Associations of clinical features with kidney tubular biomarker trajectories in individuals with type 1 diabetes

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Abstract

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Background

Identification of risk factors of tubular injury and dysfunction among individuals with type 1 diabetes (T1D) may provide insight into the mechanisms underlying tubulointerstitial pathology and allow for improved kidney health prognostication and treatment.

Methods

We examined associations of clinical characteristics and drug interventions on tubular biomarker trends in two T1D cohorts: (1) the Renin Angiotensin System Study (RASS, n=283) including adults with early T1D and no clinical evidence of kidney disease, randomized to enalapril, losartan, or placebo; and (2) the Preventing Early Renal Loss in Diabetes Study (PERL, n=530) including adults with longstanding T1D and chronic kidney disease (CKD) or at risk of kidney disease progression, randomized to allopurinol or placebo. Biomarkers were measured at 3 time points (baseline, mid-trial, closeout) over 5 years follow-up in RASS and 3 years follow-up in PERL. Measurements included: KIM-1, sTNFR1, arginine-citrulline ratio in plasma; EGF, UMOD in timed urine; a composite tubular secretion score reflecting clearances of 8 proximal tubular secreted solutes.

Results

At baseline, RASS participants had a mean age of 30 years and 47% were male, with mean diabetes duration 11 years, hemoglobin A1c (HbA1c) 8.6%, iohexol-derived glomerular filtration rate (iGFR) 128 ml/min/1.73m^2, and albumin excretion rate (AER) 6 ug/min. PERL participants had a mean age of 51 years and 66% were male, with mean diabetes duration 35 years, HbA1c 8.2%, iGFR 68 ml/min/1.73m^2, and AER 286 ug/min. We observed significant changes in tubular biomarkers across both cohorts, suggesting progressive tubular injury and dysfunction.

We identified baseline HbA1c and AER as factors associated with changes in multiple tubular biomarkers. Higher baseline HbA1c was associated with faster rise in KIM-1 (4.8 pg/mL increase per year, [95% CI 2.3, 7.3]), slower decline in arginine-citrulline ratio (0.02 unit slower decrease per year, [95% CI 0, 0.03]), and faster decline in EGF in RASS (194.7 ug/day decrease per year, [95% CI 6.8, 382.7]), and with faster rise in sTNFR1 in PERL (27.6 pg/mL increase per year, [95% CI 18.8, 36.5]). Higher baseline urinary albumin excretion rate was associated with faster declines in EGF (346.1 ug/day decrease per year, [95% CI 65.3, 626.8]) in RASS , and with faster rise in sTNFR1 (18.1 pg/mL increase per year, [95% CI 14.8, 21.4]) and faster declines in EGF (139.8 ug/day decrease per year [95% CI 31.9, 247.7]) and tubular secretion score (0.2 unit decrease per year, [95% CI 0.1, 0.3]) in PERL. Age, sex, and baseline age, diabetes duration, SBP, iGFR, and randomization to intervention versus placebo had limited associations with tubular biomarker trajectories.

Conclusion

Longitudinal changes in tubular biomarkers reflect progressive tubulointerstitial injury and dysfunction across the course of T1D DKD and are influenced by baseline glycemia and albuminuria.

INTRODUCTION

Diabetic kidney disease (DKD), defined as an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73m^2 or urinary albumin excretion rate (AER) >20 μg/min, affects 30-40% of people with type 1 diabetes (T1D) and is associated with increased risk of cardiovascular events, morbidity, and mortality.[1] Traditionally, DKD has been viewed as a glomerular disease process characterized by abnormalities in glomerular structural features and biomarkers of glomerular function (eGFR, AER). Increasingly, tubular injury and dysfunction are being recognized as important drivers of kidney disease onset and progression, with several biomarkers of kidney tubular injury, inflammation, and function garnering interest as potential predictors of long-term adverse kidney outcomes.[2–4]

Kidney injury molecule-1 (KIM-1) is a transmembrane protein that is expressed in the proximal tubule in response to injury.[5] Soluble tumor necrosis factor receptor 1 (sTNFR1) is a circulating inflammatory marker associated with kidney injury and dysfunction.[6] Increased plasma KIM-1 and sTNFR1 reflect kidney tubular injury and inflammation, respectively, and are associated with adverse kidney outcomes.[5,6] Arginine is synthesized from citrulline in the proximal tubule, uromodulin (UMOD) is produced in the thick ascending limb of the loop of Henle, and epidermal growth factor (EGF) is produced in the distal tubule.[7–9] These biomarkers reflect the synthetic capacity and viability of their respective tubular cell segments. The ratio of plasma arginine to citrulline has been used as a marker of tubular function, and high plasma citrulline concentrations have been associated with chronic kidney disease (CKD) risk.[10,11] Lower concentrations of UMOD and EGF in urine are associated with kidney disease progression.[8,9,12] Lastly, reduced clearance of organic solutes secreted by the proximal tubule is suggestive of proximal tubular dysfunction and has been associated with eGFR decline.[13]

While associations between tubular biomarkers and adverse long-term kidney outcomes are well-described in populations with and without diabetes, the factors contributing to changes in tubular biomarkers over time remain poorly understood.[2–4] Enhanced understanding of risk factors for progressive tubular injury and dysfunction in T1D may provide insight into the mechanisms underlying tubulointerstitial pathology, allow for improved kidney health prognostication, and highlight targets for intervention. Additionally, whether existing therapies which are utilized (angiotensin converting enzyme inhibitors [ACEi], angiotensin receptor blockers [ARB]) or have been investigated (allopurinol) in the treatment of DKD delay the progression of tubular pathology remains unknown.

Here, I identify clinical factors associated with longitudinal changes in tubular biomarkers and investigate whether treatment with renin-angiotensin-system inhibition (using ACEi or ARB) or allopurinol has a beneficial impact on tubular biomarker trajectories in T1D. I used data from two T1D randomized clinical trials: (1) the Renin Angiotensin System Study (RASS)[14] of adults with early T1D and no clinical evidence of kidney disease, in which participants were randomized to enalapril (ACEi), losartan (ARB), or placebo; (2) the Preventing Early Renal Loss in Diabetes Study (PERL)[15] of adults with longstanding T1D and CKD or risk for kidney disease progression, in which participants were randomized to allopurinol or placebo.

METHODS

Study design and population

RASS was a multicenter, randomized, double-blind, placebo-controlled trial testing the effects of the angiotensin-converting enzyme (ACE) inhibitor enalapril or the angiotensin receptor blocker (ARB) losartan on early kidney morphological changes in T1D (NCT00143949).[14] RASS recruited participants who were at least 16 years of age with T1D duration ≤ 20 years and without a history of kidney disease (defined as GFR < 90 ml/min/1.73m^2 and/or a UAER > 20 μg/min) or hypertension (defined as blood pressure > 135/85 mmHg or antihypertensive medication use). Individuals who were pregnant were excluded. A total of 285 participants were enrolled from the University of Minnesota (Minneapolis, Minnesota), McGill University (Montreal, Canada), and the University of Toronto (Toronto, Canada) and randomized to either losartan 100 mg daily, enalapril 20 mg daily, or placebo. Participants underwent kidney biopsies at baseline and at the end of the 5-year follow-up period. The primary study outcome was 5-year change in kidney mesangial fractional volume (the fraction of glomerular volume occupied by mesangium).

PERL was a multicenter, randomized, double-blind, placebo-controlled trial testing the effects allopurinol on kidney disease progression in adults with T1D and CKD (NCT02017171).[15,16] PERL recruited adults who were at least 18 years of age with T1D duration ≥ 8 years with a serum uric acid concentration of ≥ 4.5 mg/dL and evidence of kidney disease (defined as eGFR 40-99.9 ml/min/1.73m^2 plus UAER 20-3333 μg/min or eGFR decline ≥ 3 ml/min/1.73m^2/year over the preceding 3-5 years). Adults with a history of gout or kidney stones or who were pregnant or breastfeeding were excluded. A total of 530 participants were enrolled across 16 sites in the United States, Canada, and Denmark and randomized to 200-400 mg allopurinol (based on eGFR) or placebo. Participants were followed for 3 years. The primary study outcome was 3-year change in iohexol-based GFR.

Measurement of kidney tubular and inflammatory markers

Blood and timed overnight urine samples were collected in RASS annually. In PERL, blood samples were collected every 3-4 months and timed overnight urine samples were collected every 2-7 months. These specimens were processed and stored at -80°C at the University of Minnesota Advanced Research and Diagnostic Laboratory (ARDL). Blood and urine samples from RASS visits 0 (baseline), 3 (156 weeks), 5 (260 weeks), and 6 (closeout, 268 weeks; after 8 weeks drug washout) and from PERL visits 0 (baseline), 11 (mid-trial, 80 weeks), 16 (end-of-treatment, 156 weeks), and 17 (closeout, 164 weeks; after 8 weeks drug washout) were shipped to the University of Washington for kidney tubular disease biomarker measurements.

To identify and exclude results from urine samples likely to have inaccurate collection or time reporting, urinary creatinine excretion was compared to expected creatinine excretion based on sex, age, height, and weight.[17] Timed urine samples for which calculated creatinine excretions were at the upper and lower 0.5 percentiles of the predicted values, suggestive of over- or under-collections, respectively, (97 samples, 2% of PERL samples and 2% of RASS samples, from 46 total participants) were assigned missing values.

For plasma biomarkers KIM-1 and sTNFR1, we calculated intraclass correlation coefficients (ICCs) using replicate measurements: four duplicates each from two healthy controls; five duplicates from a pooled sample of individuals with CKD; duplicates from 9 RASS participants and 9 PERL participants. Plasma concentrations of KIM-1 were determined using a monoclonal antibody sandwich immunoassay (ENZO Life Sciences, Farmingdale, NY), yielding

an ICC of 0.993. Plasma concentrations of sTNFR1 were determined using a single-step monoclonal capture antibody-polyclonal reporter antibody sandwich immunoassay (R&D Systems, Minneapolis, MN). The ICC was 0.998. Unfortunately, granular data for ICC calculation are not available for urine UMOD, urine EGF, plasma arginine and citrulline, plasma and urine secretory solutes.

For urine biomarkers UMOD and EGF, inter-assay coefficients of variation (CVs) were calculated across 30 batches, with a high concentration and low concentration each measured in duplicate in each batch (total 60 measurements). Urinary concentrations of UMOD were determined using a polyclonal antibody sandwich immunoassay (MD Bioproducts, Oakdale, MN). Samples were diluted 750-fold with assay diluent prior to analysis. Inter-assay CVs ranged from 18.4% to 35.1%. Urinary concentrations of EGF were determined using a monoclonal capture antibody-polyclonal reporter antibody sandwich immunoassay (R&D Systems, Minneapolis, MN). Samples were diluted 200-fold with assay diluent prior to analysis. Inter-assay CVs ranged from 10.9% to 11.2%.

For plasma arginine and citrulline, as well as for the 8 plasma and urine secretory solutes, inter-assay CVs were calculated across 26 batches, with a high concentration and low concentration each measured in duplicate in each batch (total 52 measurements). Plasma concentrations of arginine and citrulline were quantified using liquid chromatography-tandem mass spectrometry, as previously described.[18] The imprecision of the assay was 10% CV at 37 μM and 12% at 60 μM for citrate and 11% at 74 μM and 12% CV at 195 μM for arginine. All assays were conducted as per manufacturer's instructions. Plasma and urine concentrations of a panel of 8 secretory solutes (cinnamoylglycine, indoxyl sulfate, isovalerylglycine, kynurenic acid, p-cresol sulfate, pyridoxic acid, tiglylglycine, xanthosine) were assayed using mass spectrometry, for which individual solute clearances were calculated [clearance = (urine concentration * urine volume)/plasma concentration)]. Clearances for each of the 8 secretory solutes were then log-transformed, min-max standardized, and averaged to create a composite tubular secretion score.[13] The imprecision of the measurements of solutes in plasma was 14-21% CV and in urine was 7-24% CV.

