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Glycosuria-mediated urinary uric acid excretion in patients with uncomplicated type 1 diabetes mellitus

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Lytvyn Y, Škrtić M, Yang GK, Yip PM, Perkins BA, Cherney DZ. Glycosuria-mediated urinary uric acid excretion in patients with uncomplicated type 1 diabetes mellitus. Am J Physiol Renal Physiol 308: F77–F83, 2015. First published November 5, 2014; doi:10.1152/ajprenal.00555.2014.—Plasma uric acid (PUA) is associated with metabolic, cardiovascular, and renal abnormalities in patients with type 2 diabetes but is less well understood in type 1 diabetes (T1D). Our aim was to compare PUA levels and fractional uric acid excretion (FEUA) in patients with TID vs. healthy controls (HC) during euglycemia and hyperglycemia. PUA, FEUA, blood pressure (BP), glomerular filtration rate (GFR-inulin), and effective renal plasma flow (ERPF-paraaminobipurate) were evaluated in patients with TID (n = 66) during clamped euglycemia (glucose 4–6 mmol/l and hyperglycemia (9–11 mmol/l), and in HC (n = 41) during euglycemia. To separate the effects of hyperglycemia vs. increased glycosuria, parameters were evaluated during clamped euglycemia in a subset of T1D patients before and after sodium glucose co-transporter 2 (SGLT2) inhibition for 8 wk. PUA was lower in T1D vs. HC (228 ± 62 vs. 305 ± 75 μmol/l, P < 0.0001). In T1D, hyperglycemia further decreased PUA (228 ± 62 to 199 ± 65 μmol/l, P < 0.0001), which was accompanied by an increase in FEUA (7.3 ± 3.8 to 11.6 ± 6.7, P < 0.0001). In T1D, PUA levels correlated positively with SBP (P = 0.029) and negatively with ERPF (P = 0.031) and GFR (P = 0.028). After induction of glycosuria with SGLT2 inhibition while maintaining clamped euglycemia, PUA decreased to 65 ± 65 μmol/l at 24 wk. In HC, PUA was lower in T1D vs. HC and positively correlates with SBP and negatively with GFR and ERPF in T1D. Glyceria rather than hyperglycemia increases uricosuria in T1D. Future studies examining the effect of uric acid-lowering therapies should account for the impact of ambient glyceria, which causes an important uricosuric effect.

HUMANS HAVE HIGHER URIC ACID (UA) levels compared with other mammals due to mutational silencing of the enzyme uricase, which results in UA remaining the end product of purine metabolism (29). Additionally, about 90% of filtered UA is reabsorbed by the S1 segment of the proximal convoluted tubule, in a process regulated by intracellular anion transporters on the basolateral membrane, such as urate transporter 1 (URAT1) and the more recently discovered glucose transporter 9 (GLUT9) (29). The lack of uricase, combined with the high reabsorptive capacity in the kidney, predisposes humans to the development of hyperuricemia.

UA has recently emerged as an inflammatory factor that increases oxidative stress and promotes activation of the renin-angiotensin-aldosterone system (RAAS) (29). From a clinical perspective, higher UA levels are associated with metabolic abnormalities (insulin resistance, hyperglycemia), cardiovascular disease (hypertension, endothelial dysfunction, arterial stiffness, cardiac diastolic dysfunction), and kidney injury (28, 29) and thus could be involved in the onset and progression of diabetic nephropathy, a common microvascular complication of diabetes mellitus (DM). Plasma UA (PUA) levels could therefore serve as a biomarker and an effective therapeutic target to supplement current clinical targets such as hemoglobin A1c (HbA1c), cholesterol, and blood pressure.

Evidence from rodent models suggests an association between high UA levels and markers of high intraglomerular pressure such as hyperfiltration, and with subsequent increases in proteinuria, glomerular sclerosis, and tubulointerstitial fibrosis, leading to chronic kidney disease (28). More recently, in an animal model of type 1 DM (T1D), UA lowering reduced proteinuria, preserved glomerular filtration rate (GFR), and suppressed renal expression of inflammatory interleukins (37). In patients with T1D, UA is associated with impaired renal function, even when UA levels are in the normal range (28, 35). For example, in 355 T1D participants from the second Joslin Study on the Natural History of Microalbuminuria, baseline UA (within the normal range) showed a significant association with early GFR loss of >3.3%/yr over a 6-yr follow-up period (15). UA also increases the risk of developing proteinuria in T1D patients (23). For example, in 652 normoalbuminuric T1D patients recruited into the Coronary Artery Calcification in Type 1 Diabetes Study, each 60 μmol/l increment in UA from baseline increased the risk of micro- or macroalbuminuria by 80% after a 6-yr follow-up period (23). Although observational associations between higher UA levels and renal outcomes show consistency among independent cohorts (29), UA levels are not clearly defined in the T1D populations.

