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Research Article

# SGLT2 inhibition versus sulfonylurea treatment effects on electrolyte and acid–base balance: secondary analysis of a clinical trial reaching glycemic equipoise: Tubular effects of SGLT2 inhibition in Type 2 diabetes

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Sodium–glucose transporter (SGLT)2 inhibitors increase plasma magnesium and plasma phosphate and may cause ketoacidosis, but the contribution of improved glycemic control to these observations as well as effects on other electrolytes and acid–base parameters remain unknown. Therefore, our objective was to compare the effects of SGLT2 inhibitors dapagliflozin and sulfonylurea gliclazide on plasma electrolytes, urinary electrolyte excretion, and acid–base balance in people with Type 2 diabetes (T2D). We assessed the effects of dapagliflozin and gliclazide treatment on plasma electrolytes and bicarbonate, 24-hour urinary pH and excretions of electrolytes, ammonium, citrate, and sulfate in 44 metformin-treated people with T2D and preserved kidney function. Compared with gliclazide, dapagliflozin increased plasma chloride by 1.4 mmol/l (95% CI 0.4–2.4), plasma magnesium by 0.03 mmol/l (95% CI 0.01–0.06), and plasma sulfate by 0.02 mmol/l (95% CI 0.01–0.04). Compared with baseline, dapagliflozin also significantly increased plasma phosphate, but the same trend was observed with gliclazide. From baseline to week 12, dapagliflozin increased the urinary excretion of citrate by  $0.93 \pm 1.72$  mmol/day, acetoacetate by 48  $\mu$ mol/day (IQR 17–138), and  $\beta$ -hydroxybutyrate by 59  $\mu$ mol/day (IQR 0–336), without disturbing acid–base balance. In conclusion, dapagliflozin increases plasma magnesium, chloride, and sulfate compared with gliclazide, while reaching similar glucose-lowering in people with T2D. Dapagliflozin also increases urinary ketone excretion without changing acid–base balance. Therefore, the increase in urinary citrate excretion by dapagliflozin may reflect an effect on cellular metabolism including the tricarboxylic acid cycle. This potentially contributes to kidney protection.

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## Introduction

Sodium–glucose transporter (SGLT)2 inhibitors was the first glucose lowering drug-class to improve cardiovascular and kidney outcomes in people with Type 2 diabetes (T2D) at high risk for cardiovascular disease [1]. SGLT2 inhibitors lower plasma glucose levels by reducing sodium-coupled glucose reuptake in the proximal tubule [2]. Although glycosuria is the main change induced by SGLT2 inhibition, it also alters the tubular handling of other electrolytes including sodium. SGLT2 inhibitors have been found to increase certain plasma electrolytes, including magnesium and phosphate [3]. SGLT2 inhibition also predisposes people with diabetes to euglycemic diabetic ketoacidosis (DKA) [1]. However, an integrative assessment of the effects of SGLT2 inhibition on electrolyte and acid–base balance in people with T2D is lacking. Plasma glucose concentrations also influence tubular transport of different solutes and water [4]. Therefore, the aim of the present study was to analyze the effects of SGLT2 inhibition on electrolyte and acid–base balance compared with sulfonylurea treatment with equal glucose lowering. This allowed us to dissect the direct tubular effects of SGLT2 inhibition from those related to glycaemia.

## Materials and methods

### Trial design

This is a pre-specified secondary analysis of the RED (Renoprotective Effects of Dapagliflozin in Type 2 Diabetes) trial; a phase-4, monocenter, randomized, double-blind, comparator-controlled, parallel-group, intervention trial [5]. The trial was originally designed to determine treatment-induced changes in gold-standard measured renal hemodynamics after 12-week dapagliflozin and gliclazide therapy in people with T2D. After a 4-week run-in period, participants were randomized to dapagliflozin 10 mg or gliclazide 30 mg (block-size of 4, performed by an independent trial pharmacist using computer-generated numbers), and treated for 12 weeks. Before and after treatment, participants collected urine during a 24-h period that ended on the night before a visit to the clinical research unit. Participants were given oral and written instructions on how to collect a 24-h urine-sample, and were instructed to postpone collection in case of fever or urinary tract infection, and to refrain from strenuous exercise during the collection-period. After an overnight fast, blood samples were obtained at 07:30 AM. The week before the test visits, participants adhered to normal sodium (9–12 g/day) and protein (1.5–2.0 g/kg/day) diets, in order to minimize variation. Written informed consent was obtained from all participants before any trial-related activities. The study complied with the Declaration of Helsinki and Good Clinical Practice guidelines and was registered at ClinicalTrials.gov (ID: NCT02682563) [5].

### Study population

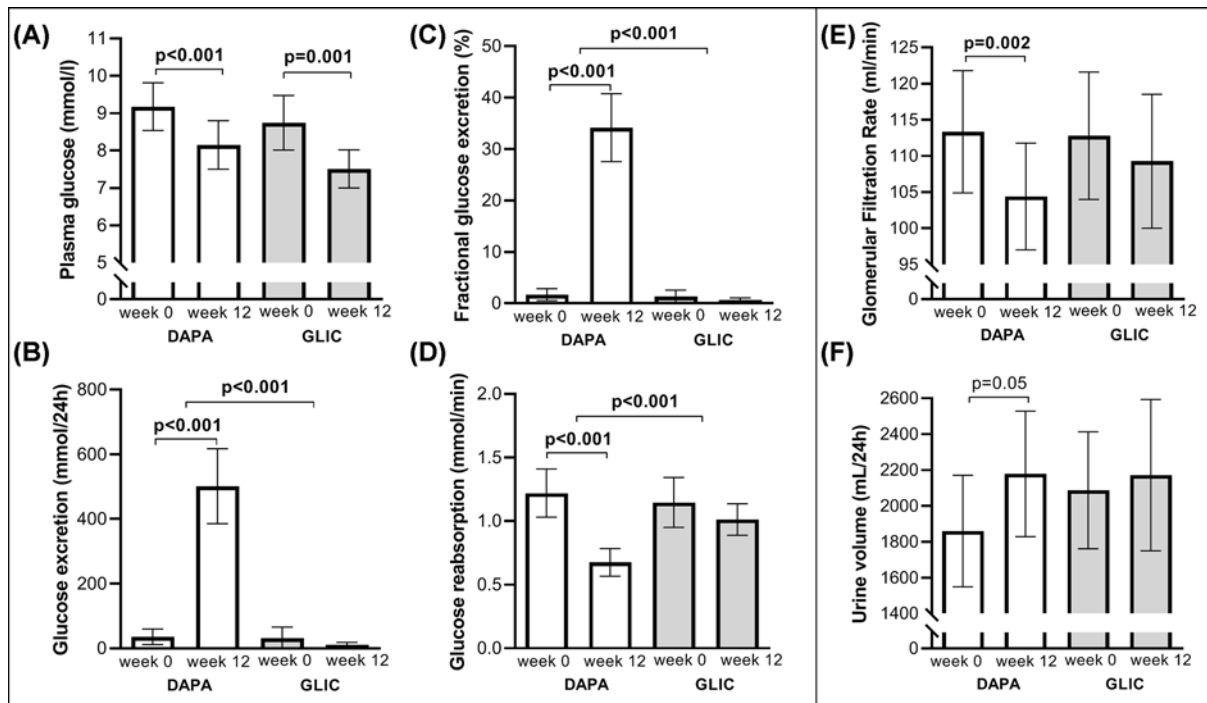
Participants were recruited from our study database and by advertisements in local newspapers. As described [5], we included Caucasian (to maximize homogeneity) men and postmenopausal women, aged 35–75 years, with an HbA1c from 6.5% to 9.0% (48–75 mmol/mol) and a body mass index >25 kg/m<sup>2</sup>. Inclusion criteria included metformin monotherapy (stable dose for ≥ 3 months) and a well-controlled blood pressure (i.e., <140/90 mm Hg). Exclusion criteria included the use of diuretics, an estimated glomerular filtration rate (GFR) <60 ml/min/1.73 m<sup>2</sup>, and macro-albuminuria (i.e. ACR >300 mg/g).

