Renal Handling of Ketones in Response to Sodium–Glucose Cotransporter 2 Inhibition in Patients With Type 2 Diabetes

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OBJECTIVE

Pharmacologically induced glycosuria elicits adaptive responses in glucose homeostasis and hormone release, including decrements in plasma glucose and insulin levels, increments in glucagon release, enhanced lipolysis, and stimulation of ketogenesis, resulting in an increase in ketonemia. We aimed at assessing the renal response to these changes.

RESEARCH DESIGN AND METHODS

We measured fasting and postmeal urinary excretion of glucose, β-hydroxybutyrate (β-HB), lactate, and sodium in 66 previously reported patients with type 2 diabetes and preserved renal function (estimated glomerular filtration rate ≥60 mL · min^{-1} · 1.73 m^{-2}) and in control subjects without diabetes at baseline and following empagliflozin treatment.

RESULTS

With chronic (4 weeks) sodium–glucose cotransporter 2 inhibition, baseline fractional glucose excretion (<2%) rose to 38 ± 12% and 46 ± 11% (fasting vs. postmeal, respectively; $P < 0.0001$) over a range of BMIs (range 23–41 kg/m^2) and creatinine clearance (65–168 mL · min^{-1} · m^{-2}). Excretion of β-HB (median [interquartile range]: 0.08 [0.10] to 0.31 [0.43] μmol · min^{-1}), lactate (0.06 [0.06] to 0.28 [0.25] μmol · min^{-1}), and sodium (0.27 [0.22] to 0.36 [0.16] mEq · min^{-1}) all increased ($P \leq 0.001$ for all) and were each positively related to glycosuria ($P \leq 0.001$). These parameters changed in the same direction in subjects without diabetes, but changes were smaller than in the patients with diabetes. Although plasma N-terminal pro–B-type natriuretic peptide levels were unaltered, plasma erythropoietin concentrations increased by 31 (64)% ($P = 0.0078$).

CONCLUSIONS

We conclude that the sodium–glucose cotransporter 2 inhibitor–induced increase in β-HB is not because of reduced renal clearance but because of overproduction. The increased lactate excretion contributes to lower plasma lactate levels, whereas the increased natriuresis may help in normalizing the exchangeable sodium pool. Taken together, glucose loss through joint inhibition of glucose and sodium reabsorption in the proximal tubule induces multiple changes in renal metabolism.

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Sodium–glucose cotransporter 2 (SGLT2) inhibitors reduce the proximal tubule reabsorption of filtered glucose, thereby causing glycosuria and decreases in plasma glucose concentrations (1). When large quantities of glucose are pharmacologically forced into urinary excretion, whole-body metabolism undergoes adaptive changes, involving glucose fluxes, hormonal responses, fuel selection, and energy expenditure. In previous work (2), we used empagliflozin to investigate the physiological response to forced glycosuria in patients with type 2 diabetes (T2D). By combining a mixed meal with the double-tracer technique and indirect calorimetry, we found that following acute or chronic empagliflozin administration, endogenous glucose production rose, tissue glucose disposal decreased, and lipid use increased. Subsequently, we showed that the SGLT2-induced increase in lipid mobilization and oxidative use was associated with increased plasma ketone (β-hydroxybutyrate [β-HB]) levels and reduced plasma lactate concentrations (3). Recently, episodes of ketoacidosis have been reported in some patients receiving SGLT2 inhibitors (SGLT2i), especially on the background of insulin treatment (4,5). Though infrequent (6), this serious complication of SGLT2 inhibition has led both the U.S. Food and Drug Administration (7) and European Medicines Agency (8) to include a warning on the product label.

The question, what is the role of renal clearance in the rise in ketonemia associated with SGLT2 inhibition, has not been addressed. With fasting prolonged for 24 days, circulating ketone concentrations rise progressively, whereas ketonuria initially increases then falls (9). Older studies suggested the presence of both saturable and nonsaturable components of renal ketone handling (10), by which low filtered loads are completely reabsorbed, whereas ketonuria occurs as plasma ketone levels rise further. On the basolateral surface of tubular cells along the S2 segment, multiselective organic anion transporters transfer ketones from the plasma to the cell cytoplasm (11), whereas multidrug-resistant anion transporters effect the extrusion of ketones from the cytoplasm into the proximal tubular lumen (12). However, the regulation of these processes is not well understood, and human data on the renal handling of ketones under more physiologic circumstances are lacking (13).

