Plasma apoM and S1P levels are inversely associated with mortality in African Americans with type 2 diabetes mellitus

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Abstract apoM is a minor HDL apolipoprotein and carrier for sphingosine-1-phosphate (S1P). HDL apoM and S1P concentrations are inversely associated with atherosclerosis progression in rodents. We evaluated associations between plasma concentrations of S1P, plasma concentrations of apoM, and HDL apoM levels with prevalent subclinical atherosclerosis and mortality in the African American-Diabetes Heart Study participants (N = 545). Associations between plasma S1P, plasma apoM, and HDL apoM with subclinical atherosclerosis and mortality were assessed using multivariate parametric, nonparametric, and Cox proportional hazards models. At baseline, participants' median (25th percentile, 75th percentile) age was 55 (49, 62) years old and their coronary artery calcium (CAC) mass score was 26.5 (0.0, 346.5). Plasma S1P, plasma apoM, and HDL apoM were not associated with CAC. After 64 (57.6, 70.3) months of follow-up, 81 deaths were recorded. Higher concentrations of plasma S1P [odds ratio (OR) = 0.14, *P* = 0.01] and plasma apoM (OR = 0.10, *P* = 0.02), but not HDL apoM (*P* = 0.89), were associated with lower mortality after adjusting for age, sex, statin use, CAC, kidney function, and albuminuria. We conclude that plasma S1P and apoM concentrations are inversely and independently associated with mortality, but not CAC, in African Americans with type 2 diabetes after accounting for conventional risk factors.—Liu, M., C. Frej, C. D. Langefeld, J. Divers, D. W. Bowden, J. J. Carr, A. K. Gebre, J. Xu, B. Larsson, B. Dahlbäck, B. I. Freedman, and J. S. Parks. **Plasma apoM and S1P levels are inversely associated with mortality in African Americans with type 2 diabetes mellitus.** ***J. Lipid Res.*** **2019.** 60: **1425–1431.**

Supplementary key words apolipoproteins • high density lipoprotein • atherosclerosis • clinical studies • ethnicity • apolipoprotein M • sphingosine-1-phosphate

Increasing numbers of patients with type 2 diabetes mellitus and associated diabetes-related complications contribute to the escalating social and economic burden on patients, families, and healthcare systems around the world. Identifying and managing risk factors for diabetes-associated cardiovascular and renal complications is critical to reducing mortality; however, this has proven to be challenging.

apoM is a dedicated carrier of sphingosine-1-phosphate (S1P), which is a signaling lipid that affects in vivo cellular/tissue function, including vascular development, lymphocyte trafficking, lymphopoiesis, cell growth and survival, cytoskeleton rearrangement, cell motility, invasion, and angiogenesis (1–3). apoM circulates in plasma primarily anchored to HDL particles via its retained signal peptide (4); apoB lipoproteins also bind small amounts of apoM (5). HDL apoM may be atheroprotective by stimulating preβ HDL formation (6, 7), promoting cellular cholesterol efflux (6–8), and thereby increasing HDL's antioxidant activity (7–9) and S1P-mediated vascular benefit (10–15). Understanding of the role of apoM in atherosclerosis progression in mouse models has been confounded by differences in outcomes

This work was supported by National Institutes of Health Grants R01 DK071891 (B.I.F.) and R01 HL119962 (J.S.P.) and the Swedish Research Council (B.D.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors declare no conflicts of interest related to this work.

Manuscript received 20 August 2018 and in revised form 27 April 2019.

Published, JLR Papers in Press, May 27, 2019
DOI https://doi.org/10.1194/jlr.P089409

Abbreviations: AA-DHS, African American-Diabetes Heart Study; CAC, coronary artery calcium; CHD, coronary heart disease; eGFR, estimated glomerular filtration rate; GHb, glycosylated hemoglobin; HR, hazard ratio; OR, odds ratio; S1P, sphingosine-1-phosphate; S1PR, S1P receptor; UACR, urine albumin:creatinine ratio.

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that appear related to genetic background and atherosclerosis site (7, 16, 17).

How apoM and S1P affect CVD risk in humans is also unclear. Two case-control studies showed that plasma apoM concentrations did not predict risk of developing coronary heart disease (CHD) (18). With respect to S1P, one study demonstrated that it was a strong predictor of occurrence and severity of coronary stenosis (19), whereas others found an inverse association between CHD and HDL S1P (20, 21). However, less than 30% of participants in these studies had diabetes and most were of European ancestry. In addition, studies did not examine HDL apoM levels.

