Kidney Medicine

Urine Epidermal Growth Factor and Kidney Function Decline in Middle-Aged Adults

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Rationale & Objective: The diagnosis and prognostication of chronic kidney disease (CKD) largely rely on glomerular measures that may not reflect tubular damage. We investigated the associations of urine kidney tubule biomarkers with estimated glomerular filtration rate (eGFR) change among middle-aged adults, when chronic diseases typically emerge.

Study Design: An observational cohort study.

Setting & Participants: A total of 1,145 participants of the Coronary Artery Risk Development in Young Adults (CARDIA) study without CKD, hypertension, or cardiovascular disease at the year 20 visit.

Exposures: Seven different biomarkers of tubular health: urine epidermal growth factor (EGF), alpha-1-microglobulin (α1m), interleukin-18, kidney injury molecule-1, monocyte chemoattractant protein-1, uromodulin, and chitinase-3-like protein 1.

Outcomes: Ten-year eGFR change and incident reduced eGFR (new onset of eGFR < 60 mL/min/1.73 m^2).

Analytical Approach: We examined associations of tubular health biomarkers with 10-year eGFR change and incident reduced eGFR with linear mixed models and interval-censored proportional hazards regression models, respectively. Both minimally and fully adjusted models were controlled for urine creatinine levels.

Results: The mean age of participants was 44.8 ± 3.7 years, with 39% African American and 56% female. The average 10-year change in eGFR was -18.6 mL/min/1.73 m^2 (95% CI, -19.4 to -17.8). In contrast to the other tubular biomarkers, which showed conflicting results, EGF demonstrated strong, consistent associations with both kidney outcomes. Each 1-standard deviation (SD) higher EGF was associated with a 2.37 mL/min/1.73 m^2 (95% CI, 0.64-4.10) smaller 10-year decrease in eGFR and a 42% (95% CI, 4%-64%) lower risk of incident reduced eGFR in the fully adjusted model.

Limitations: Observational design, measurements of eGFR were done only at 5-year intervals during follow-up.

Conclusions: In middle-aged, community-dwelling adults without hypertension, cardiovascular disease or CKD, higher urine EGF concentrations are associated with slower eGFR decline, whereas other kidney tubule biomarkers lacked a consistent association with kidney function decline.

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Chronic kidney disease (CKD) is a highly prevalent disease in adults in the United States that carries significant risks of morbidity and mortality.[1,2] Although more than half of the kidney's mass consists of proximal tubules, and the vast majority of the kidney's energy consumption occurs within the tubules, it is noteworthy that current CKD measures predominantly center on glomerular filtration (estimated glomerular filtration rate [eGFR]) and injury (albuminuria).[3] This glomerulocentric approach incompletely captures the potential role of kidney tubule damage in CKD development and progression.[4-6]

In a healthy population without CKD (kidney donors who underwent kidney biopsy), tubular atrophy was common and more severe with older age despite these individuals having normal glomerular filtration rates.[7] Among individuals with CKD, previous studies have found evidence of interstitial fibrosis and tubular atrophy in kidney biopsies to be among the strongest predictors of progression to kidney failure.[8-10] Although the role of kidney tubule measures in the diagnosis and prognostication of CKD has been investigated in several cohorts, it remains unknown whether differences in measures of kidney tubule health are associated with differential risk for the development of CKD in relatively healthy middle-aged adults, a period of life when chronic diseases emerge.[11-18]

In the current study, we examined 7 urine biomarkers of kidney tubule health capturing tubular injury (kidney injury molecule-1 [KIM-1], and interleukin-18 [IL-18]), tubular inflammation and fibrosis (monocyte chemoattractant protein-1 [MCP-1] and chitinase-3-like protein 1 [YKL-40]), reabsorption (alpha-1-microglobulin [α1m]), and synthetic function and regeneration (uromodulin [UMOD], and epidermal growth factor [EGF]) to investigate their associations with subsequent 10-year changes in eGFR and incidence of reduced eGFR among participants in the Coronary Artery Risk Development in Young Adults (CARDIA) study who were free of CKD, hypertension, and cardiovascular disease (CVD).