This prompted us to measure urinary excretion of the main ketone body, β-HB, along with lactate and sodium, in a group of well-characterized patients with T2D and in subjects without diabetes under short-term fasting and postprandial conditions at baseline and then following acute and chronic SGLT2 inhibition.

RESEARCH DESIGN AND METHODS

Population

Sixty-six patients with T2D were recruited into the study; their inclusion criteria, which are detailed in Ferrannini et al. (3), included an estimated glomerular filtration rate (GFR) $\geq$60 mL · min^{-1} · 1.73 m^2. Twenty-five subjects without diabetes (12 with normal glucose tolerance [NGT] and 13 with impaired glucose tolerance [IGT] as defined by the American Diabetes Association) served as control subjects (Supplementary Table 1). The glucose excretion data for the patients with T2D have been reported (2) and are repeated in this study for comparison purposes; the circulating β-HB and lactate levels have been reported (3) and are used in this study to calculate fractional urinary excretion rates. The study (ClinicalTrials.gov identifier NCT01248364; EudraCT no. 2010-018708-99) was carried out at three sites (Pisa, Italy; Neuss, Germany; and Graz, Austria); the protocol was approved by the Institutional Review Board at each site. All participants provided informed written consent.

Design

Participants with T2D and those with IGT underwent three open-label studies: baseline, acute (single dose of 25-mg empagliflozin), and chronic (25 mg/day for 28 days); subjects with NGT did not participate in the chronic study. Each study consisted of a 3-h basal period followed by a 5-h meal tolerance test combined with a double-tracer technique (2). The meal consisted of 1 egg, 50 g parmesan cheese, 50 g white bread, and 75 g glucose in water. In both studies, empagliflozin was ingested 30 min before starting tracer infusion (i.e., at time −210 min). Blood was drawn at timed intervals for the measurement of hormones and substrates. Urine was collected separately during the basal period and during the meal; urine volume was measured, and urine aliquots were frozen for later analysis.

Measurements

All measurements were performed at the Metabolism Laboratory of the University of Pisa; samples from all three studies of each subject were assayed together to reduce intrasubject variability. Plasma and urine glucose, sodium, lactate, and β-HB concentrations were measured on a Synchron system CX4 (Beckman Coulter, Fullerton, CA). HbA_{1c}, hematocrit, red and white blood cell count, reticulocyte count, serum, and urine creatinine and plasma albumin concentrations were measured by standard clinical chemistry methods. Plasma insulin and C-peptide were assayed on a Cobas e411 (Roche, Indianapolis, IN); erythropoietin (EPO) was assayed by ELISA (Quantikine Human Erythropoietin ELISA Immunoassay; R&D Systems, Minneapolis, MN) (normal range 3.1–14.9 IU/L, sensitivity <0.6 IU/L); and plasma N-terminal pro–B-type natriuretic peptide was measured by the monoclonal electrochemiluminescence immunoassay method using the automated Cobas e411 platform (14).

Calculations

Urinary solute (creatinine, glucose, β-HB, lactate, and sodium) excretion rate was calculated as the product of urine solute concentration and urine volume; renal solute clearance rate was calculated as the ratio of urine solute excretion to plasma solute concentration. Solute filtered rate was calculated as the product of creatinine clearance (CrCl) and plasma solute concentration. Fractional solute excretion was obtained as the ratio of urinary solute excretion to solute filtered load. Area under the curve was calculated (by the trapezium rule) for each urine collection time interval during the 3-h basal fasting period and 5-h postmeal period; mean values were then calculated at 30-min intervals.