African Americans with type 2 diabetes are at higher risk for CVD than their counterparts of European ancestry (22). Yet how apoM and S1P affect mortality and CVD outcomes in non-European populations is unknown. The African American-Diabetes Heart Study (AA-DHS) was initiated to identify inherited and environmental risk factors contributing to mortality, subclinical CVD, and bone and kidney disease in a cohort with type 2 diabetes. The present analyses were designed to test whether plasma concentrations of S1P or apoM and HDL apoM are correlated with subclinical CVD and mortality in this high-risk and understudied population.

RESEARCH DESIGN AND METHODS

Study populations

The AA-DHS recruited unrelated African Americans at the Wake Forest School of Medicine from 2007 to 2010. Recruitment criteria and objectives were previously reported (23). Type 2 diabetes mellitus was clinically diagnosed based on disease onset after the age of 30 years in the absence of diabetic ketoacidosis and with active glucose-lowering treatment (insulin and/or oral agents), a fasting glucose ⩾7.0 mmol/l (126 mg/dl), nonfasting glucose ⩾11.1 mmol/l (200 mg/dl), or glycosylated hemoglobin (GHb) 48 mmol/mol (6.5%). Individuals with prior serum creatinine concentrations ⩾176.8 μmol/l (2 mg/dl) were not eligible. The study abides by the Declarations of Helsinki Principles and was approved by the Wake Forest School of Medicine Institutional Review Board and all participants provided written informed consent. Baseline assessments consisted of interviews for medical history, anthropometric measurements, blood pressures, fasting blood (glucose, serum creatinine, GHb, lipid panels), imaging, and a spot urine test for urine albumin:creatinine ratio (UACR). Estimated glomerular filtration rate (eGFR) was computed using the creatinine-based CKD-EPI equation (24).

Vascular imaging

Subclinical atherosclerosis was assessed as calcified atherosclerotic plaque in the coronary arteries [coronary artery calcium (CAC)] using single and multidetector computed tomography systems. As reported earlier, a standardized scanning protocol based on the Multi-Ethnic Study of Atherosclerosis protocol was employed (25). Scoring parameters included a 90 Hounsfield unit threshold and two adjacent pixels to define maximum calcified lesion sizes. Because traditional Agatston (calcium) scores add noise to the computed tomography measurement of calcified plaque compared with volume-based measures, we used the calcium mass score (milligrams of calcium) derived from the volume score and accounting for density of calcified plaque on a pixel-by-pixel basis (25). Log(CAC + 1) was used as the covariate, to include participants without evidence of calcification. We excluded any quantitative CAC mass scores from participants who had previously undergone coronary artery bypass grafting, stenting, or angioplasty because these procedures could impact CAC. However, in sensitivity analyses, we imputed CAC mass scores for these individuals and repeated all analyses to ensure that data from participants with prior coronary artery procedures did not drive the results (see the Statistical analysis section).

Vital status

Vital status was assessed through December 31, 2016 using the National Death Index.

HDL isolation

Frozen (−80°C) plasma samples from 543 participants at the AA-DHS baseline visit were used for HDL isolation. Seventy microliters of plasma were adjusted to 0.25 ml using saline. The density was adjusted to 1.21 g/ml using saturated KBr. Samples were then overlaid with 1.44 ml of 1.21 g/ml KBr and centrifuged for 4 h at 435,000 *g* at 15°C using a TLA 120.1 rotor. Centrifuge tubes were sliced and top fractions (⩽0.4 ml) were collected. The slicer and tubes were rinsed twice with 457 μl of saline and solutions were combined with the top fractions before being overlaid with 686 μl 1.063 g/ml KBr and spun for 4 h at 435,000 *g* at 15°C as above. Bottom fractions containing HDL were collected and weighed, and volumes were calculated based on the final density of the fraction (1.082 g/ml). An HDL sample volume equivalent to 20 μl of the original plasma for each sample was aliquoted, diluted with Triton BSA buffer, and stored at −80°C before being shipped frozen to Lund University (Malmo, Sweden), along with aliquots of frozen plasma, for apoM and S1P quantification.

apoM ELISA

apoM ELISA was performed on plasma and HDL samples following previously reported methods (26).

