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Effects of dapagliflozin on volume status and systemic haemodynamics in patients with chronic kidney disease without diabetes: Results from DAPASALT and DIAMOND

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Abstract

Aims: To assess the effect of sodium-glucose cotransporter-2 inhibitor dapagliflozin on natriuresis, blood pressure (BP) and volume status in patients with chronic kidney disease (CKD) without diabetes.

Materials and methods: We performed a mechanistic open-label study (DAPASALT) to evaluate the effects of dapagliflozin on 24-hour sodium excretion, 24-hour BP, extracellular volume, and markers of volume status during a standardized sodium diet (150 mmol/d) in six patients with CKD. In parallel, in a placebo-controlled double-blind crossover trial (DIAMOND), we determined the effects of 6 weeks of dapagliflozin on markers of volume status in 53 patients with CKD.

Results: In DAPASALT (mean age 65 years, mean estimated glomerular filtration rate [eGFR] 39.4 mL/min/1.73 m^2, median urine albumin:creatinine ratio [UACR] 111 mg/g), dapagliflozin did not change 24-hour sodium and volume excretion during 2 weeks of treatment. Dapagliflozin was associated with a modest increase in 24-hour glucose excretion on Day 4, which persisted at Day 14 and reversed to baseline after discontinuation. Mean 24-hour systolic BP decreased by −9.3 (95% confidence interval [CI] −19.1, 0.4) mmHg after 4 days and was sustained at Day 14 and at wash-out. Renin, angiotensin II, urinary aldosterone and copeptin levels increased from baseline. In DIAMOND (mean age 51 years, mean eGFR 59.0 mL/min/1.73 m^2, median UACR 608 mg/g), compared to placebo, dapagliflozin increased plasma renin (38.5 [95% CI 7.4, 78.8]%), aldosterone (19.1 [95% CI −5.9, 50.8]%), and copeptin levels (7.3 [95% CI 0.1, 14.5] pmol/L).

Conclusions: During a standardized sodium diet, dapagliflozin decreased BP but did not increase 24-hour sodium and volume excretion. The lack of increased natriuresis and diuresis may be attributed to activation of intra-renal compensatory mechanisms to prevent excessive water loss.

KEYWORDS
adaptive response, dapagliflozin, kidney, SGLT2 inhibitor

1 | INTRODUCTION

Sodium-glucose cotransporter-2 (SGLT2) inhibition with dapagliflozin reduces the risk of end-stage kidney disease and heart failure hospitalization, and prolongs survival in patients with chronic kidney disease (CKD) with and without type 2 diabetes (T2D).[1-4] These benefits appear to be independent of glycaemic control, which implicate non-glycaemic factors contributing to the kidney and cardio-protective effects.[5]

The underlying mechanisms for these benefits remain incompletely understood. One of the main hypothesis is that SGLT2 inhibitors provide protection through haemodynamic effects including volume contraction secondary to glycosuria and natriuresis.[6] Indeed, several studies in patients with T2D suggest that SGLT2 inhibitors promote a modest osmotic/natriuretic diuresis, which can contribute to the reduction in blood pressure and increase in haematocrit observed with these therapies.[7] In addition, blood pressure reductions and increased haematocrit concentrations have been reported in patients with CKD without diabetes treated with dapagliflozin, indicating that diuretic effects are retained in patients without diabetes.[8] However, prior studies were constrained by sodium excretion estimates from spot urine samples in lieu of repetitive 24-hour urine collections and detailed recordings of dietary intake of sodium.[9-13]

The DAPASALT trial was a carefully conducted mechanistic study in patients with and without T2D and preserved or impaired kidney function. Participants were followed during a strictly standardized sodium diet with multiple consecutive 24-hour urine collections. An analysis of this study in the stratum of patients with T2D and preserved kidney function reported a reduction in 24-hour blood pressure without significant change in 24-hour sodium excretion or volume excretion.[14] These data suggest that, in patients with preserved kidney function, compensatory kidney-specific mechanisms are activated to maintain body fluid volume and prevent dehydration. Whether these findings hold true in patients with CKD without T2D in whom blood pressure and haemodynamic status are more often sodium-dependent remains unknown. Accordingly, we studied another stratum of the DAPASALT trial consisting of patients with CKD without T2D to assess the effect of dapagliflozin on natriuresis, extracellular volume and 24-hour blood pressure regulation. Additionally, we aimed to characterize kidney adaptive responses to dapagliflozin to prevent excessive loss of water and sodium by analysing data from two prospective clinical trials, DAPASALT and DIAMOND, which enrolled patients with CKD without diabetes.