Statistical Analysis

Data are given as mean ± SD (or median [interquartile range] for nonnormally distributed variables). Acute and chronic treatment responses were compared with the respective baseline responses by paired *t* test or Wilcoxon signed rank test depending on the underlying data distribution. Because of the overall similarity of results in subjects with NGT and IGT, we compared patients with T2D with all 25 subjects without diabetes (i.e., NGT + IGT). Group differences were tested by the unpaired *t* test or Mann-Whitney *U* test. Regression analyses were carried out by using mixed linear models. A two-tailed

P value ≤0.05 was considered statistically significant.

RESULTS

Influence of Obesity

CrCl was progressively higher from lean (BMI 23.8 ± 0.6 kg/m^2) to overweight (BMI 28.0 ± 1.3 kg/m^2) to obese subjects (BMI 34.7 ± 2.8 kg/m^2) both in the fasting state and during meal absorption. In a multivariate model of the pooled data from all study subjects, absolute values of baseline fasting CrCl (in mL/min) were directly related to BMI (increasing by 2.6 mL/min for every BMI unit; *P* = 0.027, over a range of 23.0–41.1 kg/m^2) after adjusting for gender, age, and glucose tolerance. Similar associations were found for baseline meal CrCl and for fasting and meal CrCl measured during acute or chronic empagliflozin administration. In contrast, fractional urinary glucose excretion after chronic drug administration was similar across BMI categories, both in the fasting state and following the meal (Fig. 1).

Patients With T2D

In the fasting state, administration of a single dose of empagliflozin increased urine output by 20% while decreasing CrCl by 10% (Supplementary Fig. 1). Renal excretion, clearance rate, and fractional excretion of glucose rose ~100-fold; except for urine output, which returned to baseline levels, these responses were maintained with chronic administration (Table 1). Following the meal, baseline urine output was lower as compared with the fasting condition, whereas CrCl, renal excretion, clearance, and fractional excretion of glucose were all higher. Both acute and chronic drug dosing were associated with changes in these responses in the same direction as in the fasting state except that they were all significantly higher. Thus, postmeal fractional glucose excretion averaged ~45 vs. ~35% of the fasting condition, translating into ~6 vs. 3 g/h lost through the urine.

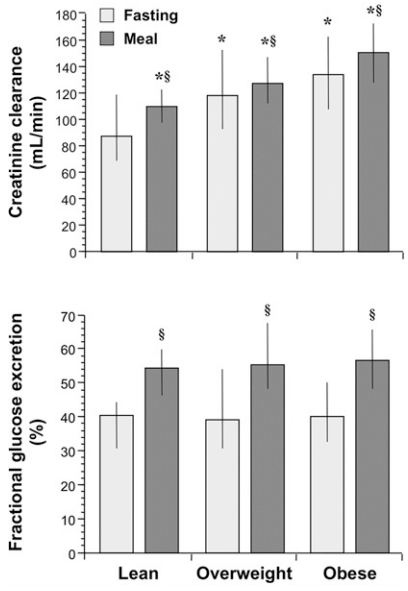

Figure 1—CrCl (top) and fractional glucose excretion (bottom) during fasting and following meal ingestion in lean, overweight, and obese subjects. Data are median and interquartile range from the chronic treatment study. *$P \leq 0.05$ vs. lean; §$P \leq 0.05$ vs. fasting.

In the fasting state, renal β-HB excretion rose two- and threefold with acute and chronic dosing, respectively; the corresponding clearance and fractional excretion rose ~35 and ~70% (Table 2). Likewise, renal lactate excretion increased three and five times with acute and chronic dosing, with similar fold changes of lactate clearance and fractional excretion. In contrast, sodium excretion was increased by ~20% only with the first empagliflozin dose, whereas with chronic treatment, sodium excretion was, if anything, reduced (Fig. 2).

Postmeal urinary β-HB excretion was similar to the fasting rate at baseline and after single-drug dosing, whereas with chronic dosing, it was significantly less than the fasting rate. Drug treatment raised postmeal β-HB excretion both acutely and, more so, chronically. Lactate excretion, in contrast, was higher than in the fasting state, and the effects of acute and chronic empagliflozin on lactate excretion parameters were proportionally increased. At baseline, postmeal sodium excretion was significantly lower than in the fasting state, an expected consequence of meal-induced antinatriuresis. With drug treatment, postmeal sodium excretion was greater than at baseline both acutely and chronically (Fig. 2 and Table 2). The time course of solute excretion is depicted in Supplementary Fig. 2. By pooling fasting and postmeal rates, excretions of β-HB, lactate, and sodium were each positively correlated with glycosuria following chronic empagliflozin administration (Supplementary Fig. 3).

