Renal Hyperfiltration Is Associated With Glucose-Dependent Changes in Fractional Excretion of Sodium in Patients With Uncomplicated Type 1 Diabetes

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OBJECTIVE
Renal hyperfiltration is a common abnormality associated with diabetic nephropathy in patients with type 1 diabetes (T1D). In animal models, increased proximal tubular sodium reabsorption results in decreased distal sodium delivery, tubuloglomerular feedback activation, afferent vasodilatation, and hyperfiltration. The role of tubular factors is less well understood in humans. The aim of the current study was therefore to compare the fractional sodium excretion (FENa) in hyperfiltrating (T1D-H) versus normofiltering (T1D-N) patients and healthy control (HC) subjects, as well as the role of ambient hyperglycemia on FENa.

METHODS
Blood pressure, renal function (inulin for glomerular filtration rate [GFR], and para-aminohippurate for effective renal plasma flow), FENa, and circulating neurohormones were measured in T1D-H (n = 28, GFR ≥135 mL/min/1.73 m²), T1D-N (n = 30), and HC subjects (n = 35) during clamped euglycemia. Studies were repeated in a subset of patients during clamped hyperglycemia.

RESULTS
During clamped euglycemia, T1D-H exhibited lower FENa than T1D-N and HC subjects (0.64 ± 0.06% vs. 0.91 ± 0.12% and 0.90 ± 0.10%, P < 0.05). During clamped hyperglycemia, FENa increased (Δ + 0.88 ± 0.22% vs. Δ + 0.02 ± 0.21%, between-group effect, P = 0.01) significantly in T1D-H, whereas FENa did not change in T1D-N. When treated as continuous variables, elevated GFR values were associated with hyperglycemia-induced increases in FENa (R² = 0.20, P = 0.007).

CONCLUSIONS
Patients with uncomplicated T1D-H exhibit lower FENa under euglycemic conditions, which may help to identify patients with hyperfiltration outside of a controlled laboratory setting. Increased FENa in T1D-H but not T1D-N under clamped hyperglycemic conditions suggests that the mechanisms responsible for increased sodium reabsorption leading to hyperfiltration can be saturated.

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Diabetic nephropathy is the leading cause of end-stage renal disease and occurs in \(\sim 20-30\%\) of patients with type 1 diabetes (T1D) (1). Although the pathogenesis of early diabetic renal injury remains incompletely understood in humans, changes in renal hemodynamic function characterized by glomerular hyperfiltration may play a role and have been primarily attributed to activation of neurohormonal pathways such as the renin-angiotensin-aldosterone system (RAAS) (2,3). Hyperfiltration is common, affecting \(\sim 50\%\) of T1D patients, defined by a glomerular filtration rate (GFR) \(\geq 135\) mL/min/1.73 m\(^2\) (4). Importantly, hyperfiltration is common in patients with type 2 diabetes aged younger than 40 years, affecting 50% of afflicted patients (5). Moreover, hyperfiltration has been implicated as a common pathogenic mechanism leading to chronic kidney disease progression in other conditions such as obesity-related glomerulopathy (50% prevalence) (6) sickle cell disease (30-40% prevalence) (7), low birth weight, and reduced nephron number (8). In addition, hyperfiltration is associated with systemic vascular abnormalities, which may be associated with increased risk of microalbuminuria and retinopathy (9-11). However, the primary factors responsible for hyperfiltration remain unclear.