2 | MATERIALS AND METHODS

2.1 | Patients and study design

The DAPASALT trial is a mechanistic, nonrandomized, open-label study in three strata of participants consisting of patients with CKD without T2D and patients with T2D with preserved and impaired kidney function on a strict standardized sodium diet to evaluate the effects of dapagliflozin on 24-hour sodium excretion, 24-hour blood pressure, and extracellular and intracellular volume.[14] Here, we report the results of the stratum consisting of patients with CKD without T2D. In this stratum, six participants were enrolled to treatment with 10 mg dapagliflozin during a strict standardized sodium diet of 150 mmol/d. Participants started their sodium-controlled diets 6 days before dapagliflozin initiation. During the study, blood and 24-hour urine samples were collected, and body weight, 24-hour blood pressure and intra- and

extracellular volume were recorded. Participants were treated with dapagliflozin for 14 days and subsequently proceeded to a 4-day wash-out period (Figure S1). The primary outcome of the study was mean change in 24-hour sodium excretion from baseline, which was defined as the mean sodium excretion value from Day −3 to Day −1, to the start of treatment, which was defined as the mean sodium excretion value from Day 2 to Day 4.[14]

Eligible participants for this stratum of the DAPASALT study were aged between 18 and 80 years, were White, Asian or of Middle Eastern origin, had no child-bearing potential, did not have a diagnosis of diabetes, were using a stable dose of an angiotensin receptor blocker (ARB) for at least 6 weeks before enrolment, and had an estimated glomerular filtration rate (eGFR) of ≥25 and ≤50 mL/min/1.73 m^2 at the screening visit. Additionally, participants were required to have stable 24-hour urinary sodium excretion on two consecutive days prior to randomization, defined as a between difference <20%.

The DIAMOND trial was a randomized, double-blind, placebo-controlled crossover study with dapagliflozin to assess the effects of 6-week treatment with dapagliflozin on surrogate measures of kidney protection in nondiabetic participants at risk of progressive kidney function decline. Results of this study have been published previously.[8] In brief, 53 participants were randomized in a 1:1 ratio to either first treatment with dapagliflozin 10 mg/d and then placebo or vice versa (Figure S2). Eligible participants were aged between 18 and 75 years, had a diagnosis of CKD, a 24-hour urinary protein excretion value of between 500 and <3500 mg/24-hour, and an eGFR of 25 mL/min/1.73 m^2 or greater. Participants had to be on a stable dose of an angiotensin-converting enzyme inhibitor or an ARB for at least 4 weeks before randomization. Patients with type 1 diabetes or T2D were excluded. At each study visit, blood and 24-hour urine samples were collected, GFR was measured via iohexol clearance and blood pressure was recorded. The primary endpoint of the study was difference in 24-hour proteinuria between dapagliflozin and placebo.[8]

The DAPASALT and DIAMOND trials were approved by an ethics committee at each participating site and conducted according to the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. Both trials are registered with clinicaltrials.gov (NCT03152084 and NCT03190694).

2.2 | Measurements

Blood and urine samples collected in the DAPASALT trial were measured by standard in-house assays at COVANCE (Geneva, Switzerland), Amsterdam University Medical Centre and Ziekenhuisgroep Twente, as described in detail previously.[14] In the DIAMOND study, clinical chemistry measurements were performed in local laboratories as described previously.[8] Copeptin, osmolality and fractional lithium excretion were measured in the clinical chemistry laboratory of the University Medical Center, Groningen, using standard in-house assays. In both clinical trials, markers of the renin-angiotensin-aldosterone system (RAAS) were measured in the same central laboratory (Erasmus University Medical Center, Rotterdam). Extracellular and intracellular volume and body water were measured using bioimpedance spectroscopy (ImpediMed Ltd, Pinkenba, Queensland, Australia).

