Renin-angiotensin-aldosterone system activation in long-standing type 1 diabetes

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BACKGROUND. In type 1 diabetes (T1D), adjuvant treatment with inhibitors of the renin-angiotensin-aldosterone system (RAAS), which dilate the efferent arteriole, is associated with prevention of progressive albuminuria and renal dysfunction. Uncertainty still exists as to why some individuals with long-standing T1D develop diabetic kidney disease (DKD) while others do not (DKD resistors). We hypothesized that those with DKD would be distinguished from DKD resistors by the presence of RAAS activation.

METHODS. Renal and systemic hemodynamic function was measured before and after exogenous RAAS stimulation by intravenous infusion of angiotensin II (ANGII) in 75 patients with prolonged T1D durations and in equal numbers of nondiabetic controls. The primary outcome was change in renal vascular resistance (RVR) in response to RAAS stimulation, a measure of endogenous RAAS activation.

RESULTS. Those with DKD had less change in RVR following exogenous RAAS stimulation compared with DKD resistors or controls (19%, 29%, 31%, P = 0.008, DKD vs. DKD resistors), reflecting exaggerated endogenous renal RAAS activation. All T1D participants had similar changes in renal effluent arteriolar resistance (9% vs. 13%, P = 0.37) irrespective of DKD status, which reflected less change versus controls (20%, P = 0.03). In contrast, those with DKD exhibited comparatively less change in afferent arteriolar vascular resistance compared with DKD resistors or controls (33%, 48%, 48%, P = 0.031, DKD vs. DKD resistors), indicating higher endogenous RAAS activity.

CONCLUSION. In long-standing T1D, the intrarenal RAAS is exaggerated in DKD, which unexpectedly predominates at the afferent rather than the efferent arteriole, stimulating vasoconstriction.

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Introduction

Diabetic kidney disease (DKD) is the leading cause of end-stage renal disease and dialysis in North America (1–3) and is a strong predictor of cardiovascular disease and mortality (4, 5). The cumulative lifetime incidence of DKD in type 1 diabetes (T1D) is approximately 50% (6, 7), which means that a subset of patients with T1D do not develop clinical DKD (6, 7). Renal and systemic hemodynamic mechanisms that protect against DKD in long-standing T1D in some patients (DKD resistors), but not in others (those with DKD), are poorly understood. Although DKD resistors may have better cardiometabolic risk factor profiles, including better glycemic, lipid, and blood pressure control, DKD risk may also be determined by differences in intrarenal hemodynamic function (8, 9). Intrarenal hemodynamic function is strongly
influenced by the renin-angiotensin-aldosterone system (RAAS) (10). Chronic activation of the renal and systemic RAAS, principally by angiotensin II (ANGII$_{1–8}$), is considered a key factor in the pathogenesis of DKD. Chronic RAAS activation raises intraglomerular pressure locally within the kidney and also initiates tissue injury and stimulates fibrosis, the generation of reactive oxygen species, and proinflammatory effects intrarenally and systemically (11, 12).

Current nephroprotective strategies that emphasize optimal control of multiple risk factors, including hyperglycemia, dyslipidemia, and hypertension, only partially protect against DKD in T1D. RAAS inhibitors remain the current standard of care for nephroprotection in DKD. From a hemodynamic perspective, however, these agents exert only partial renal protection through dilation of the efferent arteriole (11, 13). Although beneficial, RAAS inhibitors are not without risk and are associated with acute kidney injury and hyperkalemia, especially in the elderly (13). Moreover, from a physiological perspective, organ-specific measurement of RAAS activation has not been undertaken in older cohorts of patients with long-standing diabetes. Ultimately, RAAS inhibitors are limited as cardiorenal protective therapies since dual RAAS blockade exerts either no benefit or harm (12, 14–16), and agents that have been evaluated as possible add-on therapies to RAAS inhibition are largely ineffective (17, 18). In the setting of T1D, primary renal disease prevention with RAAS inhibitors is ineffective, and only a limited number of ongoing trials are examining novel pharmacotherapies to treat DKD in T1D (19). While many reasons may underlie our current inability to prevent or slow the progression of DKD, it is due in part to our limited understanding of changes in renal physiology that promote DKD. Filling in current knowledge gaps around DKD pathophysiology and identifying factors that distinguish DKD versus DKD resistor status may elucidate the role of potential novel therapeutic targets to prevent or slow DKD progression in T1D.

The primary objective of this study, the Canadian Study of Longevity in Type 1 Diabetes, was to better characterize pathways implicated in the pathophysiology of T1D complications. Specifically, we aimed to identify key determinants of factors that conferred protection against development of DKD in individuals without overt evidence of renal injury (estimated glomerular filtration rate modification of diet in renal disease [eGFR$_{MDRD}$] ≥60 ml/min/1.73 m$^2$ and 24-hour urine albumin excretion <30 mg/d), despite prolonged durations of T1D. We determined whether intrarenal hemodynamic responses to exogenous RAAS stimulation (intravenous infusion of ANGII), as a measure of baseline endogenous RAAS activation, differed in adults with T1D for ≥50 years, based on the presence or absence of DKD, and compared them with those of healthy age- and sex-matched controls. Secondary objectives were to determine whether systemic hemodynamic function, as reflected by changes in blood pressure and arterial stiffness, differed at baseline and in response to exogenous RAAS between participants with prolonged T1D and in controls. We hypothesized that adults with T1D and DKD, but not DKD resistors, would have significantly less change in renal hemodynamic functional parameters in response to exogenous RAAS stimulation, reflecting high endogenous intrarenal RAAS activation at baseline during clamped euglycemia compared with nondiabetic controls. We also hypothesized that exogenous RAAS stimulation would not modify systemic hemodynamic function in those with DKD, reflecting heightened endogenous RAAS activation, compared with DKD resistors and controls.

