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Longitudinal Trajectories of Biomarkers of Kidney Tubular Function in Type 1 Diabetes

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Introduction: Tubular biomarkers may shed insight into progression of kidney tubulointerstitial pathology complementary to traditional measures of glomerular function and damage.

Methods: We examined trajectories of tubular biomarkers in the Diabetes Control and Complications Trial and the Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC Study) of type 1 diabetes (T1D). Biomarkers were measured in a subset of 220 participants across 7 time points over 26 years. Measurements included the following: kidney injury molecule 1 (KIM-1), soluble tumor necrosis factor 1 (sTNFR1) in serum or plasma, epidermal growth factor (EGF), monocyte chemoattractant protein-1 (MCP1) in timed urine, and a composite tubular secretion score. We described biomarker trajectories and examined how these were affected by intensive glucose-lowering therapy and glycemia.

Results: At baseline, participants had a mean age of 28 years, 45% were women, and 50% were assigned to intensive glucose-lowering therapy. The mean estimated glomerular filtration rate (eGFR) was 125 ml/min per 1.73 m^2 and 90% of participants had a urinary albumin excretion rate (AER) <30 mg/24h. Mean changes in biomarkers over time (percent/decade) were: KIM-1: 27.3% (95% confidence interval [CI]: 21.4–33.5), sTNFR1: 16.9% (14.5–19.3), MCP1: 18.4% (8.9–28.8), EGF: −13.5% (−16.7 to −10.1), EGF-MCP1 ratio: −26.9% (−32.2 to −21.3), and tubular secretion score −0.9% (−1.8 to 0.0), versus −12.0% (CI: −12.9 to −11.1) for eGFR and 10.9% (2.5–20.1) for AER. Intensive versus conventional glucose-lowering therapy was associated with slower increase in sTNFR1 (relative difference in change: 0.94 [0.90–0.98]). Higher HbA1c was associated with faster increases in sTNFR1 (relative difference in change: 1.06 per 1% higher HbA1c [1.05–1.08]) and KIM-1 (1.09 [1.05–1.14]).

Conclusion: Among participants with T1D and normal eGFR at baseline, kidney tubular biomarkers changed significantly over long-term follow-up. Hyperglycemia was associated with larger increases in serum or plasma sTNFR1 and KIM-1, when followed-up longitudinally.

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Diabetic kidney disease (DKD), defined as persistent albuminuria and/or a reduction in eGFR, affects approximately 30% of people with T1D.[1] Despite advances in treatment strategies to improve glycemic control and control blood pressure, DKD remains highly prevalent in T1D, may result in kidney failure, and is associated with increased morbidity and mortality.[2] Early DKD diagnosis and improved prognostication may allow for more timely and targeted delivery of effective therapies.

Several biomarkers of kidney tubular function encompassing tubular injury and inflammation have emerged as both potential early indicators of kidney disease and predictors of eGFR decrease in people with diabetes. KIM-1 is produced by injured proximal

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tubular cells, and higher concentrations in both blood and urine are associated with risk of eGFR decrease in diabetes and nondiabetes cohorts.[3-5] sTNFR1 has been demonstrated to have immunomodulatory functions, and circulating concentrations are strongly correlated with progression of chronic kidney disease.[5] Urinary MCP-1 is an inflammatory cytokine expressed in tubular cells in response to injury, which promotes macrophage-specific chemotaxis.[6] Higher urinary MCP-1 is associated with increased kidney disease severity and risk of kidney function decline.[7,8] Urinary EGF is produced in the ascending limb of the loop of Henle and distal tubular cells and is believed to reflect functional tubular mass.[9] Lower urinary EGF is associated with increased risk of kidney disease progression. The ratio of urinary EGF to MCP-1 has also been observed to correlate inversely with kidney function decline, notably more strongly than its individual components.[7,10] In addition, proximal tubules play a critical role in the clearance of small organic solutes, especially those that are highly protein-bound and poorly filtered at the glomerulus, and lower urinary clearance of secreted small molecules has been associated with eGFR decrease.[11]

Although numerous studies have described associations of single tubular biomarker measurements with subsequent kidney outcomes, little is known about how these biomarkers change over time. Understanding how biomarkers of kidney tubular function change throughout the clinical course of T1D may provide insight into the rate of progression of tubulointerstitial pathology in DKD, which has been associated with kidney failure risk.[12,13] Specifically, tubular biomarker trajectories may allow for differentiation between tubulointerstitial pathology and glomerular pathology, reflected by albuminuria and eGFR. Understanding how clinical factors, such as hyperglycemia, contribute to tubular biomarker trajectories may elucidate underlying mechanisms of tubulointerstitial pathology. In addition, knowledge of tubular biomarker trajectories and influencing factors is essential for facilitating clinical interpretation of repeated measurements for disease monitoring, risk stratification, and application to clinical trials.

Here, we examine trajectories of biomarkers of inflammation and tubular function assessed over 26 years in adults with T1D from the DCCT/EDIC study.

METHODS

Study Design and Participants

In the DCCT, a total of 1441 adults with T1D were randomized to either intensive ($n = 711$) or conventional ($n = 730$) glucose-lowering therapy.[14] Intensive therapy aimed to safely reduce glycemia to nondiabetic levels using 3 to 4 daily insulin injections or continuous subcutaneous insulin with pumps, each adjusted by frequent self-monitored blood glucose. In contrast, conventional therapy was aimed at avoiding symptoms of hyperglycemia and hypoglycemia and consisted of 1 to 2 daily insulin injections without specific glycemic targets. The DCCT study population was comprised of 2 cohorts. The primary prevention cohort consisted of participants with 1 to 5 years of diabetes duration at baseline, no retinopathy as assessed with fundus photography, and <40 mg/24h of albuminuria. The secondary intervention cohort consisted of participants with 1 to 15 years of diabetes duration, up to moderate nonproliferative diabetic retinopathy, and <200 mg/24h of albuminuria at baseline. Participants were followed-up with for a mean of 6.5 years.