Accordingly, the first goal of this study was to compare PUA levels in healthy control patients (HC) with patients with T1D. It was hypothesized that, even within the normal range, PUA
levels would be higher in the T1D cohort and that higher PUA levels will be associated with deleterious hemodynamic profiles such as higher blood pressure and changes in renal hemodynamic function. The second goal was to examine the relationship between clamped hyperglycemia, hemodynamic parameters, and PUA levels to determine if this acute physiological stimulus, which promotes deleterious hemodynamic effects such as increased blood pressure, influences PUA levels.

RESEARCH DESIGN AND METHODS

Subject inclusion criteria and study preparation. Forty-one HC and 66 T1D patients underwent detailed physiological examinations. In brief, inclusion criteria were: 18–40 yr of age, blood pressure <140/90, normoalbuminuria on a 24-h urine collection, diabetes duration >1 yr, no history of renal or cardiovascular complications, and no intake of concomitant medications that would alter blood pressure or cardiovascular outcomes. Study visits were performed after a controlled diet for 7 days consisting of ≥150 mmol/day sodium and ≤1.5 g·kg⁻¹·day⁻¹ protein. The sodium-replete diet was used to avoid circulating volume contraction, RAAS activation, and between-subject heterogeneity. Prestudy protein intake was modest to avoid the hyperfiltration effect of high-protein diets (24). All studies were approved by the University Health Network Research Ethics Board, and all subjects gave written informed consent.

Experimental procedures. Patients with T1D were studied on two consecutive days during euglycemia and hyperglycemia. Euglycemic (4–6 mmol/l) and hyperglycemic (9–11 mmol/l) conditions in T1D were maintained using a modified glucose clamp technique as previously described (8). Blood glucose levels were stable for at least 2 h before the measurement of the study end points and were maintained 3–5 h for the rest of the study day. HC were studied during normoglycemic conditions at the Renal Physiology Laboratory at the Toronto General Hospital. GFR and effective renal plasma flow (ERPF) were estimated using inulin and paraaminohippurate (PAH) steady-state infusion clearance techniques (5), respectively, using previously described methods (9). The results of the two clearance periods were averaged. Brachial artery blood pressure measurements were obtained at 20-min intervals throughout the study days (Critikon, Tampa, FL). In a post hoc analysis undertaken to understand the relative effect of hyperglycemia vs. increased glycosuria, the effect of sodium glucose cotransporter 2 (SGLT2) inhibition on PUA and urinary UA was examined using frozen, archived samples. The aim of this analysis was to induce glycosuria while maintaining euglycemia to determine whether effects on PUA were due to increased urinary excretion. For this analysis, we analyzed urine and plasma samples (n = 40) obtained during baseline clamped euglycemic conditions and at follow-up after treatment with 25 mg qd empagliflozin for 8 wk in the Adjunctive-To-Inulin and Renal MechAnistic pilot trial of empagliflozin in T1D (ATIRMA trial, ClinicalTrials.gov NCT01392560). The primary and secondary outcomes from this trial have been published (9).

Sample collection and analytical methods. After clamped euglycemia was achieved for at least 2 h, blood was collected for measurements of inulin, PAH, sodium, PUA, and RAAS mediators [angiotensinogen, plasma renin activity (PRA), aldosterone, and angiotensin II], and urine samples were collected for UA, sodium, glucose, and creatinine measurements.

The blood samples were immediately centrifuged at 3,000 rpm at 4°C for 10 min. Plasma was extracted, placed on ice, and stored at −70°C. Inulin and PAH were measured in serum by colorimetric assays using anthrone and N-(1-naphthyl)ethylenediamine, respectively (16). All hemodynamic measurements were adjusted for body surface area. Filtration fraction (FF) represented the ratio of GFR to ERPF. Renal blood flow (RBF) was derived as ERPF/(1 − hematocrit). Renal vascular resistance (RVR) was derived by dividing mean arterial pressure by the RBF.