Results

Baseline characteristics. Of the 75 participants with T1D, 50 were DKD resistors and 25 had DKD (Figure 1). Response to ANGII was not measured in 1 control subject and in 13 participants with T1D due to elevations in blood pressure or during the study protocol, therefore the final protocol analysis set was made up of 74 controls and 62 T1D participants ($n$ = 136, Figure 1). The mean age of the 74 controls was similar to that of the 62 T1D participants (65 ± 8 vs. 65 ± 7 years, $P$ = 0.84) as was sex distribution (58% and 55% female, $P$ = 0.70). RAAS inhibitor use was present in 51 (82%) of T1D and 10 (14%) of controls. Other clinical and biochemical characteristics of the 136 study participants are summarized in Table 1, stratified by group. Baseline systolic blood pressure (SBP) was highest in DKD group, and heart rate was higher in T1D (but similar between DKD resistors and DKD subgroups). Glycemic control was similar between DKD resistors and those with DKD (glycated hemoglobin [HbA1c] 7.2% ± 0.8% vs. 7.6% ± 1.1%, $P$ = 0.08), though morning blood glucose levels were higher in DKD ($P$ = 0.02). Based on stratification, DKD had low eGFR$_{MDRD}$ and high 24-hour urine albumin excretion. eGFR$_{MDRD}$ was similar between controls and DKD resistors, but 24-hour urine albumin excretion was higher in DKD resistors. Those with DKD had higher renin and plasma uric acid concentrations compared with both controls and DKD resistors, while aldosterone concentrations were highest in controls but similar between T1D groups ($P$ = 0.06).
Renal and systemic hemodynamic function at baseline and response to exogenous RAAS stimulation with ANGII. Baseline renal hemodynamic function and systemic hemodynamic parameters (prior to ANGII infusions) are shown in Table 2. Among the measured variables, the DKD subgroup had the lowest baseline glomerular filtration rate (GFRINSULIN) and effective renal plasma flow (ERPFPAH), compared with DKD resistors and controls. Compared with controls, DKD resistors had similar baseline GFR INSULIN, ERPFPAH, and mean arterial pressure (MAP). Among the calculated variables, compared with the DKD subgroup, DKD resistors had higher renal blood flow (RBF; \( P < 0.001 \)), similar glomerular hydrostatic pressure (\( P_{GLO} \)), lower renal afferent arteriolar resistance (\( R_A; \ P < 0.001 \)), and lower renal efferent arteriolar resistance (\( R_E; \ P = 0.02 \)). The filtration fraction (FF) tended to be highest in those with DKD, although this trend did not reach significance compared with DKD resistors (\( P = 0.055 \)). Compared with controls, DKD resistors had higher \( P_{GLO} \), lower \( R_A \), and higher \( R_E \). Baseline renal vascular resistance (RVR), the variable used in the calculation of the primary study outcome, was similar between controls and DKD resistors but was markedly higher in participants with DKD (11.5 ± 2.9, 11.8 ± 2.5, and 15.4 ± 2.6 mmHg/l/min•100, respectively, \( P < 0.001 \)).

Responses in renal and systemic hemodynamic function due to exogenous RAAS stimulation with low- and high-dose ANGII infusion are summarized in Table 3. The primary study endpoint, the change in RVR in response to exogenous RAAS stimulation (\( \Delta RVR \), middle of table), was decreased in those with DKD when compared with DKD resistors (19.0% ± 9.4% vs. 28.8% ± 13.8%, \( P = 0.008 \)). Notably, \( \Delta RVR \) was similar between DKD resistors and controls. This same pattern — high response in controls and DKD resistors and comparatively low response in those with DKD — was observed for \( \Delta R_A \).
contrast, though ΔR was higher in controls, significant differences were not observed between DKD resistors and the DKD subgroup. A trend toward reduced ΔR was observed across controls, DKD resistors, and DKD subgroups (Table 3). The patterns of ΔRVR, ΔRA, and ΔRE are shown in Figure 2, with similar patterns of response in Figure 2, A and B (representing ΔRVR and ΔRA), but a different pattern of response in Figure 2C (representing ΔRE).

The response of blood pressure to exogenous RAAS stimulation followed a pattern similar to ΔRVR (Figure 3A), represented by ΔMAP. In contrast, heart rate markedly increased in those with DKD but not in DKD resistors or controls (i.e., change was close to 0). This different pattern of response is shown in Figure 3B.

Vascular studies. No statistically significant differences in augmentation index (AIx) were observed among the groups (Table 4 and Figure 4). At baseline, carotid-radial pulse wave velocity (PWV) and carotid-femoral PWV were lowest in controls (P = 0.026, P < 0.001, respectively, Table 4). Baseline carotid-femoral PWV was higher in those with DKD compared with DKD resistors (P = 0.016). In response to exogenous RAAS stimulation, carotid-radial PWV was significantly increased in the DKD subgroup compared with DKD resistors (Table 5 and Figure 4). In contrast, in response to RAAS stimulation, carotid-femoral PWV was significantly decreased in those with DKD compared with DKD resistors and controls (Table 5 and Figure 4).
Table 2. Renal hemodynamic function and systemic hemodynamic measurements at baseline