In addition to the role of neurohormonal activation, studies in animals and humans have highlighted the possible role of tubular factors leading to hyperfiltration. In the tubulocentric view of hyperfiltration, a primary increase in sodium reabsorption in the proximal tubule via sodium glucose cotransport-2 (SGLT2), results in reduced distal tubular sodium delivery at the macula densa, leading to afferent vasoconstriction and hyperfiltration (12). On the basis of this theory, studies in diabetic animal models demonstrated that SGLT1/2 inhibition with nonselective phlorizin and, subsequently, with selective SGLT2 inhibitors showed a significant attenuation of hyperfiltration (13), highlighting the involvement of SGLT2 in hyperfiltration (14). To determine whether the tubular hypothesis is also relevant in humans, we used a selective SGLT2 inhibitor in patients with T1D and demonstrated that this physiological maneuver results in a dramatic reduction in GFR in patients with hyperfiltration (T1D-H; GFR \(\geq 135\) mL/min/1.73 m\(^2\)) but no effect in patients with normofiltration (T1D-N; GFR < 135 mL/min/1.73 m\(^2\)) (15). Although these findings proved for the first time that the tubular hypothesis exists in T1D-H, the underlying cause of hyperfiltration remains unclear. Furthermore, the identification of patients with T1D-H remains difficult, due to methodological bias of GFR measured using creatinine or even cystatin C compared with direct inulin-derived measures in our previous work (16).

Despite what is known about the potential role of increased proximal tubular sodium reabsorption in T1D (17,18), the effect of renal hyperfiltration on simple measures of sodium handling, such as fractional sodium excretion (\(\text{FE}_{\text{Na}}\)) has not been clearly defined (13,14). Furthermore, given the role of hyperglycemia and subsequent glycosuria leading to increased SGLT2 activity and reduced sodium excretion, it is important to define the effect of euglycemia and hyperglycemia on renal sodium handling. Accordingly, we examined a cohort of young patients with T1D, either with renal hyperfiltration or normofiltration (4,19,20), and compared them with a similar cohort of healthy control (HC) participants under clamped euglycemic conditions. We then repeated studies the next day in a subgroup of the T1D cohort using a hyperglycemic clamp. We hypothesized that the \(\text{FE}_{\text{Na}}\) would be lower in T1D-H compared with T1D-N and HC subjects, suggestive of a tubular cause of glomerular hyperfiltration. Second, we hypothesized that under hyperglycemic conditions, \(\text{FE}_{\text{Na}}\) would be further reduced in the T1D groups as a result of primary stimulation of proximal tubular SGLT2 activity.

**RESEARCH DESIGN AND METHODS**

**Subject Inclusion Criteria and Experimental Procedure**

The University Health Network Research Ethics Board approved all research protocols and all subjects gave written informed consent. The study included 35 HC subjects and 58 participants with uncomplicated T1D (30 T1D-N and 28 T1D-H). Hyperfiltration was defined as GFR \(\geq 135\) mL/min/1.73 m\(^2\), as described previously (3,4). In brief, inclusion criteria were duration of T1D > 1 year, age 18-35 years, blood pressure < 140/90 mmHg, normalalbuminuria on a 24-h urine collection, no history of renal disease or macrovascular disease or regular medications other than insulin, including oral contraceptives. Visits for female subjects were scheduled to coincide with the follicular phase of the menstrual cycle, determined by cycle day and measurement of 17β-estradiol levels.

All of the study participants were involved with the first part of the study under clamped euglycemia on day 1 to target capillary blood glucose levels of 4–6 mmol/L (Fig. 1). To assess the effect of clamped hyperglycemia on \(\text{FE}_{\text{Na}}\), all study parameters were repeated the next day under clamped hyperglycemia in a subset of the patients (21 T1D-N and 14 T1D-H) who were invited to undergo this second set of experiments on day 2, where the target capillary blood glucose was 9–11 mmol/L.

Participants adhered to a high sodium (>140 mmol/day) diet for the 7-day period before each experiment to maintain suppression of endogenous RAAS activity and to standardize study conditions, as previously described (21). Brachial artery blood pressure (Critikon, Tampa, FL) and renal hemodynamic parameters were obtained after a 6-h modified clamp on experimental days (21). GFR and effective renal plasma flow (ERPF) were estimated using inulin and paraaminohippurate (PAH) clearance techniques, respectively, as previously described (4). After a 90-min equilibration period, blood was collected for inulin, PAH, and hematocrit measurements. Blood was further collected at 30- and 60-min intervals for inulin and PAH measurements. GFR and ERPF were estimated by a steady-state infusion of inulin and PAH, respectively (22).