Measurement of glomerular filtration rate and urinary albumin excretion rate

GFR was measured via rate of plasma disappearance of injected iohexol (iGFR). Iohexol-based GFR was assessed annually in RASS and at visits 0, 11, 16, and 17 in PERL. Plasma iohexol was measured using high-performance liquid chromatography (HPLC) in both studies. UAER was measured from timed urine collections. In RASS, UAER measurements were performed annually using a sensitive fluorescence immunoassay. In PERL, UAER measurements were performed at visits 0, 7, 9, 11, 13, 15, 16, 17 using an immunoturbidimetric method.[19] These laboratory measurements were performed at the University of Minnesota ARDL.

Clinical characteristics and covariates

In both RASS and PERL, demographic information and medical history were ascertained at baseline using interviewer-administered questionnaires.[16] Systolic and diastolic blood pressures were measured after the participant was seated in a quiet room for five minutes using an automatic blood pressure device. Hemoglobin A1c was measured from plasma using standard HPLC methods at the University of Minnesota ARDL.

Statistical analyses

Correlations among biomarkers were assessed using the Spearman rank-order method. Kidney tubular biomarker slopes and 95% confidence intervals were calculated by fitting linear mixed models with random effects fort participant and time since randomization. Biomarker slopes were calculated as both absolute and relative (percent) changes per year. Median biomarker concentrations for the overall study period and at each time point were also calculated. Multivariable linear mixed models with random effects for participant and time since randomization were fit to determine associations of associations of sex (male/female), age (per year), diabetes duration (per year), systolic blood pressure (per 5 mmHg higher), hemoglobin A1c (per 1% higher), iGFR (per 10 ml/min/1.73m^2 higher), UAER (per doubling) with subsequent tubular biomarker slope. Specifically, the following model was fit: biomarker ~ exposure * study * time + (1+time|study_id). A series of models were fit: (1) an unadjusted model; (2) a minimally-adjusted model including age and sex; (3) a fully-adjusted model including age, sex, hemoglobin A1c, diabetes duration, iGFR, and UAER. 95% confidence intervals on the estimated biomarker slope sizes were also calculated.

Linear mixed models with random effects for participant and time since randomization were likewise fit to estimate effects of enalapril or losartan versus placebo (in RASS) or allopurinol versus placebo (in PERL) with subsequent tubular biomarker slope. Specifically, the following model was fit: biomarker ~ intervention * study * time + (1+time|study_id). Given randomization of the study intervention, only unadjusted models were fit. 95% confidence intervals on the estimated biomarker slope sizes were also calculated.

RESULTS

Participant characteristics

A total of 283 out of 285 RASS participants and all 530 PERL participants were included in the study (Table 1). At baseline in RASS, participants had a mean age of 30 years, 47% were male, and 98% self-identified as White. RASS participants had a mean diabetes duration of 11 years, HbA1c 8.6%, iGFR 128 ml/min/1.73m^2, and AER 6 ug/min. Participants were randomized to losartan, enalapril, or placebo. At baseline in PERL, participants had a mean age of 51 years, 66% were male, 84% identified as White, 11% identified as Black, and 4% identified as Hispanic or Latino. PERL participants had a mean diabetes duration of 35 years, HbA1c 8.2%, iGFR 68 ml/min/1.73m^2, and AER 286 ug/min. Participants were randomized to allopurinol or placebo.

Tubular biomarker trajectories

Across both RASS and PERL, longitudinal absolute and percent changes in tubular biomarker concentrations reflected worsening tubular injury and dysfunction over time (Table 2). Overall, in PERL compared to RASS, plasma KIM-1 and sTNFR1 concentrations were higher and plasma arginine-citrulline ratio, urinary EGF excretion, and tubular secretion score were lower, consistent with more severe kidney disease. Additionally, both absolute and percent changes in biomarker concentrations per year were generally of greater magnitude in PERL compared to RASS.

KIM-1 and sTNFR1 had the greatest absolute changes in concentration per year, with KIM-1 increasing annually in RASS and PERL by 5.7 pg/mL (5.4% increase/year) and 7.5 pg/mL (2.9% increase/year), respectively, and sTNFR1 increasing annually in RASS and PERL by 14.2 pg/mL (1.3% increase/year) and 75.0 pg/mL (3.4% increase/year), respectively.

Arginine-citrulline ratio decreased annually in RASS by 0.03 (1.7% decrease/year), while this measure remained stable over time in PERL. Urinary UMOD excretion decreased annually by 3.4 mg/day (5.3% decrease/year) in PERL, with no significant absolute changes in RASS over time. Meanwhile, urinary EGF excretion decreased annually by 0.3 µg/day (1.8% decrease/year) in RASS, with no significant absolute changes in PERL over time. Tubular secretion score demonstrated no significant absolute change over time in RASS and declined by 0.5 units (1.1% decrease/year) annually in PERL. By contrast, iGFR declined annually by 1.4 ml/min/1.73m^2 (1.2% decrease/year) and 1.8 ml/min/1.73m^2 (3.4% decrease/year) in RASS and PERL, respectively, and AER only rose substantially in PERL over time, by 45 µg/min (7.0% increase/year) each year.

Associations of baseline characteristics with tubular biomarker trajectories

The extent to which baseline clinical characteristics influenced biomarker slopes is shown in Table 3. Notably, baseline HbA1c and AER in particular were associated with the changes in the most tubular biomarker trajectories compared to other examined clinical characteristics, adjusting for baseline and clinical variables.

Overall, tubular biomarker concentrations were similar by baseline HbA1c in RASS, while in PERL higher baseline concentrations of KIM-1 and sTNFR1 were seen with higher baseline HbA1c (Supplementary Tables 1, 2). In RASS, higher baseline HbA1c was associated with faster rise of KIM-1 (4.8 pg/mL increase per year per 1% higher HbA1c), [95% CI 2.3, 7.3]), faster decrease in urinary EGF excretion (194.7 ug/day decrease per year per 1% higher HbA1c, [95% CI 6.8, 382.7]), and slower decrease in arginine-citrulline ratio (0.02 unit slower decrease per year per 1% higher HbA1c, [95% CI 0, 0.03]). In PERL, higher baseline HbA1c was associated with faster rise in sTNFR1 (27.6 pg/mL increase per year per 1% higher HbA1c, [95% CI 18.8, 36.5]).

In RASS, all participants were normoalbuminuric at baseline (Supplementary Table 3). In PERL, baseline concentrations of KIM-1 and sTNFR1 were higher with macroalbuminuria versus microalbuminuria versus normoalbuminuria (Supplementary Table 4). In RASS, higher baseline AER was associated with faster decrease in urinary EGF excretion (346.1 ug/day decrease per year per doubling in AER, [95% CI 65.3, 626.8]). In PERL, higher baseline AER was associated with faster increases in sTNFR1 (18.1 pg/mL increase per year per doubling in AER, [95% CI 14.8, 21.4]) and AER (16.8 ug/min increase per year per doubling in AER, [95% CI 12.0, 21.6]), and faster decreases in summary secretion score (0.2 unit decrease per year per doubling in AER, [95% CI 0.1, 0.3]) and iGFR (0.3 ml/min/1.73m^2 decrease per year per doubling in AER, [95% CI 0.2, 0.4]).

Age, SBP, and iGFR had few associations with tubular biomarker trends (Table 3). Baseline age was not associated with changes in tubular markers in RASS. In PERL, older age was associated with slower rise in sTNFR1 (1.7 pg/mL slower rise per year per 1 year older age, [95% CI 0.7, 2.7]) and AER (3.6 slower rise per year per 1 year older age, [95% CI 2.0, 5.2]), and slower decline in iGFR (0.04 ml/min/1.73m^2 slower decrease per year per 1 year older age, 95% CI 0.01, 0.07]) (Supplementary Tables 5, 6). Higher baseline SBP was associated with faster decrease in arginine-citrulline ratio (0.008 units decrease per year per 5 mmHg higher, [95% CI 0.001, 0.016]) in RASS and with faster rise in sTNFR1 (5.9 pg/mL per 5 mmHg higher, [95% CI 2.1, 9.6]) and faster decrease in iGFR (0.2 ml/min/1.73m^2 decrease per year per 5 mmHg higher, [95% CI 0.03, 0.3]) in PERL (Supplementary Tables 7, 8). Higher baseline iGFR

was associated with faster decrease of iGFR in RASS (0.4 ml/min/1.73m^2 decrease per year per 10 ml/min/1.73m^2 higher iGFR, [95% CI 0.3, 0.6]), and with no changes in tubular biomarkers. In PERL, higher baseline iGFR was associated with slower rise in sTNFR1 (22.4 pg/mL slower rise per year per 10 ml/min/1.73m^2 higher iGFR, [95% CI 16.3, 28.6]), and AER (12.5 ug/min slower rise per year per 10 ml/min/1.73m^2 higher iGFR, [95% CI 2.3, 22.7]), and faster decrease in iGFR (0.3 ml/min/1.73m^2 decrease per year per 10 ml/min/1.73m^2 higher iGFR, [95% CI 0.1, 0.5]) (Supplementary Tables 9, 10).

There were no associations of diabetes duration or sex with absolute change in tubular biomarker concentrations per year in either RASS or PERL (Table 3; Supplementary Tables 11-14). However, in PERL, longer diabetes duration was associated with slower rise in AER (-2.5 ug/min increase per year per 1 year longer diabetes duration [95% CI -3.8, -1.1]) and male sex was associated with faster rise in AER (51.1 ug/min increase per year for male versus female sex [95% CI 14.2, 87.9]).

Associations of interventions with tubular biomarker trajectories

Baseline tubular biomarker concentrations did not differ by treatment assignment in either RASS or PERL (Supplementary Tables 15, 16). In RASS, there were no significant effects of either losartan or enalapril on tubular biomarker trajectories compared to placebo (Table 4). Meanwhile, in PERL, there were no significant effects of allopurinol with tubular biomarker trajectories compared to placebo. However, use of allopurinol had the effect of a faster rise in AER versus placebo (58.0 ug/min rise per year; [95% CI 29.6, 86.3]).

DISCUSSION

We observed longitudinal changes in multiple tubular biomarkers over 5 and 3 years of follow-up in the RASS and PERL clinical trials of people with T1D, respectively. In RASS, plasma KIM-1 and sTNFR1 increased while plasma arginine-citrulline ratio and urinary EGF excretion decreased over time. In PERL, plasma KIM-1 and sTNFR1 increased and urinary UMOD excretion and tubular secretion score decreased over time. We identified baseline HbA1c and AER as factors associated with changes in multiple tubular biomarkers. Specifically, higher HbA1c was linked to early worsening tubular function in T1D before clinical kidney disease is apparent, whereas higher AER was associated with worsening tubular function in established T1D and CKD. In contrast, sex, and baseline age, diabetes duration, SBP, and iGFR had limited associations with tubular biomarker trajectories. Additionally, randomization to losartan or enalapril versus placebo in RASS, and to allopurinol versus placebo in PERL did not significantly impact tubular biomarker trajectories.

Overall, in PERL compared to RASS, concentrations of tubular injury markers (plasma KIM-1 and sTNFR1) were higher and concentrations of tubular function markers (plasma arginine-citrulline ratio, urinary EGF and UMOD, tubular secretion score) were lower, consistent with more severe baseline kidney dysfunction. Longitudinal absolute and percent changes in tubular biomarkers were generally of greater magnitude in PERL than in RASS, suggestive of more rapid progression of tubular pathology in later versus early stages of CKD in people with T1D. Notably, however, while tubular biomarkers worsened more rapidly in PERL than in RASS, absolute rates of iGFR decline were similar (-1.4 ml/min/1.73m^2 in RASS, -1.8 ml/min/1.73m^2 in PERL).