Plasma and urine samples were measured for UA, sodium, creatinine, glucose, and urea on the Architect c8000 Clinical Chemistry System using the manufacturer’s reagents (Abbott Diagnostics, Abbott Park, IL). In addition, UA excretion was expressed as fractional excretion (FEUA), derived using (UrA × PCr)/(UCr × PCr) × 100 where UrA, PCr, UCr, and PCr are urinary UA, plasma creatinine, urinary creatinine, and PUA concentrations, respectively. Similarly, sodium excretion was expressed as fractional excretion (FENa), derived using (Una × PCr)/(UCr × PNa) × 100 where Una and Pna are urinary sodium and plasma sodium concentrations, respectively.

Aldosterone was measured using a Coat-A-Count radioimmunoassay system. PRA was measured using a radioimmunoassay kit (Diasorin, Stillwater, MN). HbA1c was measured by high-performance liquid chromatography with the Variant II system (Bio-Rad Laboratories, Hercules, CA).

Statistical analysis. Data are presented as means ± SD. To assess for between-group differences, analysis of variance with post hoc Tukey’s test was used. To compare within-group differences (responses to hyperglycemia or SGLT2 inhibition), a paired Student’s t-test was used. Linear regression analysis was used to determine correlations between responses and PUA levels. Statistical significance was defined as P < 0.05. All statistical analyses were performed using SAS v9.1.3 and GraphPad Prism software (version 6.0).

RESULTS

Baseline characteristics. Baseline parameters were similar between HC and T1D patients (Table 1). Participants were young, normotensive, and normoalbuminuric, and the two groups were similar in age and body mass index.

During euglycemia, heart rate was significantly higher, but still within the normal range, in the T1D vs. HC, and no significant differences in systolic (SBP) or diastolic blood pressure were observed. During hyperglycemic conditions, SBP significantly increased and heart rate decreased compared with euglycemia in the T1D group. As expected, T1D participants had significantly lower levels of circulating RAAS mediators compared with HC (aldosterone, PRA, and angiotensin II) (41). During hyperglycemia, aldosterone and PRA levels further decreased.

As expected, T1D subjects exhibited higher GFR, ERPF, and RBF and lower RVR compared with HC (P < 0.0001 for all comparisons). Out of the 66 T1D patients, 29 exhibited normofiltration (44%) and 37 hyperfiltration (56%), where hyperfiltration was defined as GFR ≥135 ml·min⁻¹·1.73 m⁻². In response to clamped hyperglycemia, GFR tended to increase in T1D (147 ± 40 to 159 ± 39 ml·min⁻¹·1.73 m⁻², P = 0.064) and RVR decreased (0.069 ± 0.021 to 0.055 ± 0.016 mmHg·l⁻¹·min⁻¹, P < 0.0001). No significant changes to ERPF, FF, or RBF were observed in response to hyperglycemic conditions.

Sodium, glucose, and UA handling at baseline. During clamped euglycemic conditions, PUA levels were lower in the T1D group vs. HC (228 ± 62 vs. 305 ± 75 µmol/l, P < 0.0001) (Table 1 and Fig. 1). PUA negatively correlated with FEUA in T1D patients (r = −0.60, P < 0.0001). Urine glucose (Uglucose) excretion levels were also greater in T1D vs. HC during clamped euglycemia, but there was no significant difference in the urine uric acid-to-creatinine ratio, FEUA or FEUA between HC and T1D.

Compared with levels during clamped euglycemia, PUA decreased further in response to clamped hyperglycemia.
The decline in PUA levels in T1D patients during hyperglycemia was accompanied by significant increases in $U_{\text{glucose}}$ (1.4 ± 3.2 to 9.8 ± 10.4 mmol/l, $P < 0.0001$) and $\text{FE}_{\text{UA}}$ (0.87 ± 0.56 to 1.63 ± 0.89, $P < 0.001$). $U_{\text{glucose}}$ correlations with hemodynamic parameters. PUA levels were positively correlated with SBP in T1D ($r = 0.27$, $P = 0.029$) under euglycemic conditions, but not during hyperglycemia (Fig. 2). During euglycemia, PUA levels negatively