Following the end of the DCCT in 1993, all participants were encouraged to pursue intensive therapy and were referred to their primary care providers for subsequent clinical care. In 1994, 96% of the surviving cohort enrolled in the observational EDIC follow-up study, with 96% of the surviving cohort still actively participating in the study as of 2006.[15]

Of the 1441 participants originally enrolled in the DCCT, 1264 consented to the use of their biological samples for follow-up studies. For the current study, a random sample of 220 participants with at least 1 available serum or plasma sample and 4-hour timed urine sample was selected. For each participant, biomarker measurements were performed on serum or plasma samples and timed urine for up to 7 time points spanning from 1983 to 2010: DCCT baseline, DCCT year 1, DCCT closeout, EDIC years 3 and 4, EDIC years 7 and 8, EDIC years 11 and 12, and EDIC years 15 and 16. Serum and plasma samples were used interchangeably depending on their availability.

Tubular Biomarkers

Blood samples were collected yearly and stored as serum or plasma during DCCT and EDIC. Serum was preferentially used for biomarker measurements when available, otherwise plasma was used. Urine was collected annually during DCCT, then every other year during EDIC using supervised, 4-hour timed collections. Samples were shipped frozen to the Central Biochemistry Laboratory (University of Minnesota, Minneapolis) where they were stored at −80 °C.

Serum or plasma KIM-1 and sTNFR1 and urine MCP1 and EGF were measured using enzyme-linked immunosorbent assay as per manufacturer instructions (assay details in Supplementary Table S1). In addition, the following 11 biomarkers of tubular secretion were assayed using mass spectrometry in both urine and serum or plasma: 1, 3, 7-trimethyluric

acid; 1, 7-dimethyluric acid; 2-furoylglycine; adipic acid; cinnamoylglycine; hippuric acid; indoxyl sulfate; m-hydroxy Hippurate; p-cresol sulfate; tiglylglycine; and phenylacetylglutamine. In this analysis, trimethyluric acid, furoylglycine, and m-hydroxyhippurate were excluded due to many serum samples falling below the analytical limit of quantitation resulting in high within-subject and between-subject variability.

The number of measurements below or above the lowest measurable value for each biomarker are shown in Supplementary Table S2. Three different approaches were considered for addressing measurements below the lowest measurable value, as follows: (i) discard observations below the lowest measurable value, (ii) set these values to lowest measurable value divided by 2, or (iii) use a maximum likelihood estimation to obtain estimates accounting for the truncation due to the limit of detection or quantitation. Biomarker coefficients of variation and intraclass correlation coefficients (ICCs) calculated using the 3 methods are shown in Supplementary Table S3. Coefficients of variation and ICCs using the 3 approaches were very similar, and method 2 was used for measurements below the lowest measurable value in the subsequent analyses. Because very few values were above the detectable limit (<0.01% for any given biomarker exceeded the analytical measurement range), those measurements were dropped from this analysis.

Biomarkers were log-transformed for analyses to account for their right-skewed distribution and change over time was examined using a common metric (percent change per decade). For urinary biomarkers (MCP1 and EGF), 24-hour urinary biomarker excretion was used in primary analyses, and urine biomarker to creatinine ratios were examined in secondary analyses to allow comparison to other studies. For each tubular secretion biomarker assayed using mass spectrometry, clearance was calculated by dividing the biomarker excretion rate (biomarker urine concentration x urine flow) by the biomarker serum concentration. Each clearance was then standardized on a scale of 1 to 100 as follows: (log Clearance − min[log Clearance])/(max[log Clearance] − min [log Clearance]) × 100. Finally, a tubular secretion score was calculated as the mean of the standardized clearances.[11]

Traditional Measures of Kidney Function and Damage

Serum creatinine was measured annually in both DCCT and EDIC. An automated Jaffe kinetic method was used up through May 2007, then starting from June 2007, creatinine was measured with an enzymatic IDMS-traceable creatinine method.[14,15] Creatinine results obtained with the Jaffe method were recalibrated to the IDMS-traceable enzymatic method.[16] Glomerular filtration rate was estimated from serum creatinine using the 2009 Chronic Kidney Disease-Epidemiology Collaboration equation.[17] Of note, 96% of the subcohort studied identified as Non-Hispanic White. Urine albumin was quantified from 4-hour timed urine samples collected annually during DCCT and at alternate EDIC years using solid-phase fluoroimmunoassay. AER was expressed in terms of 24-hour urinary albumin excretion, and urine creatinine was assayed using similar methods as in serum.

Covariates

Demographic information and medical history were obtained using standardized methods. Blood pressure was measured after at least 5 minutes of rest by trained research staff. HbA1c was assessed quarterly in DCCT and annually in EDIC using high-performance ion-exchange liquid chromatography.[14,15] The mean updated HbA1c is the time-weighted average of all prior measurements using weights proportional to the time interval between visits (i.e., 1/4 for DCCT, 1 for EDIC). Two measures of glycemia were employed. First, analyses for the associations of tubular biomarker levels with total previous glycemic exposure used the mean-updated HbA1c up to and including the time of the biomarker measurement. Second, analyses for the long-term effects of glycemia on tubular biomarkers used the mean updated HbA1c during DCCT only as a predictor of the biomarker measurements during EDIC.

Statistical Analysis

The characteristics of participants at DCCT baseline and at EDIC year 16 were described using mean and SD for continuous variables (e.g., HbA1c), and percentages for categorical variables (e.g., sex assigned at birth). Biomarker distributions were described using medians and quartiles. To ascertain biomarker variability over the duration of the study period, coefficients of variation both within and between participants, and ICCs were estimated using a linear mixed model with a random intercept and time effect to account for within-participant correlation among the repeated values over time. Percent biomarker change per decade was estimated using the coefficient for time in a linear mixed model with repeated measures.