Associations of HbA1c with rate of change in multiple tubular biomarkers are consistent with results from the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) study of T1D, in which higher HbA1c was associated with significantly greater increases in KIM-1 and sTNFR1 over time and randomization to intensive versus conventional glucose lowering therapy was associated with slower long-term rise in sTNFR1.[20] The DCCT/EDIC study, similar to RASS, included participants with T1D and normal GFR without albuminuria at baseline. We found that higher HbA1c was associated a greater number of tubular biomarkers in RASS than PERL (where participants already had kidney disease at enrollment), which suggests that HbA1c plays an important role in tubular dysfunction in T1D early in the course of DKD. Associations of glycemia and insulin resistance with KIM-1 and sTNFR1 have also been described in smaller studies of adults with and without diabetes.[21–23] Our results strengthen prior research identifying an association between hyperglycemia and kidney tubular injury and dysfunction, perhaps via induction of oxidative stress, inflammation, and mitochondrial dysfunction.[24]

Associations of baseline AER with change in tubular biomarkers, particularly in PERL where participants already had kidney disease at study start, may reflect common activation of pathological pathways in the glomerulus and tubulointerstitium resulting in injury to both of these compartments. Hyperglycemia-mediated endothelial dysfunction, inflammation, and fibrosis have been implicated in both glomerular and tubulointerstitial diabetes-related pathology.[25] Elevations in urinary tubular markers reflecting tubulointerstitial injury sometimes precede albuminuria onset.[26,27] Albuminuria may also contribute to tubular injury through direct toxic effects, autophagy and lysosomal dysfunction, and inflammation.[28,29] One prior study found tubular biomarkers to be associated with subsequent eGFR decline in type 2 diabetes only in people with albuminuria.[30] Moreover, some of the kidney-protective effects of sodium-glucose cotransporter 2 inhibitors have been attributed to changes in proximal tubular albumin uptake and metabolism ultimately resulting in reduced oxidative stress and fibrosis.[31]

We generally failed to observe tubular biomarker trends associated with sex, age, diabetes duration, SBP, and iGFR. This suggests that these factors have little impact on tubular function both early and later in the course of DKD. Moreover, neither enalapril nor losartan compared to placebo affected tubular biomarker trends in RASS, nor did allopurinol compared to placebo influence tubular biomarker trends in PERL. This is consistent with results from the main RASS and PERL trials, where none of these agents were found to slow kidney disease progression in their respective cohorts.

Strengths of our study include use of two well-characterized T1D randomized clinical trials encompassing a wide range of kidney disease, assessment of multiple complementary measurements of tubulointerstitial injury and function, and use of plasma and urine measurements collected prospectively in a pre-specified manner and processed using standardized laboratory assays to examine longitudinal changes over time. Our study also has several limitations. RASS study participants were predominantly of White race, limiting applicability to other races and ethnicities. We were not able to assess effects of antihypertensives or other medications started during the study period on tubular biomarker trends. Data on long-term kidney outcomes, such as incident kidney failure, are not available in RASS and PERL.

Understanding tubular biomarker changes over time and the factors that influence them is important for establishing their applicability in clinical care and research. Longitudinal

measurement of tubular biomarkers may be valuable not only for monitoring kidney disease progression, but also for assessing risk of long-term risk of adverse kidney outcomes. Identification of HbA1c and AER as potential risk factors for progressive tubular dysfunction in DKD in T1D adds to the mechanistic understanding of disease. These associations highlight the importance of glycemic control and monitoring and treatment of albuminuria for preserving tubular health. Overall, our findings point towards potential utility of long-term tubular biomarker assessment in the evaluation and prognostication of kidney disease in T1D.

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TABLES

Table 1. Selected baseline characteristics of RASS and PERL participants.

	RASS (n=283)	PERL (n=530)
Age, years	29.7 (9.7)	51.1 (10.9)
Male	132 (46.6)	351 (66.2)
Race		
Asian	-	6 (1.1)
Black	-	58 (10.9)
Hawaiian	-	1 (0.2)
Indian	-	2 (0.4)
Multi-race	-	11 (2.1)
Prefer not to answer	-	3 (0.6)
Unknown or not reported	-	3 (0.6)
White	277 (97.9)	446 (84.2)
Ethnicity		
Hispanic or Latino	-	23 (4.3)
Non-Hispanic or Non-Latino	-	504 (95.1)
Unknown/Undisclosed	-	3 (0.6)
Diabetes duration	11.2 (4.76)	34.57 (12.33)
Hypertension history	0 (0)	491 (92.6)
Cardiovascular disease history		
No	-	398 (75.1)
Other	-	2 (0.4)
Yes	-	103 (19.4)
RASi use at baseline	-	477 (90.0)
Statin use at baseline		
No	-	257 (48.5)
Yes	-	215 (40.6)
BMI (kg/m^2)	25.7 (3.7)	29.47 (6.02)
SBP (mmHg)	119.7 (11.5)	125.98 (14.2)
DBP (mmHg)	70.21 (8.4)	71.25 (10.2)
Hemoglobin A1c (%)	8.56 (1.6)	8.15 (1.3)
iGFR ($ml/min/1.73m^2$)	128.61 (20.1)	68.00 (16.9)
eGFR, creatinine-based 2021 ($ml/min/1.73m^2$)	112.94 (15.2)	77.07 (19.6)
AER (ug/min)	6.39 (5.9)	284.50 (679.5)
Randomized to intervention (%)		
Placebo	95 (33.6)	263 (49.6)
Losartan	96 (33.9)	-
Enalapril	92 (32.5)	-
Allopurinol	-	267 (50.4)

Continuous variables presented as mean (SD) unless otherwise stated. Missing values in PERL, N (%): Cardiovascular disease history: 27 (5.1); retinopathy history: 35 (6.6); statin use at baseline: 58 (10.9)

Table 2. Tubular biomarker median values and changes over time in the RASS and PERL study populations.

Biomarker*	Median across all time points (Q1 median, Q3 median)**		Absolute change per year (95% CI)		Percent change per year (95% CI)	
	RASS	PERL	RASS	PERL	RASS	PERL
KIM-1 (pg/mL)	38.0 (28.8, 56.0)	114.3 (69.8, 204.9)	5.7 (1.6, 9.8)	7.5 (3.3, 11.8)	5.4 (4.0, 6.7)	2.9 (1.6, 4.1)
sTNFR1 (pg/mL)	894.5 (794.4, 1023.6)	1587.1 (1254.0, 2079.9)	14.2 (3.6, 24.8)	75.0 (64.6, 85.4)	1.3 (0.8, 1.8)	3.4 (2.9, 3.9)
UMOD excretion (mg/day)***	56.3 (24.4, 110.7)	67.3 (34.1, 111.0)	0.6 (-1.5, 2.8)	-3.4 (-6.1, -0.7)	0.2 (-2.8, 3.3)	-5.3 (-8.8, -1.7)
EGF excretion (µg/day)***	25.9 (20.5, 33.7)	10.5 (6.7, 15.7)	-0.30 (-0.58, -0.02)	-0.25 (-0.55, 0.05)	-1.8 (-3.3, -0.2)	-4.4 (-6.1, -2.7)
Arginine-citrulline ratio	1.9 (1.6, 2.2)	1.5 (1.2, 1.9)	-0.03 (-0.05, -0.01)	0.01 (-0.02, 0.03)	-1.7 (-2.7, -0.8)	0.1 (-1.0, 1.3)
Tubular secretion score***	61.3 (57.8, 64.0)	56.3 (50.9, 60.7)	0.04 (-0.17, 0.25)	-0.53 (-0.8, -0.27)	0.1 (-0.3, 0.5)	-1.1 (-1.6, -0.6)
iGFR (ml/min/1.73 m^2)	126.0 (113.0, 136.8)	65.0 (51.8, 77.5)	-1.4 (-1.7, -1.1)	-1.8 (-2.2, -1.5)	-1.2 (-1.6, -0.7)	-3.4 (-3.8, -2.9)
AER (µg/min)***	4.7 (3.7, 6.8)	55.3 (9.1, 253.9)	0.6 (-15.3, 16.4)	45.0 (30.7, 59.3)	-3.1 (-6.0, -0.1)	7.0 (4.1, 10.1)

* Overall median biomarker measures calculated by calculating each individual's median over time, then calculating the median of medians and Q1, Q3 of medians.

** All biomarkers are log-transformed to calculate percent change per year. Percent change per year was calculated from linear mixed models accounting for time since randomization.

*** Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Table 3. Associations of baseline clinical characteristics with tubular biomarker trends, RASS and PERL participants

	RASS	PERL
Baseline clinical characteristics with Biomarker outcomes	Difference in absolute change/year (95% CI)	Difference in absolute change/year (95% CI)
Sex (ref: male)		
KIM-1 (pg/mL)	1.7 (-6.3, 9.6)	6.0 (-2.6, 14.6)
sTNFR1 (pg/mL)	2.0 (-18.1, 22.2)	14.4 (-8.9, 37.7)
UMOD (mg/day)*	2.6 (-1.7, 6.9)	5.5 (-0.7, 11.7)
EGF (µg/day)*	194.7 (-392.3, 781.6)	-269.0 (-987.2, 449.2)
Arginine-citrulline ratio	-0.01 (-0.04, 0.03)	0.00 (-0.04, 0.05)
Summary secretion score*	0.0 (-0.4, 0.4)	-0.4 (-1.0, 0.3)
iGFR (ml/min/1.73m^2)	0.4 (-0.3, 1.0)	-0.1 (-0.9, 0.7)
AER (µg/min)*	0.9 (-36.2, 38.0)	51.1 (14.2, 87.9)
Age (per 1 year greater)		
KIM-1 (pg/mL)	0.1 (-0.3, 0.5)	0.1 (-0.3, 0.5)
sTNFR1 (pg/mL)	0.2 (-0.8, 1.2)	-1.7 (-2.7, -0.7)
UMOD (mg/day)*	0.0 (-0.2, 0.3)	-0.2 (-0.5, 0.1)
EGF (µg/day)*	-18.1 (-48.2, 12.0)	3.6 (-27.5, 34.7)
Arginine-citrulline ratio	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)
Summary secretion score*	-0.01 (-0.03, 0.02)	0.02 (-0.01, 0.04)
iGFR (ml/min/1.73m^2)	-0.03 (-0.06, 0.0)	0.04 (0.01, 0.07)
AER (µg/min)*	-0.02 (-1.91, 1.87)	-3.59 (-5.17, -2.01)
Diabetes duration (per 1 year greater)		
KIM-1 (pg/mL)	-0.7 (-1.5, 0.1)	0.1 (-0.2, 0.4)
sTNFR1 (pg/mL)	-0.3 (-2.4, 1.8)	-0.4 (-1.3, 0.5)
UMOD (mg/day)*	0.3 (-0.2, 0.7)	-0.1 (-0.3, 0.2)
EGF (µg/day)*	15.1 (-46.8, 77.0)	11.1 (-15.7, 37.9)
Arginine-citrulline ratio	0 (0, 0)	0 (0, 0)
Summary secretion score*	0.01 (-0.03, 0.06)	0.02 (0, 0.04)
iGFR (ml/min/1.73m^2)	-0.03 (-0.1, 0.04)	0.01 (-0.02, 0.04)
AER (µg/min)*	0.01 (-3.87, 3.89)	-2.46 (-3.84, -1.09)
SBP (per 5 mmHg greater)		
KIM-1 (pg/mL)	-0.7 (-2.4, 1.0)	-0.6 (-2.0, 0.8)
sTNFR1 (pg/mL)	-0.9 (-5.2, 3.3)	5.9 (2.1, 9.6)
UMOD (mg/day)*	0.3 (-0.6, 1.2)	0.5 (-0.6, 1.5)
EGF (µg/day)*	41.1 (-83.4, 165.7)	-75.7 (-194.0, 42.7)
Arginine-citrulline ratio	-0.008 (-0.016, -0.001)	0.001 (-0.007, 0.008)
Summary secretion score*	0.03 (-0.06, 0.12)	-0.05 (-0.15, 0.06)
iGFR (ml/min/1.73m^2)	0.10 (-0.04, 0.24)	-0.16 (-0.28, -0.03)
AER (µg/min)*	-0.03 (-7.96, 7.89)	5.16 (-0.87, 11.20)
HbA1c (per 1% higher)		
KIM-1 (pg/mL)	4.8 (2.3, 7.3)	-0.3 (-3.6, 3.0)
sTNFR1 (pg/mL)	3.8 (-2.5, 10.1)	27.6 (18.8, 36.5)
UMOD (mg/day)*	-0.9 (-2.2, 0.5)	-0.4 (-2.8, 2.0)
EGF (µg/day)*	-194.7 (-382.7, -6.8)	-96.4 (-378.3, 185.5)
Arginine-citrulline ratio	0.02 (0, 0.03)	0 (-0.02, 0.02)
Summary secretion score*	-0.05 (-0.19, 0.09)	-0.17 (-0.41, 0.07)
iGFR (ml/min/1.73m^2)	-0.2 (-0.4, -0.03)	-0.3 (-0.6, 0.0)
AER (µg/min)*	0.7 (-11.3, 12.7)	15.1 (0.4, 29.8)
iGFR (per 10 ml/min/1.73m^2 higher)		