Subjects Without Diabetes

In subjects without diabetes, all excretion parameters were essentially similar to those measured in the patients with T2D and changed in the same direction in response to drug administration (Supplementary Tables 2 and 3). Treatment-induced changes in hematocrit and blood cell counts were minimal and none statistically significant in either group of study subjects. Plasma albumin concentrations were consistently lower during meal than in the fasting state and increased significantly with SGLT2 inhibition (Supplementary Table 4).

Fasting serum EPO concentrations increased (by 31 [64]%; *P* = 0.0078) between baseline and 4 weeks, with no significant difference between patients with T2D and subjects without diabetes (Supplementary Fig. 4). Among blood parameters, blood hemoglobin and plasma albumin were found to be reciprocally related to EPO concentrations in the pooled data from all subjects at 4 weeks (Supplementary Fig. 5). Serum plasma N-terminal pro–B-type natriuretic peptide levels did not differ between patients and control subjects and were not significantly altered by chronic empagliflozin treatment (Supplementary Table 4).

CONCLUSIONS

Baseline urinary β-HB excretion was low, amounting to <1% of the filtered load both in the fasting state and postmeal. This confirms the great efficiency of the tubular kidney to reabsorb β-HB (similar to those of glucose and sodium, possibly through the joint action of multiple transporters, e.g., SGLT1 for glucose and Na-H, Na-K chloride pumps, and epithelial sodium channel for sodium [15]). With empagliflozin administration, especially chronic, β-HB excretion, clearance rate, and fractional excretion all increased both in the fasting and postprandial state. Therefore, the observed rise in plasma β-HB concentrations was not because of reduced renal elimination, although a decrease in muscle ketone use cannot be ruled out (16). It should be noted that net β-HB excretion, as measured by the product of urine volume and urine β-HB concentration, could include not only the fraction of filtered β-HB escaping reabsorption but also secreted β-HB. Indeed, there is evidence that the kidney can synthesize ketone bodies under certain circumstances (17) or convert β-HB into acetoacetate during prolonged fasting (18). Also of interest is the fact that ketones significantly enhance renal plasma

Table 1—Effect of empagliflozin on renal glucose handling in patients with T2D

	Baseline	Acute	Chronic	P^1	P^2
Fasting					
Plasma glucose (mmol · L^{-1})	8.7 ± 1.6	8.9 ± 1.5	7.6 ± 1.1	NS	<0.0001
CrCl (mL · min^{-1} · 1.73 m^{-2})	103 ± 25	97 ± 25	93 ± 23	NS	0.05
Glucose filtration rate (μmol · min^{-1})	976 ± 338	804 ± 251	744 ± 222	<0.0001	<0.0001
Glucose excretion rate (μmol · min^{-1})	3 ± 14	249 ± 101	287 ± 112	<0.0001	<0.0001
Renal glucose clearance (mL · min^{-1})	0.3 ± 1.1	34 ± 13	43 ± 15	<0.0001	<0.0001
Fractional glucose excretion (%)	0.2 ± 1.0	31 ± 10	38 ± 12	<0.0001	<0.0001
Meal					
Plasma glucose (mmol · L^{-1})	11.0 ± 2.1[a]	9.5 ± 1.5[a]	9.5 ± 1.3[a]	<0.0001	<0.0001
CrCl (mL · min^{-1} · 1.73 m^{-2})	114 ± 22[b]	111 ± 23[a]	105 ± 22[a]	NS	0.007
Glucose filtration rate (μmol · min^{-1})	1,497 ± 454[a]	1,200 ± 322[a]	1,195 ± 329[a]	<0.0001	<0.0001
Glucose excretion rate (μmol · min^{-1})	33 ± 72[a]	555 ± 165[a]	548 ± 180[a]	<0.0001	<0.0001
Renal glucose clearance (mL· min^{-1})	2.4 ± 4.4[a]	58 ± 15[a]	58 ± 17[a]	<0.0001	<0.0001
Fractional glucose excretion (%)	1.8 ± 3.0[a]	46 ± 9[a]	46 ± 11[a]	<0.0001	<0.0001

