Acute SGLT inhibition normalizes O2 tension in the renal cortex but causes hypoxia in the renal medulla in anaesthetized control and diabetic rats

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O’Neill J, Fasching A, Pihl L, Patinha D, Franzén S, Palm F. Acute SGLT inhibition normalizes O2 tension in the renal cortex but causes hypoxia in the renal medulla in anaesthetized control and diabetic rats. Am J Physiol Renal Physiol 309:F227–F234, 2015. First published June 3, 2015; doi:10.1152/ajprenal.00689.2014.—Early stage diabetic nephropathy is characterized by glomerular hyperfiltration and reduced renal tissue PO2. Recent observations have indicated that increased tubular Na+–glucose linked transport (SGLT) plays a role in the development of diabetes-induced hypoxia. The aim of the present study was to determine how inhibition of SGLT impacts upon PO2 in the diabetic rat kidney. Diabetes was induced by streptozotocin in Sprague-Dawley rats 2 wk before experimentation. Renal hemodynamics, excretory function, and renal O2 homeostasis were measured in anesthetized control and diabetic rats during baseline and after acute SGLT inhibition using phlorizin (200 mg/kg ip). Baseline arterial pressure was similar in both groups and unaffected by SGLT inhibition. Diabetic animals displayed reduced baseline PO2 in both the cortex and medulla. SGLT inhibition improved cortical PO2 in the diabetic kidney, whereas it reduced medullary PO2 in both groups. SGLT inhibition reduced Na+ transport efficiency [tubular Na+ transport (TNa)/renal O2 consumption (QO2)] in the control kidney, whereas the already reduced TNa/QO2 in the diabetic kidney was unaffected by SGLT inhibition. In conclusion, these data demonstrate that when SGLT is inhibited, renal cortical PO2 in the diabetic rat kidney is normalized, which implies that increased proximal tubule transport contributes to the development of hypoxia in the diabetic kidney. The reduction in medullary PO2 in both control and diabetic kidneys during the inhibition of proximal Na+ reabsorption suggests the redistribution of active Na+ transport to less efficient nephron segments, such as the medullary thick ascending limb, which results in medullary hypoxia.

Diabetes; oxygen consumption; renal hypoxia; sodium-glucose linked transport; sodium transport

Diabetes affects up to 220 million people worldwide (15). Diabetic nephropathy is a renal complication of type 1 and type 2 diabetes and is a major cause of morbidity and mortality affecting up to 40% of diabetic patients (9). More recently, Na+-glucose linked transport (SGLT) inhibition has become a frontline pharmacological target in the treatment of diabetes because of its ability to lower blood glucose levels by promoting the excretion of glucose by the kidney.

Indeed, in a healthy kidney, 99% of filtered glucose is reabsorbed, mostly via high-capacity SGLT2, which is expressed in the brush-border membrane of the proximal tubule in the S1 segment (39), and, to a lesser extent, via low-capacity SGLT1, which is expressed in the S3 segment of the proximal tubule (2). Glucose is transported out of proximal tubules and into the surrounding interstitium via glucose transporter 2. The reabsorption of glucose from the tubule into the cell is dependent on the simultaneous reabsorption of Na+. The reabsorption of Na+ is, in turn, driven by the steep concentration gradient between the tubule and cell, and this gradient is maintained by the Na+-K+-ATPase pump on the basolateral side of the cell. Thus, glucose reabsorption via SGLT is secondarily an energy-dependent process, and, when energy is used, O2 is consumed (16).

It has been widely reported that the early stages of diabetic nephropathy are characterized by glomerular hyperfiltration and proteinuria (9, 19, 31). Indeed, previous studies have shown that the genetic ablation of SGLT2 and chronic nonselective pharmacological inhibition of SGLT in diabetic rats and mice not only reverse hyperglycemia but also attenuate hyperfiltration and proteinuria (16, 34, 37, 40). Furthermore, others have observed that renal tubular SGLT2 and glucose transporter 2 are upregulated in the proximal tubule of diabetic rats (6, 42, 21, 30, 22, 1). Together, these data suggest that SGLT activity and expression are increased in the diabetic kidney and are directly related to the development of glomerular hyperfiltration and proteinuria. The mechanisms underlying the relationship between diabetes-induced glomerular hyperfiltration and increased proximal reabsorption via SGLTs have yet to be fully understood, but impaired tubuloglomerular feedback regulation (39) and changes in intratubular pressure have been proposed (34).