**Sample Collection and Analytical Methods**

After the desired glycemic clamp was achieved, baseline blood samples were also collected for the measurement of aldosterone and plasma renin activity (PRA). All measurements and samples were taken in the same warm (25°C) temperature-controlled rooms after the subject rested 10 min in the supine position. Sample storage and the measurement for circulating hemodynamic factors were previously described (4). Aldosterone was measured using a Coat-A-Count radioimmunoassay kit.
Hemoglobin A1c (HBA1c) was measured by high-performance liquid chromatography, and plasma insulin levels were measured using standard techniques (23). Plasma and urine samples were collected after achievement of clamped euglycemia or hyperglycemia to measure sodium and creatinine levels. FENa was derived using 

\[
\text{FENa} = \left( \frac{U_{\text{Na}} \times P_{\text{Cr}}}{U_{\text{Cr}} \times P_{\text{Na}}} \right) \times 100
\]

where \(U_{\text{Na}}\), \(P_{\text{Cr}}\), \(U_{\text{Cr}}\), and \(P_{\text{Na}}\) are urinary sodium, plasma creatinine, urinary creatinine and plasma sodium concentrations, respectively.

Blood samples collected for inulin and PAH measurements were immediately centrifuged at 3,000 rpm for 10 min at 4°C. Plasma was separated, placed on ice, and stored at \(-70^\circ\)C before the assay. Inulin and PAH were measured in serum by colorimetric assays using anthrone and \(N\)-(1-naphthyl) ethylene-diamine, respectively (24–26). The mean of two baseline clearance periods represents GFR and ERPF, expressed as per 1.73 m². Renal blood flow (RBF) was derived using ERPF/(1 − hematocrit), and renal vascular resistance (RVR) was derived using MAP/RBF, as described previously (21,27). All renal hemodynamic measurements were adjusted for body surface area (24,26).

**Statistical Analysis**

Data are presented as mean ± SD. ANOVA with post hoc Tukey tests was used to assess for between-group differences, and significance was defined as \(P < 0.05\). To compare responses to hyperglycemia within groups, a paired student t test was used, and \(P < 0.05\) was considered significant. Linear regression analysis was performed to determine the relationship between GFR and change in FENa (Fig. 3C). All statistical analyses were performed using GraphPad Prism v6.0 and IBM SPSS Statistics v22.0.

**RESULTS**

**Baseline Parameters of Participants**

Participants were young and otherwise healthy, with urinary sodium reflective of adherence to the prescribed diet (Table 1). All participants were normotensive and normoalbuminuric, the three groups exhibited similar values for weight and BMI, and gender distribution was comparable. The two diabetes groups exhibited similar diabetes duration and HbA1c. As illustrated in Fig. 1, the target ranges for the glycemic clamps were successfully maintained, and glucose levels were similar in T1D-N versus T1D-H. Plasma sodium during euglycemic conditions was 1 mmol/L lower in HC subjects versus T1D-N and T1D-H (Table 1). For urine glucose, levels were undetectable in 67% of HC subjects and significantly lower during clamped euglycemia compared with T1D-N and T1D-H (Table 1). Urine glucose excretion was similar in the two T1D groups during clamped euglycemia.

As expected, heart rate and systolic and diastolic blood pressures were higher during clamped euglycemia but still within the normal range in the T1D-H group versus the T1D-N group (Table 1) (4). In addition, heart rate and blood pressure were also significantly elevated in T1D-H group compared with the HC group. However, these differences between T1D-H and T1D-N were not statistically significant under clamped hyperglycemia (Table 2).

Under euglycemic clamp conditions, the T1D-H group exhibited expected higher ERPF, GFR, and RBF and a lower RVR compared with the HC group. In addition, in both euglycemic and hyperglycemic states, ERPF, GFR, and RBF were significantly higher in the T1D-H group compared with the T1D-N group. RVR was significantly lower in T1D-H compared with T1D-N in clamped euglycemia. Aldosterone and PRA were significantly lower in the two diabetes groups compared with the HC group (Table 1). PRA was significantly lower in T1D-H compared with T1D-N only under hyperglycemic conditions.