KIM-1 (pg/mL)	1.8 (-0.2, 3.7)	-1.3 (-3.6, 1.1)
sTNFR1 (pg/mL)	-1.5 (-6.0, 3.0)	-22.4 (-28.6, -16.3)
UMOD (mg/day)*	-0.1 (-1.2, 1.0)	0.5 (-1.3, 2.2)
EGF (µg/day)*	63.1 (-88.4, 214.6)	-23.3 (-221.8, 175.1)
Arginine-citrulline ratio	0.004 (-0.005, 0.012)	0.002 (-0.011, 0.015)
Summary secretion score*	0.01 (-0.11, 0.12)	-0.01 (-0.18, 0.16)
iGFR (ml/min/1.73m^2)	-0.4 (-0.6, -0.3)	-0.3 (-0.5, -0.1)
AER (µg/min)*	0.3 (-9.3, 9.9)	-12.5 (-22.7, -2.3)
AER (per µg/min doubling)		
KIM-1 (pg/mL)	0.4 (-3.5, 4.3)	0.4 (-0.9, 1.7)
sTNFR1 (pg/mL)	1.8 (-6.8, 10.3)	18.1 (14.8, 21.4)
UMOD (mg/day)*	-0.7 (-2.7, 1.4)	-0.4 (-1.3, 0.6)
EGF (µg/day)*	-346.1 (-626.8, -65.3)	-139.8 (-247.7, -31.9)
Arginine-citrulline ratio	0.01 (-0.01, 0.02)	0.0 (-0.01, 0.0)
Summary secretion score*	-0.3 (-0.5, -0.1)	-0.2 (-0.3, -0.1)
iGFR (ml/min/1.73m^2)	0.03 (-0.28, 0.34)	-0.31 (-0.42, -0.19)
AER (µg/min)*	0.5 (-14.1, 15.2)	16.8 (12.0, 21.6)

Models are adjusted for age, sex, diabetes duration, HbA1c, SBP, iGFR, AER, intervention. Significant differences are bolded for clarity.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Table 4. Effects of enalapril or losartan versus placebo in RASS and allopurinol versus placebo in PERL with tubular biomarker trends.

	RASS		PERL
	Difference in absolute change/year (95% CI)		Difference in absolute change/year (95% CI)
	Losartan vs placebo	Enalapril vs placebo	Allopurinol vs placebo
KIM-1 (pg/mL)	0.3 (-9.9, 10.4)	2.3 (-7.9, 12.5)	2.4 (-6.1, 10.9)
sTNFR1 (pg/mL)	10.8 (-15.2, 36.8)	6.6 (-19.7, 32.8)	1.7 (-19.2, 22.5)
UMOD (mg/day)*	-1.9 (-7.1, 3.3)	-2.1 (-7.3, 3.1)	1.1 (-4.3, 6.5)
EGF (µg/day)*	0.4 (-0.3, 1.1)	0.3 (-0.4, 1.0)	0.3 (-0.3, 0.9)
Arginine-citrulline ratio	-0.04 (-0.08, 0.01)	0.01 (-0.03, 0.06)	0.04 (0, 0.08)
Summary secretion score*	0.1 (-0.4, 0.6)	-0.01 (-0.53, 0.51)	0.2 (-0.3, 0.8)
iGFR (ml/min/1.73m^2)	-0.3 (-1.0, 0.4)	0.3 (-0.4, 1.0)	0.2 (-0.4, 0.9)
AER (µg/min)*	1.9 (-36.2, 39.9)	0.1 (-38.2, 38.4)	58.0 (29.6, 86.3)

Models are unadjusted.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

SUPPLEMENTARY TABLES

Supplementary Table 1. Tubular biomarker concentrations and trends by baseline HbA1c in RASS.

	Baseline biomarker concentration (95% CI)			Absolute change/year (95% CI)			Difference in absolute change/year (95% CI)		
	HbA1c 7-8%	HbA1c 8-9%	HbA1c 9-10%	HbA1c 7-8%	HbA1c 8-9%	HbA1c 9-10%	Unadjusted model	Minimally-adjusted model	Fully-adjusted model
KIM-1 (pg/mL)	37.5 (-2.6, 77.6)	38.3 (3.7, 72.9)	56.5 (10.1, 103.0)	2.5 (-5.2, 10.1)	2.4 (-4.1, 9.0)	4.2 (-4.4, 12.8)	4.8 (2.2, 7.4)	4.8 (2.2, 7.4)	4.8 (2.3, 7.33)
sTNFR1 (pg/mL)	900.8 (782.0, 1019.6)	901.1 (798.8, 1003.4)	888.9 (751.8, 1026.0)	12.2 (-8.5, 32.9)	6.2 (-11.5, 23.9)	17.1 (-6.1, 40.4)	4.2 (-1.9, 10.3)	4.2 (-1.9, 10.3)	3.8 (-2.5, 10.1)
UMOD (mg/day) *	86.5 (68.5, 104.5)	78.9 (62.9, 94.9)	66.7 (45.0, 88.5)	0.7 (-3.8, 5.3)	-0.1 (-4.1, 3.8)	3.0 (-2.3, 8.2)	-0.9 (-2.2, 0.5)	-0.9 (-2.2, 0.5)	-0.9 (-2.2, 0.5)
EGF (µg/day) *	27.3 (24.8, 29.9)	27.0 (24.7, 29.2)	28.2 (25.2, 31.2)	-0.5 (-1.1, 0.1)	0.02 (-0.51, 0.55)	-0.82 (-1.52, -0.12)	-0.19 (-0.38, 0)	-0.19 (-0.38, 0)	-0.19 (-0.38, -0.01)
Arginine-citrulline ratio	2.2 (2.0, 2.4)	2.2 (2.0, 2.3)	1.9 (1.7, 2.1)	-0.02 (-0.06, 0.01)	-0.05 (-0.08, -0.02)	-0.03 (-0.07, 0.01)	0.02 (0.0, 0.03)	0.02 (0, 0.03)	0.02 (0.0, 0.03)
Summary secretion score*	60.0 (58.1, 61.9)	60.2 (58.6, 61.9)	61.3 (59, 63.5)	-0.2 (-0.6, 0.3)	0.2 (-0.2, 0.6)	-0.2 (-0.7, 0.3)	-0.04 (-0.18, 0.10)	-0.04 (-0.18, 0.10)	-0.05 (-0.19, 0.09)
iGFR (ml/min/ 1.73m^2)	125.1 (120.8, 129.4)	132.2 (128.4, 135.9)	127.5 (122.6, 132.5)	-1.0 (-1.6, -0.4)	-2.0 (-2.5, -1.5)	-1.5 (-2.1, -0.8)	-0.2 (-0.4, -0.1)	-0.2 (-0.4, -0.1)	-0.2 (-0.4, -0.03)
AER (µg/min) *	5.4 (-122.5, 133.3)	6.8 (-104.7, 118.3)	7.0 (-143.1, 157.1)	-0.2 (-33.0, 32.6)	-0.4 (-28.5, 27.8)	-0.1 (-37.5, 37.3)	0.8 (-9.1, 10.7)	0.7 (-9.2, 10.7)	0.7 (-11.3, 12.7)

Minimally-adjusted model includes age and sex. Fully-adjusted model includes age, sex, diabetes duration, SBP, iGFR, AER, intervention.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Supplementary Table 2. Tubular biomarker concentrations and trends by baseline HbA1c in PERL.

	Baseline biomarker concentration (95% CI)			Absolute change/year (95% CI)			Difference in absolute change/year (95% CI)		
	HbA1c 7-8%	HbA1c 8-9%	HbA1c 9-10%	HbA1c 7-8%	HbA1c 8-9%	HbA1c 9-10%	Unadjusted model	Minimally-adjusted model	Fully-adjusted model
KIM-1 (pg/mL)	131.4 (105.6, 157.3)	175.3 (147.2, 203.5)	259.9 (220.9, 298.8)	3.0 (-3.5, 9.5)	5.9 (-0.9, 12.8)	13.5 (3.4, 23.7)	-0.5 (-3.8, 2.8)	-0.4 (-3.7, 2.9)	-0.3 (-3.6, 3.0)
sTNFR1 (pg/mL)	1569.9 (1493.3, 1646.5)	1654.5 (1571.0, 1738.0)	1897.5 (1782.0, 2012.9)	44.1 (25.8, 62.5)	76.9 (57.7, 96.2)	130.1 (101.4, 158.8)	28.0 (19.3, 36.8)	28.0 (19.2, 36.8)	27.6 (18.8, 36.5)
UMOD (mg/day) *	105.5 (93.4, 117.7)	82.4 (69.4, 95.5)	85.4 (67.1, 103.8)	-6.3 (-11.3, -1.4)	1.0 (-4.2, 6.3)	-8.2 (-16.0, -0.3)	-0.3 (-2.7, 2.2)	-0.3 (-2.7, 2.2)	-0.4 (-2.8, 2.0)
EGF (µg/day)*	14.2 (12.5, 15.8)	12.5 (10.7, 14.4)	13.2 (10.7, 15.7)	-0.5 (-1.1, 0.1)	0.1 (-0.5, 0.7)	-0.7 (-1.6, 0.2)	-0.1 (-0.4, 0.2)	-0.1 (-0.4, 0.2)	-0.1 (-0.4, 0.2)
Arginine-citrulline ratio	1.6 (1.5, 1.7)	1.7 (1.6, 1.8)	1.5 (1.3, 1.6)	0.007 (-0.029, 0.044)	-0.005 (-0.043, 0.034)	0.005 (-0.053, 0.062)	0 (-0.02, 0.02)	0 (-0.02, 0.02)	0 (-0.02, 0.02)
Summary secretion score*	57.0 (55.8, 58.3)	56.7 (55.4, 58.1)	57.8 (55.9, 59.7)	-0.7 (-1.2, -0.2)	-0.4 (-1.0, 0.1)	-1.2 (-2.0, -0.4)	-0.2 (-0.4, 0.1)	-0.2 (-0.4, 0.1)	-0.2 (-0.4, 0.1)
iGFR (ml/min/ 1.73m^2)	68.8 (66.0, 71.6)	70.0 (66.9, 73.1)	65.1 (60.8, 69.4)	-1.7 (-2.3, -1.1)	-2.3 (-2.9, -1.6)	-2.1 (-3.1, -1.1)	-0.32 (-0.62, -0.03)	-0.32 (-0.62, -0.03)	-0.34 (-0.64, -0.04)
AER (µg/min) *	185.1 (103.7, 266.4)	260.1 (172.5, 347.7)	621.4 (498.4, 744.3)	14.4 (-11.8, 40.5)	61.0 (33.6, 88.5)	137.2 (95.6, 178.8)	15.0 (2.0, 28.1)	14.7 (1.6, 27.8)	15.1 (0.4, 29.8)

Minimally-adjusted model includes age and sex. Fully-adjusted model includes age, sex, diabetes duration, SBP, iGFR, AER, intervention.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Supplementary Table 3. Tubular biomarker concentrations and trends by baseline AER in RASS.

