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The Relationships between Markers of Tubular Injury and Intrarenal Hemodynamic Function in Adults with and without Type 1 Diabetes: Results from the Canadian Study of Longevity in Type 1 Diabetes

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Abstract

Objective: Our aim was to define the relationships between plasma biomarkers of kidney injury and intrarenal hemodynamic function (glomerular filtration rate [GFR], effective renal plasma flow [ERPF], renal vascular resistance [RVR]) in adults with type 1 diabetes (T1D).

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Methods: The study sample consisted of patients with longstanding T1D (>50 years duration); 44 Diabetic Kidney Disease (DKD) resistors (eGFR >60mL/min/1.73m² and <30mg/day urine albumin excretion) and 22 with DKD, in addition to 73 controls. GFR_{INULIN} and ERPF_{PAH} were measured, RVR calculated, and afferent (R_A)/efferent (R_E) arteriolar resistances were derived from Gomez equations. Plasma neutrophil gelatinase-associated lipocalin (NGAL), β 2 microglobulin (B2M), osteopontin (OPN) and uromodulin (UMOD) were measured using immunoassay kits from Meso Scale Discovery.

Results: Plasma NGAL, B2M and OPN were higher and UMOD lower in DKD versus DKD resistors and non-diabetic controls. In participants with T1D, plasma NGAL inversely correlated with GFR ($r=-0.33$, $p=0.006$), ERPF ($r=-0.34$, $p=0.006$) and positively with R_A ($r=0.26$, $p=0.03$) and RVR ($r=0.31$, $p=0.01$). In participants without T1D, NGAL and B2M inversely correlated with GFR (NGAL $r=-0.18$, $p=0.13$; B2M $r=-0.49$, $p<0.0001$), ERPF (NGAL $r=-0.19$, $p=0.1$; B2M $r=-0.42$, $p=0.0003$) and positively with R_A (NGAL $r=0.19$, $p=0.10$; B2M $r=0.3$, $p=0.01$) and RVR (NGAL $r=0.20$, $p=0.09$; B2M $r=0.34$, $p=0.003$). Differences were significant after adjusting for age, sex, HbA1c, SBP and LDL. There were statistical interactions between T1D status, B2M and intrarenal hemodynamic function ($p<0.05$).

Conclusions: Elevated NGAL related to intrarenal hemodynamic dysfunction in T1D, whereas elevated NGAL and B2M related to intrarenal hemodynamic dysfunction in adults without T1D. These data may define a diabetes-specific interplay between tubular injury and intrarenal hemodynamic dysfunction.

Keywords

biomarkers; diabetic kidney disease; type 1 diabetes

Introduction:

Diabetic kidney disease (DKD) continues to cause significant morbidity and mortality in type 1 diabetes (T1D) (1). DKD is characterized by both glomerular and tubular disease (2, 3). Compared to diabetic glomerulopathy, diabetic tubulopathy has received less attention from the research community.

The Kidney Injury Panel 5 from Meso Scale Diagnostics include assays for the quantitative determination of neutrophil gelatinase-associated lipocalin (NGAL/Lipocalin-2), β 2 microglobulin (B2M), osteopontin (OPN), and uromodulin (UMOD). NGAL is a protein expressed in the tubular epithelium of the distal nephron and released in response to kidney injury. Similarly, OPN is a marker of tubular damage. B2M is filtered by the glomerular basement membrane and reabsorbed by the proximal tubule, and is a marker of both tubular and glomerular damage. UMOD is a glycoprotein produced in the tubular cells and released in the tubular lumen. Prior studies have shown that UMOD concentrations decrease as renal function decreases (4). We and others have demonstrated that these kidney injury biomarkers relate to acute and chronic tubular and glomerular injury in patients with diabetes (5). In fact, we demonstrated that elevated NGAL confer greater odds of elevated albumin excretion and impaired GFR over 12 years in adults with T1D (6). While diabetic tubulopathy is

thought to precede glomerulopathy the interplay between tubular and glomerular disease in T1D remains poorly understood (7, 8).