METHODS

Study Population and Design

We conducted a study nested within CARDIA, a cohort study that enrolled 5,115 African American and White participants in 1985-1986 from 4 US locations

PLAIN LANGUAGE SUMMARY

Current measures of chronic kidney disease (CKD) rely on markers of glomerular health and function. This approach inadequately captures the role of kidney tubule health, a known histopathological predictor of CKD development. We investigated associations of 7 biomarkers of kidney tubule health with 10-year estimated glomerular filtration rate (eGFR) change and incident reduced eGFR. Among 7 biomarkers, only epidermal growth factor showed persistent and inverse associations with both 10-year eGFR change and incident reduced eGFR. These findings suggest that epidermal growth factor has an association with kidney function changes and might play a protective role in kidney disease development.

(Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA). Recruitment was purposely balanced for race, age (18-24 and 25-30 years), sex (defined as a biological classification based on an individual's reproductive anatomy and physiology, with two primary categories: female and male), and education (high school or less versus more than high school). The parent study's design, recruitment, and participant characteristics have been published previously.[19] Participants attended in-person follow-up visits at years 2, 5, 7, 10, 15, 20, 25, and 30. The current analysis used data from the 20-, 25-, and 30-year visits. A total of 3,547 (72% of the surviving cohort) attended the year 20 visit, which was the baseline for these analyses. Among these participants, we excluded participants without available serum cystatin C measurements at the year 20 visit (n = 25); those who had prevalent hypertension, defined as taking antihypertensive medications, or having a systolic blood pressure (SBP) ≥ 130 mm Hg and/or diastolic BP (DBP) ≥ 80 mm Hg (n = 1,269); those with CVD (history of heart failure, myocardial infarction, coronary revascularization, angina, stroke, transient ischemic attack or peripheral vascular disease, n = 251); those missing follow-up BP values (n = 124); and those without adequate urine samples for biomarker measurements (n = 733) at the year 20 visit. No individuals in this subset had an eGFR below 60 mL/min/1.73 m^2 at the year 20 visit; thus, no one was excluded from the study because of prevalent eGFR < 60 mL/min/1.73 m^2. The resulting sample included 1,145 participants. The study was approved by local institution review boards. Participants signed written, informed consent at each clinic visit.

Primary Exposures

The primary exposures comprised urine concentrations of kidney tubule biomarkers, including EGF, α1m, IL-18, KIM-1, MCP-1, UMOD, and YKL-40. Urine samples were obtained as single, untimed samples on the day of the follow-up examination after an overnight fast. All biomarkers were measured at the University of Alabama at Birmingham-University of California, San Diego O'Brien Center for Acute Kidney Injury Research Bioanalytical Core located in San Diego, CA.[20] All samples underwent a single freeze-thaw, and all biomarkers were measured using multiplex assays on the Meso Scale Discovery platform, except for α1m, which was measured using an enzyme-linked immunosorbent assay (Abcam ab108884).[20] The assay controls adhered with the 2-standard deviation (SD) Levey-Jennings control rule, and overall inter- and intra-assay coefficients of variation were 1.2%-5.6% and 3.1%-13.5%, respectively. Each biomarker was measured in duplicate, and results were averaged to improve precision. Laboratory personnel who conducted the measurements were blinded to clinical outcomes.

Outcomes

We modeled longitudinal trajectories of eGFR using measures from the year 20, 25, and 30 visits, and the primary outcome for this study was eGFR change over 10 years of follow-up. Briefly, eGFR was calculated using the 2012 CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) cystatin C equation and was measured using nephelometry (Siemens, Munich, Germany) at the year 20 visit at the University of Minnesota and at the year 25 and 30 visits at the Kidney Health Research Collaborative (KHRC) biomarker laboratory (San Francisco VA Healthcare System).[21] As in prior studies, cystatin C values were calibrated to the reference standard.[22] Repeat measures of 50 specimens from year 20 at the KHRC laboratory demonstrated no assay drift across visits.[23] This study's secondary outcome was incident reduced eGFR, defined as the development of eGFR < 60 mL/min/1.73 m^2 at either the year 25 or 30 visit.