<table>
<thead>
<tr>
<th></th>
<th>Controls n = 74</th>
<th>DKD resistors n = 42</th>
<th>DKD n = 20</th>
<th>P for trend</th>
<th>P for controls vs. DKD resistors</th>
<th>P for DKD resistors vs. DKD</th>
</tr>
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<tbody>
<tr>
<td>Measured</td>
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</tr>
<tr>
<td>GFR (ml/min/1.73 m²)</td>
<td>105.3 ± 18.9</td>
<td>108.7 ± 15.9</td>
<td>95.9 ± 14.6</td>
<td>0.034</td>
<td>0.33</td>
<td>0.004</td>
</tr>
<tr>
<td>ERPF&lt;sub&gt;p&lt;/sub&gt; (ml/min/1.73 m²)</td>
<td>495.6 ± 131.3</td>
<td>491.4 ± 94.5</td>
<td>392.8 ± 71.1</td>
<td>0.002</td>
<td>0.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>84.8 ± 10.0</td>
<td>86.8 ± 6.2</td>
<td>89.0 ± 6.9</td>
<td>0.13</td>
<td>0.19</td>
<td>0.22</td>
</tr>
<tr>
<td>Hematocrit (l/l)</td>
<td>0.38 ± 0.04</td>
<td>0.35 ± 0.03</td>
<td>0.34 ± 0.03</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.069</td>
</tr>
<tr>
<td>Plasma protein (g/l)</td>
<td>61 ± 4</td>
<td>56 ± 6</td>
<td>58 ± 4</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.087</td>
</tr>
<tr>
<td>RA (dyne•s•cm&lt;sup&gt;–5&lt;/sup&gt;)</td>
<td>4,440 ± 2,068</td>
<td>4,084 ± 1,273</td>
<td>5,414 ± 1,326</td>
<td>0.028</td>
<td>0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FF (%)</td>
<td>0.219 ± 0.038</td>
<td>0.225 ± 0.033</td>
<td>0.2498 ± 0.049</td>
<td>0.01</td>
<td>0.42</td>
<td>0.055</td>
</tr>
<tr>
<td>P&lt;sub&gt;GLO&lt;/sub&gt; (mmHg)</td>
<td>44.6 ± 2.8</td>
<td>49.5 ± 4.2</td>
<td>49.3 ± 3.8</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.91</td>
</tr>
<tr>
<td>R&lt;sub&gt;S&lt;/sub&gt; (dyne•s•cm&lt;sup&gt;–5&lt;/sup&gt;)</td>
<td>4,440 ± 2,068</td>
<td>4,084 ± 1,273</td>
<td>5,414 ± 1,326</td>
<td>0.028</td>
<td>0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>R&lt;sub&gt;E&lt;/sub&gt; (dyne•s•cm&lt;sup&gt;–5&lt;/sup&gt;)</td>
<td>1,217 ± 269</td>
<td>2,244 ± 406</td>
<td>2,645 ± 671</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SD. Significant values are shown in bold (P < 0.05). DKD, diabetic kidney disease; ERPF<sub>p</sub>, effective renal plasma flow<sub>p</sub>; ANGII, angiotensin II; FF, filtration fraction; GFR<sub>INSULIN</sub>, glomerular filtration rate<sub>INSULIN</sub>; MAP, mean arterial pressure; P<sub>GLO</sub>, glomerular hydrostatic pressure; R<sub>S</sub>, renal afferent arterial resistance; R<sub>E</sub>, renal efferent arterial resistance; RBF, renal blood flow; RVR, renal vascular resistance.

**Autonomic dysfunction.** Compared with participants with T1D (both DKD and DKD resistors subgroups), controls had significantly greater heart rate variability (HRV, Table 1). There were no significant differences observed in root mean square of successive difference (RMSSD) or standard deviation of normal-to-normal intervals (SDNN) between those with DKD or DKD resistors. The ratio of SDNN to RMSSD (SDNN/RMSSD) as a surrogate for sympathetic/parasympathetic activity was significantly greater in DKD resistors (2.0 ± 0.8) compared with controls (1.3 ± 0.5, P < 0.001) and the DKD subgroup (1.5 ± 0.7, P < 0.001), suggesting greater sympathetic activity in DKD resistors. A similar pattern was observed for the ratio of low-power to high-power frequencies of R to R intervals (LF/HF ratio), though values for DKD and DKD resistors were similar. HRV was associated with ΔRVR in response to low-dose ANGII (RMSSD: r = 0.41, P = 0.009; SDNN: r = 0.31, P = 0.051; LF/HF ratio: r = –0.40, P = 0.012) and high-dose ANGII (RMSSD: r = 0.36, P = 0.01; LF/HF ratio: –0.48, P = 0.002) in DKD resistors, but significant relationships were not observed in the DKD subgroup (Supplemental Table 1; supplemental material available online with this article; https://doi.org/10.1172/jci.insight.96968DS1). Furthermore, SDNN/RMSSD was negatively associated with ΔSBP in response to low-dose ANGII (r = –0.47, P = 0.035) but not high-dose ANGII (Supplemental Table 1) in the DKD subgroup but not DKD resistors. SDNN/RMSSD was not significantly associated with ΔSBP in response to low-dose ANGII or high-dose ANGII (Supplemental Table 1) in either T1D subgroup.

**Discussion**

Activation of the intrinsic RAAS is physiologically important in humans for regulating arterial blood pressure and for maintaining sodium and electrolyte hemostasis. In the setting of chronic hyperglycemia, overactivation of the RAAS is strongly implicated in the initiation and progression of DKD (10, 20–24). Organ-specific measurement of RAAS activation, however, has not been previously characterized in cohorts with long-standing T1D within defined subgroups of those with and without DKD (10).

In experimental work, animals with T1D and hyperfiltration exhibit a decrease in R<sub>S</sub>, possibly on the basis of changes in tubuloglomerular feedback, with overall similar levels of R<sub>E</sub>, depending on the amount of dietary protein intake (25). In young patients with T1D and hyperfiltration and in patients with type 2 diabetes (T2D), abnormalities in both R<sub>S</sub> and R<sub>E</sub> have been reported (20, 26). Less, however, is known about the effect of exogenous ANGII on renal segmental resistance changes in humans, which may depend on a variety of clinical factors, including age (27) and weight (28). In this set of mechanistic studies, our first major observation was that, in adults with T1D, DKD resistors had similar responses in RVR to RAAS...
stimulation compared with age- and sex-matched controls without diabetes, suggesting that these groups have relatively similar levels of endogenous RAAS activation at baseline. In contrast, those with DKD had minimal changes in RVR responses to exogenous RAAS stimulation, reflecting exaggerated intrarenal RAAS activation at baseline. When analyzed on the basis of afferent (RA) versus efferent (RE) arteriolar vascular resistance, we observed that the effect of endogenous baseline RAAS activation unexpectedly predominated at the RA in the DKD subgroup. Evidence of exaggerated endogenous RAAS activation was also present in the systemic circulation in the DKD but not the DKD resistor subgroup.