Table 1. Baseline subject characteristics and UA, sodium, glucose handling in HC and patients with T1D during euglycemia and hyperglycemia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HC ($n = 41$)</th>
<th>Euglycemia</th>
<th>Hyperglycemia</th>
</tr>
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<tbody>
<tr>
<td>Males</td>
<td>19 (43%)</td>
<td>35 (53%)</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>28.4 ± 7.1</td>
<td>25.0 ± 6.0</td>
<td>17.0 ± 6.6</td>
</tr>
<tr>
<td>Diabetes duration, yr</td>
<td>7.0 ± 11.8</td>
<td>73.9 ± 13.7</td>
<td></td>
</tr>
<tr>
<td>Height, m</td>
<td>1.74 ± 0.09</td>
<td>1.73 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>23.3 ± 3.0</td>
<td>24.8 ± 3.9</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin $A_1c$, mmol/mol (%)</td>
<td>4.1 ± 0.87</td>
<td>6.7 ± 6.1</td>
<td>8.2 ± 1.5</td>
</tr>
<tr>
<td>24-h Urine sodium, mmol/day</td>
<td>177 ± 61</td>
<td>169 ± 85</td>
<td></td>
</tr>
<tr>
<td>24-h Protein intake, g/kg· day$^{-1}$</td>
<td>1.1 ± 0.3</td>
<td>1.0 ± 0.3</td>
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Sodium, glucose, uric acid handling

<table>
<thead>
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<th>Hyperglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma uric acid, mmol/l</td>
<td>305 ± 75</td>
<td>228 ± 62*</td>
<td>199 ± 65†</td>
</tr>
<tr>
<td>Urine uric acid-to-creatinine ratio</td>
<td>248 ± 170</td>
<td>257 ± 121</td>
<td>339 ± 161†</td>
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<tr>
<td>$\text{FE}_{\text{UA}}$, %</td>
<td>6.1 ± 4.1</td>
<td>7.3 ± 3.8</td>
<td>11.6 ± 6.7†</td>
</tr>
<tr>
<td>$\text{FE}_{\text{CS}}$, %</td>
<td>0.84 ± 0.60</td>
<td>0.87 ± 0.56</td>
<td>1.63 ± 0.89†</td>
</tr>
<tr>
<td>Urine glucose-to-creatinine ratio</td>
<td>0.02 ± 0.03</td>
<td>1.4 ± 3.2*</td>
<td>9.8 ± 10.4†</td>
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Systemic hemodynamic function

<table>
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<th>Hyperglycemia</th>
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<tr>
<td>HR, beats/min</td>
<td>60 ± 9</td>
<td>74 ± 13*</td>
<td>72 ± 11†</td>
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<tr>
<td>SBP, mmHg</td>
<td>112 ± 12</td>
<td>115 ± 10</td>
<td>117 ± 11†</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>67 ± 8</td>
<td>66 ± 6</td>
<td>66 ± 8</td>
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Renal hemodynamic function

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<tr>
<td>ERPF, ml·min$^{-1}$·1.73 m$^2$</td>
<td>653 ± 157</td>
<td>824 ± 276*</td>
<td>853 ± 253</td>
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<tr>
<td>GFR, ml·min$^{-1}$·1.73 m$^2$</td>
<td>116 ± 12</td>
<td>147 ± 40*</td>
<td>159 ± 39†</td>
</tr>
<tr>
<td>Filtration fraction</td>
<td>0.19 ± 0.04</td>
<td>0.19 ± 0.06</td>
<td>0.19 ± 0.05</td>
</tr>
<tr>
<td>RBF, ml·min$^{-1}$·1.73 m$^2$</td>
<td>1.063 ± 259</td>
<td>1.310 ± 434*</td>
<td>1.305 ± 419</td>
</tr>
<tr>
<td>$\text{RVR}$, mmHg·1·min$^{-1}$†</td>
<td>0.081 ± 0.020</td>
<td>0.069 ± 0.021*</td>
<td>0.055 ± 0.016†</td>
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Circulating neurohormones