**FENa Under Euglycemic Clamp**

To determine whether renal sodium handling was different between the study groups, FENa for all participants was examined during a euglycemic clamp. There were no differences in FENa between the HC subjects and the
T1D-N groups. In contrast, FE\textsubscript{Na} was significantly lower in the T1D-H group than in the HC and T1D-N groups (Fig. 2).

**FE\textsubscript{Na} and the Response to Clamped Hyperglycemia**

A subset of T1D patients subsequently underwent the hyperglycemic clamp study, and their baseline parameters during euglycemia were comparable to the larger cohort (Table 2). In this subset, FE\textsubscript{Na} tended to be lower in the T1D-H group (0.66 ± 0.10%) compared with the T1D-N group (0.96 ± 0.15%; Fig. 3A) during clamped euglycemia, but this did not reach statistical significance (P = 0.15). In response to clamped hyperglycemia, the change in FE\textsubscript{Na} was exaggerated in T1D-H versus T1D-N (Δ = 0.88 ± 0.22% vs. Δ = 0.02 ± 0.21%; between-group effect, P = 0.01). Under hyperglycemic conditions, 11 of 14 (79%) of the T1D-H group and 10 of 21 (48%) of the T1D-N group exhibited an increase in FE\textsubscript{Na} (Fig. 3B). A significant correlation was also observed with a linear regression analysis of GFR and change in FE\textsubscript{Na} between hyperglycemia and euglycemia (Fig. 3C). In addition to between-group differences for the FE\textsubscript{Na} response, the increase in glucosuria tended to be greater in T1D-H vs. T1D-N with induction of clamped hyperglycemia (Δ = 65 ± 36 mmol/L vs. Δ = 41 ± 64 mmol/L; between-group effect, P = 0.36).

**CONCLUSIONS**

Our first novel observation in this study was that during clamped euglycemia, T1D-H had lower FE\textsubscript{Na} levels compared with T1D-N and HC subjects. Between-group differences during euglycemia were consistent with previous observations in animals, which have been attributed to an increase in SGLT2 activity (28,29). The resulting decrease in sodium delivery to the distal tubule could then lead to renal hyperfiltration through reduced sodium transport by macula densa cells, causing less adenosine to be generated, leading to reduced tubuloglomerular feedback and afferent vasodilatation (30–32). This difference in FE\textsubscript{Na} under euglycemia between T1D-N and T1D-H is, however, expected under steady-state conditions because the two groups exhibited similar salt intake, serum sodium levels, and comparable 24-h sodium excretion. Therefore, the higher GFR in T1D-H must be associated with a level of higher sodium reabsorption and lower FE\textsubscript{Na} to maintain total body sodium equilibrium. Because of the established relationship between renal sodium and glucose reabsorption in diabetes and the relevant physiological mechanisms at the proximal tubule and macula densa, the discussion will focus on the potential role of these nephron segments. We recognize that conclusions about segmental tubular sodium reabsorption in intact human organs are limited, but future human mechanistic studies might consider the use of sodium/lithium clearance techniques to gain further insight.

On the basis of what is known about the tubular hypothesis in animals, we hypothesized that clamped hyperglycemia would further increase proximal tubular delivery of glucose, resulting in exaggerated sodium-glucose cotransport, further lowering FE\textsubscript{Na} and resulting in a subsequent increase in GFR (33,34).
Instead, we observed an increase in FeNa with clamped hyperglycemia in T1D-H and no change in T1D-N, in conjunction with a nonsignificant exaggerated rise in glucosuria in T1D-H. In addition, an increase in GFR under hyperglycemic conditions was only observed in T1D-N, as previously described (4). This finding may seem counterintuitive because increased tubular delivery of glucose due to ambient hyperglycemia should increase SGLT2 function and result in increased proximal sodium reabsorption and further elevations in GFR (35).

