Kidney function is associated with an altered protein composition of high-density lipoprotein

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Patients with chronic kidney disease (CKD) exhibit a myriad of metabolic derangements, including dyslipidemia characterized by low plasma concentrations of high-density lipoprotein (HDL)–associated cholesterol. However, the effects of kidney disease on HDL composition have not been comprehensively determined. Here we used a targeted mass spectrometric approach to quantify 38 proteins contained in the HDL particles within a CKD cohort of 509 participants with a broad range of estimated glomerular filtration rates (eGFRs) (CKD stages I-V, and a mean eGFR of 45.5 mL/min/1.73m^2). After adjusting for multiple testing, demographics, comorbidities, medications, and other characteristics, eGFR was significantly associated with differences in four HDL proteins. Compared to participants with an eGFR of 60 mL/min/1.73m^2 or more, those with an eGFR under 15 mL/min/1.73m^2 exhibited 1.89-fold higher retinol-binding protein 4 (95% confidence interval 1.34-2.67), 1.52-fold higher apolipoprotein C-III (1.25-1.84), 0.70-fold lower apolipoprotein L1 (0.55-0.92), and 0.64-fold lower vitronectin (0.48-0.85). Although the HDL apolipoprotein L1 was slightly lower among African Americans than among Caucasian individuals, the relationship to eGFR did not differ by race. After adjustment, no HDL-associated proteins associated with albuminuria. Thus, modest changes in the HDL proteome provide preliminary evidence for an association between HDL proteins and declining kidney function, but this needs to be replicated. Future analyses will determine if HDL proteomics is indeed a clinical predictor of declining kidney function or cardiovascular outcomes.

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Chronic kidney disease (CKD) leads to marked metabolic derangements, including a distinct profile of dyslipidemia that worsens with the progressive loss of kidney function. Dyslipidemia of CKD is classically characterized by hypertriglyceridemia and low circulating concentrations of high-density lipoprotein–associated cholesterol (HDL-C).[1–3] Notably, although serum HDL-C concentrations exhibit a strong, inverse association with cardiovascular disease (CVD) risk in the general population, this association is attenuated and eventually abrogated as the glomerular filtration rate (GFR) declines.[4–6] Furthermore, the clinical efficacy of statin therapy is no longer observed among patients with end-stage renal disease. These findings suggest that kidney disease confers complex changes in lipid and lipoprotein metabolism that are not completely assessed by standard serum lipid measurements.

HDLs encompass a complex group of heterogeneous particles that play important roles in vascular function and CVD risk. Previous studies have suggested alterations in specific HDL proteins among chronic dialysis patients.[7–11] However, these studies are limited by small sample sizes, comparisons of end-stage renal disease patients with healthy controls, untargeted measurement strategies, and the potential for confounding by comorbidities that are associated with kidney disease. To date, no studies have characterized potential differences in HDL composition across the spectrum of kidney function in a well-characterized clinical CKD cohort that is not undergoing dialysis.

We employed a targeted mass spectrometric approach to quantify HDL-associated proteins in 509 CKD patients across the complete spectrum of kidney function. The identification of key changes in HDL composition that occur with GFR loss could be critical for better understanding changes in lipoprotein metabolism that result from impaired kidney function, thereby generating a more comprehensive understanding of the metabolic derangements that are consequent to CKD. Moreover, this line of investigation could yield novel insights into the unique dyslipidemia of CKD, as well as into the lack of clinical efficacy of standard lipid-lowering therapies among advanced CKD patients. It might also promote the development of more effective treatment strategies. We hypothesized that progressive changes in the HDL proteome would be evident across a spectrum of GFRs in CKD patients.

RESULTS

Study participants

Among the 509 Seattle Kidney Study subjects who were included in this study, the mean age was 58 ± 13.9 years, and the mean estimated GFR (eGFR) was 45.5 ± 26.4 ml/min per 1.73 m^2 (Table 1). The prevalence of diabetes ranged from 41.3% among subjects with an eGFR of ≥60 ml/min per 1.73 m^2 to 61.8% among those with an eGFR of <15 ml/min per 1.73 m^2. Study subjects exhibited mild dyslipidemia, with a mean serum HDL-C concentration of 41.6 ± 17.1 mg/dl and mean serum triglyceride concentration of 162.2 ± 118.7 mg/dl. Serum HDL-C concentrations were modestly lower among subjects with an eGFR <15 ml/min per 1.73 m^2; however, this distinction was not statistically significant (Figure 1). There was no association between serum triglyceride concentrations and eGFR (Supplementary Figure S1).

Associations of eGFR with HDL-associated proteins

After accounting for multiple testing, eGFR was significantly associated with differences in 5 HDL-associated proteins ($P < 0.0015$; Figure 2; Supplementary Table S1). Specifically, lower eGFR was associated with higher HDL concentrations of apolipoprotein C-III (apoC-III) and retinol-binding protein 4 (RBP4) and with lower HDL concentrations of cholesteryl ester transfer protein, vitronectin, and apolipoprotein L1 (apoL1).