S1P quantification

S1P was quantified as previously described (27). Briefly, S1P standard solutions were made by diluting S1P (Avanti Polar Lipids, Alabaster, AL) in 4% BSA with concentrations ranging from 0.0037 to 0.9 μM S1P. Human plasma was diluted 6.5 times with TBS [50 mM Tris-HCl (pH 7.5), 0.15 mM NaCl]. We added 200 μl of precipitation solution (methanol containing 20 nM internal standard) to 65 μl of standard or diluted human plasma; the solution was vortexed for 30 s followed by centrifugation at 17,000 *g* for 2 min. Supernatant (5 μl) was injected for the liquid chromatography tandem mass spectrometry analysis. Data were interpreted using Analyst software 1.6 (AB Sciex).

Statistical analysis

Demographic and clinical data were summarized using the 25th, 50th (median), and 75th percentile values. To test for differences between those still alive versus deceased on December 31, 2016, we computed contingency table chi-square tests for independence of categorical variables and Wilcoxon rank sum tests for continuous variables. Vascular calcium and UACRs were natural logarithm-transformed after adding 1 to their value to retain 0 values. Transformations were used to better approximate distributional assumptions of the models or minimize effects of outliers.

Plasma S1P, plasma apoM, and HDL-associated apoM were tested for associations with quantitative CAC mass score (natural logarithm of CAC + 1), presence versus absence of CAC (CAC >10 present; CAC ⩽10 absent), all-cause mortality (i.e., dead vs. alive), and survival time adjusted for age, sex, statin use, eGFR, and log

CAC (except when CAC was the outcome). For the natural logarithm of CAC + 1, we created a Tobit regression model to account for the significant number of CAC mass scores of 0. The Tobit model is a regression model that explicitly accounts for a count of observations at 0 and the remaining data following an approximate conditional normal distribution, in this case, the natural logarithm of CAC + 1 transformation. We tested associations between S1P and apoM measures and presence versus absence of CAC using logistic regression.

Quantitative CAC association analyses were repeated with inclusion of previously excluded participants by giving them an imputed CAC value. The imputation algorithm identified the median CAC (logCAC >3.3) and employed regression imputation to predict log(CAC + 1) based on age, sex, and log measures of aortic and carotid calcified atherosclerotic plaque.

We used logistic regression to test associations of all-cause mortality with plasma concentrations of S1P and apoM, and HDL-associated apoM levels using logistic regression. Finally, we used Cox proportional hazards models to test associations among S1P and apoM concentrations and mortality. In each model tested, model assumptions were examined.

RESULTS

The cohort included 545 unrelated African Americans with type 2 diabetes; 543 had apoM and S1P measurements available for this analysis. Median follow-up through December 31, 2016 was 63.6 (57.6, 70.3) months. During this period, 81 deaths were recorded. Causes of death reported on death certificates were CVD in 23.5% (N = 19), cancer in 28.4% (N = 23), diabetes in 6.2% (N = 5), infection in 7.4% (N = 6), and other in 34.6% (N = 28). **Table 1** displays baseline demographic and clinical characteristics in the full sample and stratified by living and deceased status. Participants were 56.7% female with median age 55 (49, 62) years. Deceased individuals tended to be older, male, and with a history of smoking. They also had higher rates of angina, stroke, and coronary artery bypass surgery. **Table 2** displays baseline biochemical and radiographic measurements in the cohort. Median baseline CAC was 26.5 (0.0, 346.5) mg Ca^{2+}, eGFR 91.3 (76.1, 111.3) ml/min/1.73 m^2, UACR 1.47 (0.47, 6.01) mg/mmol, plasma S1P 0.81 (0.69, 0.93) μM, plasma apoM 0.76 (0.64, 0.89) μM, and HDL apoM 0.42 (0.31, 0.52) μM. Most biochemical and radiographic measures differed in deceased individuals compared with living individuals, except fasting glucose, GHb, HDL, and triglycerides.

Plasma apoM and HDL apoM level did not correlate with CAC

Table 3 presents results of CAC multivariate risk factor association analyses. The model adjusted for age, sex, statin use, eGFR, and UACR. Although sex (male risk) and increasing albuminuria were significantly associated with CAC, we found no significant associations between CAC and plasma S1P ($P = 0.76$), plasma apoM ($P = 0.48$), or HDL apoM ($P = 0.97$). Inferences remained consistent when including imputed CAC values (see the Statistical analysis section) among those initially excluded due to presence of clinical coronary artery disease (results not shown).