Epidemiologic studies are, however, limited by estimations of glomerular filtration rate (GFR), and are without data on other parameters of intrarenal hemodynamic function including effective renal plasma flow (ERPF), renal blood flow (RBF), renal vascular resistance (RVR), filtration fraction (FF), glomerular pressure (P_{GLO}), afferent (R_A) and efferent (R_E) arteriolar resistances. In the *Canadian Study of Longevity in Type 1 Diabetes*, we recently demonstrated that intrarenal renin-angiotensin-aldosterone system (RAAS) activation is exaggerated which predominates at the afferent rather than the efferent arteriole (9). It is unknown how biomarkers of tubular injury relate to intrarenal hemodynamic function in T1D. Accordingly, the aim of this study was to examine the relationship between plasma tubular markers and intrarenal hemodynamic function in adults with and without T1D of long duration. We hypothesized that tubular injury markers would be elevated in adults with T1D, and that elevated tubular injury markers would relate to afferent arteriolar vasoconstriction in the *Canadian Study of Longevity in Type 1 Diabetes*.

Methods:

Study Design

Cross-sectional evaluation of 75 participants with 50 years of T1D duration with and without diabetic kidney disease (DKD and DKD resistors) and 75 age- and sex-matched controls to determine mechanisms of nephropathy resistance, and to define the clinical phenotype of nephropathy, retinopathy, neuropathy, and macrovascular disease for future biomarker studies. This represents an exploratory analysis within the second phase of the *Canadian Study of Longevity in Type 1 Diabetes* (funded by JDRF, Operating Grant No. 17–2013-312). The demographics and composition of this cohort have been previously described (10–12). All participants provided written informed consent, and the study and its procedures were approved by the institutional ethics board of the University Health Network and Mount Sinai Hospital in Toronto, ON, Canada. Sixty-six participants with T1D and 73 non-diabetic controls had data available for intrarenal hemodynamic function, lean and fat mass and were included in the analyses.

Measurement of Intrarenal Hemodynamic Function

All participants underwent RAAS inhibitor washout 30 days prior to renal measurements, which occurred on Study Day 2 after a screening visit (Study Day 1) (13, 14). For seven days prior to the infusion, participants were instructed to maintain a minimum sodium intake of 150 mmol/day and a protein diet of 1.5 g/Kg/day. Participants with T1D and controls without diabetes underwent the same procedures, except that participants with T1D underwent a euglycemic clamp during the morning of Study Day 2, prior to and during measurements of renal hemodynamic function. During the euglycemic clamp, blood glucose was measured every 10–15 min, and the insulin and dextrose infusions were titrated to achieve a blood glucose between 4–6 mmol/L for approximately 4h prior to and during the renal measurements.

Patients arrived at the Renal Physiology Laboratory (University Health Network, University of Toronto, Toronto, ON, Canada), at ~08:00 hrs. Blood pressure was measured with an automated sphygmomanometer (Welch-Allyn Inc, 53000 Vital Signs Monitor) at the same time that blood was drawn for renal hemodynamic parameters. The average of two values was used to assess systolic, diastolic and mean arterial blood pressure at each time point. Renal hemodynamic function was measured using inulin and PAH clearance techniques standardized per 1.73m^2 of body surface area, which represent $\text{GFR}_{\text{INULIN}}$ and effective renal plasma flow (ERPF_{PAH}), respectively as described in detail previously (15, 16). Filtration fraction (FF) was determined by dividing the $\text{GFR}_{\text{INULIN}}$ by the ERPF_{PAH} . Renal blood flow (RBF) was calculated by dividing the ERPF_{PAH} by (1-hematocrit). Renal vascular resistance (RVR) was derived by dividing the mean arterial pressure (MAP) by the RBF. Intrarenal hemodynamics (R_A , R_E , P_{GLO}) were estimated using Gomez formulae, as described elsewhere (17).

Determination of DKD Resistor Status

For analysis, participants with T1D were categorized as DKD resistors if they had $\text{eGFR}_{\text{MDRD}} < 60 \text{ ml/min/1.73m}^2$ and 24-hour urine albumin excretion $< 30 \text{ mg/day}$, otherwise they were assigned to the DKD group. The non-diabetic controls were assigned to the control group irrespective of their $\text{eGFR}_{\text{MDRD}}$ and 24-hour urine albumin excretion results.