	Baseline biomarker concentration (95% CI)	Absolute change/year (95% CI) (95% CI)	Difference in absolute change/year (95% CI)		
	Normoalbuminuria	Normoalbuminuria	Unadjusted model	Minimally-adjusted model	Fully-adjusted model
KIM-1 (pg/mL)	43.8 (24.0, 63.6)	5.7 (1.5, 9.9)	0.3 (-3.7, 4.3)	0.3 (-3.7, 4.3)	0.4 (-3.5, 4.3)
sTNFR1 (pg/mL)	893.7 (838.8, 948.5)	14.2 (4.9, 23.4)	2.2 (-6.7, 11.0)	2.2 (-6.7, 11.1)	1.8 (-6.8, 10.3)
UMOD (mg/day)*	78.3 (69.7, 86.9)	0.7 (-1.5, 2.8)	-0.8 (-2.8, 1.3)	-0.7 (-2.8, 1.3)	-0.7 (-2.7, 1.3)
EGF (µg/day)*	28.3 (27.1, 29.4)	-0.30 (-0.59, -0.02)	-0.35 (-0.62, -0.07)	-0.35 (-0.63, -0.08)	-0.35 (-0.63, -0.07)
Arginine-citrulline ratio	2.1 (2.0, 2.1)	-0.03 (-0.05, -0.01)	0.01 (-0.01, 0.02)	0.006 (-0.011, 0.024)	0.007 (-0.010, 0.024)
Summary secretion score*	60.3 (59.4, 61.3)	0.03 (-0.18, 0.24)	-0.3 (-0.5, -0.1)	-0.3 (-0.5, -0.1)	-0.3 (-0.5, -0.1)
iGFR (ml/min/1.73m^2)	128.8 (126.8, 130.8)	-1.4 (-1.7, -1.1)	0.02 (-0.26, 0.3)	0.02 (-0.26, 0.29)	0.03 (-0.28, 0.34)
AER (µg/min)*	6.4 (-40.5, 53.3)	0.5 (-14.8, 15.8)	0.6 (-14.2, 15.5)	0.6 (-14.3, 15.4)	0.5 (-14.1, 15.1)

Minimally-adjusted model includes age and sex. Fully-adjusted model includes age, sex, diabetes duration, HbA1c, SBP, iGFR, intervention.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Supplementary Table 4. Tubular biomarker concentrations and trends by baseline AER in PERL.

	Baseline biomarker concentration (95% CI)			Absolute change/year (95% CI)			Difference in absolute change/year (95% CI)		
	Normo-albuminuria	Micro-albuminuria	Macro-albuminuria	Normo-albuminuria	Micro-albuminuria	Macro-albuminuria	Unadjusted model	Minimally-adjusted model	Fully-adjusted model
KIM-1 (pg/mL)	97.7 (75.3, 120.1)	164.2 (139.9, 188.6)	399.2 (367.1, 431.4)	3.4 (-3.0, 9.8)	10.7 (3.7, 17.8)	9.5 (-0.1, 19.2)	1.0 (-0.4, 2.4)	1.0 (-0.4, 2.4)	0.4 (-0.9, 1.7)
sTNFR1 (pg/mL)	1458.1 (1396.1, 1520.0)	1719.7 (1652.4, 1787.0)	2019.0 (1930.1, 2107.9)	32.2 (17.7, 46.7)	64.9 (48.8, 81)	184.3 (162.4, 206.2)	18.1 (14.9, 21.3)	18.1 (14.9, 21.3)	18.1 (14.8, 21.4)
UMOD (mg/day) *	103.5 (93.4, 113.5)	86.5 (75.2, 97.7)	77.2 (62.4, 91.9)	-2.3 (-6.4, 1.7)	-3.9 (-8.5, 0.7)	-4.0 (-10.1, 2.2)	-0.2 (-1.1, 0.7)	-0.2 (-1.1, 0.7)	-0.4 (-1.3, 0.6)
EGF (µg/day)*	13.9 (12.5, 15.2)	13.1 (11.6, 14.7)	11.0 (9.0, 13.0)	0.2 (-0.3, 0.6)	-0.5 (-1.0, 0)	-0.8 (-1.5, -0.1)	-0.1 (-0.2, 0.0)	-0.1 (-0.2, 0.0)	-0.1 (-0.2, 0.0)
Arginine-citrulline ratio	1.6 (1.5, 1.7)	1.6 (1.5, 1.7)	1.6 (1.5, 1.7)	-0.003 (-0.035, 0.028)	0.039 (0.005, 0.074)	-0.037 (-0.084, 0.010)	0 (-0.01, 0.01)	-0.001 (-0.008, 0.006)	-0.004 (-0.011, 0.003)
Summary secretion score*	56.7 (55.6, 57.7)	56.5 (55.3, 57.6)	56.1 (54.6, 57.6)	-0.2 (-0.6, 0.2)	-0.6 (-1.0, -0.1)	-1.2 (-1.8, -0.6)	-0.15 (-0.23, -0.06)	-0.15 (-0.23, -0.06)	-0.1649 (-0.2574, -0.0723)
iGFR (ml/min/1.73m^2)	70.8 (68.5, 73.1)	67.5 (65.0, 70.1)	63.0 (59.6, 66.3)	-1.1 (-1.6, -0.7)	-1.7 (-2.2, -1.2)	-3.7 (-4.5, -3.0)	-0.3 (-0.4, -0.2)	-0.3 (-0.4, -0.2)	-0.3 (-0.4, -0.2)
AER (µg/min)*	12.9 (-37.8, 63.6)	160.7 (103.5, 217.9)	1099.8 (1024.6, 1174.9)	8.2 (-12.7, 29.1)	43.0 (19.3, 66.7)	149.7 (117.5, 181.9)	16.2 (11.6, 20.9)	16.2 (11.5, 20.8)	16.8 (12.0, 21.6)

Minimally-adjusted model includes age and sex. Fully-adjusted model includes age, sex, diabetes duration, HbA1c, SBP, iGFR, intervention.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Supplementary Table 5. Tubular biomarker concentrations and trends by baseline age in RASS.

	Baseline biomarker concentration (95% CI)			Absolute change/year (95% CI)			Difference in absolute change/year (95% CI)		
	Age 15-20 years	Age 20-25 years	Age 25-30 years	Age 15-20 years	Age 20-25 years	Age 25-30 years	Unadjusted model	Minimally-adjusted model	Fully-adjusted model
KIM-1 (pg/mL)	38.6 (-15.5, 92.7)	35.0 (-16.8, 86.8)	43.3 (-7.5, 94.1)	7.3 (-3.0, 17.6)	3.0 (-6.9, 12.8)	4.3 (-5.3, 14.0)	0.1 (-0.3, 0.5)	0.1 (-0.3, 0.5)	0.1 (-0.3, 0.5)
sTNFR1 (pg/mL)	871.2 (727.9, 1014.4)	862.2 (724.9, 999.5)	897.5 (762.4, 1032.7)	3.4 (-22.5, 29.2)	19.6 (-5.3, 44.4)	20.3 (-4.1, 44.7)	0.2 (-0.9, 1.3)	0.2 (-0.9, 1.3)	0.2 (-0.8, 1.2)
UMOD (mg/day)*	55.0 (32.6, 77.42)	64.4 (43.8, 85.1)	81.0 (61.5, 100.5)	-1.7 (-7.3, 3.9)	1.8 (-3.3, 6.9)	-0.3 (-5.1, 4.5)	0.04 (-0.18, 0.26)	0.04 (-0.18, 0.26)	0.03 (-0.19, 0.25)
EGF (µg/day)*	33.4 (30.4, 36.4)	30.5 (27.7, 33.3)	28.8 (26.1, 31.4)	-0.6 (-1.3, 0.2)	0.8 (0.1, 1.4)	-0.4 (-1.0, 0.3)	-0.02 (-0.05, 0.01)	-0.02 (-0.05, 0.01)	-0.02 (-0.05, 0.01)
Arginine-citrulline ratio	2.1 (1.9, 2.3)	2.1 (1.9, 2.3)	2.1 (1.9, 2.3)	-0.045 (-0.089, 0.0)	-0.043 (-0.086, 0.0)	-0.024 (-0.065, 0.018)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Summary secretion score*	57.4 (55.0, 59.8)	60.7 (58.5, 62.8)	60.1 (58, 62.1)	-1.1 (-1.9, -0.4)	-1.0 (-1.7, -0.3)	-1.2 (-1.9, -0.6)	0.0 (-0.03, 0.02)	0.0 (-0.03, 0.02)	-0.01 (-0.03, 0.02)
iGFR (ml/min/1.73m^2)	135.6 (130.7, 140.5)	131.0 (126.3, 135.7)	129.4 (124.8, 134)	-0.3 (-39.9, 39.3)	1.6 (-35.3, 38.4)	0.9 (-34.5, 36.2)	-0.03 (-0.06, 0)	-0.03 (-0.06, 0)	-0.03 (-0.06, 0)
AER (µg/min)*	8.0 (-144.5, 160.4)	5.8 (-136.8, 148.3)	7.4 (-128.7, 143.4)	-0.3 (-43.4, 42.9)	1.3 (-40.1, 42.6)	1.1 (-39.3, 41.5)	-0.02 (-1.61, 1.57)	-0.02 (-1.62, 1.57)	-0.02 (-1.91, 1.87)

Minimally-adjusted model includes sex. Fully-adjusted model includes sex, diabetes duration, HbA1c, SBP, iGFR, AER, intervention.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Supplementary Table 6. Tubular biomarker concentrations and trends by baseline age in PERL.

