

Table 3. Adjusted* 5-Year Change in Albumin-to-Creatinine Ratio (mg/g) by Quartile of Soluble Endothelial Cell–Selective Adhesion Molecule

	Entire Cohort (N=990)	eGFR ≥60 (N=678)	eGFR <60 (N=303)
Q1	Ref	Ref	Ref
Q2	7.92 (*P*=0.4298)	2.26 (*P*=0.8255)	21.68 (*P*=0.6827)
Q3	11.44 (*P*=0.3424)	−0.91 (*P*=0.9358)	14.06 (*P*=0.7608)
Q4	54.47 (*P*<0.0001)	40.35 (*P*=0.0041)	63.21 (*P*=0.1458)
P overall	0.0001	0.0143	0.2148

Threshold *P* value for significance=0.0017. *P* overall gives results of test of heterogeneity. ACR indicates albumin to creatinine ratio; eGFR, estimated glomerular filtration rate; Q, quartile; and Ref, reference.

*Adjusted for age, race, sex, hypertension, pack-years, type 2 diabetes mellitus, high-density lipoprotein, low-density lipoprotein, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, statins, high-sensitivity C-reactive protein, and baseline ACR.

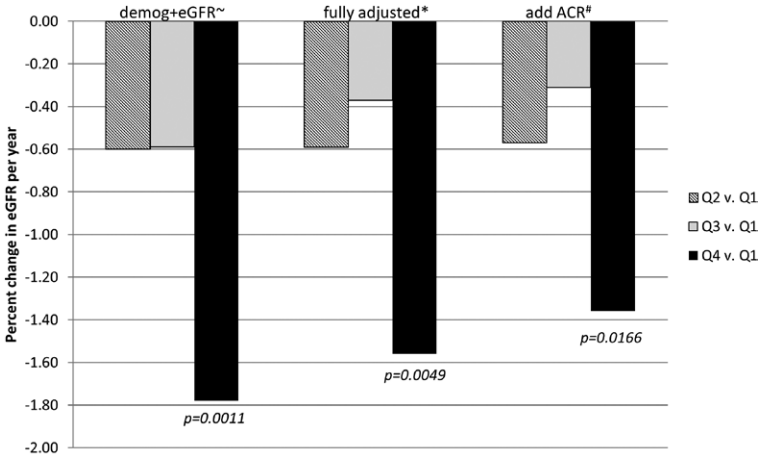

Figure. Quartiles of soluble endothelial cell–selective adhesion molecule after sequential adjustment for demographics, covariates, and albumin-to-creatinine ratio and associations with annual percent change in estimated glomerular filtration rate.

~ Adjusted for demographics and baseline eGFR only
*Adjusted for age, race, sex, htn, BMI, smoking, T2DM, HDL, LDL, ACEI/ARB, statins, CRP, baseline eGFR
Above covariates plus ACR
Threshold p-value for significance 0.0017

individuals with established atherosclerosis. CKD is a frequent complication of CVD[17–19] and accelerates risk of cardiovascular mortality in this population.[20] Previous work by Gold et al[21] has shown that sESAM is associated cross-sectionally with CKD. It is possible that sESAM is associated with CKD and kidney function decline in our study only as a result of reduced kidney filtration of this molecule. However, we adjusted for baseline kidney function and for known confounders, and our associations remained significant. Furthermore, the strong association of sESAM with ACR and change in ACR was independent of kidney filtration function assessed by eGFR, which implicates endothelial dysfunction in glomerular capillary disruption and consequent increases in ACR.[4]

sESAM regulates vascular permeability, and sESAM may regulate albumin extravasation at the level of the glomeruli in hyperglycemia.[11] In a longitudinal study of 88 individuals with type 2 diabetes mellitus, Kacso et al[22] found an increase in albuminuria over 12 to 24 months in individuals with lower sESAM levels. This finding is in contrast to our study. However, our study was larger and included only 26% diabetic subjects, and these relationships may differ in the absence of overt hyperglycemia.[11] Our study detected a much larger magnitude of change in ACR and had a longer follow-up time. Furthermore, the Kacso study[22] did not observe associations of sESAM with a change in eGFR. Decreased sESAM expression may play a role in early diabetic nephropathy,[11] and our study could not address different stages of diabetes mellitus. Our study adjusted for the presence of diabetes mellitus, and our associations remained strong.