Laboratory Measurements and Kidney Injury Biomarkers

Kidney injury biomarkers (Kidney Injury Panels 3 and 5, Meso Scale Diagnostics) were measured on stored fasting baseline plasma samples (samples stored at -80°C). The assays were performed at the University of Colorado Anschutz Medical Campus (Aurora, Colorado). The Meso Scale Discovery (MSD®) Human Kidney Injury Panel 5 Assay Kits (Gaithersburg, Maryland) were used to measure the following plasma biomarkers: B2M, NGAL/Lipocalin-2, OPN, and UMOD. The Human Kidney Injury Panels use a chemiluminescence immunoassay technology with a broad detection range for each of these biomarkers from the lowest limits of detectability in picograms/milliliter (pg/ml) to $>200,000 \text{ pg/ml}$. The assays were run according to the manufacturer's protocol on newer-thawed samples. Average intra- and inter-assay precision for the biomarkers range between 3–8%, respectively. Whereas these kits were originally designed by MSD® to monitor drug-induced kidney toxicity, they have since been successfully applied to predict DKD and non-DKD (6).

Statistical Analysis

Statistical analyses were performed using SAS version 9.4 for Windows (SAS Institute, Cary, NC). Continuous variables were assessed for normality (Shapiro-Wilk test and inspection of histograms). Comparisons of clinical characteristics between controls, DKD resistors, and DKD subgroups were made using ANOVA, the Kruskal-Wallis test, or the χ^2 -test, depending on variable distribution. Pearson's correlation and generalized linear regression models (univariable and multivariable adjustments for age, sex, HbA1c, systolic blood pressure (SBP) and low-density lipoprotein (LDL) were used to examine the relationships between biomarkers and intrarenal hemodynamic function. In sensitivity

analyses, we further adjusted for high-density lipoprotein (HDL), triglycerides, BMI and RAAS inhibitors. We examined means of GFR, ERPF and RVR across tertiles of NGAL and B2M. We also evaluated for whether T1D status and DKD resistor status were effect modifiers on the relationships between the plasma biomarkers and parameters of intrarenal hemodynamic function. Analyses were considered exploratory and hypothesis generating and adjustments for multiple comparisons were not employed. An α -level of 0.05 was used for tests of statistical significance.

Results:

Baseline characteristics of the study cohort

The baseline characteristics of the cohort, stratified by subgroup, are shown in Supplemental Table 1. As compared to controls, DKD resistors and participants with DKD were similar in age, sex distribution and weight. The use of RAAS inhibition at baseline was highest in those with T1D (91% in DKD, 78% in DKD resistors and 14% in controls, P value for trend <0.001). Glycated hemoglobin (HbA1c) and fasting blood glucose levels were higher among participants with DKD as compared to DKD resistors (HbA1c $7.6 \pm 1.0\%$ vs. $7.3 \pm 0.8\%$ and glucose 9.4 ± 4.1 mmol/L vs. 8.1 ± 3.4 mmol/L, $p=0.03$ for both comparisons). Blood pressure was similar among DKD resistors and participants with DKD. Controls and DKD resistors had similar eGFR and UACR, whereas participants with DKD had lower eGFR and uACR with a mean eGFR of 57 ± 14 mL/min/1.73m² and UACR of 9.5 mg/mmol, interquartile range 5.1, 16.3 mg/mmol.

Measured and derived parameters of intrarenal hemodynamic function are shown in Supplemental Table 2. Glomerular filtration rate (GFR_{INULIN}) and effective renal plasma flow (ERPF_{PAH}) were similar among controls and DKD resistors, and lowest in those with DKD. Controls, DKD resistors and participants with DKD had similar mean arterial pressures (MAP). DKD resistors had significantly lower renal vascular resistance (RVR, $p<0.001$), renal afferent arteriolar resistance (R_A, $p=0.01$) and renal efferent arteriolar resistance (R_E, $p=0.01$) as compared to participants with DKD, and higher renal blood flow (RBF 742 ± 163 mL/min/1.73m² in DKD resistors vs. 584 ± 112 mL/min/1.73m² in DKD, $p=0.002$). Filtration fraction (FF) was similar in all subgroups, although there was a trend towards a higher FF in those with DKD.

The clinical and biochemical characteristics of the study cohort, as well as the measured and derived parameters of intrarenal hemodynamic function as shown in Supplemental Tables 1 and 2 have also been published in previous work from our group (9).

Differences in tubular markers in study participants

Tubular markers, stratified by subgroup, are shown in Table 1. Plasma tubular markers NGAL, B2M, OPM and UMOD were lowest in DKD resistors. NGAL and B2M concentrations were significantly lower in DKD resistors as compared to controls (NGAL, $p=0.007$ and B2M $p=0.001$) and participants with DKD (NGAL, $p<0.0001$ and B2M $p<0.0001$). Similar trends were seen for OPN, although these did not achieve statistical

significance. UMOD concentrations were lower in participants with DKD as compared to controls ($p<0.0001$), but similar to DKD resistors ($p=0.83$).