### Outcome measures

The endpoints of this pre-specified secondary analysis of the RED trial [5] were dapagliflozin- and gliclazide-induced changes in plasma concentrations and urinary excretion of electrolytes and ketones, and acid–base balance.

### Measurements and calculations

GFR was measured by plasma inulin/iohexol clearances, as described [5]. Fractional excretions were calculated using fasting plasma and 24 h urine creatinine concentrations. Plasma and urinary sodium, potassium, chloride, magnesium, calcium, phosphate, urea, creatinine, bicarbonate, glucose, and uric acid were all measured using routine chemistry methods on a Cobas 8000 routine analyser (Roche diagnostics, Mannheim Germany). Creatinine was measured using the enzymatic method. Urine pH was measured by with the Edge benchtop pH-meter HI2020-02 with CAL Check. Plasma pH and bicarbonate were measured in heparinized venous blood by the ABL800 FLEX blood gas analyzer (type ABL825; Radiometer, Zoetermeer, the Netherlands). Plasma and urinary sulfate were measured by means of a validated ion-exchange chromatography assay with conductivity detection (Metrohm, Herisau, Switzerland) [6]. Citrate in urine was determined by an enzymatic method (Instruchemie, Delfzijl, The Netherlands) on a ABX Pentra 400 clinical chemistry analyzer (Horiba, Montpellier, France). Urinary ammonium was measured in triplicate using the Berthelot method [7,8]. Plasma and urine acetoacetate was measured in plasma and urine samples by an automated spectrophotometric enzymatic method on a Beckman UniCel<sup>®</sup> DXC600 Synchron<sup>®</sup> Analyzer (Fullerton,



**Figure 1. The effects of dapagliflozin and gliclazide on glucose handling**

(A) fasting plasma glucose, (B) absolute urinary glucose excretion, (C) fractional urinary glucose excretion, (D) glucose reabsorption, (E) glomerular filtration rate, (F) urine volume. Data were analyzed with paired *t*-tests within-group and by baseline corrected linear regression analysis between-group. Data are represented as mean and 95% confidence intervals, and only statistically significant *P*-values are shown. Abbreviations: DAPA, dapagliflozin; GLIC, gliclazide.

CA), and  $\beta$ -hydroxybutyrate (BHB) concentrations were measured on a Synchron system CX4 (Beckman Coulter, Fullerton, CA) [9].

The milliequivalents of each measured anion and cation in the electrogram (Figure 4) were calculated by multiplying the charge of the ion, if needed calculated from *pK* of the ion and urine pH, with the absolute excretion in the 24-h urine collections.

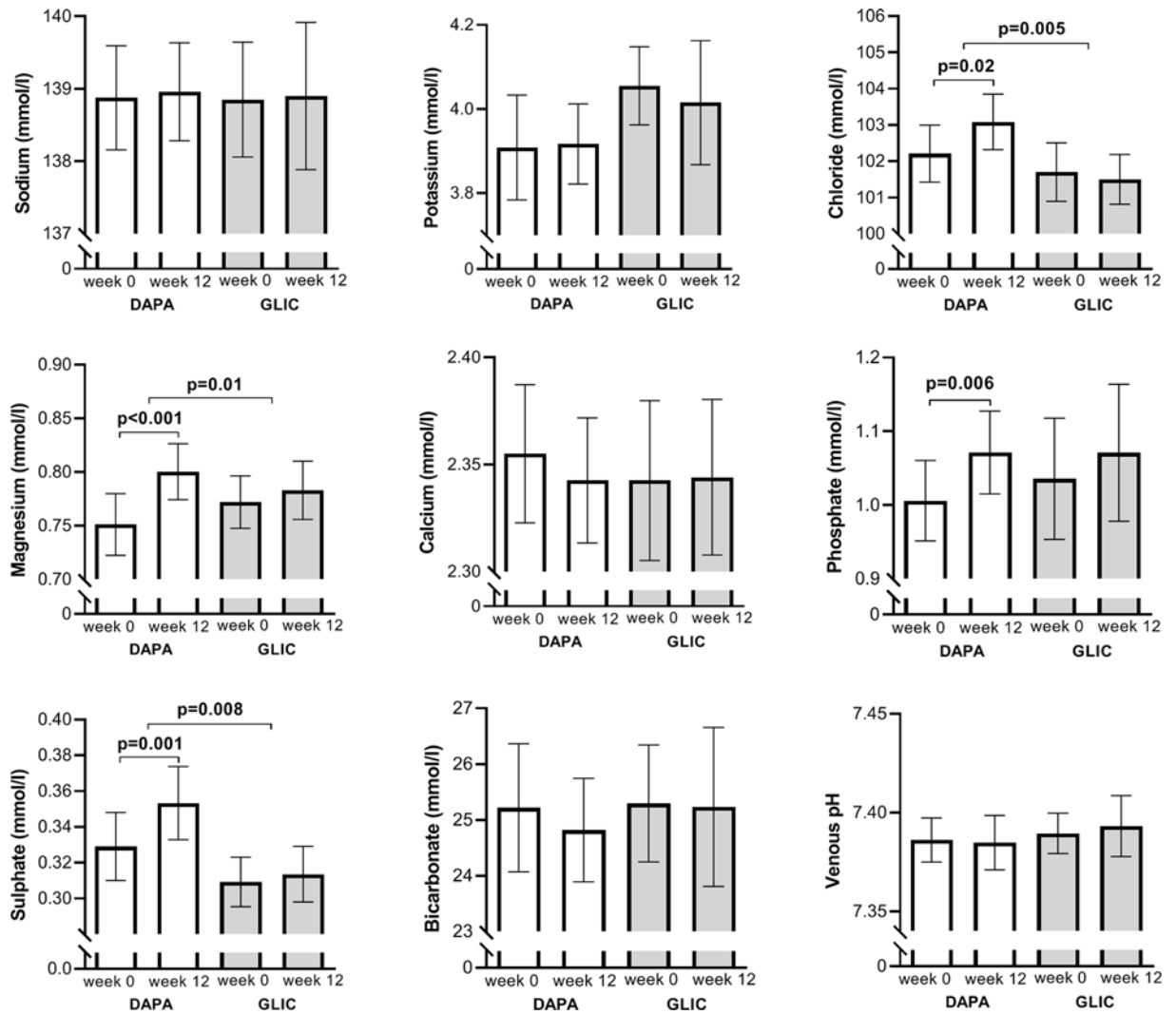
## Statistical analysis

Statistical analyses were performed in the per protocol population using SPSS 24.0 (IBM SPSS, Chicago, IL). Multivariable linear regression models were used to analyze and compare the effects of dapagliflozin and gliclazide. Corresponding baseline values were added as independent variables to correct for potential between-group baseline differences. Within-group comparisons were performed using paired samples *t*-tests (normally distributed data) or Wilcoxon signed-rank tests (non-normally distributed data). Significance was set at a 2-sided  $\alpha$ -level of < 0.05. Statistically non-significant *P*-values are not shown in figures. Data are presented as mean  $\pm$  SD for normally distributed variables, median (IQR) for non-normally distributed variables, or as baseline corrected mean difference with a two-sided 95% confidence interval (CI) unless otherwise specified. Since we aimed to provide an inclusive overview of the treatment effects, we did not correct for multiple testing to minimize the risk of type 2 error.