Covariates

The following covariates were obtained from the year 20 visit: age, sex, race, urine creatinine, smoking status, diabetes mellitus, SBP, DBP, high-density lipoprotein cholesterol (HDL-c) level, low-density lipoprotein cholesterol (LDL-c) level, triglyceride level, body mass index (BMI), physical activity intensity score, annual household income, education level, and urine albumin level. Age, sex, race, smoking status, education level, and income were self-reported by participants. Physical activity was determined through a questionnaire and reported as exercise units.[24,25] We defined diabetes mellitus as fasting glucose ≥ 126 mg/dL or the use of medications to treat diabetes mellitus. Urine albumin and urine creatinine were measured in spot urine samples using nephelometry and the Jaffé method, respectively.

Statistical Analysis

We calculated correlations among the urine biomarkers using Spearman correlations adjusted for urine creatinine

levels. Urine biomarkers were log-transformed to correct for rightward skew and standardized to the same scale (mean 0, SD 1) before analysis.

We investigated the associations of urine biomarkers with eGFR changes over time with linear mixed models, using random intercepts and slopes to account for intra-individual correlations. These models included biomarker-by-time interactions to determine whether biomarker concentrations were associated with changes in eGFR over time. We modeled biomarkers as continuous, linear predictors (per 1-SD greater). Also, we categorized the biomarkers into tertiles, setting the lowest as the reference category. We further tested 3-way interactions of each biomarker by race and time (biomarker *x* race *x* time) to determine whether urine biomarker associations with eGFR changes differed by race. Moreover, we performed interaction testing for sex, baseline eGFR, and baseline urinary albumin-creatinine ratio (UACR). Additionally, we generated least squares mean estimates of eGFR at examination years 20 (baseline), 25 and 30 by tertile of urine EGF in fully adjusted models. The minimally adjusted model accounted for age, sex, race, and urine creatinine levels. Rather than indexing for urine creatinine levels, we adjusted our models for urine creatinine levels. Indexing for urine creatinine levels may force both kidney tubule biomarkers and urine creatinine levels to share the same regression coefficient in our regression models, which would imply that kidney tubule biomarkers and urine creatinine levels have the same relationship with our outcomes. This assumption might oversimplify the complex relationships among these variables. To address this potential oversimplification, we used an alternative statistical model in which we independently modeled the contributions of urine kidney tubule biomarkers and urine creatinine levels to study outcomes. Therefore, the fully adjusted model adjusted for urine albumin levels, smoking status, diabetes mellitus, SBP, DBP, HDL-c levels, LDL-c levels, triglyceride levels, BMI, physical activity intensity scores, annual household income, and education level. Next, we modeled the tubular biomarkers in combination, using adaptive least absolute shrinkage and selection operator (LASSO) regression to determine whether any biomarkers were jointly associated with 10-year changes in eGFR.[26] This method has the ability to shrink regression coefficients to zero and selects predictors by imposing a penalty on their size. All tubular biomarkers were simultaneously included as candidate covariates, whereas sociodemographic and clinical characteristics (as listed above) were treated as unpenalized covariates and were retained in all models. We used cross-validation to determine the number of included biomarkers and the degree of coefficient shrinkage to avoid over-fitting. Our final model was estimated for the LASSO-selected markers using linear mixed-effects models, retaining only markers that remained statistically significant.

Finally, we modeled associations of urine biomarker levels with incident reduced eGFR. Because the exact date of developing reduced eGFR was unknown, we used interval-censored proportional hazards regression models.[27] We modeled the scaled biomarkers as continuous, linear predictors (per 1-SD higher) and also categorized into tertiles, setting the lowest as the reference value. As described above, models were constructed in stages. Additionally, we tested biomarker-by-race interactions to assess whether urine biomarker associations differed between African American versus White participants.

Adaptive LASSO regression was performed using the ncvreg package for R. All other analyses were conducted using SAS (Version 9.4, Cary, NC).