Relationships between tubular markers and intrarenal hemodynamics in participants with and without T1D

The relationships between tubular markers and intrarenal hemodynamic function in participants with and without T1D are shown in Tables 2 and 3, respectively. Among participants with T1D, plasma NGAL inversely correlated with GFR_{INULIN} ($r=-0.33$, $p=0.006$) and $ERPF_{PAH}$ ($r=-0.34$, $p=0.006$) and positively correlated with RVR ($r=0.31$, $p=0.01$). These correlations remained statistically significant after adjustment for age, sex, HbA1c, systolic blood pressure and LDL. NGAL was also inversely correlated with P_{GLO} ($r=-0.27$, $p=0.03$) and positively correlated with R_A ($r=0.26$, $p=0.03$) in univariable analyses. Among those without T1D, plasma NGAL was inversely associated with GFR_{INULIN} and $ERPF_{PAH}$, and positively associated with RVR and R_A after adjusting for age, sex, HbA1c, SBP and LDL (Table 2). Further, in sensitivity analyses with additional adjustments for HDL, triglycerides, BMI RAAS inhibitors, the relationships between NGAL, GFR, ERPF, RVR and R_A remained significant (data not shown). B2M was also inversely correlated with GFR_{INULIN} , $ERPF_{PAH}$ and P_{GLO} and positively correlated with RVR and R_A in adults without T1D. T1D status was a significant effect modifier in the relationship between B2M and intrarenal hemodynamic function ($p<0.05$ for interaction). In contrast, DKD resistor status was not a significant effect modifier in the relationship between B2M and intrarenal hemodynamic function. For NGAL, T1D status nor DKD resistor status were effect modifiers on the relationship with parameters of intrarenal hemodynamic function. In adults with and without T1D, correlations between OPN and UMOD and intrarenal hemodynamic function were not statistically significant.

The relationships between NGAL and intrarenal hemodynamic function in adults with T1D are shown Figure 1. GFR_{INULIN} and $ERPF_{PAH}$ were significantly lower in the highest NGAL tertile as compared to both the low and mid tertiles. Conversely, higher RVR was seen in the high NGAL tertile, as compared to the low and mid tertiles. The relationships between NGAL and GFR_{INULIN} , $ERPF_{PAH}$ and RVR remained significant after adjustment for confounders.

Discussion:

There is increasing evidence to show that the renal tubule plays an important role in the pathogenesis of DKD (2, 18). Clinical studies in T1D and T2D demonstrate that RAAS inhibition is inadequate in preventing the progression of DKD (19, 20). Additionally, DKD can also occur without significant albuminuria (21, 22), which highlights the potential importance of diabetic tubulopathy and glomerulopathy in the development and progression of DKD.

Our first major observation was that participants with DKD have significantly elevated plasma biomarkers of tubular injury as compared to participants without DKD (DKD resistors) and controls without diabetes. We measured NGAL, UMOD and OPN, markers of tubular injury, as well as B2M, a marker of both tubular and glomerular damage in this study

(4, 23, 24). In patients with T1D and DKD, NGAL, B2M and OPN were elevated as compared to controls. Furthermore, we observed that DKD resistors had lower NGAL and B2M values as compared to patients with DKD. Interestingly, DKD resistors also had the lowest absolute values of plasma biomarkers, which were also lower than controls without diabetes. Although speculative, this finding may be due to the use of RAAS inhibitors in conjunction with excellent kidney function in DKD resistors. Additionally, the non-diabetic comparator group selected in this study may have had early tubular injury that was not yet manifested when measuring typical parameters such as GFR or albuminuria. Finally, and importantly, there may be protective adaptations that occur at the level of the renal tubule in certain patients early in DKD, which may explain the DKD resistor status of these individuals.

Our findings are concordant with existing studies demonstrating that makers of tubular and glomerular injury are elevated in patients with DKD. Plasma NGAL has been shown to be elevated in DKD related to both type 1 and type 2 diabetes and has been associated with the development of elevated albumin excretion, rapid decline in GFR and in some studies, progression of DKD (25–28). Similar associations have also been demonstrated with plasma OPN and B2M (6, 29). In a prior study of patients with T1D, elevations in UMOD conferred a protective effect in the development of DKD (6), whereas in our study, patients with DKD did have lower UMOD concentrations as compared to controls, although UMOD levels were statistically similar among DKD resistors and participants with DKD.