Participant-specific biomarker changes per decade (i.e., biomarker slopes) were estimated using a linear mixed model with within-participant random slopes and intercepts. Spearman correlations were then used to assess the correlations between biomarkers as well as between participant-specific biomarker slopes.

The relative differences in change in biomarkers for diabetes therapy group (intensive vs. conventional),

Table 1. Clinical characteristics of a sample of the DCCT/EDIC cohort randomly selected for longitudinal measurements of kidney tubular functions

Characteristics	DCCT at baseline (n = 220)	Years 15 and 16 of EDIC (n = 209)
Age (yr)	28.0 (23.3–33.9)	50.6 (45.9–55.9)
Female sex	98 (44.6%)	93 (44.5%)
Race (% Non-Hispanic White)	212 (96.4%)	201 (96.2%)
Diabetes duration (yr)	4.4 (2.5–9.4)	27.6 (24.5–32.8)
Intensive therapy group	110 (50%)	106 (50.7%)
Primary cohort	103 (46.8%)	99 (47.4%)
Any proliferative diabetic retinopathy	0	49 (23.4%)
Any clinically significant macular edema	0	61 (29.2%)
Current smoker	30 (13.6%)	25 (12.0%)
Current alcohol use	1 (0.5%)	7 (3.4%)
Body mass index (kg/m^2)	23.0 (21.5–25.2)	27.7 (25.0–30.4)
Blood pressure (mm Hg)		
Systolic	114 (106–122)	122 (112–130)
Diastolic	74 (68–80)	73 (67–79)
Hypertension history	0	128 (61.2%)
Albumin excretion rate (mg/24h)	10.8 (7.2–17.3)	11.5 (7.2–23.0)
Albumin excretion rate category (%)		
≥30 mg/24h <300	21 (9.6%)	31 (14.8%)
≥300 mg/24h	0	12 (5.7%)
Serum creatinine (mg/dl)	0.68 (0.58–0.77)	0.83 (0.73–0.96)
eGFR (ml/min per 1.73 m^2)	123.6 (117.6–134.0)	95.6 (83.8–103.8)
eGFR category (%)		
eGFR ≥90	219 (99.6%)	139 (66.5%)
eGFR 75–89	0	45 (21.5%)
eGFR 60–74	1 (0.5%)	12 (5.7%)
eGFR <60	0	13 (6.2%)
HbA1c (%)	8.7 (7.7–9.7)	7.6 (7.0–8.2)
Mean updated HbA1c (%)	8.7 (7.7–9.7)	7.8 (7.2–8.5)
Medication Use		
ACEi or ARB	0	115 (55.0%)
Beta-blocker	0	16 (7.8%)
Statin	0	126 (60.3%)

ACEi, angiotensin-converting-enzyme inhibitors; ARB, angiotensin receptor blockers; DCCT, Diabetes Control and Complications Trial; EDIC, Epidemiology of Diabetes Interventions and Complications Study; eGFR, estimated glomerular filtration rate.
Continuous characteristics presented as median (Q1, Q3), whereas categorical characteristics are presented in percentages. By year 15/16 of EDIC, 61 of 106 (58%) participants in the intensive treatment group were using ACEi or ARB and 60 of 103 (58%) participants in the conventional treatment group were using ACEi or ARB.

cohort (secondary vs. primary), sex (females vs. males), and HbA1c levels were estimated using a linear mixed model that included the risk factor (e.g., therapy group), follow-up time (in decades), and an interaction between follow-up time and the risk factor.

All analyses were conducted using SAS 9.3 (SAS Institute, Inc., Cary, NC). P-values <0.05 were considered nominally significant.

RESULTS

Participant Characteristics

Forty-five percent of participants in this sub-study were female and 96% self-identified as Non-Hispanic White (Table 1). Approximately one-half were randomized to intensive glucose-lowering therapy. At baseline, participants had a mean age of 28 years, diabetes duration of 6 years, body mass index of 23 kg/m^2, no hypertension history, and a mean HbA1c of 8.9%. At EDIC years 15 and 16, participants had a median age of 51 years, diabetes duration of 28 years, body mass index of 28 kg/m^2, 61% of participants had developed hypertension, and the mean HbA1c was 7.8%. The majority of participants had normal traditional measures of kidney function and damage at baseline, with a median eGFR of 124 ml/min per 1.73 m^2 and AER 11 mg/24h (10% with AER ≥30 mg/24h). By EDIC years 15 and 16, the median eGFR was 96 ml/min per 1.73 m^2, 6% of participants had developed an eGFR <60 ml/min per 1.73m^2, and the median AER was 12 mg/24h (21% with AER ≥30 mg/24h). At EDIC years 15 and 16, 55% of participants were using angiotensin-converting-enzyme inhibitors or angiotensin receptor blockers, 8% were using beta blockers, and 60% were using statins, with these medications not allowed at DCCT entry (and angiotensin-converting-enzyme inhibitors and angiotensin receptor blockers were not prescribed during the DCCT).

Biomarker Trajectories and Variability

Biomarker measurements were performed for each participant at up to 7 time points spanning a median of 21.1 years. A total of 206 (94%) participants had blood and urine biomarker measurements performed at 6 or more time points.

Kidney tubular function biomarkers generally changed monotonically, reflecting worse function or increased damage over time (Figure 1 and Supplementary Figure S1). The distributions of biomarker slopes are shown in Figure 2. Serum or plasma sTNFR1 and KIM-1 concentrations and urinary MCP1 excretion increased significantly by 16.9%, 27.3%, and 18.4% per decade, respectively (Table 2). Urinary EGF and EGF-MCP1 excretion ratio decreased significantly by 13.5% and 26.9% per decade, respectively; and the tubular secretion score decreased by 0.9% per decade. When assessed individually, 4 of the 8 biomarkers comprising the tubular secretion score were significantly decreased (−3.0% to −1.6% change per decade) and 1 was significantly increased (4.8% change per decade) (Supplementary Table S4). In comparison, eGFR decreased by 12% per decade and AER increased by 11% per decade.