Data are mean ± SD. NS, not significant. P^1, acute vs. baseline P value; P^2, chronic vs. baseline P value (Wilcoxon signed rank test). [a]$P < 0.0001$; [b]$P < 0.001$, meal vs. fasting (Wilcoxon signed rank test).

flow and GFR (19), thereby countering the CrCl reduction that results from hemodynamic changes (Supplementary Fig. 1).

At baseline, lactate excretion was similar to β-HB excretion in absolute terms, but fractional excretion was 10 times lower because of the higher lactate than β-HB levels in the plasma. The tubular nephron can generate lactate from aerobic glycolysis, particularly in the pars recta, and use lactate for gluconeogenesis (20). The net contribution of this intrarenal Cori cycle to the lactate eventually excreted into the urine versus the fraction of filtered lactate that escapes reabsorption at the luminal side is undetermined in humans.

In any event, as in the case of β-HB excretion, empagliflozin dosing was associated with progressive increments in lactate excretion, clearance rate, and fractional excretion, similarly under fasting and postprandial conditions. It should be noted that during SGLT2 inhibition, plasma ketones increase, whereas plasma lactate decreases significantly (3). The latter change could be because of the sum of decreased glucose disposal (2), increased hepatic/renal extraction (to support the augmentation of gluconeogenic glucose production [2]), and increased renal excretion, in relative proportions that only a combined catheterization/tracer study could quantify.

Sodium excretion was consistently lower postmeal than at baseline, an expected consequence of oral glucose (21). Sodium excretion was acutely increased by single-drug administration both in the fasting and postprandial state (Fig. 2). Importantly, postprandial natriuresis remained 35% higher than at baseline during chronic treatment, confirming that glycosuria imposes an added natriuretic pressure (as also predicted by a recent detailed mathematical model [22]). In a recent study using 25-mg empagliflozin in patients with T2D on a controlled diet with standardized sodium, food, and fluid intake, 24-h natriuresis increased acutely and returned to baseline after 5 days (23). Acute blockade of

Table 2—Effect of empagliflozin on renal solute handling in patients with T2D

	Baseline	Acute	Chronic	P^1	P^2
Fasting					
β-HB excretion rate (μmol · min^{-1})	0.08 (0.10)	0.15 (0.10)	0.31 (0.43)	<0.0001	<0.0001
β-HB clearance rate (mL · min^{-1})	0.56 (0.68)	0.84 (0.46)	1.08 (1.06)	0.02	0.0003
β-HB fractional excretion (%)	0.56 (0.57)	0.75 (0.55)	0.95 (0.82)	0.01	<0.0001
Lactate excretion rate (μmol · min^{-1})	0.06 (0.06)	0.20 (0.16)	0.28 (0.25)	<0.0001	<0.0001
Lactate clearance rate (mL · min^{-1})	0.05 (0.05)	0.16 (0.13)	0.25 (0.23)	<0.0001	<0.0001
Lactate fractional excretion (%)	0.04 (0.03)	0.14 (0.12)	0.24 (0.20)	<0.0001	<0.0001
Na^+ excretion rate (mEq · min^{-1})	0.45 (0.27)	0.55 (0.25)	0.41 (0.25)	0.0005	0.02
Na^+ excretion (mEq · 180 min)	82 (48)	100 (45)	74 (45)	0.0005	0.02
Meal					
β-HB excretion rate (μmol · min^{-1})	0.08 (0.10)	0.15 (0.13)	0.22 (0.32)[b]	<0.0001	<0.0001
β-HB clearance rate (mL · min^{-1})	0.87 (0.92)	1.03 (0.69)[c]	1.33 (0.73)[c]	0.03	0.003
β-HB fractional excretion (%)	0.66 (0.67)	0.78 (0.44)	1.04 (0.77)	0.008	0.0008
Lactate excretion rate (μmol · min^{-1})	0.10 (0.17)[a]	0.38 (0.43)[a]	0.42 (0.34)[a]	<0.0001	<0.0001
Lactate clearance rate (mL · min^{-1})	0.07 (0.12)[a]	0.26 (0.25)[a]	0.29 (0.30)[b]	<0.0001	<0.0001
Lactate fractional excretion (%)	0.05 (0.07)[c]	0.22 (0.18)[c]	0.29 (0.23)	<0.0001	<0.0001
Na^+ excretion rate (mEq · min^{-1})	0.27 (0.22)[a]	0.45 (0.20)[a]	0.36 (0.16)	<0.0001	0.001
Na^+ excretion (mEq · 300 min)	80 (67)	136 (60)[a]	108 (48)[a]	<0.0001	0.0001