Plasma S1P and apoM levels, but not HDL apoM, are inversely associated with all-cause mortality

We created a multiple logistic model to test for associations of plasma S1P, plasma apoM, and HDL apoM with mortality (**Table 4**), adjusting for age, sex, statin use, CAC, eGFR, and UACR. As expected, female sex and statin use were associated with lower mortality, and higher levels of CAC and higher UACRs were associated with higher mortality. In this multivariate model, higher plasma S1P [$P = 0.01$; odds ratio (OR) = 0.14] and apoM ($P = 0.02$; OR = 0.10) levels were significantly associated with lower mortality. In contrast, HDL apoM levels were not associated with mortality ($P = 0.87$).

A parallel Cox proportional hazards model was computed to test for association of plasma S1P, plasma apoM, and HDL apoM with overall survival (**Table 5**), adjusting for age, sex, statin use, CAC, eGFR, and UACR. The correlation between plasma S1P and plasma apoM ($r = 0.24$; $P < 0.0001$) dampened the effect of each on overall survival. In the model including plasma S1P, plasma apoM, and HDL apoM, plasma S1P [$P = 0.13$; hazard ratio (HR) = 0.29] and plasma apoM ($P = 0.10$; HR = 0.26) showed trends toward being associated with survival. When plasma S1P was removed from the model, plasma apoM

TABLE 1. Baseline demographic and clinical characteristics of the AA-DHS cohort

	AA-DHS Cohort						
Variables	Full Sample	N	Living	N	Deceased	N	*P*
Age (y)	55 (49, 62)	545	55 (49, 61)	464	58 (52, 67)	81	0.0258
Female (%)	56.7	545	60.1	464	37.0	81	0.0001
Smoking (%)		545		464		81	0.0101
Never	41.5		43.8		28.4		
Former	35.2		34.9		37.0		
Current	23.3		21.3		34.6		
Follow-up time (months)	63.6 (57.6, 70.3)	545	63.2 (57.6, 69.6)	464	65.3 (59.9, 75.3)	81	0.0246
Angina (%)	14.8	515	13.4	441	23.0	74	0.0313
Heart attack (%)	10.0	353	9.5	461	11.7	77	0.5590
Stroke (%)	7.8	539	6.9	461	12.8	78	0.0732
Coronary artery bypass surgery (%)	3.4	545	3.2	464	7.4	81	0.0717
Coronary angioplasty (%)	13.1	543	13.0	463	13.8	80	0.8463
Statin use (%)	49.8	544	50.8	463	44.4	81	0.2946

Data are expressed as median (25th percentile, 75th percentile) for continuous variables or percent. *P* for comparisons of living versus deceased participants.

TABLE 2. Baseline biochemical and radiological characteristics of the AA-DHS cohort

Variables	AA-DHS Cohort						
	Full Sample	N	Living	N	Deceased	N	*P*
Fasting glucose (mmol/l)	7.41 (5.94, 9.71)	540	7.38 (5.94, 9.66)	459	7.60 (6.16, 10.32)	81	0.3955
GHb (%)	7.7 (6.6, 9.1)	531	7.7 (6.6, 9.1)	453	7.7 (6.7, 9.3)	78	0.9787
Serum creatinine μmol/l	80.0 (70.7, 97.2)	540	79.6 (70.7, 97.2)	459	88.4 (70.7, 97.2)	81	0.0060
HDL cholesterol (mmol/l)	1.19 (0.98, 1.40)	540	1.17 (0.98,1.40)	459	1.19 (0.98, 1.42)	81	0.6909
LDL cholesterol (mmol/l)	2.67 (2.15, 3.39)	529	2.72 (2.20, 3.44)	449	2.42 (1.83, 3.03)	80	0.0019
Triglycerides (mmol/l)	1.18 (0.87, 1.65)	540	1.18 (0.87, 1.65)	459	1.13 (0.86, 1.56)	81	0.4587
Coronary artery CP mass (mg Ca^+)	26.5 (0.0, 346.5)	463	17.5 (0.0, 256)	398	184 (7.0, 854)	65	0.0002
Coronary artery CP >10 mg Ca^+ (%)	56.6	463	54.0	398	72.3	65	0.0058
Carotid artery CP mass (mg Ca^+)	4.0 (0.0, 104)	537	2.5 (0.0, 87.0)	458	52.5 (0.0, 284.5)	79	0.0003
Carotid artery CP >10 mg Ca^+ (%)	45.6	537	42.6	458	63.3	79	0.0006
Aorta CP mass (mg Ca^+)	1,049 (28, 6,368)	535	839 (17, 5575)	456	2663 (400, 11764)	79	0.0003
Aorta CP >10 mg Ca^+ (%)	78.5	535	77.0	456	87.3	79	0.0384
Vascular beds with CP, N	2 (1, 3)	540	2 (1, 2)	460	2 (1, 3)	80	0.0050
eGFR (ml/min/1.73 m^2)	91.3 (76.1, 111.3)	540	91.4 (76.6, 113.0)	459	88.8 (70.8, 104.0)	81	0.0765
eGFR <60 ml/min/1.73 m^2 (%)	9.6	540	8.5	459	16.0	81	0.0336
UACR (mg/mmol)	1.47 (0.47, 6.01)	535	1.24 (0.46, 4.97)	455	4.64 (0.64, 15.0)	80	0.0002
UACR >3.39 mg/mmol (%)	36.3	535	32.5	455	57.5	80	<0.0001
Plasma apoM (μM)	0.76 (0.64, 0.89)	544	0.78 (0.65, 0.91)	463	0.68 (0.53, 0.80)	81	<0.0001
HDL apoM (μM)	0.42 (0.31, 0.52)	544	0.42 (0.33, 0.53)	464	0.34 (0.22, 0.48)	80	0.0008
S1P	0.81 (0.69, 0.93)	544	0.83 (0.70, 0.94)	463	0.74 (0.63, 0.87)	81	0.0003