Our findings are of interest for several reasons. Although this study focused on the association of CVD with development and progression of CKD, the increased cardiovascular risk in individuals with preexisting CKD is substantial, and the mediators remain incompletely characterized.[23] Candidate mechanisms for CKD to CVD risk association include inflammation, abnormal bone mineral calcification, and other conditions associated with CKD.[24,25] Endothelial dysfunction is considered a prodromal phase of atherosclerosis in CKD. However, atherosclerosis often precedes CKD diagnosis,[26] and efforts to identify early parallel processes of vascular dysregulation may be a useful framework for identifying common mechanisms for both diseases. As a marker of endothelial function,

Table 4. Associations of Quartiles of Soluble Endothelial Cell–Selective Adhesion Molecule With Rapid Loss (>3% Per Year) in Kidney Function

	Unadjusted OR; 99.83% CI; *P* Value	Adjusted* OR; 99.83% CI; *P* Value	Adjusted* Plus ACR; 99.83% CI; *P* Value
Q1	Ref	Ref	Ref
Q2	1.18; 0.44–3.12; 0.6018	1.09; 0.38–3.13; 0.8056	1.12; 0.37–3.36; 0.7489
Q3	1.16; 0.43–3.13; 0.6359	0.94; 0.3–2.93; 0.8702	0.99; 0.31–3.19; 0.9869
Q4	2.52; 0.99–6.41; 0.0019	2.13; 0.65–7.01; 0.0465	2.01; 0.58–6.93; 0.0783
P overall	0.0043	0.0659	0.0157
Q4 vs Q1–Q3	2.28; 1.11–4.65; 0.0003	2.11; 0.86–5.22; 0.0095	1.94; 0.76–4.93; 0.0265

Threshold *P* value for significance=0.0017. *P* overall gives results of test of heterogeneity. ACR indicates albumin-to-creatinine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; OR, odds ratio; Q, quartile; and Ref, reference.

*Adjusted for age, race, sex, hypertension, pack-years, type 2 diabetes mellitus, high-density lipoprotein, low-density lipoprotein, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, statins, high-sensitivity C-reactive protein, and baseline eGFR.

sESAM may help to explain the early pathogenesis of kidney abnormalities in the setting of known CVD.

Our study has several strengths. The Heart and Soul Study cohort is a unique cohort in which to examine the relationship between CVD and CKD. This cohort has robust measurements of sESAM and repeated measures of eGFR and ACR in 990 individuals. The coefficients of variation of biomarker measurement were not perfect but were comparable to those of other studies and are low for a new biomarker.[27] Also, our samples were all measured in concert, minimizing risks of drift among biomarker samples.

Our study has several limitations. First, the endothelial function biomarkers are only available on baseline serum samples. Although we have robust measurements of kidney function at baseline and at 5 years of follow-up, this cohort has a relatively low prevalence and incidence of kidney disease. We had inadequate power to stratify by race, and there were relatively few women in this cohort. Exploratory analyses suggest that further investigations into racial differences may be revealing, especially in light of higher prevalence of apolipoprotein L1 risk variants among the black population.[28] Finally, we were underpowered to detect associations with more severe changes in kidney function, such as rapid kidney function loss, decline in eGFR >50%, incident end-stage renal disease, or increase in ACR >50%. Application of the Bonferroni correction suggests that our results for changes in eGFR may be attributable to chance.

In conclusion, elevated sESAM may indicate increased risk of progressive albuminuria and rapid kidney function loss in individuals with known atherosclerotic disease. The accelerated kidney function loss is independent of baseline reduced eGFR, albuminuria, hypertension, and diabetes mellitus. This study indicates a possible role for endothelial function in the development and progression of CKD among individuals with preexisting CVD. Future investigations should focus on the pathophysiology of atherosclerosis and endothelial dysfunction as determinants of kidney function decline.

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Disclosures

None.

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Significance

The risk of kidney disease in individuals with established cardiovascular disease is high. Mechanisms of onset of kidney disease are unclear and do not seem to be explained exclusively by traditional risk factors such as diabetes mellitus and hypertension. Endothelial dysfunction may be a pathogenic mechanism in the onset of kidney disease in systemic atherosclerosis. In the Heart and Soul Study, a cohort of individuals with established ischemic heart disease, we evaluated associations between soluble endothelial cell–selective adhesion molecule, a marker of endothelial dysfunction, and kidney function assessed by estimated glomerular filtration rate and albuminuria. Our findings suggest a role for endothelial dysfunction in the onset of kidney disease in this population.