Injury to the tubulointerstitium may be a better predictor of GFR decline than structural changes of the glomerulus (2). Although tubular injury is not always a precipitant or cause of tubulointerstitial injury, it has been shown that tubular injury occurs early in DKD (30, 31). Furthermore, in T1D, regression of elevated albumin excretion is associated with lower levels of tubular biomarkers, supporting the important role of the tubule early in the pathogenesis of DKD (32). Various mechanisms of injury, primarily to the proximal tubule, have been described in DKD. First, under normal conditions, the proximal tubule is responsible for greater than two thirds of the sodium reabsorption in the kidney, a process which is metabolically demanding (33). In the setting of hyperglycemia, there is an increase in the reabsorption of sodium and glucose due to upregulation of sodium-glucose cotransporter 2 (SGLT2) in the proximal tubule (34). These increases in metabolic demands predispose the proximal tubule epithelial cells (PTECs) to hypoxia, which in turn, stimulates a cascade of mediators leading to fibrosis and apoptosis (18, 35–37). Second, the increase in glucose reabsorption that occurs in PTECs in the setting of diabetes also results in the generation of advanced glycosylation end-products (AGEs). AGEs and the high glucose milieu have been shown to trigger the generation reactive oxygen species (ROS) as well as pro-fibrotic and pro-inflammatory pathways, ultimately leading to glomerulopathy and progressive DKD (38, 39). Similarly, increased albumin reabsorption by the proximal tubule is also toxic to the PTECs, promoting a similar cascade of cytokines and pro-inflammatory mediators contributing to tubulointerstitial injury (38–40). Due to the cross-sectional design of our study, early changes in tubular markers in participants with DKD could not be ascertained. Nevertheless, the biomarker profile observed in DKD resistors, as compared to DKD and controls, provide important insights into the role of tubular injury in DKD.

In addition to diabetic tubulopathy, it has been well established that increased intrarenal RAAS activity plays a central role in the pathogenesis of DKD (14, 41), yet the interplay between tubular injury and intrarenal hemodynamic function is not well understood. Previous mechanistic work from our group in the same study population, has shown that activation of the intrarenal RAAS is exaggerated in patients with long-standing T1D and DKD, which is manifested primarily at the afferent arteriole (R_A), rather than at the efferent arteriole (R_E) (9). In this study, we demonstrated that in individuals with and without diabetes, serum NGAL was inversely correlated with GFR_{INULIN} and $ERPF_{PAH}$ and positively correlated with RVR. Furthermore, when we evaluated the relationship between NGAL and intrarenal hemodynamic function in the entire study cohort with T1D, the highest NGAL tertiles were associated with significantly lower GFR_{INULIN} , $ERPF_{PAH}$ and higher RVR. Interestingly, in univariable models in patients with T1D and in multivariable models in controls without diabetes, NGAL was associated with a higher afferent arteriolar tone (R_A). These findings, in light of our prior work demonstrating that intrarenal RAAS activity predominates at the R_A , suggests a potential important interplay between tubular injury and intrarenal RAAS activity in individuals with long-standing diabetes. Although the mechanism is unclear, tubular injury in the setting of diabetes may result in an increase in local angiotensinogen production by proximal tubular cells and subsequent increase in local RAAS activity. This putative mechanism, supported by existing experimental studies (42–47), warrants further human study.

Interestingly, when evaluating the relationship between B2M and intrarenal hemodynamic function, we observed important differences among patients with and without diabetes. The presence of T1D modified this relationship, whereby in adults without diabetes, increases in B2M were inversely correlated with GFR_{INULIN} and $ERPF_{PAH}$ and positively correlated with R_A and RVR. However, these relationships were not seen in adults with diabetes. NGAL is predominantly a marker of proximal tubular damage, whereas B2M is filtered by the glomerular basement membrane (GBM) and reabsorbed by the proximal tubule (4, 38). Although speculative, this observation suggests potential differences in the mechanisms of injury and relative contribution of proximal tubular injury in the pathogenesis of diabetic vs. non-diabetic renal disease. Under normal circumstances, B2M is freely filtered across the GBM and almost completely catabolized by cells of the proximal tubule through endocytosis (48). The proximal tubular injury that occurs in the setting of diabetes may impair tubular reabsorption, endocytosis and catabolism of B2M, which may lead to an increase in urinary excretion of B2M. Although our study did not specifically test this hypothesis, this putative mechanism may explain the inverse correlation between plasma B2M and GFR_{INULIN} which we only observed in adults without diabetes.