Data are median (interquartile range). P^1, acute vs. baseline P value; P^2, chronic vs. baseline P value (Wilcoxon signed rank test). [a]$P < 0.0001$; [b]$P < 0.001$; [c]$P < 0.05$, meal vs. fasting (Wilcoxon signed rank test).

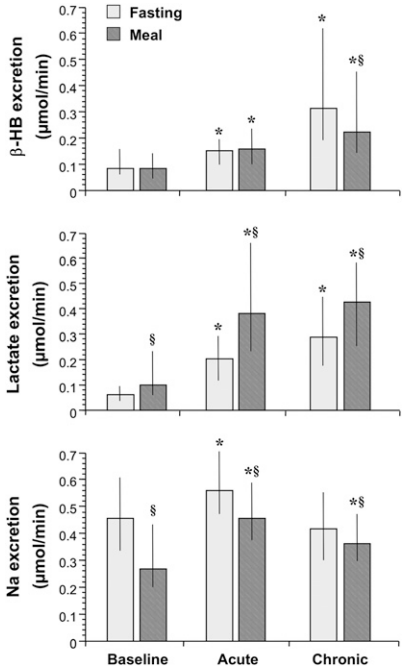

Figure 2—Urinary β-HB (top), lactate (middle), and Na excretion rate (bottom) during fasting and following meal ingestion at baseline and after acute and chronic empagliflozin administration in patients with T2D. Plots are median and interquartile range. *$P \leq 0.01$ vs. baseline; §$P \leq 0.05$ vs. fasting.

proximal sodium absorption delivers an excess of sodium (and chloride) to the macula densa, thereby triggering the release of vasoconstrictive molecules (adenosine, prostaglandin H2, and thromboxane A2) and vasoconstriction of the afferent glomerular arterioles (24). It has been argued that this resetting of the tubuloglomerular feedback is the likely mechanism underlying the acute decrement in GFR observed in patients with type 1 diabetes (25). In contrast, under everyday life conditions, even mild natriuresis induced by the glycosuric effect of each daily SGLT2 dose should gradually reduce the body pool of exchangeable sodium (~2.5–2.7 mol), which is characteristically expanded (by ~10%) in patients with hyperglycemia regardless of whether they are hypertensive or normotensive (26). In this regard, it is of note that excretion of all three measured solutes—β-HB, lactate, and sodium—correlated with glucose excretion, which is further evidence for the central role of glycosuria in the changes in renal solute handling following SGLT2 inhibition.

The present analysis of SGLT2i-induced glycosuria yielded some unanticipated findings. Firstly, whereas CrCl was 35% lower in lean (mean BMI of 23.8 kg/m^2) than in obese participants (mean BMI of 34.7 kg/m^2) (Fig. 1), renal glucose clearance in response to chronic empagliflozin administration was only 23% lower in the former than the latter in the fasting state (34 [10] vs. 44 [23] mL · min^{-1}) and 16% lower during the meal (46 [9] vs. 55 [27] mL · min^{-1}). This quantitative difference between GFR and SGLT2i-induced renal glucose clearance would translate into a difference of only 18% in 24-h glycosuria between the lean and the obese (e.g., 96 vs. 117 g using the mean fasting and postmeal glucose levels measured in our patients with T2D). However, an obese 60-year-old man has an estimated calorie consumption of 2,375 kcal/day, whereas a lean 60-year-old woman consumes 1,715 kcal/day (27); assuming 50% carbohydrate in both of their diets, those urinary glucose losses would amount to 34% of carbohydrate intake in the obese man and 45% of carbohydrate intake in the lean woman. This calculation highlights the fact that SGLT2 inhibition induces a degree of glycosuria, relative to the filtered glucose load, that is essentially independent of body size (Fig. 1), thereby exposing the lean person to a substantially higher degree of carbohydrate deficit than the obese individual. This may represent an additional circumstance predisposing less obese patients with T2D on SGLT2i treatment on a low-carbohydrate diet to an augmented reliance on fat use for energy production (3), hyperketonemia, and, occasionally, ketoacidosis (5,6).