Data are expressed as median (25th percentile, 75th percentile) for continuous variables or percent. *P* for comparisons of living versus deceased participants. CP, calcified plaque.

became significant ($P = 0.0280$; HR = 0.18); when plasma apoM was removed from the model, plasma S1P became significant ($P = 0.0373$; HR = 0.20). These results suggest that plasma S1P and apoM independently contribute to survival. HDL apoM did not approach significance in any models.

DISCUSSION

The present study tested to determine whether plasma S1P, plasma apoM, and HDL apoM concentrations are associated with subclinical atherosclerosis and mortality in African Americans with type 2 diabetes mellitus. After adjusting for age, sex, statin use, eGFR, and UACR, we observed no significant associations among plasma S1P, plasma apoM, or HDL apoM and CAC, a measure of subclinical atherosclerosis. In contrast, higher concentrations of plasma S1P and plasma apoM (but not HDL apoM) were associated with lower mortality. Conventional CVD risk factors were also associated with mortality in this study, including male sex, increasing CAC, higher levels of albuminuria, and no statin therapy. Our results suggest that plasma concentrations of S1P and plasma apoM contribute independently to risk of mortality in the AA-DHS cohort, after accounting for age, sex, statin use, CAC, kidney function, and albuminuria.

TABLE 3. Prevalent subclinical atherosclerosis, as measured by natural logarithm of CAC, on multivariate risk factor analyses

Parameter	Estimate	Standard Error	T Value	*P*
Intercept	−4.941	1.422	−3.47	0.0005
Age	0.148	0.015	9.83	<0.0001
Female sex	−0.702	0.259	−2.71	0.0067
Statin use	0.487	0.260	1.87	0.0610
eGFR	−0.00008	0.0060	−0.13	0.8993
Log UACR	0.292	0.097	3.00	0.0027
Plasma apoM	−0.643	0.905	−0.71	0.4773
HDL apoM	−0.044	1.132	−0.04	0.9687
S1P	0.200	0.668	0.30	0.7647
σ	2.805	0.103	—	—

Tobit regression analysis to account for CAC = 0; analyses transformed CAC to ln(CAC + 1) and UACR to ln(UACR + 1).