<table>
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<th>Euglycemia</th>
<th>Hyperglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldosterone, ng/dl</td>
<td>245 ± 254</td>
<td>45 ± 31*</td>
<td>31 ± 10†</td>
</tr>
<tr>
<td>PRA, ng·ml$^{-1}$·h$^{-1}$</td>
<td>1.34 ± 1.14</td>
<td>1.53 ± 0.43*</td>
<td>0.35 ± 0.27†</td>
</tr>
<tr>
<td>Angiotensinogen, ng/ml</td>
<td>1.264 ± 1,000</td>
<td>1.092 ± 722</td>
<td>1.076 ± 742</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>11.6 ± 8.4</td>
<td>3.1 ± 3.5*</td>
<td>2.2 ± 2.2</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of subjects. Twenty-four-hour protein intake was estimated by the formula [(urine urea × 0.18) + 14]/wt in kg. $\text{FE}_{\text{UA}}$, fractional excretion of uric acid (UA); $\text{FE}_{\text{CS}}$, fractional excretion of sodium; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; ERPF, effective renal plasma flow; GFR, glomerular filtration rate; RBF, renal blood flow; $\text{RVR}$, renal vascular resistance; PRA, plasma renin activity; HC, healthy controls; T1D, type 1 diabetic patients. *$P < 0.05$ for HC vs. T1D. †$P < 0.05$ when comparing parameters of T1D between hyperglycemia and euglycemia states.

Fig. 1. Plasma uric acid (PUA, A), fractional uric acid excretion ($\text{FE}_{\text{UA}}$, B), and urine glucose/creatinine (C) levels in healthy control (HC, $n = 41$) and type 1 diabetes (T1D, $n = 66$) subjects during euglycemic (EU) and hyperglycemic (HYPER) conditions. The bars above cohorts represent significance levels of $P < 0.05$. 

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correlated with ERPF ($r = -0.27, P = 0.031$), and $\text{FE}_{UA}$ positively correlated with ERPF ($r = 0.30, P = 0.017$) in T1D patients. During hyperglycemia, PUA negatively correlated with GFR in T1D ($r = -0.27, P = 0.028$).

**Sodium, glucose, and uric acid handling upon empagliflozin SGLT2 inhibition.** SGLT2 inhibitors are a new class of agents for the treatment of type 2 diabetes (T2D) that block proximal renal tubular glucose reabsorption, leading to increased glucose excretion. Therapeutically, this translates into important plasma glucose-lowering effects (6). Trials with SGLT2 inhibitors in patients with T2D have reported consistent and clinically relevant decreases in PUA levels (14, 40); however, the mechanisms responsible were never clearly elucidated. Accordingly, to better understand whether PUA lowering with hyperglycemia is due to systemic effects leading to decreased production or renal effects causing increased uricosuria, plasma and urine UA levels were measured before and after SGLT2 inhibition while maintaining clamped euglycemia in 40 T1D patients.

During clamped euglycemic conditions, after empagliflozin treatment, the anticipated increase in urine glucose-to-creatinine ratio ($1.3 \pm 3.2$ to $42.9 \pm 17.8, P < 0.0001$) was accompanied by a decline in PUA ($225 \pm 65$ to $191 \pm 62$ mmol/L, $P < 0.0001$) and increases in urine UA-to-creatinine ratio ($290 \pm 110$ to $327 \pm 103$ mmol/mmol, $P = 0.0075$) and $\text{FE}_{UA}$ ($8.2 \pm 3.6$ to $11.1 \pm 5.1, P < 0.0001$) (Fig. 3).

**DISCUSSION**

Observational associations between higher UA levels and metabolic abnormalities, cardiovascular disease, and kidney dysfunction show consistency among independent healthy and disease state cohorts, in both animals and humans (29). The potential renal protective effects of UA lowering in T1D patients are being studied as part of the National Institutes of Health-funded Protecting Early Renal Function Loss or “PERL” study (NCT02017171) (30), highlighting the promising future role for UA-based therapies in T1D. However, UA levels during euglycemia compared with hyperglycemia have not been clearly defined in otherwise healthy T1D patients. Our first goal was to compare PUA levels in HC with levels in patients with T1D. Our second goal was to determine if acute clamped hyperglycemia, which promotes deleterious hemodynamic effects such as increased blood pressure, influences PUA levels.

Due to the strong association between PUA levels and cardiovascular and renal abnormalities, especially in the context of diabetes, it was initially hypothesized that T1D patients would have higher PUA levels compared with HC. Our first major observation, however, was that T1D patients had lower PUA levels under euglycemic conditions compared with HC, in conjunction with increased urinary glucose that did not correlate with the degree of UA excretion. Hyperglycemia in T1D patients was associated with a significant increase in urinary sodium, glucose, and UA excretion and thus a further PUA decrease, highlighting an important physiological link between renal handling of UA, glucose, and sodium (6). Furthermore, the negative correlation between PUA and $\text{FE}_{UA}$ during euglycemia and hyperglycemia suggests that PUA decreased as a result of increased renal excretion. The lack of elevated UA excretion in T1D compared with HC under euglycemic conditions may suggest that T1D patients produce less UA in plasma or consume less UA-containing products,

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**Fig. 2.** Linear regression analysis of PUA with systolic blood pressure (SBP) in T1D during euglycemia (A), with effective renal plasma flow (ERPF) in T1D during euglycemia (B), and with glomerular filtration rate (GFR) in T1D during hyperglycemia (C). T1D $n = 40$.