Within-participant coefficients of variation for tubular function biomarkers ranged from 10.0% to 75.4%, compared with 9.1% and 278.8% for eGFR and albuminuria, respectively (Table 2). ICC ranged from 0.11 to 0.52, compared with 0.33 and 0.37 for eGFR and albuminuria, respectively. The highest ICC values were observed for the composite tubular secretion score (0.52), sTNFR1 (0.46), and KIM-1 (0.43).

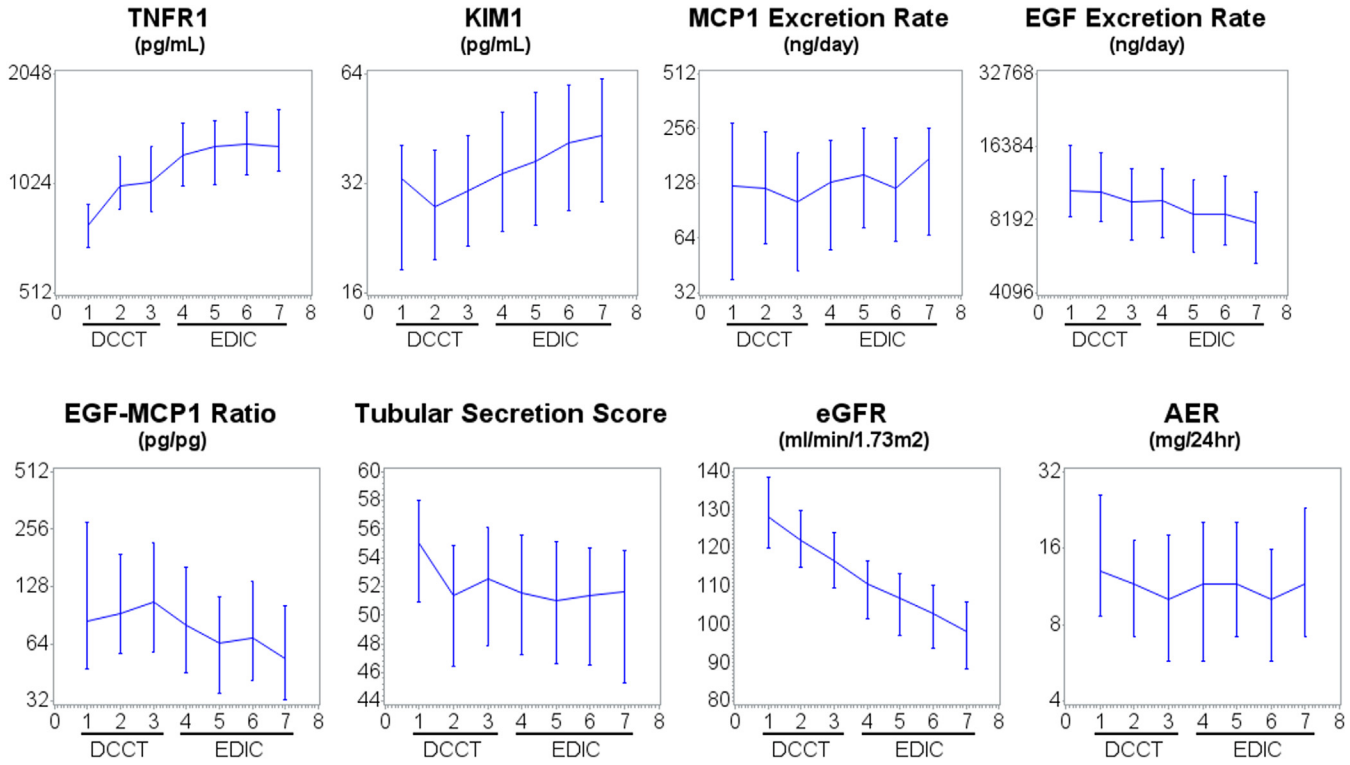

Figure 1. Biomarker trends over time in DCCT/EDIC. AER, urinary albumin excretion rate; DCCT, Diabetes Control and Complications Trial; EDIC, Epidemiology of Diabetes Interventions and Complications Study; EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate; KIM1, kidney injury molecule 1; MCP1, monocyte chemoattractant protein-1; TNFR1, tumor necrosis factor 1. Median and interquartile range for each biomarker are shown. Values on x-axis correspond to the following: 1: DCCT baseline, 2: DCCT year 1, 3: DCCT closeout, 4: EDIC years 3 and 4, 5: EDIC years 7 and 8, 6: EDIC years 11 and 12, 7: EDIC years 15 and 16.

Biomarker Correlations

Correlations between kidney tubular function biomarkers and traditional measures of kidney function and damage were weak to moderate (Figure 3a and Supplementary Table S5). eGFR correlated most strongly with serum or plasma sTNFR1 ($r = -0.40$, $P < 0.001$),