RESULTS

Study Population and Baseline Characteristics

The baseline characteristics of the study population were presented across tertiles of EGF/Cr (Table 1). Compared with tertiles 1 and 2, study participants in tertile 3 were more likely to be female and less likely to be African American, and they had lower median SBP, DBP, and triglyceride levels, lower BMI, higher HDL-c levels, and slightly higher UACR. Median follow-up time was 10.0 years (interquartile range, 9.8-10.2). Among 1,152 participants in our analysis, 82% attended both follow-ups for kidney function measures, and 18% had 1 follow-up.

Associations of Urine Biomarkers With 10-Year eGFR Change

Intercorrelations among the urine tubule biomarkers, urine albumin levels, and eGFR are summarized in Table S1. After adjustment for urine creatinine levels, the urine biomarkers showed weak to moderate correlations with one another, with correlation coefficients ranging from −0.11 to +0.49.

The average 10-year change in eGFR in the overall studied cohort was -18.6 mL/min/1.73m^2 (95% CI, -19.4 to -17.8). Table 2 summarizes the associations of urine biomarkers with 10-year eGFR changes. In minimally and fully adjusted models, each 1-SD higher EGF was associated with a 2.96 mL/min/1.73 m^2 (95% CI, 1.23-4.70) and 2.37 mL/min/1.73 m^2 (95% CI, 0.64-4.10) smaller decrease in eGFR over 10 years, respectively. The results of the regression model indexed for urine creatinine are depicted in Table S2. When EGF was examined in tertiles, the highest EGF tertile was associated with a lesser decrease in eGFR per decade compared with the lowest EGF tertile in the minimally adjusted model but was modestly attenuated and no longer statistically significant in the fully adjusted model (Table S3).

Among the other biomarkers, we observed that each 1-SD higher α1m level was associated with a 1.21 mL/min/1.73 m^2 (95% CI, 0.20-2.22) and 1.26 mL/min/1.73 m^2 (95% CI, 0.29-2.23) smaller decrease in eGFR per decade in the minimally and fully adjusted models, respectively. This relationship also appeared to change linearly across α1m tertiles (Table S3). There was no statistically

Table 1. Summary of Demographic and Clinical Characteristics at the Year 20 Visit of CARDIA, Stratified by Tertile of EGF/Creatinine

Range	EGF/cr Tertile 1 (4-82)	EGF/cr Tertile 2 (82-121)	EGF/cr Tertile 3 (121-356)
N	N = 381	N = 382	N = 382
Parameter			
Age, y	46 (42-48)	45 (41-48)	45 (42-48)
Female	144 (38%)	199 (52%)	298 (78%)
African American	198 (52%)	154 (40%)	94 (25%)
Smoking status			
Current	67 (18%)	66 (17%)	49 (13%)
Past	63 (17%)	79 (21%)	82 (22%)
Never	246 (65%)	236 (62%)	245 (65%)
Diabetes mellitus	19 (5%)	20 (5%)	13 (3%)
Systolic BP, mm Hg	112 (105-118)	108 (103-115)	106 (101-114)
Diastolic BP, mm Hg	68 (63-73)	67 (61-71)	66 (60-71)
LDL-c, mg/dL	109 (90-129)	109 (91-130)	104 (84-126)
HDL-c, mg/dL	51 (40-61)	51 (43-61)	57 (47-68)
Triglycerides, mg/dL	86 (61-137)	84 (60-118)	75 (55-109)
BMI, kg/m^2	27 (25-31)	27 (24-31)	26 (23-30)
Physical activity intensity score	340 (170-576)	322 (154-582)	294 (140-498)
Annual Household Income			
<$50,000/year	130 (35%)	127 (34%)	96 (25%)
$50,000-99,000/year	121 (32%)	130 (34%)	157 (41%)
≥$100,000/year	124 (33%)	120 (32%)	126 (33%)
Education level			
≤ High school	80 (21%)	89 (23%)	67 (18%)
College	210 (55%)	196 (51%)	204 (53%)
Graduate School	91 (24%)	97 (25%)	111 (29%)
eGFR, mL/min/1.73 m^2	112 (102-117)	110 (104-116)	112 (106-117)
UACR, mg/g	4.0 (2.7-5.7)	3.9 (2.7-5.6)	4.5 (3.3-6.7)
Urine creatinine, mg/dL	121 (55-192)	139 (83-185)	125 (77-179)