## Results

### Clinical trial results

As previously described [5], 44 people were randomized to 12-week treatment with dapagliflozin (*n*=24) or gliclazide (*n*=20). Baseline characteristics were well balanced between treatment groups (Table 1) and medication remained unchanged during the treatment period. No (serious) adverse events, including hypoglycemic events, episodes of acute kidney injury or electrolyte disturbances occurred in either group and no participants dropped out after treatment commenced. There were five genital fungal infections in the dapagliflozin group versus none in the gliclazide group.



**Figure 2.** The effects of dapagliflozin and gliclazide on fasting plasma electrolyte and bicarbonate concentrations. Data were analyzed with paired *t*-tests within-group and by baseline corrected linear regression analysis between-group. Data are represented as mean and 95% confidence intervals, and only statistically significant *P*-values are shown. Abbreviations: DAPA, dapagliflozin; GLIC, gliclazide.

**Table 1** Baseline characteristics

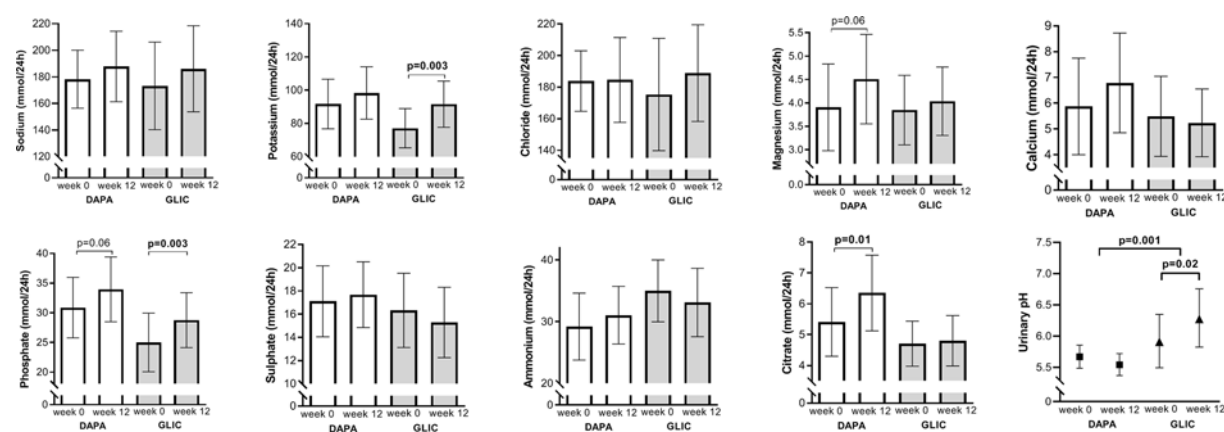
Characteristic	Dapagliflozin (n=24)	Gliclazide (n=20)
Age, years	63 ± 7	63 ± 7
Male, n (%)	19 (79)	15 (75)
Current smoker, n (%)	3 (13)	1 (5)
Alcohol intake, units/week	5 (IQR 2–13)	4 (IQR 2–8)
ASCVD, n (%)	4 (17)	1 (5)
Hypertension, n (%)	16 (67)	16 (80)
eGFR (CKD-EPI), ml/min/1.73 m <sup>2</sup>	85 ± 13	89 ± 19
UACR, mg/mmol	11 (IQR 6–17)	12 (IQR 4–17)
Diabetes duration, years	9.8 ± 4.1	10.7 ± 7.3
Metformin dose, mg	1556 ± 736	1585 ± 765
Statin, n (%)	16 (67)	14 (70)
RAS inhibitor, n (%)	16 (67)	16 (80)

Data are represented as mean ± SD, frequency, or median (IQR). Abbreviations: ASCVD, atherosclerotic cardiovascular disease; eGFR, estimated glomerular filtration rate; CKD-EPI, chronic kidney disease epidemiology collaboration; UACR, Urinary albumin-creatinine ratio; RAS, renin-angiotensin system.

**Table 2** Metabolic and cardiovascular measures

Variable	Dapagliflozin (n=24)			Gliclazide (n=20)			Corrected mean difference dapagliflozin – gliclazide (95% CI) and P-value	
	Week 0	Week 12	Within-group	Week 0	Week 12	Within-group		
Body weight, kg	96.6 ± 17.9	93.7 ± 16.9	<b>P&lt;0.001</b>	98.5 ± 17.9	99.6 ± 18.3	<b>P=0.001</b>	-4.03 (-2.81 to -5.24)	<b>P&lt;0.001</b>
Body mass index, kg/m <sup>2</sup>	30.8 ± 3.9	29.8 ± 3.7	<b>P&lt;0.001</b>	31.6 ± 3.9	31.9 ± 4.1	<b>P=0.001</b>	-1.30 (-0.93 to -1.67)	<b>P&lt;0.001</b>
Systolic blood pressure, mmHg	137.7 ± 13.6	129.2 ± 10.7	<b>P=0.001</b>	131.6 ± 11.4	131.1 ± 11.8	<b>P=0.83</b>	-5.3 (-10.9 to 0.2)	<b>P=0.06</b>
Diastolic blood pressure, mmHg	84.4 ± 5.7	80.4 ± 5.6	<b>P&lt;0.001</b>	81.7 ± 5.4	80.9 ± 6.5	<b>P=0.51</b>	-2.6 (-5.3 to 0.2)	<b>P=0.06</b>
Heart rate, BPM	65.0 ± 9.9	63.2 ± 7.9	<b>P=0.27</b>	69.4 ± 11.6	69.2 ± 11.3	<b>P=0.90</b>	-3.1 (-7.2 to 1.0)	<b>P=0.14</b>
Hematocrit, %	40.7 ± 3.3	42.5 ± 2.9	<b>P&lt;0.001</b>	40.2 ± 2.5	40.2 ± 2.8	<b>P=0.89</b>	1.8 (0.9 to 2.7)	<b>P&lt;0.001</b>
Fasting insulin, pmol/l	72.7 ± 58.3	54.3 ± 31.2	<b>P&lt;0.05</b>	55.5 ± 19.6	58.2 ± 23.1	<b>P=0.45</b>	-12.8 (-3.3 to -22.3)	<b>P=0.01</b>
HbA1c, %	7.39 ± 0.66	6.92 ± 0.56	<b>P&lt;0.001</b>	7.36 ± 0.60	6.71 ± 0.49	<b>P&lt;0.001</b>	0.19 (-0.05 to 0.43)	<b>P=0.12</b>
Fasting plasma glucose, mmol/l	9.2 ± 1.5	8.2 ± 1.5	<b>P&lt;0.001</b>	8.8 ± 1.6	7.5 ± 1.1	<b>P=0.001</b>	0.4 (-0.3 to 1.1)	<b>P=0.23</b>
Glomerular filtration rate, ml/min	113 ± 20	104 ± 17	<b>P&lt;0.05</b>	113 ± 19	109 ± 20	<b>P=0.12</b>	-5 (-12 to 1)	<b>P=0.11</b>

Multivariable linear regression models were used to examine week 0-corrected dapagliflozin- compared with gliclazide-induced effects. Paired *t*-tests or Wilcoxon signed rank tests were used for within-group comparisons. Data are represented as mean ± SD. Significant differences indicated in bold font. Abbreviation: HbA1c, glycated hemoglobin.