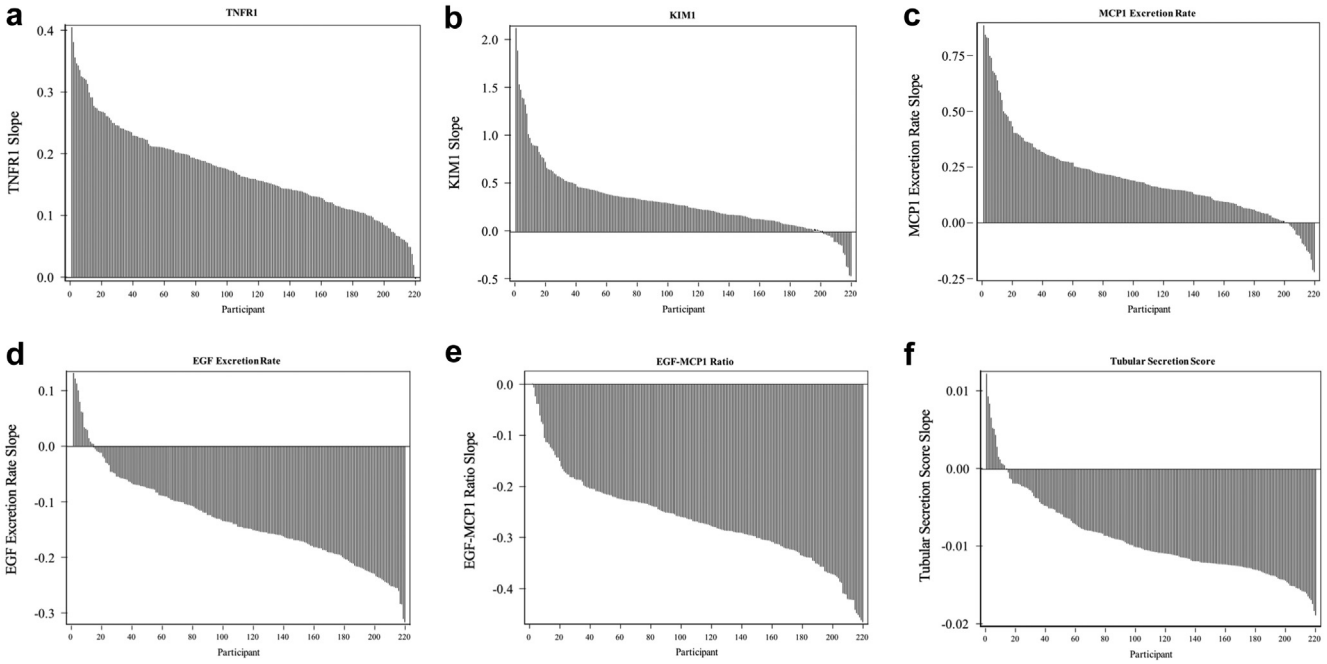

Figure 2. Waterfall plots depicting the distribution of slopes for (a) sTNFR1, (b) KIM-1, (c) urinary MCP-1 excretion, (d) urinary EGF excretion, (e) urinary EGF-MCP1 ratio, and (f) the tubular secretion score. EGF, epidermal growth factor; KIM1, kidney injury molecule 1; MCP1, monocyte chemoattractant protein-1; TNFR1, tumor necrosis factor 1. Slopes presented as percent change per decade.

Table 2. Characteristics of kidney biomarkers measured repeatedly over time in the DCCT/EDIC cohort

Characteristics	Median (Q1, Q3) from all time points	CV within participant	CV between participant	Intraclass correlation	Mean change over time (95% CI) (percent per decade)
sTNFR1 (pg/ml)	1174 (953–1468)	24.7	26.8	0.46	16.9 (14.5–19.3)
KIM-1 (pg/ml)	34 (23–52)	75.4	87.3	0.43	27.3 (21.4–33.5)
Urinary MCP1 excretion rate (ng/day)	128 (57–235)	56.5	105.5	0.22	18.4 (8.9–28.8)
Urinary EGF excretion rate (ng/day)	9325 (6501–12,776)	32.6	49.1	0.31	−13.5 (−16.7 to −10.1)
EGF-MCP1 ratio (ng/ng)	73 (42–156)	82.5	240.6	0.11	−26.9 (−32.2 to −21.3)
Tubular secretion score	52 (47–55)	10.0	9.5	0.52	−0.9 (−1.8 to 0.0)
eGFR (ml/min per 1.73 m^2)	110 (99–119)	9.1	13.0	0.33	−12.0 (−12.9 to −11.1)
AER (mg/24h)	11.5 (7–19)	278.8	365.5	0.37	10.9 (2.5–20.1)

AER, urinary albumin excretion rate; CI, confidence interval; CV, coefficient of variation; DCCT, Diabetes Control and Complications Trial; EDIC, Epidemiology of Diabetes Interventions and Complications Study; EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate; KIM-1, kidney injury molecule 1; MCP1, monocyte chemoattractant protein-1; sTNFR1, soluble tumor necrosis factor 1.
Except for medians (Q1, Q3), all estimates are calculated using log-transformed biomarkers to account for their right-skewed distribution. Mean change over time is reported as percent change per decade (regardless of units of measurement for each untransformed biomarker).
CVs are estimated using a model-based covariance estimated from a linear mixed model. Change over time is calculated per 10 years from the linear mixed model beta estimate as change = exp(beta) − 1.

serum or plasma KIM-1 ($r = -0.29$, $P < 0.001$), and urinary EGF excretion ($r = 0.21$, $P < 0.001$). AER correlated most strongly with urinary MCP1 excretion ($r = 0.41$, $P < 0.001$), serum or plasma KIM-1 ($r = 0.35$, $P < 0.001$), and urinary EGF-MCP1 excretion ratio ($r = -0.34$, $P < 0.001$). The strongest correlations between kidney tubular function biomarkers were observed between serum or plasma sTNFR1 and KIM-1 ($r = 0.39$, $P < 0.001$) and between urinary excretion of MCP1 and EGF ($r = 0.35$, $P < 0.001$). Notably, urinary EGF-MCP1 excretion ratio correlated more strongly with MCP1 than EGF excretion ($r = -0.83$ vs. $r = 0.16$, respectively).

Correlations between participant-specific biomarker changes over time (i.e., slopes) generally paralleled cross-sectional correlations but were weaker than those observed in cross-sectional analyses (Figure 3b and Supplementary Table S6). Change in eGFR correlated most strongly with changes in sTNFR1 ($r = -0.33$, $P < 0.001$) and tubular secretion score ($r = -0.31$, $P < 0.001$). Change in AER correlated most strongly with changes in serum or plasma KIM-1 ($r = 0.33$, $P < 0.001$) and sTNFR1 ($r = 0.26$, $P < 0.001$).

Urinary biomarker excretion rates correlated strongly with urinary biomarker to creatinine ratios. For urinary MCP1, cross-sectional and slope correlations were $r = 0.94$ and $r = 0.91$, respectively. For urinary EGF, cross-sectional and slope correlations were $r = 0.80$ and $r = 0.78$, respectively.