Note: Year 20 is the baseline for this current study. Data displayed are n (%) or median (interquartile range).
Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; eGFR, cystatin C based estimated glomerular filtration rate; HDL-c, high-density lipoprotein cholesterol; SBP, systolic blood pressure; LDL-c, low-density lipoprotein cholesterol, T1, Tertile 1; T2, Tertile 2; T3, Tertile 3; UACR, urine albumin-creatinine ratio.

significant association between KIM-1 as a continuous variable and 10-year eGFR changes. However, the highest KIM-1 tertile was associated with a 3.20 mL/min/1.73 m^2 (95% CI, 0.58-5.83) lesser 10-year decrease in eGFR as compared with the first tertile, in the fully adjusted model (Table S3). Each 1-SD higher urine albumin was associated with a 1.61 mL/min/1.73 m^2 (95% CI, −3.15 to −0.08) greater 10-year decrease in eGFR in minimally adjusted

Table 2. Associations of Urine Biomarkers With 10-Year Changes in eGFR (mL/min/1.73 m^2)

Per 1 SD Higher Biomarker	Minimally Adjusted Model[a] Estimate (95% CI)	Fully Adjusted Model[b] Estimate (95% CI)
EGF	2.96 (1.23-4.70)	2.37 (0.64-4.10)
α1m	1.21 (0.20-2.22)	1.26 (0.29-2.23)
IL-18	0.15 (−0.98 to 1.28)	0.51 (−0.58 to 1.59)
KIM-1	0.44 (−0.80 to 1.67)	0.91 (−0.36 to 2.17)
MCP-1	−0.09 (−1.23 to 1.05)	0.16 (−1.04 to 1.36)
UMOD	0.00 (−0.85 to 0.86)	−0.39 (−1.13 to 0.35)
YKL-40	0.05 (−0.83 to 0.92)	0.07 (−0.80 to 0.93)
Urine albumin	−1.61 (−3.15 to −0.08)	−1.58 (−3.22 to 0.05)

Notes: Bolded values indicate statistically significant results. 10-year changes in eGFR was modeled by measures from the year 20, 25, and 30 visits.
Abbreviations: α1m, alpha-1-microglobulin; EGF, epidermal growth factor; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; MCP-1, monocyte chemoattractant protein-1; SD, standard deviation; UMOD, uromodulin; YKL-40, chitinase-3-like protein 1.
[a]Adjusted for age, sex, race, and urine creatinine.
[b]Additionally adjusted for urine albumin, smoking status, diabetes mellitus, systolic blood pressure,diastolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, body mass index, physical activity intensity score, annual household income, and education level.

model, although the association was slightly attenuated and no longer statistically significant in the fully adjusted model. The remaining 4 urine biomarkers were not significantly associated with the 10-year change in eGFR in any model.

When we modeled biomarkers in combination using adaptive LASSO and adjusted for sociodemographic and clinical risk factors, eGFR, and urine albumin levels, the model selected only EGF among the 7 candidate markers. Each 1-SD higher EGF was associated with a 2.37 mL/min/1.73 m^2 smaller decrease in eGFR per decade (95% CI, 0.64-4.10). Although urine albumin level was unpenalized and therefore not forced into the model, each 1-SD higher urine albumin level was associated with a 1.68 mL/min/1.73 m^2 greater decrease in eGFR per decade (95% CI, −3.32 to −0.04), independent of EGF and other risk factors.

As shown in Fig 1, higher EGF tertiles were associated with higher mean eGFR at baseline and throughout follow-up. The eGFR differences across the EGF tertiles widened during follow-up. Compared with the EGF T1, adjusted mean eGFR was 3 mL/min/1.73 m^2 and 5 mL/min/1.73 m^2 higher at the year 20 visit, 3.25 mL/min/1.73 m^2 and 6.29 mL/min/1.73 m^2 higher at year 25 visit, and 3.50 mL/min/1.73 m^2 and 7.58 mL/min/1.73 m^2 higher at year 30 visit for EGF T2 and T3, respectively.