Importantly, Körner et al. (16), in 1994, were among the first to demonstrate that glomerular hyperfiltration and proteinuria were also accompanied by elevated renal O2 consumption (QO2) in the proximal tubule of the diabetic rat kidney. This increase could be attributed to increases in intracellular Na+ accumulation via SGLT, which, in turn, increases the activity of the Na+–K+-ATPase pump and thereby proximal tubule O2 consumption (16). More recently, Palm et al. (24), in 2003, reported that increased QO2 was accompanied by a corresponding reduction in renal cortical and medullary O2 availability. These data combined certainly raise the question as to whether the attenuation of glucose reabsorption via SGLT could correct renal hypoxia in the diabetic rat kidney.

Thus, the overall aim of the present study was to determine whether there is a relationship between SGLT-mediated Na+ and glucose uptake in the proximal tubule and glomerular hyperfiltration, proteinuria, and renal hypoxia in the diabetic rat kidney. We therefore hypothesized that acute nonselective inhibition of SGLT will reverse renal tissue hypoxia in addition
MATERIALS AND METHODS

All chemicals and assay kits were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified.

Animal Model

Male Sprague-Dawley rats (8 wk old) were obtained from Charles River and maintained under a 12:12-h light-dark regime at 20 ± 3°C in the Centre for Biomedical Resources, Linköping University. Animals received free access to standard chow and water. All experimental procedures were performed under European Community Directive 86/609/EC and were approved by the local Animal Experimentation Ethical Committee. National Institutes of Health guidelines for the care of experimental animals were adhered to throughout each protocol. Diabetes was induced in conscious rats (n = 9) by the injection of streptozotocin (50 mg/kg) into the tail vein. Control animals (n = 12) received vehicle. Animals were deemed to be diabetic if blood glucose levels (MediSense, Bedford, MA) were >16 mmol/l 24 h postinduction. Animals were submitted for acute experimentation 14–18 days after the induction of diabetes. Control animals were 9 wk old and diabetic animals were 10–11 wk old when the acute experiments were carried out.

Surgical Protocol

Animals were anesthetized via an intraperitoneal injection of Inactin (control animals: 120 mg/kg and diabetic animals: 80 mg/kg) and placed on a heated pad so that body temperature was maintained at 37°C. A tracheostomy was performed (PP240 tubing) to facilitate ventilation. A cannula was inserted into the right carotid artery (PP50) and into the right femoral artery to facilitate the infusion of [3H]inulin (1 H H2O, 1 μCi, specific activity 10 mCi/mmol). Animals were allowed to recover for 45 min before the experiment. At the end of the experiment, animals were terminated via an intra-arterial KCl bolus, and the left kidney was removed and weighed.

Experimental Protocol

In vivo intraperitoneal administration of phlorizin. After the stabilization period, one 40-min clearance period was taken before and one ~30 min after the intraperitoneal administration of phlorizin (200 mg/kg). Phlorizin was dissolved in propylene glycol (~0.1 ml) and delivered directly into the peritoneum via the left subcostal flank incision site. As a control, propylene glycol (0.1 ml vehicle) was administered to a separate group of normoglycemic animals (n = 12). The vehicle per se did not affect any of the parameters involved in tubular Na+ handling or tissue O2 homeostasis (data not shown). Blood samples were collected at specific points during the experimental protocol for the measurement of blood gas parameters.

Measurement of hemodynamic parameters. MAP was measured via the right carotid artery cannula, which was connected to a blood pressure transducer and a signal transduction amplifier. MAP was continuously monitored and recorded with a PowerLab instrument (AD Instruments, Hastings, UK). Renal blood flow (RBF) was determined by [14C]para-aminiohippuric acid extraction, and glomerular filtration rate (GFR) was determined by the clearance of [3H]inulin.