apoM, an HDL apolipoprotein discovered in 1999 (5), is a member of the lipocalin family of proteins, which bind small hydrophobic lipid molecules via a β sheet "clam shell-like" pocket (28, 29). Christoffersen et al. (14) reported that HDL S1P specifically binds to human and mouse apoM, suggesting a novel function for the subset of HDL particles containing apoM. S1P signaling affects vascular development, lymphocyte trafficking, lymphopoiesis, cell growth and survival, cytoskeleton rearrangement, cell motility, invasion, and angiogenesis (1–3). We previously reported that hepatic overexpression of apoM stimulates hepatic S1P production and assembly of a subset of S1P-containing lipoproteins in the hepatocyte secretory pathway, providing a specialized and unique S1P secretory and transport system that we hypothesized would reduce atherosclerosis by lowering macrophage cholesterol content and inflammation (30). In support of this concept, there is evidence that apoM stimulates preβ HDL formation (6, 7), promotes cellular cholesterol efflux (6–8), and increases HDL antioxidant activity (7–9). Without apoM, plasma S1P is significantly reduced and detected only in the albumin fraction. Furthermore, HDL containing apoM/S1P resulted in more robust p44/42 and Akt signaling in human umbilical vein endothelial cells compared with apoM-free HDL (14). Furthermore, apoM knockout mice had defective lung endothelial barrier function compared with wild-type mice (14).

Several studies have tested apoM or S1P concentrations for association with CVD in humans. The FINRISK '92 and Copenhagen City Heart Study, two case-control studies, suggested that plasma apoM does not predict risk for CHD (18) compared with controls. In another study that used

TABLE 4. Mortality risk based on multivariate logistic regression association analysis

Parameter	Estimate	Standard Error	Wald Chi-Square	*P*	OR	95% Confidence Limits
Intercept	1.744	1.567	1.24	0.2657	—	—
Age	0.0004	0.0179	0.00	0.9838	1.00	(0.97, 1.04)
Female sex	−0.947	0.282	11.32	0.0008	0.39	(0.22, 0.67)
Statin use	−0.831	0.286	8.42	0.0037	0.44	(0.25, 0.76)
Log CAC	0.164	0.061	7.24	0.0071	1.18	(1.05, 1.33)
eGFR	−0.006	0.006	0.81	0.3681	0.99	(0.98, 1.01)
Log UACR	0.324	0.096	11.44	0.0007	1.38	(1.15, 1.67)
Plasma apoM	−2.307	0.952	5.87	0.0154	0.10	(0.02, 0.64)
HDL apoM	−0.164	1.148	0.021	0.8862	0.85	(0.09, 8.06)
S1P	−1.970	0.804	6.00	0.0143	0.14	(0.03, 0.67)

Multivariate logistic regression models dead relative to alive, with OR for a change of one unit of the independent (predictor) variable. There is no evidence of a sex-by-plasma apoM interaction ($P = 0.53$) or a sex-by-HDL apoM interaction ($P = 0.48$).

samples from subjects with or without metabolic syndrome, plasma concentrations of apoM and intima-media thickness (measured by ultrasonography) were not associated (31). Others have reported that plasma concentrations of apoM are negatively associated with inflammatory markers and positively associated with 1 and 3 year survival rates in patients with critical limb ischemia, a severe form of atherosclerosis (32).

Serum S1P was a strong predictor of occurrence and severity of coronary stenosis in one study (19), but another study using plasma found that HDL S1P was lower in subjects with myocardial infarction and stable CHD compared with control subjects (20). S1P concentrations in apoB lipoprotein-depleted serum, which does not distinguish between HDL and albumin S1P, was inversely associated with ischemic heart disease in 204 subjects from the Copenhagen Heart Study, regardless of whether subjects had low (34–39 mg/dl) or high (62–74 mg/dl) serum HDL cholesterol concentrations (21). However, using serum to measure S1P levels might not reflect the circulatory levels because activated platelets release high amounts of stored S1P (27). In the current study within the AA-DHS cohort, we detected no significant cross-sectional associations between plasma or HDL apoM or plasma S1P levels with subclinical CVD (assessed as CAC) in multivariate risk factor analyses.

S1P has complex and sometimes opposing roles in different cell types involved in atherosclerosis progression, which could explain differences in results from clinical studies. For example, S1P affects monocyte migration and endothelial cell adhesion, endothelial cell activation, smooth muscle cell proliferation, vascular tone, and cellular inflammatory signaling (33). Deletion of the S1P receptor, S1PR1, in apoE knockout mice did not increase intimal area, but macrophage and smooth muscle cell content within atherosclerotic plaque was significantly reduced compared with control mice (34). S1PR3 also was involved in S1P-mediated macrophage migration in vitro and in vivo and inflammatory responses, suggesting that macrophage S1P signaling through S1PR3 is proinflammatory (34). Whole body and bone marrow S1PR2 expression is both proatherogenic and proinflammatory (35). Several studies of S1P in atherosclerosis used FTY720, which is phosphorylated in vivo to a S1P mimetic that binds to S1PR1 (and S1PR3–5), inducing its internalization and degradation. FTY720 treatment reduced atherosclerosis in LDL receptor knockout (36) and apoE knockout mice (37). However, in another study in apoE knockout mice, FTY720 at a higher dose did not affect early or advanced atherosclerosis, despite 2.4-fold increased plasma cholesterol concentrations and lymphopenia (38). Marked differences in experimental design among these studies, as discussed by Klingenberg et al. (38), may contribute to these disparate results.