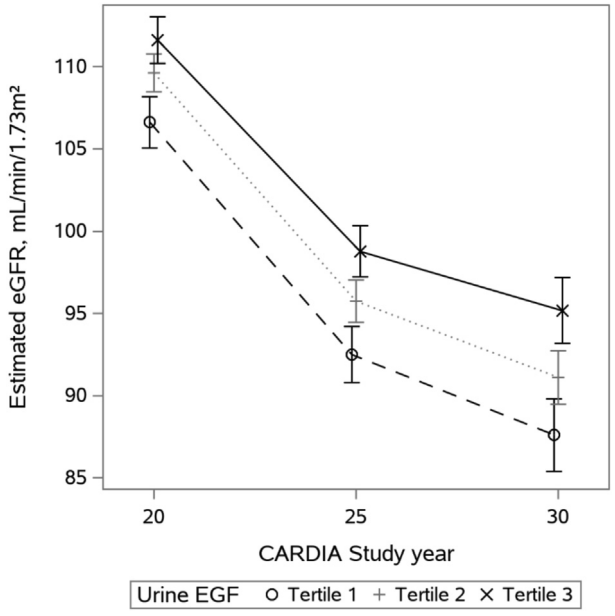

Figure 1. Adjusted mean eGFR trajectories by tertile of urine epidermal growth factor. Estimates were obtained from linear mixed models with random intercepts and slopes and were adjusted for age, sex, race, urine creatinine level, smoking status, diabetes mellitus, systolic blood pressure, diastolic blood pressure, high-density lipoprotein cholesterol level, low-density lipoprotein cholesterol level, triglyceride level, body mass index, physical activity intensity score, annual household income, education level, and urine albumin level. Abbreviations: EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate.

Associations of EGF With Incident Reduced eGFR

During follow-up, 52 (4.5%) participants developed incident reduced eGFR. The incidence rate of reduced eGFR decreased in a stepwise manner with ascending EGF tertiles (Fig S1). In the minimally adjusted model, each 1-SD higher EGF was associated with a 65% (95% CI, 49%-76%) lower risk of incident reduced eGFR and with a 42% (95% CI, 4%-64%) lower risk of incident reduced eGFR in the fully adjusted model (Table 3). Examining EGF in tertiles showed a decreased risk of incident reduced CKD in the second and third tertiles compared with the first. This associations lost statistical significance in the fully adjusted model (Table S4).

In addition, each 1-SD higher urine albumin concentration was associated with a 49% (95% CI, 3%-116%) higher risk of incident reduced eGFR in the fully adjusted model. Other urine biomarkers did not have a statistically significant association with incident reduced eGFR.

Interaction Testing of EGF With Baseline Characteristics

For the linear outcome, we observed similar associations between EGF and reduced eGFR in both African American and White participants (P for interaction = 0.89) (Table S5). However, for the outcome of incident reduced eGFR, we observed a statistically significant interaction ($P = 0.03$); each 1-SD higher EGF was associated with a 59% (95% CI, 26%-77%) lower risk among African American participants and a 24% (95% CI, −34% to 57%) lower risk among White participants.

In addition, we performed interaction analysis between EGF and sex for the 10-year eGFR decline (Table S6). We found similar associations in both male and female

Table 3. Associations of Urine Biomarkers with Incident Reduced eGFR (eGFR < 60 mL/min/1.73 m^2)

Per 1 SD Higher Biomarker	Minimally Adjusted Model[a] HR (95% CI)	Fully Adjusted Model[b] HR (95% CI)
EGF	0.35 (0.24-0.51)	0.58 (0.36-0.96)
α1m	0.90 (0.64-1.28)	0.74 (0.49-1.10)
IL-18	1.17 (0.79-1.74)	1.04 (0.68-1.60)
KIM-1	1.36 (0.84-2.19)	0.87 (0.56-1.33)
MCP-1	0.91 (0.60-1.36)	0.83 (0.51-1.36)
UMOD	0.86 (0.70-1.05)	0.93 (0.70-1.25)
YKL-40	0.88 (0.66-1.18)	0.99 (0.71-1.38)
Urine albumin	1.13 (0.79-1.62)	1.49 (1.03-2.16)