1H and 14C activities were measured in both urine and plasma by liquid scintillation. Blood gases and electrolytes were measured in blood withdrawn from the carotid artery and renal vein using the iSTAT system (Abbott Laboratories, Abbott Park, IL).

Measurement of excretory parameters. Urine flow was determined gravimetrically at baseline, during the intraperitoneal administration of phlorizin. Urinary Na+ and K+ concentrations were determined using flame spectrophotometry (model ILS43, Instrumentation Lab, Milan, Italy). Urinary protein excretion was measured using a BCA protein assay (Bio-Rad Laboratories, Sundbyberg, Sweden), and urinary albumin excretion was determined using a rat albumin ELISA (Bethyl Laboratories, Montgomery, TX). Urinary glucose was measured using a glucose hexokinase assay kit (Roche, Stockholm, Sweden).

In vivo measurement of PO2 in the renal cortex and medulla. At the end of each 40-min clearance period, a Clark-type microelectrode (Unisense, Aarhus, Denmark) was used to measure both cortical and medullary PO2, as previously described (24). In brief, a small portion of the renal capsule was removed, and the electrode was inserted 0.5–1.0 and 3.5–4.0 mm into the kidney to measure cortical and medullary PO2, respectively. This procedure was repeated three to four times before and after SGLT inhibition, and the average was calculated for each region and time point and used as N = 1. Importantly, after each set of cortical and medullary recordings, the electrode was
where blood O2 content follows: $Q_{O2}$

**Calculations:** GFR was calculated as follows: $GFR = U \times UV/P$, where $U$ is urinary $^3$H activity, $UV$ is urine volume, and $P$ is plasma $^3$H activity. Renal plasma flow (RPF) was calculated as follows: $RPF = U \times UV/14C$ extraction, A is arterial $14C$ activity and $14C$ extraction is the arteriovenous difference in $14C$ activity/arterial $14C$ activity. RBF was then calculated as follows: $RBF = RPF/(1 - hematocrit/100)$. Renal vascular resistance was determined as follows: renal vascular resistance = (MAP/RBF). In vivo $Q_{O2}$ was estimated from the arteriovenous difference in $O2$ content and was calculated as follows: $Q_{O2} = the arteriovenous difference in O2 content \times RBF$, where blood $O2$ content = $1.34 \times hemoglobin O2$ saturation $\times hemoglobin concentration + blood PO2 \times 0.003$. Tubular Na+ transport (TNa) was calculated as follows: $TNa = [PNa] \times GFR - [UNa] \times UV$, where $[PNa]$ is the plasma Na+ concentration and $[UNa]$ is the urinary Na+ concentration. Fractional Na+ excretion was calculated as follows: fractional Na+ excretion = Na+ clearance/GFR, where Na+ clearance = $[UNa] \times UV/[PNa]$.

**Statistical analysis:** Data are presented as means ± SE. All statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA). All data were analyzed using 2 × 2 repeated-measures ANOVA followed by Bonferroni post hoc test. Unpaired t-test was used where appropriate to compare baseline parameters between the control group and diabetic group. Differences were deemed to be statistically significant when $P < 0.05$.

**RESULTS**

**General Animal Characteristics**

All animals administered streptozotocin developed hyperglycemia. Control and diabetic animals had similar body weights, but both left kidney weights and kidney-to-body weight ratios were approximately twofold higher in diabetic animals relative to control animals (Table 1).

MAP and RBF were largely unaffected by acute inhibition of SGLT by phlorizin. Although phlorizin had no overall effect on RPF in either group, there was a significant interaction between groups because phlorizin had a tendency to increase this parameter in control animals and reduce it in diabetic animals (Table 2). Phlorizin had no significant effect on GFR in the control group but significantly reduced glomerular hyperfiltration in the diabetic group (Table 2).