Supplemental Table I. Biomarkers in the Heart and Soul Study and p-values of associations with longitudinal outcomes

		delta ACR	%change eGFR	rapid kidney function loss
	Fully adjusted models	p-value	p-value	p-value
1	Angiogenin	0.9803	0.916	0.4048
2	ANP propeptide	0.0652	0.0058	0.022
3	BNP	0.4116	0.011	0.0023
4	CRP	0.4911	0.1509	0.0677
5	Cystatin C	0.0103	0.05	0.1256
6	D-Dimer	0.1904	0.2828	0.3555
7	ESAM	0.0001	0.0329	0.0658
8	Galectin 3	0.2404	0.2591	0.1137
9	GDF-15	0.0037	0.009	0.0513
10	LTBR	0.0006	0.0089	0.0539
11	Mesothelin	0.0847	0.1264	0.1191
12	MPO	0.0057	0.3247	0.4616
13	Neuropilin 1	0.0252	0.6482	0.5284
14	NGAL	0.6881	0.796	0.81
15	NGAL plasma specific	0.2433	0.2479	0.1317
16	NRP-1	0.8007	0.3993	0.7574
17	NTProCNP	0.3263	0.0232	0.0038
18	Osteopontin	0.0311	0.0087	0.0501
19	Pentraxin 3	0.3468	0.6366	0.8867
20	Periostin	0.0325	0.1129	0.2631
21	PIGR	0.1493	0.0907	0.0842
22	PSAP-B	0.0308	0.0694	0.6157
23	RAGE	0.0364	0.1519	0.4358
24	ST-2	0.3566	0.0431	0.011
25	Syndecan-1	0.1514	0.7674	0.6162
26	TNFR1A	0.0081	0.0002	0.0065
27	Troy	0.003	0.0046	0.2421
28	VEGFR1	0.5642	0.5982	0.618
29	WAP4C	0.0005	0.0006	0.0209

Threshold p-value for significance 0.0017.

Materials and Methods

Participants

We used data from participants in the Heart and Soul Study. This is a prospective cohort study of patients with stable ischemic heart disease. Methods have been described in detail previously.[1] Between September 2000 and December 2002, 1024 subjects were recruited from outpatient clinics in the San Francisco Bay Area using ≥1 of the following criteria: (1) history of myocardial infarction (MI), (2) angiographic evidence of 50% stenosis in ≥1 coronary vessels (3) evidence of exercise-induced ischemia by treadmill or nuclear testing, or (4) history of coronary revascularization. At the baseline examination, individuals underwent a medical history, physical examination, and comprehensive health status questionnaire. After a 12-hour fast, morning venous blood samples were drawn. A five-year follow-up visit including repeat kidney measures was performed in over 80% of all survivors (N= 667). For this study, we excluded individuals without measures of sESAM (N=34).

Primary Predictors

Our primary predictor was the level of serum sESAM, which was measured on stored bsaeline serum samples by Luminex multiplexed bead-based immunoassays (Alere, San Diego, CA). Serum samples were obtained after a 12-hour fast and were stored at -80^{o}C with no freeze-thaw cycles before measurement. sESAM was measured along with 22 other biomarkers on the Luminex platform by sandwich assay: LTBR, Mesothelin, MPO, Neuropilin 1, Osteopontin, Pentraxin 3, Periostin, PIGR, PSAP-B, ST-2, Syndecan-1, TNFR1A, Troy, BNP, RAGE, VEGFR1, NTProCNP, ANP propeptide, Galectin 3, D-Dimer, GDF-15, NGAL plasma-specific. In addition, 6 markers were measured on separate Luminex competitive antigen platforms: Angiogenin, CRP, cystatin C, WAP4C, NGAL, and NRP-1. The range of sESAM in our study overlaps with previously reported ranges in the Dallas Heart Study.[2] The range of detection was 0.087 to 130 ng/mL and intra- and inter-assay coefficients of variation (CV) for ESAM were 5% and 6% at a concentration of 31 ng/mL.