Note: Bolded values indicate statistically significant results.
Abbreviations: α1m, alpha-1-microglobulin; EGF, epidermal growth factor; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; MCP-1, monocyte chemoattractant protein-1; SD, standard deviation; UMOD, uromodulin; YKL-40, chitinase-3-like protein 1.
[a]Adjusted for age, sex, race, and urine creatinine.
[b]Additionally adjusted for urine albumin, smoking status, diabetes mellitus, systolic blood pressure, diastolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, body mass index, physical activity intensity score, annual household income, education level, and eGFR.

participants, and we did not observe a statistically significant interaction ($P = 0.94$).

Further, we tested for an interaction with baseline eGFR and UACR. We did not observe a statistically significant interaction between EGF and 10-year eGFR decline by both baseline eGFR and UACR ($P = 0.82$ and $P = 0.22$, respectively).

DISCUSSION

In this longitudinal cohort study of middle-aged adults without CKD, hypertension, or CVD at the year 20 visit of CARDIA, we found that higher urine EGF and α1m concentrations were associated with lower 10-year mean eGFR declines, and only higher urine EGF concentration was associated with lower risk of incident reduced eGFR independent of baseline demographic characteristics, CKD risk factors, and eGFR. In addition, when examining biomarkers in parallel to identify the best set of markers using LASSO regression, EGF was the only kidney tubule biomarker that remained associated with 10-year eGFR decline. In this setting of middle-aged adults, the associations of EGF concentration with eGFR decline appeared even stronger than urine albumin level. These results support the potential role of EGF in kidney disease progression and highlight its unique protective association with kidney outcomes in contrast to most other kidney tubule biomarkers, for which higher levels correlate with adverse outcomes.

Our results are consistent with several previous studies that have identified urine EGF level as having a uniquely protective association among kidney tubule biomarkers in the pathophysiology of CKD. Ascher et al[15] evaluated 14 kidney tubule biomarkers and their associations with incident CKD in women living with HIV (human immunodeficiency virus type 1) infection. The study determined that lower urine EGF concentration and higher urine α1m excretion were mutually associated with a higher incidence of CKD. Large European cohort studies found associations between lower urine EGF concentrations and higher risk of rapid eGFR loss and incident CKD in nondiabetic populations.[28] Further, among 865 participants of Translational Research Investigating Biomarker Endpoints in AKI (TRIBE-AKI) study who underwent cardiac surgery, a lower postoperative urine EGF concentration was associated with a higher risk of a composite outcome of CKD incidence and progression.[29]

The thick ascending limb of Henle and distal convoluted tubule produce EGF, a mitogenic factor that stimulates the proliferation of fibroblasts and epithelial cells.[30] A series of experimental studies have shown that EGF-deficient mice developed attenuation of the proximal tubular brush border and kidney fibrosis; in rats, EGF promotes kidney tubule cell regeneration, and it improves kidney function recovery after acute kidney injury.[30-32] In humans, kidney biopsy studies in patients with CKD have demonstrated that lower EGF mRNA expression correlates with greater tubulointerstitial injury, whereas higher urine EGF concentrations are indicative of functional kidney tubules and higher regeneration capability.[33,34] These findings, spanning from experimental animals to multiple human populations, directionally align with the results observed in this study.

In this same group of participants from the CARDIA cohort, we previously found that lower urine EGF levels were uniquely and independently associated with a higher risk of incident hypertension.[35] In combination with our current findings, this work in CARDIA might suggest that early reductions in kidney tubule function may have a causal role in the development of both hypertension and CKD in parallel.