**Figure 3.** The effects of dapagliflozin and gliclazide on 24-h urinary excretion of electrolytes, ammonium, and citrate, and urinary pH

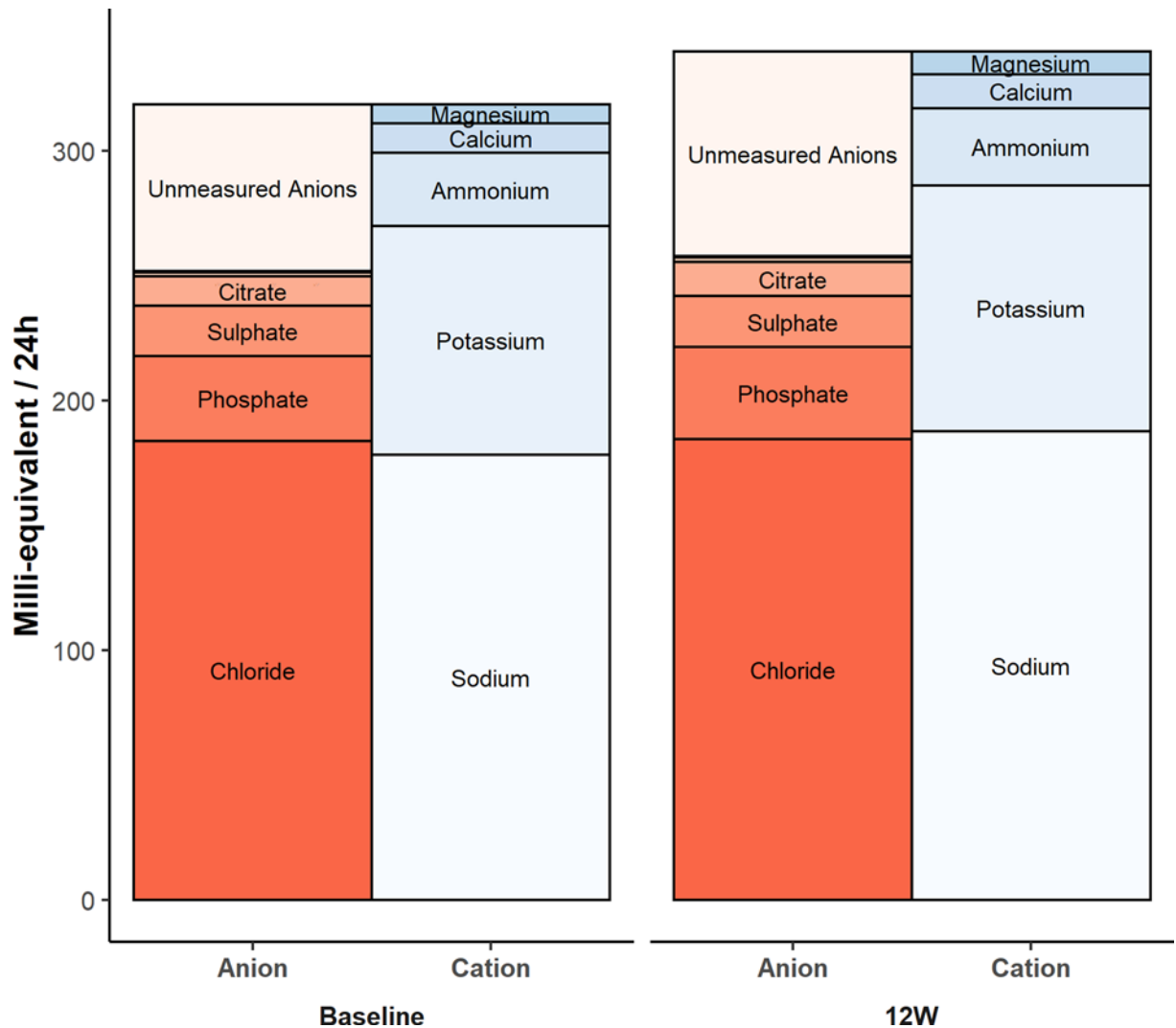
Data were analyzed with paired *t*-tests or wilcoxon signed-rank test within-group and by baseline corrected linear regression analysis between-group. Data are represented as mean, or geometric mean (pH) and 95% confidence intervals. Only statistically significant *P*-values are shown. Abbreviations: DAPA, dapagliflozin; GLIC, gliclazide.

### Dapagliflozin effects on glucose handling, urinary volume, and mGFR

Dapagliflozin and gliclazide both decreased HbA1c and fasting plasma glucose (FPG), with no between-group differences (Figure 1A and Table 2). Dapagliflozin increased both the absolute (Figure 1B) and fractional (Figure 1C) urinary excretion of glucose, and reduced glucose reabsorption by 44 ± 19% (Figure 1D; *P*<0.001 for all), both from baseline and versus gliclazide. From baseline to week 12, dapagliflozin reduced measured GFR by 9 ± 12 ml/min (*P*=0.002; Figure 1E and Table 2) [5]. Finally, dapagliflozin increased urine volume from baseline by 319 ± 756 ml/day (*P*=0.05; Figure 1F). Gliclazide had no significant effects on renal glucose handling and GFR.

### Dapagliflozin effects on plasma electrolytes, bicarbonate, and ketones

Compared with gliclazide, dapagliflozin increased plasma chloride by 1.4 mmol/l (95% CI 0.4–2.4; *P*=0.005), plasma magnesium by 0.03 mmol/l (95% CI 0.01–0.06; *P*=0.01), and plasma sulfate by 0.02 mmol/l (95% CI 0.01–0.04;



**Figure 4.** The effects of dapagliflozin on the urine electrogram

Miliequivalents of each measured anion and cation were calculated by multiplying the charge of the ion, if needed calculated from  $pK$  of the ion and urine pH, with the absolute excretion in the 24-h urine collections. The anions acetoacetate,  $\beta$ -hydroxybutyrate and uric acid are shown but not labeled in this figure due to their low excretions.