	Baseline biomarker concentration (95% CI)			Absolute change/year (95% CI)			Difference in absolute change/year (95% CI)		
	Age 50-55 years	Age 55-60 years	Age 60-65 years	Age 50-55 years	Age 55-60 years	Age 60-65 years	Unadjusted model	Minimally-adjusted model	Fully-adjusted model
KIM-1 (pg/mL)	175.0 (136.5, 213.5)	178.3 (140.4, 216.3)	119.3 (76.6, 162.1)	-0.5 (-10.4, 9.4)	8.9 (-0.8, 18.5)	5.3 (-5.5, 16.1)	-0.1 (-0.5, 0.3)	-0.1 (-0.5, 0.3)	0.1 (-0.3, 0.5)
sTNFR1 (pg/mL)	1658.9 (1556.5, 1761.3)	1730.0 (1629.1, 1830.9)	1575.6 (1461.9, 1689.2)	66.4 (42.1, 90.6)	75.2 (51.8, 98.7)	61.7 (35.3, 88.2)	-1.6 (-2.6, -0.7)	-1.6 (-2.6, -0.7)	-1.7 (-2.7, -0.7)
UMOD (mg/day) *	98.0 (81.5, 114.5)	89.8 (74.5, 105.0)	94.0 (76.9, 111.1)	-4.3 (-10.9, 2.4)	-5.5 (-11.6, 0.7)	-1.1 (-7.9, 5.8)	-0.2 (-0.5, 0.1)	-0.2 (-0.4, 0.1)	-0.2 (-0.5, 0.1)
EGF (µg/day) *	14.6 (12.4, 16.8)	12.3 (10.2, 14.3)	12.0 (9.7, 14.3)	-0.08 (-0.8, 0.7)	-0.5 (-1.1, 0.2)	0.1 (-0.6, 0.9)	0.004 (-0.025, 0.033)	0.004 (-0.025, 0.032)	0.004 (-0.028, 0.035)
Arginine-citrulline ratio	1.6 (1.5, 1.8)	1.5 (1.4, 1.7)	1.5 (1.4, 1.7)	0.02 (-0.03, 0.07)	-0.01 (-0.06, 0.04)	0.02 (-0.03, 0.08)	0.0007 (-0.0012, 0.0026)	0.021 (-0.004 0.047)	0.001 (-0.001 0.003)
Summary secretion score*	57.7 (55.9, 59.4)	56.1 (54.5, 57.7)	55.2 (53.4, 57)	-1.5 (-2.3, -0.7)	-1.6 (-2.4, -0.9)	-1.7 (-2.6, -0.9)	0.01 (-0.01, 0.04)	0.01 (-0.01, 0.04)	0.02 (-0.01, 0.04)
iGFR (ml/min/ 1.73m^2)	70.6 (67.1, 74.1)	63.5 (59.9, 67.0)	66.6 (62.6, 70.6)	24.0 (-9.8, 57.9)	40.4 (8.2, 72.6)	17.6 (-18.9, 54.1)	0.03 (0, 0.06)	0.03 (0, 0.06)	0.04 (0.01, 0.07)
AER (µg/min) *	245.8 (140.9, 350.6)	158.6 (59.6, 257.6)	110.1 (-4.8, 225.0)	58.4 (15.7, 101.2)	36.8 (-4.8, 78.5)	15.2 (-31.8, 62.2)	-3.4 (-4.7, -2.0)	-3.4 (-4.7, -2.0)	-3.6 (-5.2, -2.0)

Minimally-adjusted model includes sex. Fully-adjusted model includes sex, diabetes duration, HbA1c, SBP, iGFR, AER, intervention.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Supplementary Table 7. Tubular biomarker concentrations and trends by baseline systolic blood pressure in RASS.

	Baseline biomarker concentration (95% CI)			Absolute change/year (95% CI)			Difference in absolute change/year (95% CI)		
	SBP 110-120 mmHg	SBP 120-130 mmHg	SBP 130-140 mmHg	SBP 110-120 mmHg	SBP 120-130 mmHg	SBP 130-140 mmHg	Unadjusted model	Minimally-adjusted model	Fully-adjusted model
KIM-1 (pg/mL)	43.3 (1.9, 84.6)	41.2 (-0.3, 82.7)	45.4 (-9.1, 99.9)	6.7 (-1.2, 14.5)	3.0 (-4.8, 10.8)	6.3 (-4.0, 16.7)	-0.8 (-2.6, 1.1)	-0.8 (-2.6, 1.1)	-0.7 (-2.4, 1.0)
sTNFR1 (pg/mL)	875.8 (767.9, 983.8)	905.6 (797.4, 1013.8)	952.7 (810.6, 1094.7)	14.3 (-5.4, 34.0)	8.6 (-11.0, 28.3)	18.7 (-7.2, 44.7)	-1.1 (-5.6, 3.4)	-1.0 (-5.5, 3.5)	-0.9 (-5.2, 3.3)
UMOD (mg/day) *	82.6 (66.5, 98.7)	78.9 (62.4, 95.3)	86.4 (65.5, 107.4)	0.6 (-3.4, 4.6)	-1.0 (-5.1, 3.0)	1.8 (-3.5, 7.0)	0.3 (-0.6, 1.2)	0.3 (-0.6, 1.2)	0.3 (-0.6, 1.2)
EGF (µg/day)*	27.3 (25.1, 29.5)	30.0 (27.7, 32.2)	27.3 (24.4, 30.1)	-0.4 (-0.9, 0.1)	-0.5 (-1.1, 0)	0.2 (-0.5, 0.9)	0.04 (-0.09, 0.16)	0.04 (-0.09, 0.16)	0.04 (-0.08, 0.17)
Arginine-citrulline ratio	2.1 (1.9, 2.2)	2.0 (1.9, 2.2)	2.2 (2.0, 2.4)	-0.018 (-0.051, 0.016)	-0.031 (-0.064, 0.003)	-0.045 (-0.09, -0.001)	-0.01 (-0.02, 0)	-0.01 (-0.02, 0)	-0.01 (-0.02, 0)
Summary secretion score*	59.7 (58.0, 61.4)	61.3 (59.5, 63.0)	61.8 (59.6, 64.0)	0.08 (-0.32, 0.48)	-0.07 (-0.48, 0.33)	-0.03 (-0.55, 0.49)	0.02 (-0.07, 0.11)	0.02 (-0.07, 0.11)	0.03 (-0.06, 0.12)
iGFR (ml/min/1.73m^2)	127.5 (123.7, 131.2)	131.2 (127.4, 135)	133.6 (128.6, 138.5)	-1.6 (-2.1, -1.0)	-0.9 (-1.5, -0.4)	-1.4 (-2.1, -0.7)	0.1 (-0.03, 0.23)	0.1 (-0.03, 0.23)	0.1 (-0.04, 0.24)
AER (µg/min) *	6.4 (-109.2, 122.1)	7.0 (-108.9, 122.9)	7.4 (-139.4, 154.2)	1.3 (-28.8, 31.4)	0.05 (-29.84, 29.93)	1.0 (-37.0, 39.0)	-0.05 (-6.82, 6.72)	-0.04 (-6.82, 6.73)	-0.03 (-7.96, 7.89)

Minimally-adjusted model includes age and sex. Fully-adjusted model includes age, sex, diabetes duration, HbA1c, iGFR, AER, intervention.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Supplementary Table 8. Tubular biomarker concentrations and trends by baseline systolic blood pressure in PERL.

	Baseline biomarker concentration (95% CI)			Absolute change/year (95% CI)			Difference in absolute change/year (95% CI)		
	SBP 120-130 mmHg	SBP 130-140 mmHg	SBP 140-150 mmHg	SBP 120-130 mmHg	SBP 130-140 mmHg	SBP 140-150 mmHg	Unadjusted model	Minimally-adjusted model	Fully-adjusted model
KIM-1 (pg/mL)	146.5 (112.7, 180.3)	196.6 (161.8, 231.3)	265.2 (227.5, 302.8)	11.1 (2.4, 19.8)	4.4 (-4.6, 13.4)	4.2 (-5.4, 13.9)	-0.6 (-2.1, 0.9)	-0.6 (-2.1, 0.9)	-0.6 (-2.0, 0.8)
sTNFR1 (pg/mL)	1581.0 (1492.8, 1669.1)	1657.7 (1567.4, 1748.1)	1747.3 (1649, 1845.6)	61.1 (40.0, 82.3)	93.7 (72.0, 115.5)	103.5 (80.1, 127.0)	7.0 (3.3, 10.6)	7.0 (3.3, 10.6)	5.9 (2.1, 9.6)
UMOD (mg/day) *	98.3 (85.0, 111.5)	74.1 (60.0, 88.3)	88.2 (72.8, 103.7)	-2.9 (-8.3, 2.6)	-0.5 (-6.2, 5.1)	-1.6 (-7.9, 4.7)	0.6 (-0.3, 1.6)	0.6 (-0.3, 1.6)	0.5 (-0.6, 1.5)
EGF (µg/day) *	13.2 (11.4, 15.0)	13.2 (11.3, 15.1)	12.6 (10.6, 14.7)	0.4 (-0.2, 1.0)	-0.7 (-1.3, -0.04)	-0.6 (-1.3, 0.1)	-0.07 (-0.18, 0.04)	-0.07 (-0.18, 0.04)	-0.08 (-0.19, 0.04)
Arginine-citrulline ratio	1.5 (1.4, 1.6)	1.7 (1.6, 1.9)	1.6 (1.5, 1.7)	0.044 (0.002, 0.087)	0.01 (-0.034, 0.054)	-0.019 (-0.066, 0.028)	0 (-0.01, 0.01)	0 (-0.01, 0.01)	0 (-0.01, 0.01)
Summary secretion score*	56.6 (55.2, 58.0)	57.0 (55.5, 58.4)	55.8 (54.2, 57.4)	0.01 (-0.53, 0.56)	-0.83 (-1.39, -0.27)	-0.95 (-1.58, -0.31)	-0.05 (-0.15, 0.05)	-0.05 (-0.15, 0.05)	-0.05 (-0.15, 0.06)
iGFR (ml/min/ 1.73m^2)	69.5 (66.3, 72.6)	70.0 (66.8, 73.2)	66.2 (62.7, 69.7)	-1.2 (-1.9, -0.5)	-2.6 (-3.3, -1.9)	-2.5 (-3.3, -1.8)	-0.2 (-0.3, -0.1)	-0.2 (-0.3, -0.1)	-0.2 (-0.3, -0.03)
AER (µg/min) *	181.1 (90.3, 271.9)	370.3 (276.0, 464.7)	428.5 (324.7, 532.3)	18.6 (-10.8, 47.9)	46.6 (16.2, 77.0)	102.0 (68.3, 135.8)	4.1 (-1.0, 9.3)	4.2 (-1.0, 9.3)	5.2 (-0.9, 11.2)

Minimally-adjusted model includes age and sex. Fully-adjusted model includes age, sex, diabetes duration, HbA1c, iGFR, AER, intervention.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Supplementary Table 9. Tubular biomarker concentrations and trends by baseline iGFR in RASS.

	Baseline biomarker concentration (95% CI)			Absolute change/year (95% CI)			Difference in absolute change/year (95% CI)		
	iGFR 90-120 ml/min/1.73m^2	iGFR 120-150 ml/min/1.73m^2	iGFR 150-250 ml/min/1.73m^2	iGFR 90-120 ml/min/1.73m^2	iGFR 120-150 ml/min/1.73m^2	iGFR 150-250 ml/min/1.73m^2	Unadjusted model	Minimally-adjusted model	Fully-adjusted model
KIM-1 (pg/mL)	45.9 (5.6, 86.2)	43.1 (13.6, 72.5)	43.4 (-15.2, 102.0)	3.6 (-4.0, 11.2)	5.8 (0.2, 11.5)	10.2 (-1.1, 21.5)	1.8 (-0.3, 3.9)	1.8 (-0.2, 3.9)	1.8 (-0.2, 3.7)
sTNFR1 (pg/mL)	947.0 (870.8, 1023.1)	870.5 (814.9, 926.2)	855.4 (744.6, 966.1)	18.9 (0.5, 37.2)	15.4 (1.8, 29.0)	2.3 (-24.8, 29.3)	-1.6 (-6.6, 3.4)	-1.5 (-6.5, 3.5)	-1.5 (-6.0, 3.0)
UMOD (mg/day) *	80.9 (65.4, 96.4)	78.9 (67.4, 90.5)	63.7 (39.7, 87.8)	1.0 (-2.8, 4.9)	0.4 (-2.4, 3.3)	1.5 (-4.6, 7.5)	-0.1 (-1.2, 1.0)	-0.1 (-1.2, 1.0)	-0.1 (-1.2, 1.0)
EGF (µg/day)*	23.9 (22.0, 25.8)	29.8 (28.4, 31.2)	33.3 (30.3, 36.2)	-0.5 (-1.0, 0)	-0.2 (-0.6, 0.2)	-0.4 (-1.2, 0.4)	0.1 (-0.1, 0.2)	0.1 (-0.1, 0.2)	0.1 (-0.1, 0.2)
Arginine-citrulline ratio	2.0 (1.8, 2.1)	2.1 (2.0, 2.2)	1.9 (1.7, 2.1)	-0.02 (-0.06, 0.01)	-0.04 (-0.06, -0.02)	0.01 (-0.04, 0.06)	0 (-0.01, 0.01)	0 (-0.01, 0.01)	0 (-0.01, 0.01)
Summary secretion score*	58.9 (57.4, 60.3)	60.9 (59.8, 61.9)	62.9 (60.6, 65.2)	-0.2 (-0.5, 0.2)	0.2 (-0.1, 0.5)	-0.2 (-0.8, 0.4)	0.01 (-0.10, 0.12)	0 (-0.11, 0.11)	0.01 (-0.11, 0.12)
iGFR (ml/min/1.73m^2)	109.9 (108.0, 111.7)	132.8 (131.4, 134.1)	156.8 (154.1, 159.4)	-0.6 (-1.2, -0.1)	-1.5 (-1.8, -1.1)	-2.9 (-3.7, -2.1)	-0.4 (-0.6, -0.3)	-0.4 (-0.6, -0.3)	-0.4 (-0.6, -0.3)
AER (µg/min) *	5.7 (-104.3, 115.7)	7.2 (-74.8, 89.3)	6.9 (-161.7, 175.4)	0 (-28.5, 28.5)	0.6 (-20.7, 21.9)	1.8 (-42.6, 46.3)	0.3 (-7.9, 8.4)	0.3 (-7.8, 8.5)	0.3 (-9.3, 9.9)