SGLT inhibition increased urinary glucose excretion in both control and diabetic rats (Fig. 2A), with concomitantly increased urine flow in both groups (Fig. 2B). The reduced glucose transport by SGLT in proximal tubules did not affect total TNa in either group (Fig. 3A). However, both absolute (Fig. 3B) and fractional (Fig. 3C) urinary Na+ excretions increased after SGLT inhibition. Interestingly, SGLT inhibition did not reduce total kidney $Q_{O2}$ in either group (Fig. 4A) even though it reduced diabetes-induced hyperfiltration. TNa/ $Q_{O2}$ was significantly reduced by SGLT inhibition in controls, whereas the already reduced TNa/QO2 observed in diabetes was not significantly affected by SGLT inhibition (Fig. 4B). The reduced glucose and Na+ transport in proximal tubules was manifested as increased PO2 in the diabetic kidney cortex (Fig. 5A). However, the increased Na+ load to the more distal parts of the nephron after SGLT inhibition reduced medullary PO2 in both control and diabetic kidneys (Fig. 5B). Furthermore, the magnitude of the reduction in medullary PO2 during SGLT inhibition was significantly greater in the control group (Fig. 5B). SGLT inhibition increased urinary protein excretion in control animals, whereas the already increased baseline urinary protein excretion in diabetic animals was not significantly affected by SGLT inhibition (Fig. 6A). However, SGLT inhibition had no significant effect on urinary albumin excretion in control animals but significantly increased this parameter in diabetic animals (Fig. 6B).

### Table 1. General characteristics of control and diabetic rats at baseline

<table>
<thead>
<tr>
<th></th>
<th>Number of Animals/Group</th>
<th>Blood Glucose, mmol/l</th>
<th>Body Weight, g</th>
<th>Left Kidney Weight, g</th>
<th>Left Kidney Weight/Body Weight, 1/1000</th>
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<tr>
<td>Control rats</td>
<td>12</td>
<td>6.1 ± 0.3</td>
<td>339 ± 5</td>
<td>1.5 ± 0.1</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td>9</td>
<td>25.5 ± 0.7*</td>
<td>357 ± 12</td>
<td>2.2 ± 0.1*</td>
<td>6.2 ± 0.2*</td>
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Values are means ± SE. *P < 0.05 vs. control rats.

### Table 2. Hemodynamic and filtration effects of Na+–glucose linked transport inhibition by phlorizin in control and diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Mean Arterial Blood Pressure, mmHg</th>
<th>Renal Plasma Flow, ml/min</th>
<th>Renal Blood Flow, ml/min</th>
<th>Renal Vascular Resistance, mmHg ml 1/1000 min⁻¹</th>
<th>Glomerular Filtration Rate, ml/min</th>
<th>Filtration Fraction, %</th>
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<tbody>
<tr>
<td>Control rats</td>
<td></td>
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<tr>
<td>Baseline</td>
<td>120 ± 3</td>
<td>3.7 ± 0.4</td>
<td>6.7 ± 0.6</td>
<td>20.5 ± 2.4</td>
<td>0.95 ± 0.09</td>
<td>27 ± 2</td>
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<tr>
<td>Phlorizin</td>
<td>115 ± 3</td>
<td>5.4 ± 1.0</td>
<td>9.6 ± 1.6</td>
<td>18.9 ± 5.1</td>
<td>0.91 ± 0.11</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td></td>
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<tr>
<td>Baseline</td>
<td>118 ± 2</td>
<td>7.8 ± 1.3</td>
<td>14.6 ± 2.5†</td>
<td>16.0 ± 4.3</td>
<td>1.51 ± 0.14†</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>Phlorizin</td>
<td>116 ± 4</td>
<td>5.8 ± 1.1</td>
<td>11.8 ± 2.3</td>
<td>16.4 ± 2.9</td>
<td>1.15 ± 0.14*</td>
<td>22 ± 2</td>
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2 × 2 Repeat-measures ANOVA

<table>
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Values are means ± SE. NS, not significant. *P < 0.05 vs. corresponding baseline; †P < 0.05 vs. the corresponding control.
SGLT inhibition may have deleterious consequences for O2-induced increases in QO2, SGLT activity, and Na+ evidence for a direct and positive correlation between diabetes-renal cortex. Indeed, previous studies by others have provided present study are consistent with the observed hypoxia in the renal cortex of the diabetic rat kidney. In this regard, mitochondria are consuming more O2 in the absence of generating ATP (10, 11), which may explain the reduced TNa/QO2 observed in diabetic animals at baseline in the present study. Overall, the existing body of evidence provided by the literature, coupled with the observations of the present study, suggests that SGLT-mediated intracellular accumulation of Na+ and glucose during chronic hyperglycemia increases Na+-K+-ATPase activity and the generation of ROS. The resultant increase in QO2 results in a reduction in the availability of O2 in the renal cortex and thereby hypoxia.