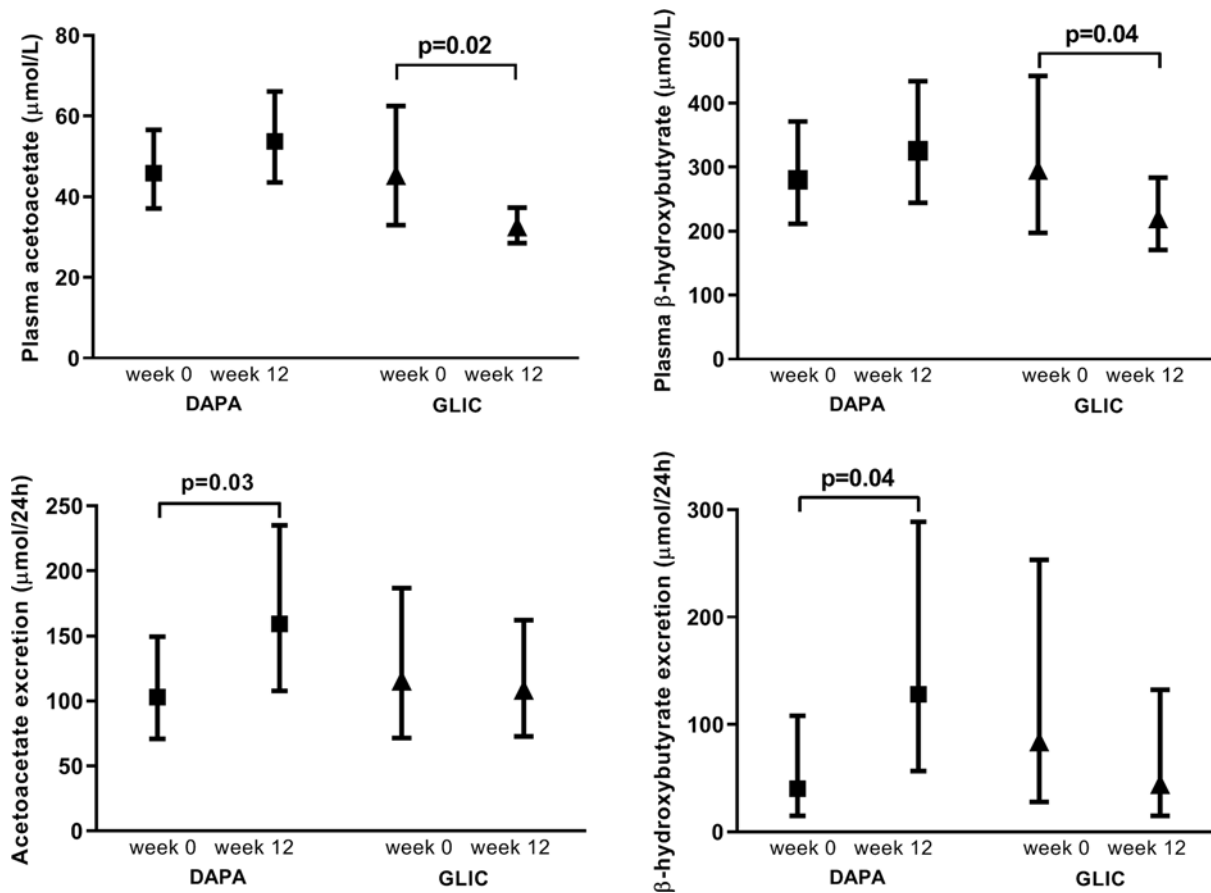
$P=0.008$ ). From baseline, dapagliflozin also increased plasma phosphate by  $0.07 \pm 0.10$  mmol/l ( $P=0.006$ ), but not versus gliclazide. Finally, no changes in plasma bicarbonate and plasma pH were observed in either group. (Figure 2).

### Dapagliflozin effects on urinary electrolyte excretion and urinary electrogram

The small changes in plasma electrolyte concentrations were not accompanied by differences in 24-h (Figure 3) or fractional (Supplementary Table S1) excretions, or urinary pH. Dapagliflozin did induce a  $0.93 \pm 1.72$  mmol/day ( $P=0.01$ ) increase in urinary citrate excretion versus baseline. Analysis of the urinary electrogram showed that dapagliflozin increased urinary solute excretion but the composition of the urinary electrogram remained unchanged (Figure 4).

### Dapagliflozin effects on plasma and urine ketones

Dapagliflozin increase plasma acetoacetate and  $\beta$ -hydroxybutyrate non-significantly, and increased urinary acetoacetate excretion by  $48 \mu\text{mol/day}$  (IQR 17–138;  $P=0.03$ ) and  $\beta$ -hydroxybutyrate by  $59 \mu\text{mol/day}$  (IQR 0–336;



**Figure 5. The effects of dapagliflozin and gliclazide on fasting plasma ketone levels and urinary ketone excretion**

Data were analyzed with Wilcoxon rank-signed tests within-group and by baseline corrected linear regression analysis between-group. Data are represented as geometric mean and 95% confidence intervals, and only statistically significant *P*-values are shown. Abbreviations: DAPA, dapagliflozin; GLIC, gliclazide.

*P*=0.04). In contrast, gliclazide significantly decreased plasma acetoacetate by 8 μmol/l (IQR -33 to 0; *P*=0.02), and β-hydroxybutyrate by 25 μmol/l (IQR -328 to 31, *P*=0.04), without affecting urinary ketone excretion (Figure 5).

## Discussion

In this pre-specified secondary analysis of the RED trial, we analyzed the effects of dapagliflozin on electrolyte and acid–base balance in people with T2D and preserved renal function. Because the effects of dapagliflozin were compared with the sulfonylurea gliclazide, we were able to study these effects while plasma glucose levels were equally lowered. We confirm that dapagliflozin causes small but significant increases in plasma magnesium and show for the first time that this is also the case for plasma chloride and sulfate. Of interest, the dapagliflozin-induced increase in plasma phosphate appeared to depend on glycemia, because the same trend was observed with gliclazide. Increases in plasma ketone concentrations were likely prevented by an increase in urinary ketone excretion with dapagliflozin. Accordingly, there were no effects on systemic acid–base balance, which was also supported by the absence of changes in urinary ammonium excretion. We did observe an increase in urinary citrate excretion, which is a novel finding. Below we will discuss possible explanations for these findings, including their potential clinical relevance.

SGLT2 inhibition has been consistently found to increase plasma magnesium concentrations in people with T2D [10,11], although the mechanism remains unclear. Hypomagnesemia and urinary magnesium wasting are associated with T2D, possibly due to reduced activity of TRPM6, a transient receptor potential cation channel in the distal convoluted tubule, and may be secondary to insulin resistance [12,13]. We recently reported that clamp-measured insulin resistance was unaffected by dapagliflozin or gliclazide, making improved insulin resistance a less likely mediator [14]. However, since we did not measure glucose disposal and endogenous glucose production separately, and



others have found SGLT2 inhibitor-induced improvements in insulin sensitivity [15], we cannot fully discard the contribution of improved insulin resistance. Glucagon reduces magnesium reabsorption in the proximal tubule, but strongly increases magnesium reabsorption in the thick ascending limb and distal convoluted tubule [16]. Furthermore, plasma insulin may cause a redistribution of magnesium from the extracellular to the intracellular compartment [17]. The increased plasma magnesium concentration upon SGLT2 inhibition could thus be caused by the increase in glucagon/insulin ratios [18]. Another contributing factor to the increased magnesium reabsorption is an increased chloride reabsorption leading to an increased lumen-positive potential and increase in paracellular reabsorption of magnesium [19]. The increase in plasma magnesium following SGLT2 inhibition is modest. Yet, the drug class is currently under investigation as a potential treatment for refractory hypomagnesemia [20].