The role of tubular factors in the pathogenesis of DKD is not yet fully understood, nor is the role of pharmacotherapies that impact tubular mechanisms of DKD. From a therapeutic perspective, tubular reabsorptive factors related to sodium-glucose cotransporter-2 (SGLT2) have recently been recognized as a potential target in patients with type 2 diabetes. Blockade with SGLT2 inhibitors have been the subject of ongoing studies in patients with DKD. SGLT2 inhibitors decrease proximal tubular sodium reabsorption, thereby increasing the delivery of sodium to the macula densa, thereby restoring tubuloglomerular feedback and vasoconstriction of the afferent arteriole (49). The resulting decrease in intraglomerular

hypertension and hyperfiltration (50) is one of the mechanisms proposed to explain the decrease in albuminuria and progression of DKD (51) observed in clinical trials of patients with type 2 diabetes treated with SGLT2 inhibitors (52–54). In experimental studies, SGLT2 inhibitors have also been shown to decrease pro-fibrotic and pro-inflammatory pathways activated in DKD, although this has yet to be shown in humans. Other therapies that may impact on tubular function and mitigate against renal tubular injury include glucagon like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors (55, 56). The potential interplay between tubular injury and intrarenal hemodynamic dysfunction that we describe herein supports the examination of tubular factors associated with renal protection, including in T1D.

Our study has important limitations worth mentioning. First, by study design, plasma biomarkers were measured at a single time point in individuals with long-standing T1D. As such, the biomarker profiles observed cannot be generalized to earlier stages of DKD or to participants with type 2 diabetes. The cross-sectional design also does not allow any prospective inference between who will and will not develop DKD. Further, the relationships observed between tubular injury markers and intrarenal hemodynamic function may reflect acute metabolic decompensation and dehydration. In addition, the mean GFR_{INULIN} of DKD resisters and participants with DKD was 108 ± 16 and 93 ± 15 ml/min/ $1.73m^2$, respectively, and therefore, our results may not be generalizable to participants with more significant reductions in GFR. There were 3 participants in the DKD resistor group with an elevated GFR_{INULIN} consistent with hyperfiltration (135 ml/min/ $1.73m^2$) and thus one could argue that they had early evidence of DKD. However, excluding these participants from our analyses did not change any of our findings. The GFR values observed in the study participants may be due to a selection bias, whereby those with longstanding diabetes and advanced renal disease may not have been eligible for study recruitment due to events such as death or critical / chronic illness. This may explain the relatively modest kidney disease observed in our DKD group, and as such they could also be considered “slow progressors”. Furthermore, the modest decrease in GFR may be age-related rather than true DKD. Second, only a subset of biomarkers were measured in this study. Other pathways of injury, in addition to the mechanisms described in this study, may be related to intrarenal hemodynamic dysfunction and important in the development of DKD. Third, concentrations of biomarkers in plasma are influenced by GFR. Thus, the findings of our study suggest an association between markers of tubular injury and intrarenal hemodynamic function as well as potential mechanistic insights, but cannot necessarily confirm causality. Furthermore, while the insulin clamp allowed us to measure parameters of intrarenal hemodynamic function while euglycemic, which is a strength of our study, it is also important to acknowledge that insulin has important effects on glomerular and tubular function. Therefore, the insulin clamp may have confounded the differences in intrarenal hemodynamic function we observed between adults with and without T1D. Fourth, although controls and participants with T1D had similar demographic and clinical characteristics, residual confounding due to unmeasured differences between subgroups may have influenced study results despite adjustment in our analyses. Fifth, RVR , RBF , FF , P_{GLO} , R_A and R_E are indirect measurements of intrarenal hemodynamic function and are based on physiological assumptions that may be altered in the setting of diabetes (17). Sixth,

classification of participants as DKD resisters or having DKD was based on a history of T1D, eGFR and albumin excretion values, rather than on histological findings from renal biopsies. As such, other causes of kidney disease cannot be excluded. Nevertheless, this study does provide important insights into the role of the renal tubule in the pathogenesis of DKD. Although prior studies have described the role of tubular (57) and glomerular factors (58) in kidney disease progression in participants with longstanding T1D, to our knowledge, this is the first study to describe the potential relationship between tubular injury and renal hemodynamics in this unique population with longstanding disease of greater than 50 years.