Secondly, over the 5 h following a mixed meal, renal glucose clearance and fractional excretion were ~50% higher than during the preceding 3 h of fasting, in both participants with T2D and without diabetes, i.e., postprandial glycosuria was larger than could be accounted for by the prevailing glycemia and CrCl. What meal-related factors may underlie this phenomenon remain unknown and should be investigated.

Finally, CrCl was consistently higher during the mixed meal than in the fasting state (Fig. 2), a well-known consequence of protein stimulation of glucagon release (along with vasopressin and urea [28]). However, both acute and chronic empagliflozin dosing were associated with a significant ~10% decrease in both fasting and postmeal CrCl, similarly in subjects with T2D and without diabetes. This finding directly confirms the drop in estimated GFR that has been consistently observed in the early stages of SGLT2i development and in subsequent clinical studies (1). This change in CrCl has been related to blood-volume depletion (29). However, in our subjects, volume contraction, as judged from the plasma albumin concentrations, was very small over the few hours following single-drug administration (Supplementary Table 4). As mentioned above, proximal tubular natriuresis could lead to vasoconstriction of the afferent glomerular arteriole by activating tubuloglomerular feedback at the macula densa (29). Additionally, the observed prompt rise in plasma glucagon and glucagon-like peptide 1 levels following single empagliflozin dosing (2) might have had a role of its own given the presence of both glucagon and glucagon-like peptide 1 receptors in the distal tubular nephron (30,31).

Finally, the finding of an increase in serum EPO after 4 weeks of empagliflozin treatment raises the possibility that part of the increase in hematocrit, which sets in rapidly and is sustained for as long as treatment continues (32), may be the result of enhanced erythropoiesis. In a previous study using dapagliflozin in 10 patients with T2D (33), a 6% increase in red blood cell mass, as measured by the direct ^{51}Cr-labeled erythrocyte technique, was detected in concomitance with a transient rise in serum EPO levels. Regulation of EPO production by interstitial renal fibroblasts involves multiple factors (oxygen sensing through hypoxia-inducible factor, adenosine, and renin among others) (34). In our data, the correlation between hemoglobin and EPO levels (Supplementary Fig. 5) does suggest that the release of the hormone responds to relative hypoxemia in the renal medulla. Thus, with SGLT2 inhibition, sodium escaping proximal reabsorption may impose a work overload on the distal tubule, resulting in a transient increase in oxygen consumption and reduction in oxygen tension in the medulla (35). Enhanced erythropoiesis could then follow and raise the oxygen-carrying capacity of perfusing blood, thereby re-establishing appropriate, if not better, kidney oxygenation (36). However, the precise sequence of

events starting from enhanced glycosuria to oxygen availability and stimulation of EPO production needs to be elucidated.

In summary, we found that the physiological response to SGLT2 inhibition in patients with T2D and preserved renal function as well as in subjects without diabetes includes an increase in the absolute and fractional excretion of β-HB, lactate, and sodium, which quantitatively track with glycosuria. These findings, and the increase in EPO production, indicate that substantial glucose loss through joint inhibition of glucose and sodium reabsorption in the proximal tubule induces multiple changes in renal metabolism that, taken together, may be beneficial for kidney function in the long term.

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Author Contributions. E.F. and E.M. designed experiments, analyzed data, performed all statistical analyses, contributed to discussions, and wrote the manuscript. S.B., S.F., B.A., E.B., and A.C. carried out all laboratory determinations. All of the authors edited the manuscript. E.F. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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