**DISCUSSION**

The present study sought to determine whether diabetes-induced renal hypoxia could be attenuated by inhibiting SGLT in the proximal tubule. Overall, the main findings of this study are that acute SGLT inhibition by phlorizin reduces Na+ and glucose reabsorption and attenuates glomerular hyperfiltration, which reverses renal cortical hypoxia in diabetic rats. On the other hand, SGLT inhibition promotes renal medullary hypoxia in both control and diabetic animals by promoting increased Na+ load to the more distal parts of the nephron. Taken together, these data clearly show that there is a link between the uptake of glucose and Na+ via SGLT in the proximal tubule, glomerular hyperfiltration, and tissue hypoxia in the renal cortex of diabetic rats. Importantly, these data also reveal that the improvement of cortical tissue PO2 observed during SGLT inhibition may have deleterious consequences for O2 metabolism in the medullary region.

The present study provides evidence for a causative link between proximal tubule cell glucose accumulation via SGLT and hypoxia in the renal cortex of the diabetic rat kidney. In agreement with a report by Palm et al. (24), the elevated renal QO2 and TNa and reduced TNa/QO2 in diabetic animals in the present study are consistent with the observed hypoxia in the renal cortex. Indeed, previous studies by others have provided evidence for a direct and positive correlation between diabetes-induced increases in QO2, SGLT activity, and Na+-K+-ATPase activity in the renal cortex of the diabetic kidney (16). More recently, it has been demonstrated that the enhanced uptake of glucose via SGLT also promotes the production of ROS, such as peroxynitrite, in the renal cortex and medulla in a rat model of type 1 diabetes (23). In the same set of experiments, phlorizin additionally had an inhibitory effect on antioxidant enzymes such as catalase and a stimulatory effect on prooxidant enzymes such as glutathione peroxidase (23).

Importantly, previous research has also demonstrated that diabetes-induced oxidative stress augments renal QO2 by stimulating mitochondrial uncoupling in proximal tubule cells (7, 8, 24). In this regard, mitochondria are consuming more O2 in the absence of generating ATP (10, 11), which may explain the reduced TNa/QO2 observed in diabetic animals at baseline in the present study. Overall, the existing body of evidence provided by the literature, coupled with the observations of the present study, suggests that SGLT-mediated intracellular accumulation of Na+ and glucose during chronic hyperglycemia increases Na+-K+-ATPase activity and the generation of ROS. The resultant increase in QO2 results in a reduction in the availability of O2 in the renal cortex and thereby hypoxia.
Interestingly, the phlorizin-induced normalization of renal cortex PO$_2$ was not accompanied by a corresponding decrease in renal QO$_2$ or TNa in either control or diabetic rat kidneys in the present study. Furthermore, SGLT inhibition had no significant effect on TNa/QO$_2$ in diabetic animals but actually reduced this parameter in control animals. These observations clearly suggest that the uptake of glucose and norepinephrine, also reduced RBF but were without effect on proximal TNa (4). However, other vasoconstrictors, such as endothelin and norepinephrine, also reduced RBF but were without effect on medullary PO$_2$ (4), indicating that factors other than a reduction in O$_2$ delivery interfere with PO$_2$ in the medulla during NOS inhibition. Furthermore, others later demonstrated that NOS inhibition does in fact increase QO$_2$ in the dog kidney (17). These observations, taken together with the findings of the present study, show that a reduction in PO$_2$ in the renal medulla during the inhibition of proximal tubule TNa is possibly due to a compensatory shift in renal TNa to less-efficient medullary regions, thereby resulting in an increase in Qo$_2$ (4).