**Fig. 3.** PUA (A), $\text{FE}_{UA}$ (B), and urine glucose/creatinine (C) levels in T1D ($n = 40$) during euglycemic conditions at baseline and after treatment with the sodium glucose cotransporter 2 (SGLT2) inhibitor empagliflozin (EMPA). The bars above cohorts represent significance levels of $P < 0.05$. 

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although the similar protein intake based on urine urea excretion in these groups suggests that differences in intake of UA-containing foods were not relevant to our findings.

Our observations support several studies showing an increase in UA excretion in response to intravenous D-glucose infusion (4, 36). More recently, an association was found between lower PUA and poor glycemic control (11, 18, 22). Previous studies have shown that insulin levels are positively correlated with PUA and insulin administration decreases UA excretion (33). However, it is not known whether this is a direct effect of insulin or the result of insulin-mediated normalization of glycemia, leading to reduced glycosuria. Worsening glycemic control resulting in hyperglycemia and glycosuria has been correlated with a decrease in PUA (20, 22). Thus, it is perhaps not surprising that epidemiological studies have shown a decreased risk of UA-related conditions, such as gout, in diabetic compared with nondiabetic individuals, especially in the context of T1D (34). The mechanisms behind the glucose-mediated PUA-lowering effects have been explained by osmotic diuresis caused by increased plasma glucose levels (36), proximal tubule alterations (18), or the effect of glucose on renal UA handling (20, 36).

Our second aim was to determine whether PUA lowering with hyperglycemia in T1D was due to systemic hyperglycemia causing decreased UA production or renal glycosuria causing increased UA excretion. SGLT2 inhibition with empagliflozin under clamped euglycemic conditions was used to increase urinary glucose excretion to determine if glycosuria during euglycemia results in a persistent decrease in PUA through increased renal UA excretion. Previous trials with SGLT2 inhibitors in patients with T2D have reported consistent and clinically relevant decreases in PUA levels; however, urine UA excretion was not measured, and the mechanisms responsible have not, to our knowledge, been clearly elucidated (14, 40). Our post hoc analysis demonstrated a decline in PUA during euglycemia with glycosuria induced with SGLT2 inhibition, an effect that was accompanied by an increase in UA excretion. Consistent with our observations, in a recent study using healthy controls, SGLT2 inhibition with luseogliflozin resulted in a positive correlation between urine UA and U(glucose) excretion (10). The results of the present study provide the first evidence in the T1D population suggesting that hyperglycemia-mediated uricosuria is likely due to renal glycosuria rather than a direct effect of systemic hyperglycemia. PUA-lowering effects reported with SGLT2 inhibition may be of clinical relevance, since this may in part explain the potential protective renal and cardiovascular physiological profile that has been linked with this emerging drug class (6).

The molecular mechanisms responsible for the uricosuric effect of glucose are not clear. PUA levels depend on the exogenous pool, which varies with dietary intake, while the endogenous pool is mainly regulated by hepatic production, intestinal secretion, and renal excretion (29). Approximately 70% of UA is excreted into urine but is easily filtered into the renal tubule, and about 90% of filtered UA is reabsorbed by the S1 segment of the proximal convoluted tubule (29). Approximately 10% of filtered UA is excreted (29). Accordingly, our HC showed a FEUA of 6.1 ± 4.1% and T1D during euglycemia 7.3 ± 3.8%. UA reabsorption occurs by intracellular anion transporters on the basolateral membrane, mainly by URAT1 and a more recently discovered GLUT9 isoform 2 (1, 2), and on the apical membrane organic anion transporter (OAT) 4 and OAT10 (3, 21). Recently, transport experiments in Xenopus oocytes showed that none of the transporters involved in UA reabsorption were influenced by luseogliflozin (10). GLUT9 isoform 2 is a facilitative glucose transporter mostly expressed in the kidney and the liver, located on the apical membrane (2). GLUT9 isoform 2 secretes UA in exchange for glucose at 10 mM (1). Additionally, GLUT9 isoform 2 is expressed in the collecting ducts where it plays a role in the reabsorption of UA (27). Plasma glucose is mostly filtered in the glomerulus and is concentrated in the proximal tubule. It is possible that during euglycemia the concentration needed for GLUT9 stimulation is not reached in the proximal tubule and the lower PUA in T1D during euglycemia vs. HC could occur by mechanisms other than glycosuria-mediated uricosuria. Based on these findings, the results of our study could be explained as follows: glycosuria during SGLT2 inhibition stimulates excretion of UA by GLUT9 isoform 2 on the apical membrane of the proximal tubule and possibly inhibits reabsorption of UA in the collecting ducts (Fig. 4).