In addition to EGF, we observed significant associations of higher urine α1m level with slower reductions in eGFR, although this association was not significant for the outcome of incident reduced eGFR. For α1m, this finding was in the opposite direction from previous studies.[14,17,36] Previous studies have reported associations of higher α1m levels and tubular toxicity, higher risk of CKD progression, and mortality in individuals with HIV.[36,37] Also, a higher preoperative α1m concentration was associated with a higher risk of acute kidney injury and CKD progression in TRIBE-AKI participants with cardiac surgery.[38] In this study, α1m was measured using an enzyme-linked immunosorbent assay and not by nephelometry, as in most prior studies; it is uncertain whether our results could be explained in part by use of a different measurement method.

Our results build on previous findings that have shown kidney tubule health measures to be associated with kidney function decline. Herein, we found EGF to have a strong and positive association with declining kidney function even among largely healthy, middle-aged individuals with low incident reduced eGFR during follow-up. Future studies should investigate the determinants of EGF production to better understand this dimension of kidney health and whether longitudinal changes in EGF have incremental associations with declining eGFR. If confirmed, EGF measurement may ultimately have utility to identify individuals for targeted prevention of CKD development.

The strengths of this study include its evaluation of a well-characterized cohort that is balanced by sex and with African American and White participants, the comprehensive and complete data on key confounding factors, and the measurement of established biomarkers. This study also has important limitations. First, measurements of eGFR were done only at 5-year intervals during follow-up. Therefore, if a study participant developed end-stage kidney disease after the year 20 visit and did not attend subsequent follow-up visits, they were not included in our analyses. Further, we estimated glomerular filtration rates using serum cystatin C based calculations, which could limit generalizability of our findings, considering that cystatin C measurement is not universally incorporated into routine clinical practice. Second, though the study cohort was balanced for self-reported African American

and White participants, other race groups or ethnicities were not enrolled. Third, measurement of α1m was performed with a different technique than previous studies, which may account for differences in the results of this study compared with prior reports. Fourth, we do not have information on the incidence of end-stage kidney disease during study follow-up, although we believe that the incidence must have been very low given that only 52 declined below an eGFR of 60 mL/min/1.73 m^2. Finally, our ancillary study selected CARDIA participants who were able to provide follow-up specimens, so we avoided CARDIA participants who died during that decade after the year 20 visit. Among the 4,925 CARDIA participants who were alive at their 20th year of follow-up, only 200 (4.1%) died between the year 20 and 30 follow-ups. Therefore, our selected subcohort is likely to be somewhat healthier than CARDIA participants overall.

We measured 7 urine biomarkers and investigated their associations with kidney function decline in middle-aged adults selected for absence of hypertension at baseline. Among them, EGF had the strongest associations with both linear eGFR decline and incident reduced eGFR, and point estimates appeared even stronger than that for urine albumin level. Although our findings should be validated in subsequent studies, EGF has the potential to develop as a useful biomarker of tubular function and eGFR decline relatively early in the life-course, even among persons without hypertension or prevalent CVD at the time of EGF measurement. The addition of urine EGF concentration to standard glomerular indicators may eventually help to capture the involvement of kidney tubules, and to facilitate early diagnosis and risk stratification for CKD in future studies.

SUPPLEMENTARY MATERIALS

Supplementary File (PDF)

Figure S1: Incidence rate of reduced eGFR by urine biomarker tertiles.

Table S1: Spearman Partial Correlation Coefficients, Adjusted for Urine Creatinine.

Table S2: Associations of Urine Biomarkers Indexed for Urine Creatinine Levels With 10-Year Changes in eGFR (mL/min/1.73 m^2).

Table S3: Associations of Tertiled Urine Biomarkers with 10-Year Changes in eGFR (mL/min/1.73 m^2).

Table S4: Associations of Tertiled Urine Biomarkers with Incident Reduced eGFR (eGFR < 60 mL/min/1.73 m^2).

Table S5: Associations of Urine Biomarkers with 10-Year Changes in eGFR, Stratified by Race.

Table S6: Associations of Urine Biomarkers with 10-Year Changes in eGFR, Stratified by Sex.

ARTICLE INFORMATION

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