The present data demonstrate that SGLT inhibition had a positive effect on O$_2$ availability in the cortex of the diabetic kidney. However, the same treatment induced medullary hypoxia in both control and diabetic rat kidneys. Indeed, these data do not give any indication as to what may happen to renal O$_2$ availability during the exclusive administration of SGLT2 inhibitors, which are used clinically to lower plasma glucose levels in diabetic patients. Whether SGLT1 activity and expression might become upregulated in this scenario in an effort to compensate is presently unknown. On the one hand, in phase II clinical trials, it has been recently demonstrated that the dual SGLT1/SGLT2 inhibitor LX-4211 did not increase urinary glucose excretion any more than what was observed when patients were just administered a specific SGLT2 antagonist (29). On the other hand, it has been widely reported that even the most potent SGLT2 inhibitors can only inhibit 30–50% of total tubular glucose reabsorption. Indeed, a recent mathematical model has predicted that SGLT1 compensation may be an underlying factor (18).

The ability of SGLT inhibition to significantly reduce diabetes-induced glomerular hyperfiltration in the present study was not surprising as this has been well established in numerous studies in diabetic rats and mice (20, 27, 39, 37, 34). These observations clearly suggest that the uptake of glucose and

![Graph A](image1.png)

![Graph B](image2.png)

**Fig. 4.** Total kidney O$_2$ consumption (A) and tubular electrolyte transport efficiency [tubular Na$^+$ transport (TNa)/renal O$_2$ consumption (QO$_2$); B] in control (n = 12) and diabetic (n = 9) rats during baseline and after SGLT inhibition by phlorizin. *P < 0.05 vs. the corresponding baseline; #P < 0.05 vs. the corresponding control.

![Graph C](image3.png)

![Graph D](image4.png)

**Fig. 5.** Renal cortical (A) and medullary tissue (B) PO$_2$ in control (n = 12) and diabetic (n = 9) rats during baseline and after SGLT inhibition by phlorizin. *P < 0.05 vs. the corresponding baseline; #P < 0.05 vs. the corresponding control.
Filtration. Indeed, it has been proposed that diabetes-induced hyperfiltration in the mouse model of type 1 diabetes. Furthermore, it was later demonstrated that SGLT inhibition by phlorizin could still attenuate glomerular hyperfiltration in the above-mentioned mouse model (34). These data certainly imply that mechanisms other than a defective tubuloglomerular feedback response cause diabetes-induced glomerular hyperfiltration. Indeed, it has been proposed that diabetes-induced increases in Na\(^+\) and glucose uptake via SGLT in the diabetic rat kidney plays a role in promoting glomerular hyperfiltration. Whether this is due to a defective tubuloglomerular feedback response or a reduction in proximal tubule hydrostatic pressure or indeed a combination of both is currently unclear.

The phlorizin-induced attenuation of glomerular hyperfiltration observed in diabetic animals in the present study was accompanied by a significant increase in the urinary excretion of glucose and Na\(^+\). This was anticipated and provides evidence that SGLTs were inhibited by phlorizin, as has been previously demonstrated by others in diabetic rats and mice (20, 34, 39). Importantly, additional studies have shown that SGLT2 activity and expression are upregulated in the diabetic rat kidney (22, 30, 42). However, this is not reflected by the present observations, whereby phlorizin produced increases in Na\(^+\) and glucose excretion of a similar magnitude in control and diabetic animals. Indeed, SGLT activity may well have been elevated at baseline in the diabetic group, but this was possibly masked by the parallel reductions in GFR and thereby the filtered load of glucose after the acute inhibition of SGLT (37, 40).