Adjusted Variables

All covariates in this analysis were taken from the baseline study visit and examination. Demographic characteristics, medical history, and smoking status were ascertained by standardized questionnaire. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Resting systolic and diastolic blood pressure (SBP and DBP) and heart rate were measured by standard, calibrated sphygmomanometer in the supine

position after 5 minutes rest by trained study personnel. Participants were asked to bring their medication bottles to all study visits, and research personnel recorded all current medications, including angiotensin converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), and statins. High-density lipoprotein (HDL) and total cholesterol were measured from fasting serum samples. The Friedewald equation was used to calculate low-density lipoprotein (LDL) cholesterol concentrations.[3] C-reactive protein (hs-CRP) was measured by the Roche Integra high-sensitivity assay.

Outcomes

Serum creatinine was measured by the rate Jaffe method (mg/dl) in baseline serum samples. Cystatin C was measured from frozen samples collected at the baseline study visit with the use of a BNII nephelometer (Dade Behring Inc., Deerfield, IL, USA) with a particle-enhanced immunonephelometric assay (N Latex Cystatin C; Dade Behring, Inc.).[4] Estimated glomerular filtration rate (eGFR) was determined by the combined creatinine-cystatin C equation ($eGFR_{cr\text{-}cys}$).[5] Baseline reduced eGFR was defined as an $eGFR_{cr\text{-}cys}$<60 ml/min/1.73 m^2 based on KDIGO definitions.[6] Rapid kidney function decline was defined as a change in $eGFR_{cr\text{-}cys}$ of greater than 3% per year as in our prior work.[7] Annualized percent change in eGFR was calculated from the difference between baseline and 5-year follow-up $eGFR_{cr\text{-}cys}$. Incident reduced eGFR was defined as a new $eGFR_{cr\text{-}cys}$<60 ml/min/1.73 m^2 at follow-up with a concomitant $eGFR_{cr\text{-}cys}$ decline of ≥1 ml/min per year. We chose this definition in order to reduce misclassification due to changes close to the eGFR threshold of 60 ml/min/1.73 m^2.

Urine albumin and creatinine were measured in a 24-hour urine collection at baseline and at the 5-year follow-up visit. At the intake appointment, participants were provided with a 3-L collection jug for urine and were asked to save all urine between the end of their intake appointment and the time when a research technician recovered the urine at the participant's home 24 hours later. Participants were instructed to keep the urine collections refrigerated at all times. The research technician arrived at the patient's home 24 hours after the timed collection was initiated to avoid over- or under-collection. If participants reported missing any urine or if the collections were <1 or >3 liters, then collections were repeated. If participants were unable to collect all urine for any reason or had urinary incontinence, then no data were recorded. A urine albumin to creatinine ratio (ACR) was calculated in mg/g from the 24-hour sample. Baseline albuminuria was defined as urine ACR ≥ 30 mg/g based on KDIGO definitions.[8] Change in ACR was calculated as the absolute difference between year 5 follow-up ACR and baseline.

Statistical Analyses

We first described the baseline characteristics of all participants by quartile of sESAM. In assessing the associations of sESAM levels with kidney outcomes, we used linear and logistic models for continuous and binary outcomes respectively. To capture possible non-linearities, we compared outcomes by quartile of sESAM. To obtain adjusted mean values of the outcome by quartile, we used regression standardization.

For all regression models, adjustment variables included demographic characteristics (age, sex, race); lifestyle characteristics (smoking, alcohol use, BMI, physical activity); and comorbid conditions (diabetes mellitus, hypertension, SBP, DBP, LDL and HDL cholesterol, medications, and hs-CRP). Covariates were selected based on evaluation of known confounders of atherosclerosis and kidney disease. In models for changes in eGFR, we adjusted for the baseline value. For changes in ACR, we also conducted stratified analyses by eGFR status. Given the irregular distribution of change in ACR, we also conducted a sensitivity analysis of the association between sESAM and change in ACR using a proportional odds model with ordinal change in ACR categories of ≤-300, -300 to -30, -30 to 30, 30 to 300, and >300 mg/g.

Our biomarker was measured on a multiplexed Luminex Alere platform. Because this platform was used in the Heart and Soul cohort to measure a total of 29 markers for disparate scientific purposes, we employed a Bonferroni correction for a threshold p-value of 0.001724 (0.05/29). For complete statistical description, we also reported the p-value for the overall test of heterogeneity of each model.

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