Associations of eGFR with these HDL-associated proteins remained significant after adjustment for demographics, body mass index, smoking, diabetes, statin use, and serum lipid concentrations (Table 2). Associations of eGFR with apoC-III, RBP4, cholesteryl ester transfer protein, vitronectin, and apoL1 were not materially altered by additional adjustment for the urine albumin:creatinine ratio. However, the association between eGFR and cholesteryl ester transfer protein was no longer met statistically significant ($P = 0.0023$). In fully adjusted models, the associations of eGFR with HDL protein concentrations appeared to be generally linear, with progressive differences in each HDL-associated protein across CKD stages (Figure 3).

To assess possible interactions between eGFR and other clinical variables, subgroup analyses were performed to compare the associations between eGFR and HDL protein abundance in subjects with and without current statin use, existing diabetes, and a history of coronary artery disease (Supplementary Table S2). No significant interactions were found between these subgroups and the associations between eGFR and HDL protein abundance.

Further characterization of apoL1 associations by race

The apoL1 concentration in HDL was lower among black compared to nonblack subjects (Figure 4a). However, the association of lower eGFR with lower HDL-associated apoL1 concentrations did not differ as a function of race (Figure 4b). There was no statistical interaction between race and the association of eGFR with the apoL1 concentration in HDL.

Associations of albuminuria with proteins in HDL

On further analysis of the targeted quantification of HDL-associated proteins and evaluations for associations with albuminuria, only apoL1 met statistical significance after accounting for multiple testing (Figure 5). Specifically, each doubling of the urinary albumin:creatinine ratio was associated with a 4% lower apoL1 concentration in HDL (95% confidence interval, 2%–6% lower; $P < 0.001$).This association remained unchanged in magnitude and statistical significance ($P = 0.0008$) after adjustment for demographic

Table 1 | Subject characteristics by estimated glomerular filtration rate (eGFR)

Characteristic	eGFR >60	eGFR = 45–60	eGFR = 30–45	eGFR = 15–30	eGFR <15
N	92	91	106	102	34
Age, yr	49.9 (±12.1)	57.3 (±11.6)	63.1 (±13.3)	60.6 (±14.9)	61.0 (±12.6)
BMI, kg/m^2	30.8 (±8.3)	31.9 (±6.8)	31.2 (±8.3)	31.3 (±7.9)	30.2 (±6.2)
eGFR (CKD-EPI), ml/min per 1.73 m^2	82.8 (±20.6)	53.9 (±9.0)	39.0 (±6.7)	22.7 (±4.7)	11.5 (±4.1)
Female sex	25 (27.2)	19 (20.9)	30 (28.3)	25 (24.5)	6 (17.6)
Prevalent diabetes	38 (41.3)	47 (51.6)	58 (54.7)	50 (49.0)	21 (61.8)
Prevalent CAD	19 (20.7)	29 (31.9)	41 (38.7)	42 (41.2)	7 (20.6)
Race					
White	56 (60.9)	59 (64.8)	74 (69.8)	60 (58.8)	22 (64.7)
Black	29 (31.5)	24 (64.8)	23 (21.7)	31 (30.4)	6 (17.6)
Other	7 (7.6)	8 (64.8)	9 (8.5)	11 (10.8)	6 (17.6)
Statin use	43 (46.7)	53 (58.2)	59 (56.2)	63 (62.4)	17 (51.5)
Current smoking	22 (24.2)	22 (24.7)	16 (15.5)	20 (20.2)	7 (21.9)
HDL-cholesterol, mg/dl	42.1 (±20.6)	38.7 (±14.9)	42.0 (±15.9)	39.2 (±14.5)	35.3 (±12.1)
LDL-cholesterol, mg/dl	113.6 (±55.1)	92.6 (±33.7)	101.4 (±41.8)	100.3 (±41.7)	90.9 (±35.9)
Triglycerides, mg/dl	157.9 (±126.7)	168.4 (±110.5)	152.1 (±109.5)	164.0 (±133.1)	167.1 (±115.0)
Total cholesterol, mg/dl	183.5 (±67.3)	161.9 (±41.9)	174.9 (±54.5)	173.4 (±61.1)	158.1 (±42.8)
Urine ACR (median, IQR)	65.3 (9.2–451.5)	30.4 (3.7–337.2)	73.1 (11.7–452.1)	189.8 (43.5–783.3)	484.0 (179.1–1510.3)
CRP (median, IQR)	1.9 (0.7–4.4)	3.0 (1.2–6.4)	2.8 (0.8–7.7)	3.0 (1.0–7.3)	2.0 (0.7–6.4)

ACR, albumin:creatinine ratio; BMI, body mass index; CAD, coronary artery disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CRP, C-reactive protein; HDL, high-density lipoprotein.
Clinical and demographic characteristics of the subjects classified by eGFR. Normally distributed data are presented as mean (±SD), and non-normally distributed data are presented as median (interquartile range; IQR). Diabetes and CAD prevalence, race, sex, smoking status, and statin use are presented as number of subjects (%). CAD is defined as any history of myocardial infarction, cardiac arrest, coronary artery bypass graft, or percutaneous coronary intervention.