A modest total proteinuria was observed in the diabetic group at baseline. Interestingly, the acute administration of phlorizin augmented urinary protein excretion, particularly in control animals. The observed phlorizin-induced increases in urinary protein excretion in the present study are supported by the findings of others in a rat model of type 1 diabetes (20). This group additionally demonstrated that this increase in urinary protein excretion was related to an independent inhibitory effect of phlorizin on the tubular reabsorption of \(\beta_2\)-microglobulin (20). Indeed, a previous study (43) in type 1 diabetic humans revealed a positive correlation between tubular flow rate and the urinary excretion of \(\beta_2\)-microglobulin. Phlorizin caused a robust diuresis in the present study in both control and diabetic animals. This observation, combined with previous findings of others (20, 43), suggests that the increases in urinary protein excretion after the acute administration of phlorizin are possibly due to flow-induced decreases in \(\beta_2\)-microglobulin reabsorption. It is unclear from the present data why phlorizin-increased urinary protein excretion is much greater in control animals, and there are currently no data in the literature to explain such observations. However, it might be tempting to speculate that diabetic nephrons are more adapted to high urine flow. It may well be that acute increases in urine flow after SGLT inhibition in the diabetic group only result in mild increases in urinary protein excretion because the tubule has adapted mechanisms at this early phase in the disease process in an effort to counteract the urinary loss of protein.

Surprisingly, the proteinuria was not accompanied by albuminuria in diabetic animals at baseline. Indeed, diabetes-induced proteinuria and specifically albuminuria have been demonstrated clinically and in animal models of type 1 and type 2 diabetes (1, 20, 25, 37, 43). On the other hand, it has been reported that when glomerular injury is mild, as may be the case in the present study, tubules still have a large capacity for albumin reabsorption but a comparably lower capacity to reabsorb larger-molecular-weight proteins, such as IgG (3). This may indeed account for the occurrence proteinuria in the absence of albuminuria in the present study. Importantly, Tucker et al. (36) demonstrated hyperfiltration in diabetic rats thereby Na\(^+\) via SGLT plays a role in promoting increased GFR in the diabetic rat kidney. The proposed mechanisms underlying this relationship are controversial. Indeed, Vallon and coworkers (39) would argue that increased proximal Na\(^+\) uptake via SGLT reduces the distal delivery of Na\(^+\) to the macula densa. This results in the inhibition of tubuloglomerular feedback, dilation of the afferent arteriole, and thereby glomerular hyperfiltration (39). This viewpoint is based on data collected from in vivo micropuncture studies carried out in a rat model of type 1 diabetes whereby intratubular administration of phlorizin reduced proximal TNa, increased distal Na\(^+\) concentration, and attenuated single nephron glomerular hyperfiltration (39). These findings were later substantiated when it was shown that diabetes-induced glomerular hyperfiltration was virtually absent in type 1 diabetic mice who were deficient in the adenosine A\(_1\) receptor and therefore lacked a functional tubuloglomerular feedback response (41).

In contrast, Sällström et al. (33) reported that genetic ablation of the adenosine A\(_1\) receptor had no effect on glomerular hyperfiltration in a mouse model of type 1 diabetes. Furthermore, it was later demonstrated that SGLT inhibition by phlorizin could still attenuate glomerular hyperfiltration in the above-mentioned mouse model (34). These data certainly imply that mechanisms other than a defective tubuloglomerular feedback response cause diabetes-induced glomerular hyperfiltration. Indeed, it has been proposed that diabetes-induced increases in Na\(^+\) and glucose uptake in the proximal tubule and the resultant reductions in the distal delivery of Na\(^+\) (35) might cause a reduction in proximal tubule hydrostatic pressure (12), a corresponding increase in net filtration pressure, and thereby GFR (26, 34). Either way, it is clear that increased Na\(^+\) and glucose uptake via SGLT in the diabetic rat kidney plays a role in promoting glomerular hyperfiltration. Whether this is due to a defective tubuloglomerular feedback response or a reduction in proximal tubule hydrostatic pressure or indeed a combination of both is currently unclear.
in the absence of albuminuria, 10 days after streptozotocin administration. Interestingly, lysine was also acutely administered to inhibit tubular albumin reabsorption. Lysine had no effect on urinary albumin excretion in the control group but caused a significant increase in this parameter in the diabetic group (36). In the context of the present study, these data clearly suggest that the filtered load of albumin may well be increased at this early stage in the disease, but albumin is not detected in the urine because it is being reabsorbed in the proximal tubule (36).