Although our observation that FeNa is lower in T1D-H under euglycemic conditions is consistent with upregulated SGLT2 activity, the increase in FeNa during hyperglycemia suggests that other factors become important under these conditions. First, if proximal tubular sodium reabsorption was maximal in T1D-H under clamped euglycemic conditions, further delivery of glucose may result in increased glycosuria and subsequent higher FeNa, as suggested by the larger but statistically nonsignificant glucosuric response in T1D-H. Such a phenomenon has been suggested in studies involving healthy dogs, because the mechanisms responsible for diabetes-induced reductions in FeNa are saturable (36). A similar effect may occur in T1D-H patients under hyperglycemic conditions, leading to elevated FeNa compared with HC subjects (37). Our results support the notion that T1D-H exhibit a state of tubular sodium avidity during clamped euglycemic conditions, supporting the tubular hypothesis for hyperfiltration. Nevertheless, the increase in FeNa with clamped hyperglycemia suggests that mechanisms responsible for increased sodium reabsorption leading to hyperfiltration can be saturated. As a consequence, interpretation of FeNa data in patients with T1D needs to be interpreted in the context of ambient plasma glucose levels. In addition, given the intrarenal hemodynamic effects of glucose on efferent vasoconstriction (21,33), changes in glycemic states could significantly alter GFR through activation of neurohormones such as the RAAS (38). A better understanding of the intricate relationship among glucose, FeNa, and GFR may aid

Figure 2—FeNa in HC subjects and T1D patients with measurements during clamped euglycemia (mean ± SD). FeNa values were calculated from urine and plasma concentrations of sodium and creatinine (n = 35 for HC subjects, n = 30 for T1D-N [GFR <135 mL/min/1.73 m²], and n = 28 for T1D-H [GFR ≥135 mL/min/1.73 m²]). *P < 0.05 comparing T1D-H with HC subjects. †P < 0.005 comparing T1D-H with T1D-N.