Clinical Factors Associated With Change in Kidney Tubular Function Biomarkers

Changes in urinary EGF and MCP1 excretion significantly differed between female and male participants (Table 3 and Supplementary Figure S2). Compared to males, female participants had a significantly slower

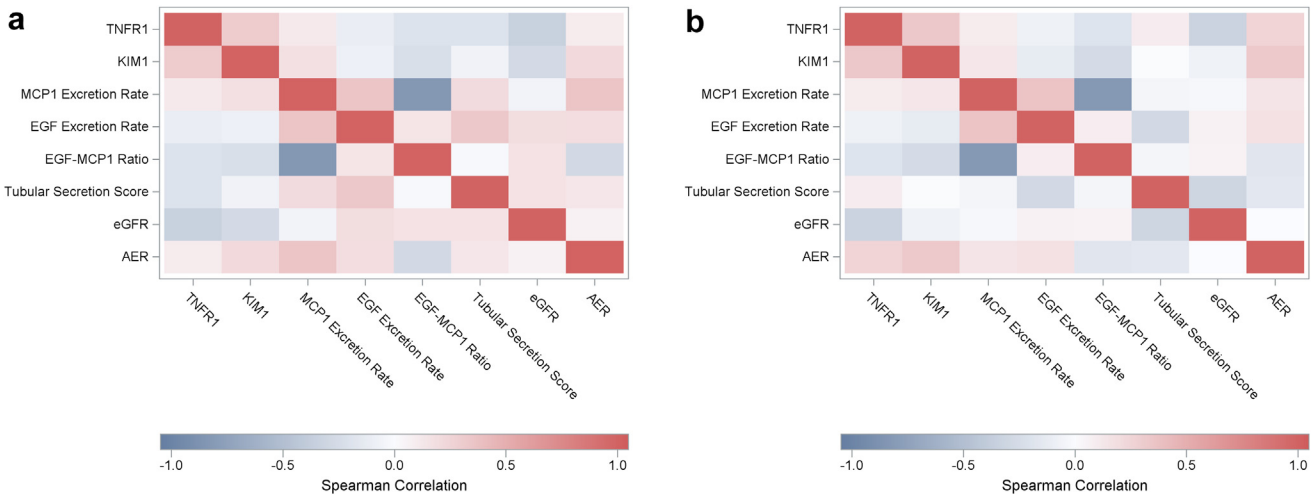

Figure 3. Spearman correlation of log-transformed (a) biomarkers and (b) biomarker slopes. AER, urinary albumin excretion rate; EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate; KIM1, kidney injury molecule 1; MCP1, monocyte chemoattractant protein-1; TNFR1, tumor necrosis factor 1.

Table 3. Associations of clinical characteristics with changes in kidney tubular function biomarkers over time

Clinical characteristics	sTNFR1	KIM-1	Urinary MCP1 excretion	Urinary EGF excretion	EGF-MCP1 ratio	Tubular secretion score	eGFR	AER
Sex								
Change among females (% change/decade)	1.18 (1.15–1.22)	1.26 (1.17–1.35)	1.04 (0.92–1.18)	0.82 (0.78–0.87)	0.79 (0.71–0.88)	0.98 (0.97–1.00)	0.88 (0.86–0.89)	1.05 (0.93–1.18)
Change among males (% change/decade)	1.16 (1.13–1.19)	1.29 (1.21–1.37)	1.31 (1.18–1.47)	0.90 (0.86–0.95)	0.69 (0.62–0.76)	1.00 (0.99–1.01)	0.88 (0.87–0.89)	1.16 (1.04–1.29)
Sex Difference in change (females compared with males)	1.02 (0.98–1.06)	0.98 (0.89–1.07)	0.79 (0.67–0.93)[b]	0.91 (0.85–0.99)[a]	1.16 (1.00–1.34)	0.99 (0.97–1.00)	0.99 (0.97–1.02)	0.91 (0.77–1.06)
DCCT therapy assignment								
Conventional glucose-lowering therapy (% change/decade)	1.21 (1.17–1.24)	1.28 (1.20–1.37)	1.09 (0.97–1.23)	0.84 (0.79–0.89)	0.77 (0.69–0.85)	0.99 (0.97–1.00)	0.88 (0.86–0.89)	1.21 (1.09–1.36)
Intensive glucose-lowering therapy (% change/decade)	1.13 (1.10–1.16)	1.27 (1.18–1.35)	1.28 (1.14–1.44)	0.89 (0.85–0.94)	0.70 (0.63–0.77)	1.00 (0.98–1.01)	0.88 (0.87–0.90)	1.01 (0.91–1.13)
Difference in change (conventional vs. intensive)	1.07 (1.02–1.11)[b]	1.01 (0.92–1.11)	0.85 (0.72–1.01)	0.94 (0.87–1.01)	1.10 (0.95–1.28)	0.99 (0.97–1.01)	0.99 (0.97–1.01)	1.20 (1.02–1.40)[a]
Hemoglobin A1c								
DCCT mean HbA1c (difference in change/1% higher HbA1c)[d]	1.03 (1.01–1.05)[b]	0.98 (0.94–1.02)	0.91 (0.84–0.99)[a]	0.98 (0.94–1.01)	1.07 (1.00–1.15)	1.00 (0.99–1.00)	0.98 (0.97–0.99)[b]	1.06 (0.98–1.14)
Mean-updated HbA1c (difference in change/1% higher HbA1c)	1.06 (1.05–1.08)[c]	1.09 (1.05–1.14)[c]	0.99 (0.92–1.06)	0.97 (0.94–1.01)	0.99 (0.93–1.06)	0.99 (0.98–1.00)	0.98 (0.97–0.99)[b]	1.26 (1.17–1.35)[c]