Minimally-adjusted model includes age and sex. Fully-adjusted model includes age, sex, diabetes duration, HbA1c, SBP, AER, intervention.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Supplementary Table 10. Tubular biomarker concentrations and trends by baseline iGFR in PERL.

	Baseline biomarker concentration (95% CI)		Absolute change/year (95% CI)		Difference in absolute change/year (95% CI)		
	iGFR 30-60 ml/min/1.73m^2	iGFR 60-90 ml/min/1.73m^2	iGFR 30-60 ml/min/1.73 m^2	iGFR 60-90 ml/min/1.73m^2	Unadjusted model	Minimally-adjusted model	Fully-adjusted model
KIM-1 (pg/mL)	259.0 (231.0, 287.0)	154.4 (132.9, 175.9)	9.4 (1.8, 17.1)	7.0 (1.5, 12.5)	-2.4 (-4.9, 0.2)	-2.3 (-4.9, 0.2)	-1.3 (-3.6, 1.1)
sTNFR1 (pg/mL)	2199.6 (2146.5, 2252.7)	1425.8 (1385.1, 1466.5)	123.6 (105.5, 141.6)	50.0 (36.9, 63.0)	-24.4 (-30.5, -18.4)	-24.3 (-30.3, -18.3)	-22.4 (-28.6, -16.3)
UMOD (mg/day)*	77.8 (66.1, 89.5)	95.9 (87.1, 104.7)	-5.0 (-9.9, 0)	-2.2 (-5.8, 1.3)	0.4 (-1.6, 2.0)	0.4 (-1.2, 2.0)	0.5 (-1.3, 2.2)
EGF (µg/day)*	7.7 (6.3, 9.1)	14.3 (13.2, 15.4)	-0.2 (-0.8, 0.3)	-0.2 (-0.6, 0.2)	-0.03 (-0.21, 0.15)	-0.03 (-0.22, 0.15)	-0.02 (-0.22, 0.18)
Arginine-citrulline ratio	1.4 (1.3, 1.5)	1.7 (1.6, 1.8)	0 (-0.038, 0.037)	0 (-0.022, 0.033)	0 (-0.01, 0.02)	0 (-0.01, 0.02)	0 (-0.01, 0.01)
Summary secretion score*	51.8 (50.7, 52.9)	58.2 (57.4, 59.0)	-0.7 (-1.2, -0.2)	-0.5 (-0.8, -0.1)	-0.02 (-0.18, 0.14)	-0.02 (-0.18, 0.14)	-0.01 (-0.18, 0.16)
iGFR (ml/min/1.73m^2)	49.8 (48.4, 51.2)	74.6 (73.5, 75.7)	-1.5 (-2.1, -0.9)	-1.9 (-2.3, -1.5)	-0.3 (-0.5, -0.1)	-0.3 (-0.5, -0.1)	-0.3 (-0.5, -0.1)
AER (µg/min)*	456.2 (378.0, 534.4)	216.4 (157.6, 275.3)	84.1 (57.9, 110.3)	36.0 (17.0, 55.0)	-12.2 (-20.9, -3.5)	-11.8 (-20.5, -3.1)	-12.5 (-22.7, -2.31)

Minimally-adjusted model includes age and sex. Fully-adjusted model includes age, sex, diabetes duration, HbA1c, SBP, AER, intervention.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Supplementary Table 11. Tubular biomarker concentrations and trends by baseline diabetes duration in RASS.

	Baseline biomarker concentration (95% CI)			Absolute change/year (95% CI)			Difference in absolute change/year (95% CI)		
	Diabetes duration 0-5 years	Diabetes duration 5-10 years	Diabetes duration 10-15 years	Diabetes duration 0-5 years	Diabetes duration 5-10 years	Diabetes duration 10-15 years	Unadjusted model	Minimally-adjusted model	Fully-adjusted model
KIM-1 (pg/mL)	39.4 (-23.0, 101.8)	42.8 (1.1, 84.6)	44.6 (8.1, 81.2)	13.4 (1.4, 25.5)	9.6 (1.6, 17.6)	2.1 (-5.0, 9.2)	-0.7 (-1.6, 0.2)	-0.7 (-1.6, 0.1)	-0.7 (-1.5, 0.1)
sTNFR1 (pg/mL)	828.2 (659.4, 996.7)	854.1 (741.3, 966.8)	905.4 (806.8, 1004.0)	20.9 (-10.4, 52.3)	15.0 (-5.9, 35.9)	12.6 (-5.8, 31.0)	-0.3 (-2.5, 1.9)	-0.3 (-2.5, 1.9)	-0.3 (-2.4, 1.8)
UMOD (mg/day) *	72.3 (46.9, 97.7)	81.8 (64.6, 99)	73.9 (59.4, 88.3)	2.0 (-4.3, 8.2)	-1.6 (-5.8, 2.6)	0.3 (-3.3, 3.9)	0.3 (-0.2, 0.7)	0.3 (-0.2, 0.7)	0.3 (-0.2, 0.7)
EGF (µg/day)*	32.3 (28.8, 35.7)	28.7 (26.4, 31.0)	28.7 (26.7, 30.6)	-0.4 (-1.3, 0.4)	-0.7 (-1.3, -0.1)	0.07 (-0.41, 0.56)	0.02 (-0.05, 0.08)	0.02 (-0.04, 0.08)	0.02 (-0.05, 0.08)
Arginine-citrulline ratio	2.1 (1.8, 2.3)	2.1 (2.0, 2.3)	2.0 (1.9, 2.2)	-0.04 (-0.09, 0.01)	-0.03 (-0.06, 0.01)	-0.02 (-0.05, 0.01)	0.001 (-0.003, 0.005)	0.001 (-0.003, 0.005)	0.001 (-0.001, 0.003)
Summary secretion score*	60.4 (57.8, 63.0)	60.9 (59.2, 62.7)	60.2 (58.8, 61.7)	0.22 (-0.41, 0.84)	-0.31 (-0.73, 0.11)	0.08 (-0.28, 0.45)	0.02 (-0.03, 0.06)	0.02 (-0.03, 0.06)	0.01 (-0.03, 0.06)
iGFR (ml/min/1.73m^2)	133.2 (127.3, 139.1)	129.1 (125.2, 133.1)	129.7 (126.3, 133.1)	0.4 (-51.1, 51.9)	0.3 (-33.8, 34.4)	0.9 (-29.0, 30.8)	-0.03 (-0.09, 0.03)	-0.03 (-0.09, 0.03)	-0.03 (-0.10, 0.04)
AER (µg/min) *	6.3 (-171.6, 184.2)	7.6 (-111.3, 126.5)	6.5 (-94.9, 108.0)	0.7 (-45.4, 46.8)	0.3 (-30.3, 30.9)	0.7 (-25.6, 27.1)	0 (-3.3, 3.3)	0.01 (-3.28, 3.3)	0.01 (-3.9, 3.9)

Minimally-adjusted model includes age and sex. Fully-adjusted model includes age, sex, HbA1c, SBP, iGFR, AER, intervention.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Supplementary Table 12. Tubular biomarker concentrations and trends by baseline diabetes duration in PERL.

	Baseline biomarker concentration (95% CI)			Absolute change/year (95% CI)			Difference in absolute change/year (95% CI)		
	Diabetes duration 0-5 years	Diabetes duration 5-10 years	Diabetes duration 10-15 years	Diabetes duration 0-5 years	Diabetes duration 5-10 years	Diabetes duration 10-15 years	Unadjusted model	Minimally-adjusted model	Fully-adjusted model
KIM-1 (pg/mL)	265.6 (210.2, 320.9)	143.2 (99.7, 186.7)	176.0 (136.4, 215.6)	9.0 (-5.4, 23.5)	20.6 (9.3, 31.9)	1.2 (-9.2, 11.7)	-0.1 (-0.4, 0.3)	-0.1 (-0.4, 0.3)	0.1 (-0.2, 0.4)
sTNFR1 (pg/mL)	1510.2 (1360.5, 1660.0)	1553.0 (1435.3, 1670.6)	1768.2 (1661.4, 1875.0)	98.9 (63.0, 134.9)	91.6 (63.5, 119.6)	67.3 (41.4, 93.2)	-0.5 (-1.3, 0.4)	-0.5 (-1.3, 0.4)	-0.4 (-1.3, 0.5)
UMOD (mg/day) *	71.7 (48.9, 94.6)	105.0 (86.9, 123.1)	96.7 (79.6, 113.9)	-3.8 (-13.0, 5.4)	-6.2 (-13.6, 1.2)	-8.4 (-15.3, -1.4)	-0.1 (-0.3, 0.1)	-0.1 (-0.3, 0.1)	-0.1 (-0.3, 0.2)
EGF (µg/day)*	14.3 (11.3, 17.4)	13.6 (11.2, 16.1)	12.4 (10.1, 14.7)	-0.3 (-1.4, 0.7)	-0.2 (-1.0, 0.7)	-0.4 (-1.2, 0.3)	0.01 (-0.01, 0.04)	0.01 (-0.01, 0.03)	0.01 (-0.02, 0.04)
Arginine-citrulline ratio	2.0 (1.8, 2.2)	1.6 (1.4, 1.7)	1.6 (1.4, 1.7)	-0.03 (-0.11, 0.04)	-0.01 (-0.06, 0.05)	0.0 (-0.05, 0.06)	0.001 (-0.001, 0.003)	0.001 (-0.001, 0.003)	0.001 (-0.001, 0.003)
Summary secretion score*	58.4 (56.1, 60.8)	58.4 (56.5, 60.2)	54.9 (53.2, 56.7)	-0.4 (-1.3, 0.5)	-1.1 (-1.8, -0.3)	-0.6 (-1.3, 0.1)	0.02 (-0.01, 0.04)	0.02 (-0.01, 0.04)	0.02 (0.0, 0.04)
iGFR (ml/min/ 1.73m^2)	76.8 (71.6, 82.0)	69.0 (64.9, 73.2)	64.5 (60.7, 68.3)	165.9 (104.5, 227.3)	25.5 (-24.5, 75.4)	27.8 (-17.4, 73.0)	0.01 (-0.02, 0.03)	0.01 (-0.02, 0.03)	0.01 (-0.02, 0.04)
AER (µg/min) *	356.4 (205.7, 507.2)	380.8 (258.8, 502.8)	191.6 (78.5, 304.7)	178.0 (130.1, 225.9)	30.9 (-8.6, 70.4)	38.8 (2.5, 75.1)	-2.5 (-3.6, -1.3)	-2.5 (-3.6, -1.3)	-2.5 (-3.8, -1.1)

Minimally-adjusted model includes age and sex. Fully-adjusted model includes age, sex, HbA1c, SBP, iGFR, AER, intervention.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Supplementary Table 13. Tubular biomarker concentrations and trends by sex in RASS.