Table 2—Baseline characteristics, biochemistry, and hemodynamic function of T1D-N and T1D-H patients during clamped euglycemia and hyperglycemia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T1D-N (n = 21)</th>
<th>T1D-H (n = 14)</th>
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<tbody>
<tr>
<td></td>
<td>Euglycemia</td>
<td>Hyperglycemia</td>
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<td></td>
<td>Hyperglycemia</td>
<td>Hyperglycemia</td>
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<tr>
<td><strong>Baseline parameters</strong></td>
<td></td>
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<tr>
<td>GFR (mL/min/1.73 m²)</td>
<td>97–133</td>
<td>135–227</td>
</tr>
<tr>
<td>Males</td>
<td>12 (57)</td>
<td>7 (47)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>23.9 ± 5.5</td>
<td>21.0 ± 3.6</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>18 ± 5</td>
<td>15 ± 4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78 ± 13</td>
<td>71 ± 12</td>
</tr>
<tr>
<td>Height (meters)</td>
<td>1.73 ± 0.09</td>
<td>1.70 ± 0.09</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26 ± 4</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>HbA1c, % (mmol/mol)</td>
<td>8.2 ± 1.3 (66 ± 14)</td>
<td>9.1 ± 1.7 (76 ± 19)</td>
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<tr>
<td>Estrogen (pmol/L - in women)</td>
<td>128 ± 91</td>
<td>148 ± 88</td>
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<tr>
<td><strong>Sodium and glucose handling</strong></td>
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<td></td>
</tr>
<tr>
<td>Sodium excretion (mmol/24 h)</td>
<td>169 ± 58</td>
<td>170 ± 97</td>
</tr>
<tr>
<td>Plasma sodium (mmol/L)</td>
<td>140 ± 2</td>
<td>141 ± 3</td>
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<tr>
<td>Urine glucose (mmol/L)</td>
<td>9 ± 13</td>
<td>14 ± 27</td>
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<tr>
<td><strong>Systemic hemodynamic function</strong></td>
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</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>67 ± 13</td>
<td>74 ± 8</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>64 ± 12</td>
<td>70 ± 7</td>
</tr>
<tr>
<td>Systolic</td>
<td>112 ± 9</td>
<td>116 ± 8</td>
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<tr>
<td>Diastolic</td>
<td>63 ± 7</td>
<td>65 ± 6</td>
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<tr>
<td><strong>Renal hemodynamic function</strong></td>
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<tr>
<td>ERPF (mL/min/1.73 m²)</td>
<td>663 ± 100</td>
<td>820 ± 189‡</td>
</tr>
<tr>
<td>GFR (mL/min/1.73 m²)</td>
<td>118 ± 10</td>
<td>151 ± 16†</td>
</tr>
<tr>
<td>Filtration fraction</td>
<td>0.18 ± 0.03</td>
<td>0.19 ± 0.04</td>
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<tr>
<td>RBF (mL/min/1.73 m²)</td>
<td>1,060 ± 172</td>
<td>1,350 ± 336†</td>
</tr>
<tr>
<td>RVR (mHg/L/min)</td>
<td>0.076 ± 0.012</td>
<td>0.063 ± 0.014†</td>
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<tr>
<td><strong>Circulating neurohormonal factors</strong></td>
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<td></td>
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<tr>
<td>Aldosterone (ng/dL)</td>
<td>73 ± 80</td>
<td>52 ± 45</td>
</tr>
<tr>
<td>PRA (ng/mL/h)</td>
<td>0.37 ± 0.34</td>
<td>0.17 ± 0.13†</td>
</tr>
</tbody>
</table>
| Continuous data are expressed as a range or as the mean ± SD and categoric data as number (%). *P < 0.05 when comparing parameters of T1D-N between hyperglycemia and euglycemia states. †P < 0.05 when comparing parameters of T1D-H between hyperglycemia and euglycemia states. §P < 0.05 when comparing T1D-H with T1D-N in euglycemia. 5P < 0.05 when comparing T1D-H with T1D-N in hyperglycemia.
in the development of more practical methods of identifying hyperfiltration in patients with T1D. To address the hypothesis of a saturated sodium reabsorption in T1D-H patients, future studies could examine the relationship between baseline GFR and thresholds for maximal renal sodium reabsorption.

Consistent with our previous work, plasma levels of RAAS mediators were suppressed in T1D and lowest in T1D-H (4), consistent with the “paradox of low renin state in diabetes,” where low plasma levels of RAAS mediators are observed in the context of an opposite increase in RAAS activation in the kidney (39). Although the exact mechanism behind this paradox requires further investigation, its existence in diabetes has been clearly elucidated through observation of exaggerated renal responses in pharmacological RAAS inhibition (40,41). Importantly, and consistent with previous work in this area, blood pressure was higher in T1D-H (4,42,43). Although this blood pressure elevation within the normal range in T1D-H could be attributed to an elevated systemic sympathetic tone (43), our results suggest that decreased FENa in euglycemic states may also contribute through increases in total body sodium (44). However, because 24-h sodium excretion was comparable between the two groups and the patients were kept on a similar high-salt diet, based on the need to maintain salt balance, one would expect the higher GFR in T1D-H to be associated with a higher fractional tubular sodium reabsorption. To better assess whether T1D-H retain more sodium and thereby contributing to volume expansion and higher blood pressures, measures of total body water and sodium content should be measured in future studies.

The potential clinical implications of our findings require some comments. In our previous work using the SGLT2 inhibitor empagliflozin, we demonstrated significant reductions in GFR, ERPF, and RBF and increases in RVR in T1D-H but no change in T1D-N, highlighting the role of the tubular hypothesis in the pathogenesis of the hyperfiltration state in humans (15). An interesting observation in this previous study was a reduction in systolic blood pressure in the T1D-H group only. In conjunction with findings from the current study, it is therefore tempting to speculate that low FENa contributes to renal hyperfiltration and to increased blood pressure within the normal range in T1D-H and that this abnormal physiology may at least be partially corrected with SGLT2 inhibition.