Dapagliflozin increased plasma sulfate and chloride. The majority of filtered sulfate is reabsorbed in the proximal tubule, where secondary active sulfate uptake takes place via sodium-coupled sulfate transport, driven by the electrochemical gradient for sodium across the luminal membrane [21]. Because SGLT2 inhibition increases the luminal sodium gradient, this may explain the increase in plasma sulfate concentrations. In patients with heart failure, sulfate clearance is related to diuretic use, creatinine clearance, and sodium excretion [22]. Thus, dapagliflozin may increase plasma sulfate, because of its diuretic properties and its effects on GFR and sodium excretion. As shown by the urine electrogram, the increase in plasma chloride concentrations is due to increased chloride reabsorption coupled to other ions than sodium. In the proximal tubule chloride is mainly reabsorbed paracellularly in the S3 segment. Therefore, a shift of solute transport from the S1/S2 segments to the S3 segment could explain the increase in plasma chloride [23]. Effects of dapagliflozin on transcellular chloride transport seem less likely, as these are coupled to bicarbonate, and we found no effects on plasma bicarbonate [24]. Although disturbances in plasma chloride levels have been implicated in worsened outcome in heart failure (hypochloremia) [25] or renal disease (hyperchloremia) [26], chloride levels remained within the normal range in our study and the observed increase is probably too small to affect clinical outcome.

Plasma phosphate levels were increased by dapagliflozin after 12 weeks of treatment. It is believed that sodium-coupled phosphate reabsorption (via NaPi2) is increased in the proximal tubule by the higher availability of sodium following SGLT2 inhibition [27]. Increases in phosphate reabsorption and serum phosphate levels, together with increases in plasma parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23) and reductions in 1,25-dihydroxyvitamin D levels, have been previously found, both in healthy volunteers treated with canagliflozin [28] and in people with T2D and CKD stage 2 to 4 treated with dapagliflozin [29]. Because plasma phosphate also increased with gliclazide, we found no between group differences, which might indicate a glucose-dependent mechanism. It is however also possible that gliclazide increases plasma phosphate levels via increasing insulin levels [30].

It has been hypothesized that increased PTH and FGF23 secretion following increased phosphate reabsorption upon SGLT2 inhibition may be detrimental for bone health [27]. However, a dedicated analysis found no effect of dapagliflozin versus placebo on markers of bone formation/resorption, bone mineral density, or fractures, while PTH and FGF23 also remained unchanged despite a slight increase in plasma phosphate [31]. Although bone fractures have been added to the label of SGLT2 inhibitor drug-class as an adverse effect, data remain conflicting. Dapagliflozin increased fracture risk versus placebo in a phase III trial including 252 people with T2D and stage 3 CKD [32], as did canagliflozin in the post-marketing CANVAS program [33]. In contrast, fracture risk was not increased in other outcome trials with SGLT2 inhibitors, including the renal CREDENCE trial using canagliflozin [34–36].

SGLT2 inhibitors increase the risk for diabetic ketoacidosis, which is a major problem in people with T1D, where absolute risks up to 6% have been found [37]. Although absolute risks are extremely low in people with T2D (0.005–0.6%), especially those with sufficient endogenous insulin production, relative risks are significantly increased [33,35,36]. In addition to increased ketogenesis [18], it has been hypothesized that SGLT2 inhibition might reduce urinary ketone excretion. The increased luminal sodium concentration could theoretically drive the reabsorption of filtered ketone bodies via the proximal tubule transporters *SLC5A8* (S3 segment) and *SLC5A12* (S1, S2, and S3 segments) [38]. In contrast to this hypothesis, we observed an increase, rather than a decrease, in urinary ketone excretion. These results are in agreement with a previous study, although this study did not report the effects on acetoacetate [9]. The increased delivery of ketone bodies to the proximal tubule seemingly contributes to attenuated diabetes associated kidney damage, potentially via correcting mTORC1 hyperactivation [39]. Furthermore, we observed no changes in plasma ketones following dapagliflozin, despite increased urinary glucose excretion and decreased plasma insulin concentrations following dapagliflozin. Accordingly, venous and urinary pH, venous bicarbonate levels, and urinary ammonium excretion were unaffected by both treatments. Dapagliflozin did increase urinary citrate excretion. The proximal tubule regulates citrate reabsorption in response to changes in systemic acid–base balance [40], but because we did not observe these changes, other explanations must apply. Since citrate can be released from bone, increased bone resorption, as discussed above, might be an explanation [41]. Of interest, citrate accumulates

in the kidney cortex of diabetic mice, and this effect is nullified by SGLT2 inhibition [42]. The normalization of accumulated tricarboxylic acid cycle intermediates such as citrate by SGLT2 inhibitors may reduce oxidative stress and thereby contribute to kidney protection. Indeed, patients with diabetes and low urinary citrate experience more rapid progression of CKD [43,44]. The use of urine metabolomics in future research may be helpful to further explore this pathway.

The strength of the present study is the randomized and double-blind design of the trial with the incorporation of an active comparator group providing the possibility to compare the effects of dapagliflozin with a control-group that reached similar glucose lowering. However, the present study also has a number of limitations. First, we excluded patients with heart failure or CKD, and those using diuretics. The results can therefore not be translated to patients with these comorbidities in which electrolyte and acid-base disorders are common. Second, we did not measure plasma citrate concentrations. Third, it is to be expected that a new electrolyte balance is reached after 12 weeks and that urinary excretion mainly depends on dietary intake. Although we standardized sodium and protein intake, diet was not fully controlled. Fourth, the study was not primarily powered for the analysis presented here. Therefore, we provide an overview of the observed effects, with statistical testing within groups and between groups, without rigorous correction for multiple testing. This increased the risk of type 1 error. Finally, we were unable to pinpoint which tubular mechanisms were responsible for the changes in plasma electrolyte concentrations, and propose that experimental studies are required to resolve this. Of note, dapagliflozin reduced blood pressure, GFR, body weight and increased hematocrit, while gliclazide did not. We have previously described the mechanisms explaining these effects [2,5], and cannot exclude that these effects might have influenced our results. Lastly, despite the fact that randomization rendered well balanced groups, blood pressure and plasma insulin levels differed somewhat at baseline. Although we statistically corrected for baseline values, this potentially affected the physiological effects of both drugs.

In conclusion, dapagliflozin caused small increases in plasma chloride, magnesium, and sulfate, while gliclazide did not despite similar glucose lowering. Dapagliflozin also increased urinary ketone and citrate excretion. Especially the increase in urinary citrate excretion by dapagliflozin is a novel finding that may reflect an effect on cellular metabolism including the tricarboxylic acid cycle. This could potentially contribute to kidney protection by this drug-class.

## Clinical perspectives

- SGLT2 inhibitors target hyperglycemia by limiting sodium-coupled glucose uptake in the proximal tubule in people with Type 2 diabetes and have been shown to improve cardiorenal outcome. They are increasingly being used in clinical practice, but an integrative assessment of the effects of SGLT2 inhibition on electrolyte and acid–base balance in people with T2D was lacking. Especially in comparison with an equally potent glucose lowering agent.
- Dapagliflozin caused small increases in plasma chloride, magnesium, and sulfate, while gliclazide did not despite similar glucose lowering. Dapagliflozin also increased urinary ketone and citrate excretion.
- Our findings help deepen our understanding of the tubular effects of this novel drug class. Especially, the increase in urinary citrate excretion by dapagliflozin is a novel finding that may reflect an effect on cellular metabolism including the tricarboxylic acid cycle. This could potentially contribute to the observed kidney protection.

## Data Availability

We are unable to share our data due to patient privacy.