In conclusion, this study demonstrates that tubular markers of injury are elevated in DKD, and DKD resisters had a similar tubular biomarker profile to that of controls without diabetes. This study also demonstrates that plasma NGAL, in adults with T1D, and NGAL/B2M in adults without T1D, are correlated to intrarenal hemodynamic dysfunction. These findings suggest an important diabetes-specific interplay between tubular injury and intrarenal hemodynamic dysfunction, which may be helpful to enrich enrollment in future clinical trials in T1D.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Duality of Interest:

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Figure 1: GFR, ERPF and RVR Across Tertiles of NGAL

* After multivariable adjustments (age, sex, HbA1c, SBP and LDL) significant difference observed between low and high tertile ($p=0.02$) and between mid and high tertile ($p=.01$). GFR: Glomerular filtration rate; NGAL: Neutrophil gelatinase-associated lipocalin.

* After multivariable adjustments (age, sex, HbA1c, SBP and LDL) significant difference observed between low and high tertile ($p=0.03$) and between mid and high tertile ($p=.03$). GFR: Glomerular filtration rate; NGAL: Neutrophil gelatinase-associated lipocalin.

* After multivariable adjustments (age, sex, HbA1c, SBP and LDL) significant difference observed between low and high tertile ($p=0.03$) and between mid and high tertile ($p=.03$). GFR: Glomerular filtration rate; NGAL: Neutrophil gelatinase-associated lipocalin.

Table 1:

Tubular Markers in the study participants

	Controls n=73	DKD Resistors n=44	DKD n=22	P for Trend	P for Controls vs DKD Resistors	P for DKD Resistors vs DKD
NGAL (ng/ml)	174.4 (158.1–192.3)	139.9 (123–158.5)	225.7 (189.1–269.3)	<0.0001	0.007	<0.0001
B2M (ng/ml)	1488.5 (1357.2–1632.5)	1158.1 (1029.1–1303.2)	1771.0 (1498.7–2092.9)	<0.0001	0.001	<0.0001
OPN (ng/ml)	88.6±53.2	76.3±64.6	106.1±70.4	0.08	0.29	0.06
UMOD (ng/ml)	163.6±74.9	61.6±67.4	65.6±59.8	<0.0001	<0.0001	0.83

Data expressed as mean ± SD, median [interquartile range].

DKD: Diabetic kidney disease; NGAL: Neutrophil gelatinase-associated lipocalin; B2M: β -microglobulin; OPM: Osteopontin; UMOD: Uromodulin

Table 2:
Relationships between Tubular Markers and Intrarenal Hemodynamic Function in Adults with Type 1 Diabetes

Variables		Type 1 Diabetes					
		GFR _{INULIN}	ERPF _{FAH}	RVR	P _{GLO}	R _A	R _E
NGAL* (per ng/ml)	r:	-0.33, p=0.006	-0.34, p=0.006	0.31, p=0.01	-0.27, p=0.03	0.26, p=0.03	0.16, p=0.21
	$\beta \pm SE^{**}$:	-10.20 $\pm$ 3.80, p=0.009	-67.83 $\pm$ 18.88, p=0.0007	14.27 $\pm$ 6.53, p=0.03	-1.58 $\pm$ 0.93, p=0.10	335.58 $\pm$ 324.00, p=0.30	195.14 $\pm$ 117.93, p=0.10
B2M* (per ng/ml)	r:	-0.02, p=0.86	-0.13, p=0.29	0.09, p=0.46	-0.05, p=0.66	0.05, p=0.71	0.08, p=0.51
	$\beta \pm SE^{**}$:	-0.60 $\pm$ 4.14, p=0.89	-26.54 $\pm$ 21.13, p=0.21	4.65 $\pm$ 6.95, p=0.51	-0.20 $\pm$ 0.98, p=0.84	26.66 $\pm$ 335.89, p=0.94	159.36 $\pm$ 122.20, p=0.20
OPN (per ng/ml)	r:	-0.02, p=0.88	-0.10, p=0.41	0.04, p=0.74	-0.11, p=0.39	0.01, p=0.93	0.12, p=0.33
	$\beta \pm SE^{**}$:	-0.01 $\pm$ 0.03, p=0.93	-0.18 $\pm$ 0.16, p=0.25	0.03 $\pm$ 0.05, p=0.62	-0.01 $\pm$ 0.00, p=0.41	0.26 $\pm$ 2.50, p=0.92	1.20 $\pm$ 0.91, p=0.19
UMOD (per ng/ml)	r:	0.21, p=0.10	0.14, p=0.25	-0.19, p=0.12	0.08, p=0.50	-0.20, p=0.11	-0.02, p=0.84
	$\beta \pm SE^{**}$:	0.05 $\pm$ 0.03, p=0.16	0.11 $\pm$ 0.17, p=0.52	0.74 $\pm$ 0.05, p=0.18	0.00 $\pm$ 0.01, p=0.72	-3.92 $\pm$ 2.61, p=0.14	0.32 $\pm$ 0.98, p=0.74