Despite the potential mechanistic importance of hyperfiltration as a risk factor for diabetic nephropathy, our ability to accurately and precisely measure hyperfiltration in large clinical trials remains limited, because estimates of GFR using creatinine or cystatin C are poor markers of renal function in this range (45). Fortunately, current “early” nephroprotection studies are now using GFR measures, such as iohexol, along with control of physiological confounders such as ambient glucose levels and dietary protein (46). With these techniques, proper assessments of early functional abnormalities, such as hyperfiltration and early renal function decline, as well as responses to renal protective therapies, are possible. However, the association between hyperfiltration and clinical renal end points, such as progression to proteinuria, advanced kidney disease, or dialysis, will remain challenging due to the long period of time required to reach these complications. Ultimately, clinical trials examining the role of hyperfiltration as a risk factor for diabetic nephropathy may need to involve enriched cohorts of patients with hyperfiltration and other “preclinical” abnormalities, such as albuminuria within the “high-normal range,” blood pressure within the “high-normal range,” or increased urinary excretion of inflammatory biomarkers, that reflect underlying renal injury.

Our study does have limitations. First, the limited number of patients in this study might have limited our ability to detect some between-group differences, including glucosuric responses. We attempted to minimize the effect of the small sample size by performing studies under carefully controlled physiological conditions, thereby accounting for important potential confounding variables, such as sodium and protein intake, that influence renal function and blood pressure. We avoided performing hyperglycemic clamps in HC subjects for two reasons: 1) hyperglycemic clamps in HC subjects require the use of octreotide, which independently influences PRA, thereby confounding the study results; and 2) acute hyperglycemia in nondiabetic HC subjects does not represent a physiological state. Salt intake before the dietary control period was not measured as part of our study, and it is possible the T1D-N normally exhibited a lower sodium intake than that in the prescribed study diet. Given the effect of high salt intake on
reducing GFR, T1D-N may only appear to “normofilter” during this experimental setting. We also instructed participants to follow a high sodium diet (>140 mmol/day target) rather than a specific, narrow range, and as such, cannot rule out effects of different sodium intakes above this threshold. The relationship between dietary salt and renal hyperfiltration is, however, beyond the scope of the current study and should be included in future work. Similarly, our results should not be generalized to patients with impaired renal function or hypertension, because these conditions may influence the relationship between hyperglycemia and FE_{Na}.

Finally, although we have attributed changes in FE_{Na} to increased SGLT2 activation causing hyperfiltration, we recognize that other mechanisms may also be operative such as primary proximal tubular cell hypertrophy due to ornithine decarboxylase, leading to increased primary sodium reabsorption via SGLT2 and sodium-hydrogen exchange (47). Alternatively, primary activation of the RAAS by hyperglycemia contributes to hyperfiltration via constriction of the efferent arteriole and also increases proximal tubular sodium reabsorption via enhanced sodium-hydrogen exchange (48). We believe that it is unlikely that the filtered load of sodium made a significant contribution to differences in FE_{Na}, although serum sodium was higher in T1D-N and T1D-H versus HC subjects, suggesting a greater filtered sodium load, FE_{Na} was only lower in T1D-H. This suggests that filtration status was a more important determinant of FE_{Na} rather than filtered sodium load.

In summary, T1D-H exhibit significantly reduced FE_{Na} compared with T1D-N and HC subjects under euglycemic conditions. Conversely, under hyperglycemic conditions FE_{Na} is significantly elevated in T1D-H compared with T1D-N. Our findings illustrate the importance of tubuloglomerular feedback as a determinant of renal sodium handling in T1D-H as well as the important role of ambient glucose levels. Future studies should determine the clinical role of blocking proximal tubular sodium reabsorption with SGLT2 inhibitors, because these agents have the potential to reduce hyperfiltration and blood pressure predominantly in T1D-H, thereby protecting against the initiation and progression of diabetic nephropathy.

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