AER, urinary albumin excretion rate; CV, coefficients of variation; DCCT, Diabetes Control and Complications Trial; EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate; KIM-1, kidney injury molecule 1; MCP1, monocyte chemoattractant protein-1; sTNFR1, soluble tumor necrosis factor 1.
[a]P-value between 0.05 and 0.01.
[b]P-value between 0.01 and 0.001.
[c]*P*-value <0.0001.
[d]Analysis uses only biomarker data collected during EDIC Study.
Relative change in biomarker over time per increase in HbA1c is reported as increase in fold-change per decade per 1% increase in HbA1c. For example, the estimated rate of change per decade for sTNFR1 with a participant with an HbA1c of 7% is 1.10, whereas for a participant with an HbA1c of 8% is 1.17, which results in a fold change of 1.17/1.10 = 1.06.
Relative change between group, sex, and cohort is reported as the ratio between groups. All estimates are generated from linear mixed models adjusting for time in decades, risk factor, and time x risk factor interaction.

increase in urinary MCP1 excretion and a faster decrease in urinary EGF excretion over time (MCP1 relative change, 0.79; 95%: CI 0.67–0.93; EGF relative change, 0.91; 95% CI: 0.85–0.99). All other biomarkers changed similarly over time in female and male participants.

Compared to participants in the intensive therapy group, participants in the conventional therapy group had a faster increase in serum sTNFR1 over time (relative change, 1.07; 95% CI: 1.02–1.11) (Table 3 and Supplementary Figure S2). Notably, this effect was observed despite sTNFR1 being higher in the intensive than in the conventional therapy group throughout DCCT, after which during EDIC the reverse was seen. In addition, both higher mean-updated HbA1c and higher DCCT mean HbA1c were associated with greater subsequent increase in sTNFR1 over time. Higher mean-updated HbA1c was also associated with a greater increase in KIM-1 (relative change, 1.09; 95% CI: 1.05–1.14). Surprisingly, higher DCCT mean HbA1c was associated with a slower increase in urinary MCP1 excretion over time (relative change, 0.91; 95% CI: 0.84–0.99).

Higher HbA1c was associated with a more rapid decrease in the urinary clearances of p-Cresol sulfate and indoxyl sulfate (Supplementary Table S7) but not with the composite secretion score. In cross-sectional analyses, concurrent and mean-updated HbA1c on biomarker concentrations were significantly positively associated with serum or plasma KIM-1 and urinary MCP1 excretion rate, and significantly negatively correlated with urinary EGF-MCP1 ratio (Supplementary Table S8).

DISCUSSION

We examined kidney tubular biomarker trajectories over 26 years of follow-up in adults from the DCCT/EDIC in order to gain insight into the course of tubular function decline in T1D. We observed significant changes in plasma KIM-1 and sTNFR1 and urinary excretion of MCP1, EGF, and EGF-MCP1 excretion ratio over time, which are consistent with worsening tubular function. Overall, changes in tubular markers paralleled the decrease in eGFR and increase in AER but the correlations were not strong. In addition, improved glycemic control, reflected by randomization to intensive versus conventional diabetes therapy and lower HbA1c, was associated with slower increases in sTNFR1 and KIM-1 over time.

Few studies have examined longitudinal changes in tubular biomarkers, particularly in diabetes cohorts. These studies were conducted over shorter follow-up periods (1–3 years) compared to our work in DCCT/EDIC, with many focusing specifically on serial

measurements of sTNFR1.[18-20] One longer-term study of 47 adults with diabetes and an eGFR >60 ml/min per 1.73 m^2 measured sTNFR1 4 times over 8 years.[21] Overall, these studies describe increasing sTNFR1 in association with concurrent or subsequent eGFR decrease, as demonstrated in our study. Other studies have investigated tubular markers in the context of acute kidney injury, evaluating repeated measurements taken over several days.[22] Our work extends over a longer period of time and across other tubular biomarkers that have been recognized to represent various important facets of kidney tubular function.

The tubular markers we assessed in this study reflect different aspects of tubular integrity and function, including proximal tubular injury (KIM-1), secretion (tubular secretion score), functional tubular cell mass (EGF), and inflammation (sTNFR1 and MCP1).[3-11] Overall, tubular biomarkers exhibited monotonic changes over time consistent with worsening of all aspects of tubular function. We also observed a similar linear decrease in eGFR, as has been previously described. Compared to eGFR and albuminuria, most of the tubular biomarkers that we assessed demonstrated proportionally larger changes over time, most notably with plasma KIM-1 increasing and urinary EGF-MCP1 ratio decreasing by 27% per decade, respectively. These significant changes in tubular markers occurred whereas eGFR and AER were largely within the normal range, before DKD was clinically apparent. This suggests that tubular function decline occurs early in DKD, and that tubular markers (for which larger absolute changes can be readily assessed) may potentially be able to serve as more sensitive indicators of disease progression than traditional glomerular markers, especially when eGFR and urinary albumin are within the "normal" range.[17,23]

We observed weak but significant correlations between tubular biomarkers, eGFR, and AER in cross-sectional and slope analyses. Overall, eGFR correlated most strongly with sTNFR1 (cross-sectional $r = -0.40$, slope $r = -0.33$) and AER correlated most strongly with KIM-1 (cross-sectional $r = 0.35$, slope $r = 0.33$). AER also correlated with MCP1 in cross-sectional analyses ($r = 0.41$); though, in slope analyses the correlation was much weaker ($r = 0.15$). These findings, represented by biomarkers in which disease pathology progresses at different rates, may reflect underlying pathologic processes common to distinct facets of kidney function decline.