## Competing Interests

E.v.B., F.G., A.P., S.B., A.H.J.D., M.v.B., M.K., J.A.J., and E.H. have no conflicts of interest. M.M. is a consultant and speaker for Eli Lilly & Co, Sanofi and Novo Nordisk; all honoraria are paid to his employer (Amsterdam UMC, location VUMC). D.T. reports grants from ZONMW and from Chiesi Pharmaceuticals. M.N. received an unrestricted investigator-initiated grant from Astra Zeneca on SGLT2i and lipid fluxes. E.F. received consultancy/speaker fees, outside the present work, from Boehringer Ingelheim, Eli Lilly, AstraZeneca, and Sanofi. D.v.R. has acted as a consultant and received honoraria from Boehringer Ingelheim and Lilly, Merck, Novo

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## CRedit Author Contribution

**Erik J.M. van Bommel:** Conceptualization, Resources, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Project administration. **Frank Geurts:** Resources, Methodology, Writing – original draft, Writing – review and editing. **Marcel H.A. Muskiet:** Conceptualization, Funding acquisition, Writing – review and editing. **Adrian Post:** Resources, Methodology, Writing – review and editing. **Stephan J.L. Bakker:** Resources, Supervision, Writing – review and editing. **A.H. Jan Danser:** Conceptualization, Resources, Writing – review and editing. **Daan J. Touw:** Resources, Validation, Writing – review and editing. **Miranda van Berkel:** Resources, Validation, Investigation, Writing – review and editing. **Mark H.H. Kramer:** Resources, Supervision, Writing – review and editing. **Max Nieuwdorp:** Conceptualization, Resources, Supervision, Writing – review and editing. **Ele Ferrannini:** Resources, Investigation, Writing – review and editing. **Jaap A. Joles:** Conceptualization, Resources, Data curation, Supervision, Writing – review and editing. **Ewout J. Hoorn:** Conceptualization, Resources, Supervision, Writing – review and editing. **Daniël H. van Raalte:** Conceptualization, Resources, Supervision, Funding acquisition, Investigation, Writing – review and editing.

## Trial Registration

Registered at ClinTrials.gov with study number NCT02682563.

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## Abbreviations

ASCVD, atherosclerotic cardiovascular disease; CKD-EPI, chronic kidney disease epidemiology collaboration; eGFR, estimated glomerular filtration rate; RAS, renin–angiotensin system; SGLT, sodium–glucose transporter; UACR, urinary albumin–creatinine ratio.

## References

- Zelniker, T.A., Wiviott, S.D., Raz, I. et al. (2019) SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet* **393**, 31–39, [https://doi.org/10.1016/S0140-6736\(18\)32590-X](https://doi.org/10.1016/S0140-6736(18)32590-X)
- van Bommel, E.J., Muskiet, M.H., Tonneijck, L. et al. (2017) SGLT2 Inhibition in the Diabetic Kidney-From Mechanisms to Clinical Outcome. *Clin. J. Am. Soc. Nephrol.* **12**, 700–710, <https://doi.org/10.2215/CJN.06080616>
- Filippatos, T.D., Tsimihodimos, V., Liamis, G. et al. (2018) SGLT2 inhibitors-induced electrolyte abnormalities: An analysis of the associated mechanisms. *Diab. Metab. Syndr.* **12**, 59–63, <https://doi.org/10.1016/j.dsx.2017.08.003>
- Pasquel, F.J. and Umpierrez, G.E. (2014) Hyperosmolar hyperglycemic state: a historic review of the clinical presentation, diagnosis, and treatment. *Diabetes Care* **37**, 3124–3131, <https://doi.org/10.2337/dc14-0984>
- van Bommel, E.J.M., Muskiet, M.H.A., van Baar, M.J.B. et al. (2020) The renal hemodynamic effects of the SGLT2 inhibitor dapagliflozin are caused by post-glomerular vasodilatation rather than pre-glomerular vasoconstriction in metformin-treated patients with type 2 diabetes in the randomized, double-blind RED trial. *Kidney Int.* **97**, 202–212, <https://doi.org/10.1016/j.kint.2019.09.013>
- Post, A., Minovic, I., van den Berg, E. et al. (2018) Renal sulfate reabsorption in healthy individuals and renal transplant recipients. *Physiol. Rep.* **6**, e13670, <https://doi.org/10.14814/phy2.13670>
- Hoge, J.H., Hazenberg, H.J. and Gips, C.H. (1974) Determination of ammonia in urine with an ammonium electrode and with a direct method. *Clin. Chim. Acta* **55**, 273–279, [https://doi.org/10.1016/0009-8981\(74\)90001-1](https://doi.org/10.1016/0009-8981(74)90001-1)
- Cunarro, J.A. and Weiner, M.W. (1974) A comparison of methods for measuring urinary ammonium. *Kidney Int.* **5**, 303–305, <https://doi.org/10.1038/ki.1974.41>