The overall aims of the Canadian Study of Longevity in Type 1 Diabetes were to examine pathophysiological mechanisms that contribute to complications in patients with prolonged durations (>50 years) of T1D, with the primary objective to characterize renal and systemic vascular phenotypes related to DKD. Recent reports from the Scottish Registry Linkage Study and from studies of the Swedish National Diabetes Register indicate that T1D is associated with a several-fold increase in mortality across all age groups (29–31). The strongest risk factor for cardiovascular disease and mortality in T1D is DKD (4, 5). The FinnDiane and Pittsburgh EDC studies reported that, in the absence of DKD, mortality is not increased in patients with T1D compared with patients without diabetes over 20-years of follow-up (32, 33). These findings support more aggressive risk factor management in T1D, especially in those at increased risk of DKD. While approximately 50% of people with T1D will resist DKD (6, 7), despite treatment optimization, a subset of people (~30%–50%) with T1D will continue to develop DKD (4, 5) for reasons that remain incompletely understood.

The current standard of care for DKD includes the use of RAAS inhibitors (34), which preferentially modify renal efferent tone through blocking the production of ANGII (ACE inhibitors) or the effect of ANGII at the ANGII type 1 (AT1) receptor expressed on vascular smooth muscle cells (with ANGII receptor blockers) (10). In T1D, hyperglycemia augments RAAS activation, including increased ambient levels of intrarenal RAAS mediators (35) and also leads to changes in AT, receptor localization, expression, and/or sensitivity (36). In the present set of experiments, we observed different intrarenal hemodynamic functional responses to acute exogenous RAAS stimulation with low- and high-dose ANGII infusion in participants with T1D and evidence of DKD compared with those without DKD. Interestingly, our analysis based on Gomez equations suggested that differences in RAAS-stimulated

Table 3. Percentage change in renal hemodynamic function and systemic hemodynamics in response to exogenous RAAS stimulation with ANGII

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>DKD resistors</th>
<th>DKD</th>
<th>P for trend</th>
<th>P for controls vs. DKD resistors</th>
<th>P for DKD resistors vs. DKD</th>
</tr>
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<tbody>
<tr>
<td><strong>Measured</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>ΔGFRINSULIN (%)</td>
<td>-2.9 ± 12.4</td>
<td>-5.6 ± 6.9</td>
<td>-6.2 ± 13.2</td>
<td>0.35</td>
<td>0.16</td>
<td>0.85</td>
</tr>
<tr>
<td>ΔERPFPAH (%)</td>
<td>-14.7 ± 7.8</td>
<td>-13.8 ± 7.2</td>
<td>-11.6 ± 6.2</td>
<td>0.28</td>
<td>0.54</td>
<td>0.27</td>
</tr>
<tr>
<td>ΔSBP (%)</td>
<td>8.6 ± 8.2</td>
<td>10.3 ± 8.2</td>
<td>5.4 ± 7.2</td>
<td>0.087</td>
<td>0.27</td>
<td>0.026</td>
</tr>
<tr>
<td>ΔDBP (%)</td>
<td>8.7 ± 9.5</td>
<td>8.6 ± 6.4</td>
<td>4.9 ± 6.8</td>
<td>0.18</td>
<td>0.94</td>
<td>0.044</td>
</tr>
<tr>
<td>ΔMAP (%)</td>
<td>8.6 ± 8.4</td>
<td>9.3 ± 6.4</td>
<td>4.6 ± 6.0</td>
<td>0.069</td>
<td>0.61</td>
<td>0.009</td>
</tr>
<tr>
<td>ΔHeart rate (%)</td>
<td>-0.3 ± 6.8</td>
<td>1.7 ± 6.6</td>
<td>6.1 ± 7.7</td>
<td>0.002</td>
<td>0.13</td>
<td>0.026</td>
</tr>
<tr>
<td>ΔHematocrit (%)</td>
<td>-2.1 ± 3.3</td>
<td>-0.7 ± 3.2</td>
<td>-1.2 ± 3.8</td>
<td>0.088</td>
<td>0.028</td>
<td>0.58</td>
</tr>
<tr>
<td>ΔProtein (%)</td>
<td>-3.1 ± 4.7</td>
<td>-1.0 ± 6.3</td>
<td>-3.5 ± 5.3</td>
<td>0.084</td>
<td>0.061</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>Derived</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>ΔRVR (%)</td>
<td>30.5 ± 18.0</td>
<td>28.8 ± 13.8</td>
<td>19.0 ± 9.4</td>
<td>0.025</td>
<td>0.61</td>
<td>0.008</td>
</tr>
<tr>
<td>ΔRBF (%)</td>
<td>-15.9 ± 8.2</td>
<td>-14.2 ± 7.6</td>
<td>-12.4 ± 6.0</td>
<td>0.19</td>
<td>0.29</td>
<td>0.36</td>
</tr>
<tr>
<td>ΔFF (%)</td>
<td>14.4 ± 15.4</td>
<td>10.0 ± 9.2</td>
<td>6.3 ± 13.8</td>
<td>0.044</td>
<td>0.062</td>
<td>0.31</td>
</tr>
<tr>
<td>ΔP GFR (%)</td>
<td>-1.7 ± 5.4</td>
<td>-1.7 ± 5.2</td>
<td>-4.3 ± 5.4</td>
<td>0.16</td>
<td>&gt;0.99</td>
<td>0.085</td>
</tr>
<tr>
<td>ΔR A (%)</td>
<td>47.9 ± 36.0</td>
<td>48.0 ± 25.7</td>
<td>32.7 ± 20.8</td>
<td>0.16</td>
<td>0.99</td>
<td>0.031</td>
</tr>
<tr>
<td>ΔR E (%)</td>
<td>19.8 ± 21.2</td>
<td>12.8 ± 11.8</td>
<td>8.8 ± 17.4</td>
<td>0.031</td>
<td>0.031</td>
<td>0.37</td>
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</table>