Finally, the phlorizin-induced increase in urinary albumin excretion in the diabetic group in this study was not anticipated given that others have shown the opposite effect in mouse models of type 1 and 2 diabetes (1, 37). This discrepancy may be related to the fact that SGLTs were chronically inhibited (12 wk) in these studies (1, 37). Furthermore, the chronic SGLT inhibition blunted diabetes-induced diuresis over time (1) in a mouse model of type 2 diabetes. Although Vallon et al. (37) appear not to have measured urine volume in their experiments in Akita mice, it may be inferred that SGLT inhibition had a similar effect on urine flow in these experiments since diabetes-induced polydipsia was also blunted after 3 mo of treatment. Taking all previous studies into account, it may well be that the acute increase in urine flow after SGLT inhibition by phlorizin in the present study was somehow interfering with the tubular reabsorption of albumin in diabetic animals. The mechanism underlying this is currently unclear but may be due to a nonspecific interaction between the drug and luminal proteins (megalin and cubulin) (35) involved in the reabsorption of albumin. Interestingly, phlorizin had no effect on albumin excretion in control animals. This may be related to the fact that filtration of albumin was not increased at baseline in the first place. On the other hand, the filtered load of albumin may well have been increased in diabetic animals, but, as Tucker et al. (36) suggest, this may have been masked by an initial reciprocal increase in the tubular reabsorption of albumin.

Indeed, the phlorizin-induced increase in albumin excretion observed exclusively in diabetic animals may also be explained by the fact that nonselective SGLT inhibitors have been shown to increase plasma levels of glucagon-like protein-1 (28). Glucagon-like protein-1 stimulates the release of atrial natriuretic peptide (ANP) (14). ANP, in turn, has been reported to selectively increase the urinary excretion of albumin in type 1 diabetic patients (44). It has been proposed that the glomerular permeability of albumin was not increased by nonselective SGLT inhibition in control subjects because the presumably intact glomerular filtration barrier in this group was not sensitive to ANP (44). These observations may explain why urinary albumin excretion was not altered by SGLT inhibition in the control animals in the present study.

Summary and Significance

Overall the data presented in this study shows that the SGLT-mediated accumulation of Na⁺ and glucose in proximal tubules of the diabetic rat kidney promote renal cortex hypoxia as well as glomerular hyperfiltration. On the other hand, the phlorizin-induced normalization of renal cortex PO₂ was paralleled by a reduction in this parameter in the renal medulla. This, coupled with the fact that phlorizin did not alter either QO₂, TNa, or TNa/QO₂ in diabetic animals, indicates that TNa may have been diverted to other nephron segments, such as the medullary thick ascending limb, where Na⁺ transport is less efficient and therefore requires more O₂. Whether this is indeed the case or not warrants further investigation. The association between SGLT activity and diabetes-induced proteinuria and particularly albuminuria is less clear and cannot be sufficiently explained by the present data. However, one might speculate that the robust diuresis induced by SGLT inhibition may have inadvertently interfered with the tubular reabsorption of certain proteins, including albumin.

These experiments are clinically relevant because SGLT2 inhibitors are now used in the treatment of diabetes and are, for the most part, renoprotective. However, the present data also show that PO₂ in the medulla becomes compromised by the acute administration of these drugs. If medullary hypoxia persists during the long-term use of these drugs, diabetic-related complications, such as hypertension, diabetic nephropathy, and chronic kidney disease, may in fact be exacerbated. However, it must be noted that the data presented in the present study are based on observations in an acute setting and are thus limited. Indeed, future studies should extend the present findings and examine the impact of chronic SGLT inhibition on intrarenal O₂ homeostasis in the diabetic kidney.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.O.N. and F.P. conception and design of research; J.O.N., A.F., L.P., D.P., and S.F. performed experiments; J.O.N. and A.F. analyzed data; J.O.N. and F.P. interpreted results of experiments; J.O.N. and F.P. drafted manuscript; J.O.N. and F.P. edited and revised manuscript; J.O.N. and F.P. approved final version of manuscript.

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