	Baseline biomarker concentration (95% CI)		Absolute change/year (95% CI)		Difference in absolute change/year (95% CI)		
	Male	Female	Male	Female	Unadjusted model	Minimally-adjusted model	Fully-adjusted model
KIM-1 (pg/mL)	42.7 (9.9, 75.5)	44.8 (14.1, 75.5)	6.5 (0.5, 12.6)	5.0 (-0.7, 10.7)	1.6 (-6.8, 9.9)	1.6 (-6.8, 9.9)	1.7 (-6.3, 9.6)
sTNFR1 (pg/mL)	926.0 (841.1, 1010.9)	864.3 (784.7, 943.9)	15.1 (-0.5, 30.7)	13.5 (-1.2, 28.1)	1.6 (-19.7, 23.0)	1.6 (-19.7, 23.0)	2.0 (-18.1, 22.2)
UMOD (mg/day)*	72.6 (59.8, 85.5)	82.3 (70.6, 94.0)	2.0 (-1.2, 5.1)	-0.4 (-3.2, 2.5)	2.3 (-2.0, 6.6)	2.4 (-1.9, 6.7)	2.6 (-1.7, 6.9)
EGF (µg/day)*	28.7 (27.0, 30.5)	28.1 (26.5, 29.7)	-0.2 (-0.6, 0.2)	-0.40 (-0.79, -0.01)	0.2 (-0.4, 0.8)	0.2 (-0.4, 0.8)	0.2 (-0.4, 0.8)
Arginine-citrulline ratio	2.0 (1.9, 2.2)	2.1 (2.0, 2.2)	-0.03 (-0.06, -0.01)	-0.03 (-0.05, 0)	-0.01 (-0.04, 0.03)	-0.01 (-0.04, 0.03)	-0.01 (-0.04, 0.03)
Summary secretion score*	61.6 (60.3, 62.9)	59.4 (58.2, 60.6)	0.03 (-0.29, 0.34)	0.04 (-0.25, 0.32)	-0.01 (-0.44, 0.42)	-0.01 (-0.44, 0.42)	0.0 (-0.44, 0.43)
iGFR (ml/min/1.73m^2)	133.9 (130.9, 136.8)	124.5 (121.8, 127.2)	-1.2 (-1.6, -0.8)	-1.6 (-2.0, -1.2)	0.4 (-0.2, 1.0)	0.4 (-0.2, 1.0)	0.4 (-0.3, 1.0)
AER (µg/min)*	5.8 (-84.6, 96.2)	7.4 (-75.5, 90.2)	1.0 (-22.3, 24.2)	0.2 (-21.0, 21.5)	0.8 (-30.7, 32.2)	1.2 (-30.4, 32.7)	0.9 (-36.2, 38.0)

Male used as reference.

Minimally-adjusted model includes age. Fully-adjusted model includes age, diabetes duration, HbA1c, SBP, iGFR, AER, intervention.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Supplementary Table 14. Tubular biomarker concentrations and trends by sex in PERL.

	Baseline biomarker concentration (95% CI)		Absolute change/year (95% CI)		Difference in absolute change/year (95% CI)		
	Male	Female	Male	Female	Unadjusted model	Minimally-adjusted model	Fully-adjusted model
KIM-1 (pg/mL)	191.9 (171.5, 212.3)	169.8 (141.2, 198.4)	9.0 (3.8, 14.2)	4.6 (-2.7, 11.9)	4.4 (-4.6, 13.3)	-0.1 (-0.4, 0.3)	6.0 (-2.6, 14.6)
sTNFR1 (pg/mL)	1652.1 (1599.4, 1704.9)	1699.3 (1625.2, 1773.4)	81.0 (68.2, 93.7)	63.2 (45.2, 81.2)	17.7 (-4.3, 39.8)	-0.5 (-1.3, 0.4)	14.4 (-8.9, 37.7)
UMOD (mg/day)*	88.8 (80.5, 97.0)	98.5 (86.9, 110.0)	-1.9 (-5.2, 1.4)	-5.6 (-10.3, -0.9)	3.7 (-2.1, 9.5)	3.6 (-2.2, 9.4)	5.5 (-0.7, 11.7)
EGF (µg/day)*	14.2 (13.1, 15.3)	10.7 (9.2, 12.0)	-0.33 (-0.71, 0.05)	-0.08 (-0.62, 0.45)	-0.24 (-0.90, 0.41)	-0.23 (-0.89, 0.420)	-0.27 (-0.99, 0.45)
Arginine-citrulline ratio	1.6 (1.6, 1.7)	1.6 (1.5, 1.7)	0.01 (-0.02, 0.031)	0.004 (-0.032, 0.040)	0.002 (-0.042, 0.046)	0.001 (-0.042, 0.046)	0.005 (-0.044, 0.054)
Summary secretion score*	57.9 (57.1, 58.7)	53.7 (52.6, 54.9)	-0.7 (-1.0, -0.3)	-0.3 (-0.7, 0.2)	-0.40 (-0.98, 0.18)	-0.40 (-0.98, 0.18)	-0.37 (-0.99, 0.25)
iGFR (ml/min/1.73m^2)	70.5 (68.6, 72.3)	63.3 (60.7, 65.8)	-2.0 (-2.4, -1.6)	-1.6 (-2.1, -1.0)	-0.4 (-1.1, 0.3)	0.01 (-0.02, 0.03)	-0.08 (-0.85, 0.70)
AER (µg/min)*	350.4 (295.3, 405.5)	167.4 (91.0, 243.8)	66.3 (48.5, 84.0)	19.7 (-5.3, 44.8)	46.6 (15.9, 77.3)	46.6 (15.9, 77.3)	51.1 (14.2, 87.9)

Male used as reference.

Minimally-adjusted model includes age. Fully-adjusted model includes age, diabetes duration, HbA1c, SBP, iGFR, AER, intervention.

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Supplementary Table 15. Tubular biomarker concentrations and trends by randomization to intervention vs placebo in RASS.

	Baseline biomarker concentration (95% CI)			Absolute change/year (95% CI)			Difference in absolute change/year (95% CI) - Unadjusted model	
	Placebo	Losartan	Enalapril	Placebo	Losartan	Enalapril	Losartan	Enalapril
KIM-1 (pg/mL)	38.8 (0, 77.5)	48.2 (9.8, 86.6)	44.5 (4.9, 84.1)	4.9 (-2.4, 12.1)	5.1 (-2.0, 12.3)	7.2 (-0.1, 14.4)	0.3 (-9.9, 10.4)	2.3 (-7.9, 12.5)
sTNFR1 (pg/mL)	891.9 (791.6, 992.2)	887.9 (788.4, 987.5)	900.4 (797.9, 1002.8)	8.4 (-10.1, 26.9)	19.2 (0.9, 37.5)	15.0 (-3.6, 33.6)	10.8 (-15.2, 36.8)	6.6 (-19.7, 32.8)
UMOD (mg/day)*	82.3 (67.4, 97.3)	72.5 (57.5, 87.5)	83.8 (68.3, 99.2)	1.9 (-1.7, 5.6)	0.1 (-3.7, 3.8)	-0.2 (-3.9, 3.6)	-1.9 (-7.1, 3.3)	-2.1 (-7.3, 3.1)
EGF (µg/day)*	27.9 (25.9, 29.9)	30.2 (28.2, 32.2)	27.1 (25.0, 29.1)	-0.5 (-1.0, -0.053)	-0.1 (-0.6, 0.4)	-0.2 (-0.7, 0.3)	0.4 (-0.3, 1.1)	0.3 (-0.4, 1.0)
Arginine-citrulline ratio	2.1 (1.9, 2.2)	2.1 (2.0, 2.2)	2.0 (1.9, 2.1)	-0.02 (-0.05, 0.01)	-0.06 (-0.09, -0.03)	-0.01 (-0.04, 0.02)	-0.04 (-0.08, 0.01)	0.01 (-0.03, 0.06)
Summary secretion score*	59.6 (58.1, 61.1)	60.9 (59.4, 62.4)	60.6 (59, 62.2)	0 (-0.4, 0.4)	0.1 (-0.3, 0.5)	0 (-0.37, 0.37)	0.1 (-0.4, 0.6)	-0.01 (-0.53, 0.51)
iGFR (ml/min/1.73m^2)	128.5 (125.0, 132.0)	130.4 (126.9, 133.9)	127.6 (124.1, 131.2)	-1.4 (-1.9, -0.9)	-1.7 (-2.2, -1.2)	-1.1 (-1.6, -0.6)	-0.3 (-1.0, 0.4)	0.3 (-0.4, 1.0)
AER (µg/min)*	6.8 (-99.1, 112.7)	6.6 (-99.5, 112.7)	6.5 (-102.8, 115.9)	-0.1 (-26.9, 26.72)	1.8 (-25.2, 28.8)	0 (-27.4, 27.4)	1.9 (-36.2, 39.9)	0.1 (-38.2, 38.4)

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)

Supplementary Table 16. Tubular biomarker concentrations and trends by randomization to intervention vs placebo in PERL.

	Baseline biomarker concentration (95% CI)		Absolute change/year (95% CI)		Difference in absolute change/year (95% CI – Unadjusted model
	Placebo	Allopurinol	Placebo	Allopurinol	
KIM-1 (pg/mL)	181.2 (157.6, 204.8)	187.7 (164.2, 211.1)	6.4 (0.5, 12.3)	8.8 (2.7, 14.9)	2.4 (-6.1, 10.9)
sTNFR1 (pg/mL)	1671.1 (1610.0, 1732.3)	1665.0 (1604.3, 1725.7)	74.2 (59.7, 88.7)	75.9 (60.9, 90.9)	1.7 (-19.2, 22.5)
UMOD (mg/day)*	89.9 (80.5, 99.4)	94.7 (85.3, 104.2)	-3.9 (-7.6, -0.1)	-2.8 (-6.7, 1.1)	1.1 (-4.3, 6.5)
EGF (µg/day)*	12.8 (11.6, 14.1)	13.0 (11.7, 14.2)	-0.4 (-0.8, 0)	-0.6 (-0.5, 0.4)	0.3 (-0.3, 0.9)
Arginine-citrulline ratio	1.6 (1.5, 1.7)	1.6 (1.5, 1.7)	-0.01 (-0.04, 0.02)	0.02 (-0.01, 0.05)	0.04 (0, 0.08)
Summary secretion score*	56.8 (55.9, 57.8)	55.9 (55.0, 56.9)	-0.6 (-1.0, -0.3)	-0.4 (-0.8, 0)	0.2 (-0.3, 0.8)
iGFR (ml/min/1.73m^2)	67.2 (65.1, 69.4)	68.8 (66.7, 71)	-1.9 (-2.4, -1.5)	-1.7 (-2.2, -1.3)	0.2 (-0.4, 0.9)
AER (µg/min)*	267.6 (204.8, 330.5)	316.9 (253.9, 379.9)	16.9 (-2.8, 36.6)	74.9 (54.5, 95.3)	58.0 (29.6, 86.3)

* Outlier urine values excluded, corresponding to 97 samples (2% of PERL samples and 2% of RASS samples, from total 46 participants)