Positive values represent increase from baseline. Significant values are shown in bold (P < 0.05). *Change in RVR in response to exogenous RAAS stimulation (high-dose ANGII) was the derived measure of inherent RAAS activation and primary endpoint in this study. DKD, diabetic kidney disease; ERPF, effective renal plasma flow para-aminohippuric acid; FF, filtration fraction; GFRINSULIN, glomerular filtration rate INSULIN; MAP, mean arterial pressure; P GFR, glomerular hydrostatic pressure; R A, renal afferent arterial resistance; RBF, renal blood flow; R E, renal efferent arterial resistance; RVR, renal vascular resistance.
RVR responses were due to an attenuated response to ANGII at the afferent renal arteriole in those with DKD versus DKD resisters due to high endogenous baseline RAAS activation at the afferent arteriole. Evidence of endogenous RAAS activation was present to a similar degree at the efferent arteriole, as there was minimal change in the $R_E$ in all T1D participants regardless of DKD status. These observations suggest that under conditions of prolonged T1D duration, patients with DKD manifest accentuated endogenous renal RAAS activation, predominantly at the afferent arteriole, leading to blunted hemodynamic responsiveness to exogenous RAAS stimulation (Figure 5). Whether this occurs due to higher local RAAS production in the renal microcirculation or due to higher expression or sensitivity of AT$_1$ receptors in renal arterioles in DKD or due to the development of resistance to ANGII is unknown but warrants further investigation. A trend toward reduced $\Delta R_E$ was observed among the DKD subgroup compared with the DKD resisters; however, this was not statistically significant. We leave open the possibility that this study may not have been adequately powered to detect an effect on $R_E$.

The hemodynamic phenotype in the systemic circulation of those with T1D typically parallels the changes in the renal microcirculation. Systemic circulatory changes in T1D have been attributed to RAAS activation (37–40), leading to increased arterial stiffness, endothelial dysfunction, hypertension, and the risk of macrovascular complications — all of which may be exaggerated with increased age (41–44). In the present study, changes in blood pressure did not differ among DKD resisters compared with the controls in response to RAAS stimulation. In contrast, in T1D adults with DKD, the vasoconstrictive blood pressure response to RAAS stimulation was comparatively attenuated, suggesting higher endogenous baseline RAAS activation in the systemic circulation in those with DKD. These observations suggest that the classical RAAS paradox, whereby intrarenal RAAS activation is exaggerated in the presence of low levels of the systemic RAAS (45), may not occur in the setting of prolonged T1D durations, since the responses to exogenous RAAS stimulation were concordantly suppressed for RVR and for systemic blood pressure in those with DKD. In keeping with these observations, baseline plasma renin concentrations were highest among those with DKD, and circulating aldosterone levels tended to be higher in those with DKD compared with those without DKD.

Preservation of renal and systemic vascular function in DKD resisters may contribute to protection against microvascular and macrovascular injury and the development of clinical complications. To further assess the vasculature in this cohort, we measured arterial stiffness and observed that, only in participants with T1D with DKD, there was an attenuated carotid-femoral PWV response compared...
with those without DKD and compared with controls with exogenous RAAS stimulation. In contrast, carotid-radial PWV responses to exogenous RAAS stimulation were exaggerated in participants with T1D and DKD. In patients with T1D without DKD, PWV responses to exogenous RAAS stimulation were similar compared with controls. These findings substantiate the hypothesis that, in the setting of prolonged T1D, DKD is associated with circulation-specific changes in PWV-derived arterial stiffness responses to exogenous ANGII.

Cardiac autonomic neuropathy is an early predictor of macrovascular disease, carotid artery disease, and mortality in diabetes mellitus (46). In this cohort, although control participants had higher HRV compared with adults with T1D, we did not observe statistically significant differences in HRV between T1D adults with and without DKD. Despite the fact that no significant differences were observed in HRV among T1D participants within the cohort, HRV was positively associated with the intrarenal hemodynamic response to exogenous RAAS stimulation only in DKD resistors, which may also reflect a relationship between lower baseline RAAS activation (e.g., accentuated RAAS stimulation response) and preservation of HRV in DKD resistors. Interestingly, those with T1D and DKD had increased heart rate upon exogenous RAAS stimulation, despite having minimal change in blood pressure upon exogenous RAAS stimulation. We are presently unaware of definitive mechanisms in humans linking exaggerated responses in heart rate to RAAS stimulation in those with DKD in the absence of increases in arterial pressure. Prior preclinical studies in baroreceptor-denervated rats revealed acute sympathoexcitatory effects in response to exogenous ANGII — effects, which may counter ANGII-stimulated increases in blood pressure, which are associated with a baroreceptor response that affects heart rate via downregulating sympathetic nerve activity (47).