Overexpression of apoM leads to atheroprotection in apoE knockout mice (16) and in some (6, 7), but not all (16), studies using LDL receptor knockout mice. Different outcomes may be due to increased proatherogenic apoB lipoprotein concentrations in atherogenic diet-fed male mice with apoM overexpression, which negates the anticipated atheroprotection (16). There may also be site-specific effects on atheroprotection, as apoM overexpression protects against aortic root atherosclerosis in both apoE knockout and LDL receptor knockout mice, whereas whole aorta atheroprotection based on en face quantification was not observed (7, 16). Only one study examined atherosclerosis in apoM knockout mice and observed decreased en face lesion area in apoM/LDL receptor double

TABLE 5. Overall survival based on Cox proportional hazards multivariate risk factor analysis

Parameter	Parameter Estimate	Standard Error	Chi-Square	*P*	HR
Age	−1.236	0.117	111.39	<0.0001	0.291
Female sex	−0.626	0.261	5.73	0.0167	0.535
Statin use	−0.902	0.287	9.91	0.0016	0.406
Log CAC	0.138	0.057	5.87	0.0154	1.148
eGFR	−0.011	0.006	3.29	0.0699	0.989
Log UACR	0.209	0.088	5.63	0.0177	1.232
Plasma apoM	−1.340	0.820	2.67	0.1021	0.262
HDL apoM	−0.162	0.957	0.03	0.8656	0.851
S1P	−1.228	0.806	2.32	0.1273	0.293

knockout mice compared with LDL receptor knockout mice (16); however, plasma cholesterol concentrations were significantly reduced in apoM/LDL receptor double knockout mice, confounding interpretation of the role of apoM in atheroprotection.

Previous studies also support a role of apoM and apoM-carried S1P in regulating endothelial barrier integrity and inflammation; these are critical determinants in the progression of endothelial dysfunction. apoM knockout mice have increased endothelial barrier leakage dependent on S1P signaling (14, 39). HDL-carried apoM/S1P also limits neuroinflammation (2) and vascular inflammation (13, 15). apoM and S1P levels are negatively associated with severity of sepsis (40–42), and apoM overexpression protects mice from organ failure and death in lipopolysaccharide-induced sepsis (43). These effects of the apoM/S1P complex could contribute to the negative associations between plasma apoM and S1P levels and all-cause mortality seen in our study.

We also found that HDL apoM levels were not significantly associated with mortality. Discordant associations between all-cause mortality and plasma concentrations of apoM and S1P versus HDL apoM may be due to beneficial (i.e., anti-inflammatory) effects of non-HDL-bound apoM. However, the use of ultracentrifugation to isolate HDL, which may lead to loss of HDL apoM, and frozen plasma samples for our analyses may introduce technical issues that limit our conclusions regarding a distinct role for HDL versus non-HDL apoM and S1P in mortality. Although our results do not establish a causal relationship between plasma apoM or S1P and mortality, our data suggest that plasma apoM or S1P may be a useful biomarker for mortality in African Americans with type 2 diabetes.

This is the first report to assess relationships between plasma concentrations of S1P and apoM and HDL apoM concentrations on subclinical CVD and mortality in African Americans with type 2 diabetes. The deep phenotyping of the AA-DHS cohort included data on kidney function, albuminuria, and multiple CVD risk factors. The AA-DHS was also enriched for participants with diabetes without advanced nephropathy at enrollment, minimizing possible confounding between kidney disease and mortality. However, we lack data on carotid intima-medial thickness in this cohort. In addition, the primary outcome was all-cause mortality, not CVD-related death. Finally, although many deaths in the cohort were likely related to cardiovascular causes, we could not confirm them based on death certificates from the National Death Index.

In conclusion, results from this well-characterized cohort of African Americans with type 2 diabetes mellitus demonstrated significant and independent inverse associations between mortality and plasma concentrations of S1P and apoM.

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