- 9 Ferrannini, E., Baldi, S., Frascerra, S. et al. (2017) Renal Handling of Ketones in Response to Sodium-Glucose Cotransporter 2 Inhibition in Patients With Type 2 Diabetes. *Diabetes Care* **40**, 771–776, <https://doi.org/10.2337/dc16-2724>
- 10 Weir, M.R., Kline, I., Xie, J. et al. (2014) Effect of canagliflozin on serum electrolytes in patients with type 2 diabetes in relation to estimated glomerular filtration rate (eGFR). *Curr. Med. Res. Opin.* **30**, 1759–1768, <https://doi.org/10.1185/03007995.2014.919907>
- 11 Tang, H., Zhang, X., Zhang, J. et al. (2016) Elevated serum magnesium associated with SGLT2 inhibitor use in type 2 diabetes patients: a meta-analysis of randomised controlled trials. *Diabetologia* **59**, 2546–2551, <https://doi.org/10.1007/s00125-016-4101-6>
- 12 Gommers, L.M., Hoenderop, J.G., Bindels, R.J. et al. (2016) Hypomagnesemia in Type 2 Diabetes: A Vicious Circle? *Diabetes* **65**, 3–13, <https://doi.org/10.2337/db15-1028>
- 13 Nair, A.V., Hocher, B., Verkaart, S. et al. (2012) Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 11324–11329, <https://doi.org/10.1073/pnas.1113811109>
- 14 van Bommel, E., Muskiet, M., van Baar, M. et al. (2019) 250-OR: Beta-Cell Rest Induced by Dapagliflozin Improves  $\beta$ -Cell Secretory Capacity in Type 2 Diabetes Patients: A Randomized Controlled Trial vs. Gliclazide. *Diabetes* **68**, Supplement 1, <https://doi.org/10.2337/db19-250-OR>
- 15 Merovci, A., Solis-Herrera, C., Daniele, G. et al. (2014) Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. *J. Clin. Invest.* **124**, 509–514, <https://doi.org/10.1172/JCI70704>
- 16 Bankir, L., Bouby, N., Blondeau, B. et al. (2016) Glucagon actions on the kidney revisited: possible role in potassium homeostasis. *Am. J. Physiol. Renal. Physiol.* **311**, F469–F486, <https://doi.org/10.1152/ajprenal.00560.2015>
- 17 Cianciolo, G., De Pascalis, A., Capelli, I. et al. (2019) Mineral and Electrolyte Disorders With SGLT2i Therapy. *JBMR Plus* **3**, e10242, <https://doi.org/10.1002/jbm4.10242>
- 18 Ferrannini, E., Baldi, S., Frascerra, S. et al. (2016) Shift to Fatty Substrate Utilization in Response to Sodium-Glucose Cotransporter 2 Inhibition in Subjects Without Diabetes and Patients With Type 2 Diabetes. *Diabetes* **65**, 1190–1195, <https://doi.org/10.2337/db15-1356>
- 19 Blaine, J., Chonchol, M. and Levi, M. (2015) Renal Control of Calcium, Phosphate, and Magnesium Homeostasis. *Clin. J. Am. Soc. Nephrol.* **10**, 1257–1272, <https://doi.org/10.2215/CJN.09750913>
- 20 Ray, E.C., Boyd-Shiwarski, C.R., Liu, P. et al. (2020) SGLT2 Inhibitors for Treatment of Refractory Hypomagnesemia: A Case Report of 3 Patients. *Kidney Med.* **2**, 359–364, <https://doi.org/10.1016/j.xkme.2020.01.010>
- 21 Markovich, D. (2001) Physiological roles and regulation of mammalian sulfate transporters. *Physiol. Rev.* **81**, 1499–1533, <https://doi.org/10.1152/physrev.2001.81.4.1499>
- 22 Koning, A.M., Meijers, W.C., Minovic, I. et al. (2017) The fate of sulfate in chronic heart failure. *Am. J. Physiol. Heart Circ. Physiol.* **312**, H415–H421, <https://doi.org/10.1152/ajpheart.00645.2016>
- 23 Teulon, J., Planelles, G., Sepulveda, F.V. et al. (2018) Renal Chloride Channels in Relation to Sodium Chloride Transport. *Compr. Physiol.* **9**, 301–342, <https://doi.org/10.1002/cphy.c180024>
- 24 Planelles, G. (2004) Chloride transport in the renal proximal tubule. *Pflügers Arch.* **448**, 561–570, <https://doi.org/10.1007/s00424-004-1309-y>
- 25 Ter Maaten, J.M., Damman, K., Hanberg, J.S. et al. (2016) Hypochloremia, Diuretic Resistance, and Outcome in Patients With Acute Heart Failure. *Circ. Heart Fail.* **9**, <https://doi.org/10.1161/CIRCHEARTFAILURE.116.003109>
- 26 Khatri, M., Zitovsky, J., Lee, D. et al. (2020) The association between serum chloride levels and chronic kidney disease progression: a cohort study. *BMC Nephrol.* **21**, 165, <https://doi.org/10.1186/s12882-020-01828-3>
- 27 Taylor, S.I., Blau, J.E. and Rother, K.I. (2015) Possible adverse effects of SGLT2 inhibitors on bone. *Lancet Diab. Endocrinol.* **3**, 8–10, [https://doi.org/10.1016/S2213-8587\(14\)70227-X](https://doi.org/10.1016/S2213-8587(14)70227-X)
- 28 Blau, J.E., Bauman, V., Conway, E.M. et al. (2018) Canagliflozin triggers the FGF23/1,25-dihydroxyvitamin D/PTH axis in healthy volunteers in a randomized crossover study. *JCI Insight* **3**, e99123, <https://doi.org/10.1172/jci.insight.99123>
- 29 de Jong, M.A., Petrykiv, S.I., Laverman, G.D. et al. (2019) Effects of Dapagliflozin on Circulating Markers of Phosphate Homeostasis. *Clin. J. Am. Soc. Nephrol.* **14**, 66–73, <https://doi.org/10.2215/CJN.04530418>
- 30 DeFronzo, R.A., Cooke, C.R., Andres, R. et al. (1975) The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. *J. Clin. Invest.* **55**, 845–855, <https://doi.org/10.1172/JCI107996>
- 31 Ljunggren, O., Bolinder, J., Johansson, L. et al. (2012) Dapagliflozin has no effect on markers of bone formation and resorption or bone mineral density in patients with inadequately controlled type 2 diabetes mellitus on metformin. *Diabetes Obes. Metab.* **14**, 990–999, <https://doi.org/10.1111/j.1463-1326.2012.01630.x>
- 32 Kohan, D.E., Fioretto, P., Tang, W. et al. (2014) Long-term study of patients with type 2 diabetes and moderate renal impairment shows that dapagliflozin reduces weight and blood pressure but does not improve glycemic control. *Kidney Int.* **85**, 962–971, <https://doi.org/10.1038/ki.2013.356>
- 33 Neal, B., Perkovic, V., Mahaffey, K.W. et al. (2017) Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. *N. Engl. J. Med.* **377**, 644–657, <https://doi.org/10.1056/NEJMoa1611925>
- 34 Perkovic, V., Jardine, M.J., Neal, B. et al. (2019) Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy. *N. Engl. J. Med.* **380**, 2295–2306, <https://doi.org/10.1056/NEJMoa1811744>
- 35 Wiviott, S.D., Raz, I., Bonaca, M.P. et al. (2019) Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. *N. Engl. J. Med.* **380**, 347–357, <https://doi.org/10.1056/NEJMoa1812389>
- 36 Zinman, B., Wanner, C., Lachin, J.M. et al. (2015) Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N. Engl. J. Med.* **373**, 2117–2128, <https://doi.org/10.1056/NEJMoa1504720>
- 37 Rosenstock, J., Marquard, J., Laffel, L.M. et al. (2018) Empagliflozin as Adjunctive to Insulin Therapy in Type 1 Diabetes: The EASE Trials. *Diabetes Care* **41**, 2560–2569, <https://doi.org/10.2337/dc18-1749>
- 38 Palmer, B.F., Clegg, D.J., Taylor, S.I. et al. (2016) Diabetic ketoacidosis, sodium glucose transporter-2 inhibitors and the kidney. *J. Diabetes Compli.* **30**, 1162–1166, <https://doi.org/10.1016/j.jdiacomp.2016.05.008>

- 39 Tomita, I., Kume, S., Sugahara, S. et al. (2020) SGLT2 Inhibition Mediates Protection from Diabetic Kidney Disease by Promoting Ketone Body-Induced mTORC1 Inhibition. *Cell Metab.* **32**, 404e406–419e406, <https://doi.org/10.1016/j.cmet.2020.06.020>
- 40 Zuckerman, J.M. and Assimos, D.G. (2009) Hypocitraturia: pathophysiology and medical management. *Rev. Urol.* **11**, 134–144
- 41 Granchi, D., Baldini, N., Olivieri, F.M. et al. (2019) Role of Citrate in Pathophysiology and Medical Management of Bone Diseases. *Nutrients* **11**, 2576, <https://doi.org/10.3390/nu11112576>
- 42 Tanaka, S., Sugiura, Y., Saito, H. et al. (2018) Sodium-glucose cotransporter 2 inhibition normalizes glucose metabolism and suppresses oxidative stress in the kidneys of diabetic mice. *Kidney Int.* **94**, 912–925, <https://doi.org/10.1016/j.kint.2018.04.025>
- 43 Sas, K.M., Kayampilly, P., Byun, J. et al. (2016) Tissue-specific metabolic reprogramming drives nutrient flux in diabetic complications. *JCI Insight* **1**, e86976, <https://doi.org/10.1172/jci.insight.86976>
- 44 Liu, J.J., Liu, S., Gurung, R.L. et al. (2018) Urine Tricarboxylic Acid Cycle Metabolites Predict Progressive Chronic Kidney Disease in Type 2 Diabetes. *J. Clin. Endocrinol. Metab.* **103**, 4357–4364, <https://doi.org/10.1210/jc.2018-00947>