Our conclusion may reflect recent in vitro data showing that stimulation of Xenopus oocytes expressing GLUT9 isoform 2 with 10 mM D-glucose resulted in UA efflux, and stimulation of the oocytes with 100 mM D-glucose (thought to be the concentration in the collecting ducts) inhibited the uptake of UA (10). Finally, increased glycosuria and uricosuria could, in the appropriate context, suggest the presence of more generalized “Fanconi-like” proximal tubular dysfunction. Because SGLT2 inhibition causes minor but statistically significant increases, rather than decreases, in serum potassium, phosphate, and bicarbonate, a proximal tubulopathy with this class of agents is very unlikely and has not been reported (38).

To examine the functional role of PUA in this otherwise healthy cohort of T1D patients, we correlated PUA with blood pressure and renal hemodynamic function. We found a signif-
icant positive correlation between PUA and SBP and negative correlations between PUA and ERPF and PUA and GFR in T1D. In contrast, PUA did not correlate with any of these measures in HC. These observations in T1D patients are consistent with the vasoconstrictive phenotype, as suggested by observational studies. For example, the independent association between PUA and blood pressure has been reported in various cohorts, including a subset of the Framingham Heart Study (12, 13). The deleterious effect of PUA on cardiovascular function may be worsened by the hypertensive effect of hyperglycemia in T1D patients (7, 19, 31). Hyperglycemia induces systemic vascular abnormalities such as endothelial dysfunction in humans (19, 31). As a result of the effects of hyperglycemia and neurohormonal activation of the RAAS, UA levels are independently associated with endothelial dysfunction, thereby promoting hypertension, even when UA levels are within the normal range (12, 26). Therefore, lower PUA levels in T1D patients, especially under hyperglycemic conditions, do not necessarily indicate that T1D patients are protected from the deleterious effects of UA. The effects of PUA may be exacerbated by hyperglycemia in T1D patients, leading to exaggerated deleterious hemodynamic consequences despite lower absolute PUA levels. From a clinical perspective, small trials have already started to show that lowering UA exerts antiproteinuric and antihypertensive effects and could prevent renal functional loss and vascular injury (13, 17, 25, 29, 32, 39). Thus, despite the lower UA levels in T1D vs. HC, which are further lowered during hyperglycemia, studying UA-lowering agents in T1D patients could be a critical step toward preventing progression of diabetes-related complications.

Our study has limitations. First, the study cohort consisted of a carefully selected group of patients with uncomplicated disease, limiting the generalizability of the data to populations outside of T1D, or to patients with existing complications. Additionally, although the similar urine urea excretion and thus protein intake suggest that differences in dietary intake of high UA-containing foods were unlikely, consumption of UA was not recorded. Fructose is another exogenous source of UA, which was not recorded in this study, and should be considered in future analyses. Finally, while we propose a possible explanation for glycosuria-mediated uricosuria, we could not determine the mechanistic basis at the molecular level. Future studies are needed to confirm our hypothesis.

In conclusion, glycosuria, rather than the direct effect of hyperglycemia, is responsible for increased uricosuria in T1D patients and may be mediated by glucose-mediated activation of GLUT9 isoform 2 on the apical membrane of the proximal tubule. Because PUA lowering may lead to renal and vascular protective effects, our data suggests that PUA lowering by SGLT2 inhibition via increased uricosuria may be clinically important. Finally, future studies examining the effect of UA-lowering therapies should account for the impact of ambient glucose levels, which cause a clinically relevant uricosuric and consequent PUA-lowering effect.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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