While it is not yet clear how our observations may translate into clinical practice, it is relevant that sodium glucose cotransporter-2 (SGLT2) inhibitors reduce cardiovascular risk, albuminuria, and progressive DKD in patients with T2D who participated in the EMPA-REG OUTCOME and CANVAS Program cardiovascular safety trials (48–50). Renal benefits with SGLT2 inhibitors in animals and in humans have been attributed in large part to proximal tubular natriuresis, leading to activation of tubuloglomerular feedback and afferent vasoconstriction (35, 51–53). In the current set of studies involving adults with long-standing DKD

Table 4. Arterial stiffness measurements at baseline

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>DKD resistors</th>
<th>DKD</th>
<th>P for trend</th>
<th>P for controls vs. DKD resistors</th>
<th>P for DKD resistors vs. DKD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic AIx (%)</td>
<td>22.6 ± 9.8</td>
<td>23.8 ± 6.4</td>
<td>26.1 ± 7.6</td>
<td>0.27</td>
<td>0.42</td>
<td>0.23</td>
</tr>
<tr>
<td>Carotid AIx (%)</td>
<td>25.4 ± 10.0</td>
<td>25.5 ± 7.6</td>
<td>27.9 ± 7.9</td>
<td>0.54</td>
<td>0.95</td>
<td>0.26</td>
</tr>
<tr>
<td>Carotid-radial PWV (m/s)</td>
<td>7.8 ± 1.2</td>
<td>8.6 ± 1.9</td>
<td>8.2 ± 1.1</td>
<td>0.11</td>
<td><strong>0.043</strong></td>
<td>0.61</td>
</tr>
<tr>
<td>Carotid-femoral PWV (m/s)</td>
<td>7.6 ± 1.9</td>
<td>10.4 ± 3.7</td>
<td>12.7 ± 4.4</td>
<td><strong>&lt;0.001</strong></td>
<td><strong>&lt;0.001</strong></td>
<td><strong>0.016</strong></td>
</tr>
</tbody>
</table>

Significant values are shown in bold (*P* < 0.05). AIx, augmentation index; DKD, diabetic kidney disease; PWV, pulse wave velocity.
T1D and DKD, RA was already increased at baseline, suggesting that renal hemodynamic effects of SGLT2 inhibition may be less effective compared with reported effects in younger T1D participants with hyperfiltration and vasoafferent dilation (54–56). Despite salutary cardiorenal outcome effects in the setting of T2D, along with possible glycemia-related benefits in patients with uncomplicated T1D (57, 58), pilot studies in patients with T1D and DKD are needed to assess the renal therapeutic potential of SGLT2 inhibitors.

Our study has limitations worth mentioning. While our sample size was small compared with larger epidemiologic cohorts, to ensure meaningful analysis we performed careful a priori sample size calculations and used robust techniques to measure renal and systemic renal hemodynamic function. While nondiabetic controls were matched based on age and sex with the T1D cohort, we cannot rule out the potential effects of subgroup differences (such as age, sex, baseline RAAS inhibitor use) between the T1D subgroups (those with DKD and DKD nonresistors) which may have confounded the present analyses. Results from this study may not be generalizable to youth with T1D or individuals with T1D of shorter durations.

To gain additional information about the human intrarenal circulation in vivo, we applied Gomez equations to measurements of GFR, RBF, ERPF, RVR, hematocrit, and serum protein to calculate R_a and R_g, glomerular pressure (P_GLO), and filtration pressure (59). However, as discussed elsewhere, the Gomez formula calculated estimates may not have captured the actual between-group differences in all hemodynamic parameters, such as P_GLO (59). Moreover, we focused on a single vascular pathway, the RAAS, and did not

Figure 4. Change in arterial stiffness in response to exogenous RAAS stimulation with ANGII. Percentage change in radial (A) and carotid (B) Aix and percentage change in radial (C) and femoral (D) PWV are shown for age- and sex-matched controls (n = 74), DKD resistors (n = 42), and DKD nonresistors (n = 20) at baseline, in response to low- and high-dose ANGII, and during recovery. Data represent mean ± SEM. ANGII, angiotensin II; Aix, augmentation index; PWV, pulse wave velocity. *P < 0.05, Student’s t test, for DKD resistors versus DKD.
investigate other vasoconstrictive or vasodilatory pathways independent of the RAAS, which may also be of importance in the differentiation of DKD resistor status in those with prolonged T1D durations. In addition, other factors, including atrial natriuretic peptide, may be involved in the regulation of renal function in the setting of diabetes, although this is likely relevant primarily during uncontrolled hyperglycemia rather than under the clamped euglycemic conditions used as part of the Canadian Study of Longevity in Type 1 Diabetes. Finally, we studied participants who were already prone to DKD, and therefore, the changes observed in endogenous RAAS activation may represent associative rather than causative DKD mechanisms. The strengths of this study included direct measures of GFR and renal plasma flow, with simultaneous measurements of systemic hemodynamic function, vascular studies, and neurohormonal and autonomic assessment under clamped euglycemic conditions. Another strength of our study is complete intrarenal and systemic hemodynamic functional testing at baseline and in response to low and high doses of ANGII in participants with prolonged durations of T1D (≥50 years) and in age- and sex-matched controls without diabetes. In contrast to studies including patients with shorter diabetes durations (10–30 years), wherein DKD resistor status may be less reliable due to the possibility of participants subsequently developing DKD, a strength of the current study is our confidence in the classification of the DKD resistor subgroup, who despite very prolonged durations of T1D did not have evidence of renal injury.

In summary, in adults with long-standing T1D, those categorized as DKD resistors had similar responses to exogenous RAAS stimulation as age- and sex-matched nondiabetic controls. In contrast, participants with long-standing T1D and evidence of DKD exhibited less change in intrarenal hemodynamic function with exogenous RAAS stimulation, particularly at the renal afferent arteriole, compared with those without DKD and controls. Differing patterns of systemic vascular responses to exogenous RAAS stimulation were also observed among DKD and DKD resistor subgroups. These observations strongly suggest that baseline endogenous RAAS activation is at least one important factor that differentiates DKD resistor status in adults with a prolonged duration of T1D. Furthermore, these results emphasize that, in adults with long-standing T1D with DKD, excessive RAAS tone and currently available methods to modify the RAAS affect the renal circulation differently than assumed, a finding which offers insights into limitations of these agents and possible future targets for therapy.