* Natural log transformed

** Adjusted for age, sex, HbA1c, SBP and LDL

r: Correlation coefficient; β : Beta coefficient; SE: Standard error; NGAL: Neutrophil gelatinase-associated lipocalin; B2M: β -microglobulin; OPM: Osteopontin; UMOD: Uromodulin; GFR: Glomerular filtration rate; ERPF: Effective renal plasma flow; MAP: Renal vascular resistance; PGLO: Glomerular pressure; R_A: Afferent arteriolar resistance; R_E: Efferent arteriolar resistance.

Relationships between Tubular Markers and Intrarenal Hemodynamic Function in Adults without Type 1 Diabetes

Table 3:

Variables		Non-diabetic Controls					
		GFR _{INULIN}	ERPF _{PAH}	RVR	P _{GLO}	R _A	R _E
NGAL* (per ng/ml)	r:	-0.18, p=0.13	-0.19, p=0.10	0.20, p=0.09	-0.06, p=0.63	0.19, p=0.10	0.21, p=0.07
	$\beta \pm SE^{**}$:	-13.35 $\pm$ 6.17, p=0.03	-88.97 $\pm$ 38.72, p=0.02	30.59 $\pm$ 11.05, p=0.007	-0.79 $\pm$ 0.97, p=0.42	1382.91 $\pm$ 579.88, p=0.02	116.72 $\pm$ 73.79, p=0.12
B2M* (per ng/ml)	r:	-0.49, p<0.0001	-0.42, p=0.0003	0.34, p=0.003	-0.22, p=0.06	0.30, p=0.01	0.18, p=0.13
	$\beta \pm SE^{**}$:	-31.74 $\pm$ 6.83, p<0.0001	-160.66 $\pm$ 45.57, p=0.0008	39.34 $\pm$ 13.60, p=0.005	-3.06 $\pm$ 1.14, p=0.009	1925.51 $\pm$ 708.20, p=0.008	2.09 $\pm$ 92.98, p=0.98
OPN (per ng/ml)	r:	0.13, p=0.27	-0.05, p=0.69	-0.05, p=0.67	0.06, p=0.64	-0.12, p=0.33	0.21, p=0.08
	$\beta \pm SE^{**}$:	0.05 $\pm$ 0.04, p=0.23	-0.01 $\pm$ 0.28, p=0.98	-0.07 $\pm$ 0.08, p=0.42	0.00 $\pm$ 0.00, p=0.47	-4.73 $\pm$ 4.17, p=0.26	0.52 $\pm$ 0.52, p=0.32
UMOD (per ng/ml)	r:	0.20, p=0.09	0.21, p=0.08	-0.24, p=0.05	-0.18, p=0.13	-0.20, p=0.09	-0.06, p=0.62
	$\beta \pm SE^{**}$:	0.05 $\pm$ 0.03, p=0.07	0.30 $\pm$ 0.19, p=0.12	-0.10 $\pm$ 0.05, p=0.06	-0.01 $\pm$ 0.00, p=0.16	-4.34 $\pm$ 2.85, p=0.13	-0.04 $\pm$ 0.36, p=0.91

* Natural log transformed

** Adjusted for age, sex, SBP and LDL

r: Correlation coefficient; β : Beta coefficient; SE: Standard error; NGAL: Neutrophil gelatinase-associated lipocalin; B2M: β -microglobulin; OPM: Osteopontin; UMOD: Uromodulin; GFR: Glomerular filtration rate; ERPF: Effective renal plasma flow; MAP: RVR: Renal vascular resistance; PGLO: Glomerular pressure; R_A: Afferent arteriolar resistance; R_E: Efferent arteriolar resistance.