We identified sex and glycemic control as factors influencing longitudinal change in tubular biomarkers. Women had a slower increase in urinary MCP1 excretion and a faster decrease in urinary EGF excretion over time than men, whereas there was no sex difference in the rate of EGF-MCP1 ratio change. Notably, trends in creatinine-normalized urinary MCP1 and EGF concentrations demonstrated sex-related differences opposite to what we observed with timed urinary excretion of these biomarkers.[24] The reason for this finding is unclear and may be related to sex-specific differences in creatinine excretion. Among healthy adults and children, higher urinary EGF normalized to urinary creatinine and body surface area has been reported in women compared to men, consistent with our findings here. In addition, among adults with T1D, sex has been found to modify the association between creatinine-normalized urinary MCP1 and kidney tubulointerstitial lesions early in DKD.[25] Overall, our findings reiterate the importance of accounting for sex as a potential confounder in tubular biomarker analyses.

We observed significantly greater increases in sTNFR1 with higher HbA1c over the entire DCCT/EDIC study duration, as well as solely within EDIC in response to poor glycemic control during DCCT. This is consistent with findings from studies in type 2 diabetes describing a correlation between HbA1c and sTNFR1.[26] Long-term benefits of intensive glycemic therapy on sTNFR1 were apparent despite higher sTNFR1 with intensive therapy during DCCT, which has been previously described (along with higher high-sensitivity C-reactive protein).[27] Shorter-term increases in sTNFR1 with improved glycemic control in DCCT may be related to insulin-induced weight gain during this period, with long-term improvements during EDIC mirroring beneficial effects on microvascular diabetes complications which have been attributed to a metabolic memory effect.[28,29] Slowed increase in sTNFR1 may also reflect long-term improvement in insulin sensitivity with prolonged glycemic control, which has been inversely correlated with sTNFR1 in studies of obese adults.[30-33] Similarly, improved long-term glycemic control and mean-updated HbA1c were both associated with slower decrease in eGFR and increase in AER, in line with results in the full cohort.[34,35] We also noted a faster increase in KIM-1 with higher HbA1c, though only when considering shorter-term HbA1c as opposed to more distant glycemic control, consistent with associations between increasing HbA1c and rising KIM-1 described in a nondiabetes cohort.[36] Unexpectedly, higher mean-updated HbA1c was associated with a slower increase in urinary MCP1 excretion, contrary to studies describing direct correlations between these variables.[8]

The mechanisms underlying the benefits of glycemic control on sTNFR1 and KIM-1 trends are likely multifactorial. Hyperglycemia is associated with kidney tubular oxidative stress and inflammation, with

beneficial effects of glycemic control on sTNFR1 and KIM-1 potentially reflecting favorable impacts on these processes.[37] In addition, improvements in these biomarkers may be mediated by reduction in albuminuria.[38] Further research is required to better understand how hyperglycemia may affect tubular function and related biomarkers.

Enhanced understanding of the trajectories of tubular biomarkers in T1D will be necessary to determine their potential utility in clinical care and to optimize their use in research settings. Considering that tubular biomarkers reflect different aspects of tubular integrity and function, knowledge of biomarker trajectories and the correlations between these biomarkers can provide biological insight into the progression of distinct tubular pathology in DKD. Linked to the clinical kidney outcomes, tubular biomarker slopes may be useful for prognostication and for assessing responses to therapy. Therefore, our future work will focus on examining associations of longitudinal tubular biomarker trajectories with incident chronic kidney disease in T1D. In research, drug effects on tubular biomarker changes over time may serve as outcomes in clinical trials targeting kidney tubular pathology.

Strengths of this study include the use of a well-characterized T1D cohort with extensive longitudinal data allowing for performance of tubular biomarker measures on blood and urine samples at up to 7 time points per participant over 26 years. This study also has several limitations. Biomarkers were assessed over long time intervals during which kidney function was also changing, making it difficult to identify the extent to which biomarker variability was due to biologic variability or progressive kidney damage. Kidney biopsy data were not available, and thus we could not confirm the presence of tubulointerstitial pathology or link this to our functional measures of tubular injury and dysfunction. Because participants were enrolled early in the course of T1D, only a small proportion of participants developed reduced eGFR or albuminuria over the duration of the study period, preventing us from extending findings of tubular biomarker changes to advanced DKD. We were also not able to assess the effects of antihypertensives started during DCCT or EDIC on biomarker trajectories.

In conclusion, among people with T1D, we observed significant changes in kidney tubular biomarkers over 26 years follow-up, despite most participants having normal eGFR and no albuminuria. These findings suggest tubular function decline occurs early in the course and before the onset of DKD when clinical disease (assessed using traditional markers of glomerular function) becomes apparent. In addition, we identified hyperglycemia as associated with faster increases in plasma sTNFR1 and KIM-1 over time, highlighting the importance of long-term glycemic control in the preservation of tubular function.

APPENDIX

List of the DCCT/EDIC Research Group

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DISCLOSURE

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DATA AVAILABILITY STATEMENT

Data collected for the DCCT/EDIC study through June 30, 2017 are available to the public through the NIDDK Central Repository (https://repository.niddk.nih.gov/studies/edic/). Data collected in the current cycle (July 2017–June 2022) will be available within 2 years after the end of the funding cycle. Dr. Barbara H. Braffett is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

AUTHOR CONTRIBUTIONS

CPL and XG wrote the manuscript. XG and IB conducted the statistical analyses. IB, JCS, GML, BAP, ABK, VLA, AP, MEM, and IHdB reviewed and edited the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Tubular secretion score component clearances trends over time.

Figure S2. Biomarker trends over time between groups with significant relative differences.

Table S1. List of renal tubular biomarkers assayed.

Table S2. Summary of the number of biomarkers measured.

Table S3. Tubular biomarkers' coefficients of variation, intraclass correlations, and index of individuality.

Table S4. Characteristics of the tubular secretion score component biomarker standardized clearances.

Table S5. Spearman Correlation of Log -transformed Biomarkers. P -values are shown below the diagonal in grey.

Table S6. Spearman Correlation of Biomarker Slopes. P -values are shown below the diagonal in grey.

Table S7. Tubular secretion score component biomarkers' difference in relative change over time by original DCCT treatment assignment, sex, cohort, and glycemic level.

Table S8. Association between biomarkers and glycemia.

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