### Methods

#### Study design

This was a cross-sectional cohort study of 75 participants with T1D of ≥50-year duration with and without DKD and 75 age- and sex-matched controls to determine mechanisms of nephropathy resistance (Figure 1). Participants with T1D were categorized as DKD resistors if they had eGFRMDRD ≥60 ml/min/1.73 m² and 24-hour urine albumin excretion <30 mg/d at their screening visit. Study
participants were categorized as DKD if they had an eGFR <60 ml/min/1.73 m² or 24-hour urine albumin excretion >30 mg/d at their screening visit. Secondary objectives included clinical phenotyping of other diabetes-related complications, including nephropathy, retinopathy, neuropathy, and macrovascular disease. This study represented the second phase of the Canadian Study of Longevity in Type 1 Diabetes. The participants were studied over the course of 2 clinical visits, approximately 2–4 weeks apart. Visits were conducted between February 2015 and September 2016. Study day 1 included informed consent procedures, clinical visit, preparation instructions for study day 2, and dual-energy x-ray absorptiometry scans for body fat measurement (Supplemental Figure 1). Among T1D participants, point-of-care nerve conduction testing (DPN-Check, Neurometrix Inc.), and retinal examination (retinal photographs and measurement of macular thickness [optical coherence tomography]) were also completed. Study day 2 included measurement of renal hemodynamic function, arterial stiffness and autonomic function, standard formal nerve conduction tests, corneal nerve measurement (Rostock Cornea Module of the Heidelberg Tomograph III, Heidelberg Engineering) as a proxy for small-fiber neuropathy, and coronary artery calcification scoring by chest computed tomography.

Figure 5. Endogenous RAAS activation in controls, DKD resistors, and DKD with T1D. AT receptors are predominately expressed at the renal efferent arteriole with less relative expression at the afferent arteriole. In controls, upon exogenous RAAS stimulation with intravenous infusion of ANGII, ANGII freely interacts with available AT receptors at the afferent and efferent arterioles, initiating vasoconstrictive responses at the RA and RE, respectively. In T1D participants without DKD (DKD resistors), locally within the kidney there is relatively more endogenous intrarenal RAAS at baseline occupying AT receptors (relative to controls), predominantly at the RE compared with the RA. Therefore, upon exogenous RAAS stimulation, ANGII can freely bind AT receptors at the RA, producing vasoconstriction to a similar degree as controls, but to a lesser degree at the RE. In contrast, in participants with DKD, there is exaggerated presence of endogenous RAAS, both at the afferent and efferent arterioles at baseline, such that upon exogenous RAAS stimulation, there are fewer AT receptors available for ANGII binding and therefore fewer vasoconstrictive changes relative to DKD resistors and controls predominantly at the RA, ANGII, angiotensin II; DKD, diabetic kidney disease; RAAS, renin-angiotensin-aldosterone system; RE, renal efferent arteriolar vasoconstriction; RVR, renal vascular resistance; T1D, type 1 diabetes.
Study population
Participants were recruited from the nation-wide registry of approximately 450 Canadians with long-standing T1D (duration ≥50 years) established during the first phase of the Canadian Study of Longevity in Type 1 Diabetes, as previously described (51, 60, 61). Search criteria for second phase of the study included residence in the Greater Toronto Area (e.g., proximity to the University Health Network and Mount Sinai Hospital in Toronto) or a willingness to travel to Toronto General Hospital, Toronto, Ontario, Canada, for the 2 study days. Age- and sex-matched controls were recruited from friends or family members of the T1D participants or were recruited through community advertisement. Inclusion criteria for the controls were (a) 1:1 sex matching as well as being within 5 years of age of a T1D participant and (b) ability to understand and cooperate with study procedures. Exclusion criteria were (a) presence of diabetes mellitus (for controls), (b) microalbuminuria or eGFR <45 ml/min/1.73 m², (c) history of hypertension or blood pressure >140/90 mmHg, and (d) current eye infection, corneal damage, severe movement disorder, or proparacaine allergy to preclude safe corneal confocal microscopy examination (for T1D participants).

Measurement of renal hemodynamic function in response to ANGII
Prestudy procedures in brief: All participants underwent RAAS inhibitor (ACE inhibitors, angiotensin receptor blockers, direct renin inhibitors, aldosterone antagonists) washout 30 days prior to study day 2. For 7 days prior to the study day 2, participants were instructed to maintain a minimum sodium intake of 150 mmol/d and a protein diet of 1.5 g/kg/d. Compliance was evaluated by measurement of 24-hour urine sodium and urea excretion on the seventh day (62, 63). During this time, study staff reviewed daily blood pressure measurements through home monitoring (ambulatory blood pressure meters were provided to participants). For participants who could not tolerate RAAS withdrawal (consistent home blood pressure readings >140/80 mmHg), calcium channel blockade with amlodipine was used if required, and evaluation of urinary albumin and serum creatinine and potassium took place at study day 2 (11, 64).

Study day procedures: Following an overnight fast, participants arrived at the Renal Physiology Laboratory (Toronto General Hospital) for measurement of renal hemodynamic function. Renal hemodynamic function was measured at baseline and in response to low- and high-dose ANGII infusion (Clinalfa; 51.2 μg/vial prepared in a 400 ng/ml solution). All study participants underwent the same experimental procedures, except that participants with T1D underwent a minimum 2-hour euglycemic clamp prior to and during measurement of renal hemodynamic function. The euglycemic clamp was maintained by measurement of venous blood glucose every 10–15 minutes, and an insulin infusion was titrated to achieve a constant blood glucose range of 4–6 mmol/l.

After a brief physical exam, peripheral intravenous catheters were placed for blood sampling, infusion of regents, and infusion of dextrose (5% dilution) or insulin (0.2 IU/ml dilution) (for euglycemic clamp). After a rest period of approximately 15 minutes, baseline blood and urine were drawn. Ad libitum water consumption was allowed during the experimental period, up to a maximum of 500 ml. Patients remained supine throughout the study and during measurements but were allowed to ambulate for subjective voiding.

Renal hemodynamic function was measured using insulin and para-aminohippurate (PAH) clearance techniques standardized per 1.73 m² of body surface area, which measures the GFR_{INSULIN} and ERPF_{PAH} respectively (65–67). FF was determined by dividing the GFR_{INSULIN} by the ERPF_{PAH}. RBF was calculated by dividing the ERPF_{PAH} by 1-hematocrit. RVR was derived by dividing MAP by RBF. Intrarenal hemodynamic resistance measurements (R_a, R_t, and P_GLO) were estimated using Gomez formulae, as described elsewhere (59, 68). Renal hemodynamic function was measured at (a) at baseline, (b) following a 0.5 ng/kg/min (low-dose) infusion of intravenous ANGII, (c) following a 1 ng/kg/min (high-dose) infusion of intravenous ANGII, and (d) during a 90-minute recovery period. Blood was collected for hematocrit, total protein, insulin, and PAH measurements. Initiation of ANGII infusion was withheld if a patient’s blood pressure increased above 150/80 mmHg, and infusion of ANGII was stopped if a study subject’s SBP increased to >160/100 mmHg during the ANGII experimental period.

Vascular studies
Right radial artery waveforms by high-fidelity micromanometer (SPC-301; Millar Instruments) and central aortic pressure waveforms (SphygmoCor, AtCor Medical Systems) were measured before and after each dose of intravenous ANGII infusion in all study participants. Systemic arterial stiffness was determined by the AIx, calculated as the difference between the second systolic peak and inflection point, expressed as a
percentage of the central pulse pressure and corrected to an average heart rate of 75 beats/min. The aortic PWV was measured by sequentially recording ECG-gated right carotid and radial artery waveforms. Our group has published and validated the use of the SphygmoCor device previously (69).

HRV
HRV, testing vagal tone (RMSSD) sympathetic activity (SDNN), and LF/HF ratios were measured in all study participants using methods we have previously described (SphygmoCor) (69).

Study endpoints
As the gold-standard measure of endogenous intrarenal RAAS activation (20, 70, 71), the primary endpoint of the study was change in RVR in response to exogenous RAAS stimulation, defined as the percentage difference between RVR measured at baseline and RVR measured after the second infusion of ANGII. The relationship between change in RVR and endogenous RAAS activation is inverse (a smaller percentage change in RVR after exogenous RAAS stimulation reflects greater endogenous RAAS activation at baseline, a greater percentage change in RVR reflects endogenous RAAS activation at baseline). The primary comparison was between the DKD resistor and DKD subgroups of the T1D participants. Secondary endpoints included changes in intrarenal hemodynamic function (R_A, R_E, P_GLO, GFR_INSULIN, ERPF_PAH, RBF, RVR, FF), systemic variables (MAP and heart rate), and vascular studies of arterial stiffness measures (AIX, PWV) and autonomic function (SDNN, RMSSD, LF, HF). Secondary comparisons included analysis of trends across all 3 subgroups (DKD, DKD resistors, and controls, respectively) and between controls and DKD resistors.

Statistics
Statistical analyses were performed using SAS version 9.2 for Windows (SAS Institute). The primary endpoint comparison was made using the 2-tailed Student’s t test. Continuous variables were assessed for normality (Shapiro-Wilk and inspection of histograms). Tests for trend of clinical characteristics and study endpoints among controls, DKD resistors, and DKD subgroups were made using ANOVA, the Kruskal-Wallis test, or the \( \chi^2 \) test, depending on variable distribution. Secondary comparisons were also made using the t test or the Wilcoxon rank-sum test. A 2-tailed P value of 0.05 was used for tests of statistical significance.

All study participants included in the final analysis underwent ANGII infusion, and analyses were done on a per protocol basis. Missing GFR_INSULIN and ERPF_PAH data existed at all 4 time points for 6 participants (3 controls and 3 T1D) due to sample contamination. Three participants had partial (<4 time points) sample contamination, and in these cases, observations were carried forward. The planned sample size was based on previous studies in T1D longevity cohorts of nephropathy resistance (72) (in which prevalence of nephropathy resistance was estimated to be one-third) and renal hemodynamics (62) (in which \( \Delta \text{RVR} \) was observed to be 0.086 ± 0.035 mmHg/l/min in participants with T1D aged >35 years and 0.057 ± 0.026 mmHg/l/min in non-diabetic controls). To achieve a difference in mean ARVR, a DKD resistor sample size of \( n = 21 \) was required to achieve 90% power with a type I error of 0.05. To recruit at minimum 21 DKD resistors, we planned a sample size of 75 T1D participants.

Study approval
All participants provided written informed consent prior to inclusion in this study, and the study was approved by the institutional research ethics boards of the University Health Network and Mount Sinai Hospital.

Author contributions
BAP and DZIC created the hypothesis and objectives, designed the study, and prepared the manuscript. JAL supervised clinical visits, collected the data, researched the data, and prepared the manuscript. GB supervised clinical visits, collected the data, and reviewed the manuscript. YL performed vascular studies and reviewed the manuscript. LEL researched the data, prepared summary tables and figures, and prepared the manuscript. PB researched the data and prepared the manuscript. MAF performed screening visits, collected the data, and reviewed the manuscript. VL, LC, and JT were the research nurses for this study and reviewed the manuscript. AO collected the data and reviewed the manuscript for scholarly content. DS, AW, HAK, MHB, NP, and VB reviewed the manuscript. DZIC is the guarantor of this manuscript and, as such, had full access to all the data in the study and takes responsiblity for the integrity and accuracy of the data analysis.
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