2.3 | Statistical analysis

Categorical variables are reported as number with percentage. Normal distributed continuous variables are reported as mean with SD or geometric mean with 95% confidence interval (CI). Variables with a skewed distribution are reported as median with interquartile range (IQR).

The sample size calculation for the DAPASALT study was performed for each of the three strata of the study separately. Enrolling 15 patients per stratum provided 80% power to detect an increase in 24-hour sodium excretion of at least 20 mmol/24-hour from baseline, assuming an SD of 25 mmol/24-hour in sodium excretion change from baseline and a two-sided alpha-level of 0.05. Because of the complex protocol and high demand on study participants, patient enrolment was ceased after six patients had completed the study. The 53 patients enrolled in the DIAMOND study provided more than 80% power to detect at least 25% difference in the primary outcome of 24-hour urine protein excretion between dapagliflozin and placebo, assuming an SD of 0.7 in log-transformed proteinuria.

Statistical analyses were performed separately in the DAPASALT and DIAMOND trials. We did not pool data from the two studies because of the differences in study design. In DAPASALT, a longitudinal repeated-measures analysis was used to estimate the change from baseline in each outcome variable. The model included a fixed effect of time, interaction between time and baseline, and continuous baseline value as covariates. An unstructured covariance matrix structure was used to model correlations among the repeated measurements. Point estimates for the least squares (LS) means at each timepoint were derived. Due to the small sample size and early termination of the DAPASALT study, no *P* values are reported but 95% CIs are provided to facilitate interpretation of the results. In the DIAMOND study a mixed effects linear regression model was used to analyse repeated measures and estimate mean differences between dapagliflozin and placebo. The model included treatment and categorical time period as fixed factors and patients as random factors. A *P* value < 0.05 was used to indicate statistical significance in the DIAMOND trial. The analyses of the DAPASALT study were prespecified and the analyses of the DIAMOND study were post hoc.

3 | RESULTS

3.1 | Patient characteristics

In DAPASALT, in the strata of patients with CKD without diabetes, 17 participants were screened, of whom six completed the study and were evaluated. In DIAMOND, 58 participants were screened, of whom 53 were randomized. During follow-up, three participants discontinued and 50 completed the study. The baseline characteristics of the six DAPASALT and 53 DIAMOND participants are shown in Table 1. The mean (SD) eGFR of DAPASALT participants was 39.4 (6.1)

TABLE 1 Characteristics of participants in the DAPASALT and DIAMOND trials

Characteristics	DAPASALT participants (n = 6)	DIAMOND participants (n = 53)
Age, years	65 (10)	51 (13)
Male sex, n (%)	4 (66.7)	36 (67.9)
Race, n (%)		
White	6 (100)	29 (55)
Asian	0 (0)	17 (32)
Other	0 (0)	7 (13)
Body weight, kg	82.9 (15.4)	83.0 (20.3)
BMI, kg/m^2	27.7 (3.9)	28.0 (5.1)
HbA1c, %	5.8 (0.4)	5.6 (0.4)
Systolic blood pressure, mmHg	125.2 (10.0)	126.0 (14.8)
Diastolic blood pressure, mmHg	72.4 (5.5)	76.2 (8.2)
eGFR, mL/min/1.73 m^2	39.4 (6.1)	59.0 (27.6)
UACR, mg/g (IQR)	111 (4, 392)	608 (387, 952)
Haemoglobin, g/L	138.1 (29.1)	134.6 (20.2)
Haematocrit, L/L	0.36 (0.20)	0.40 (0.06)
Concomitant treatment, n (%)		
ACE inhibitors or ARBs	6 (100)	53 (100)
Diuretics	0 (0)	14 (26)
Immunosuppressives	0 (0)	1 (2)
NSAIDs	0 (0)	2 (4)
Vitamin D analogues	0 (0)	12 (23)
Corticosteroids	0 (0)	4 (8)

Note: Data are reported as mean (SD), unless stated otherwise.
Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; IQR: interquartile range; UACR, urine albumin:creatinine ratio.

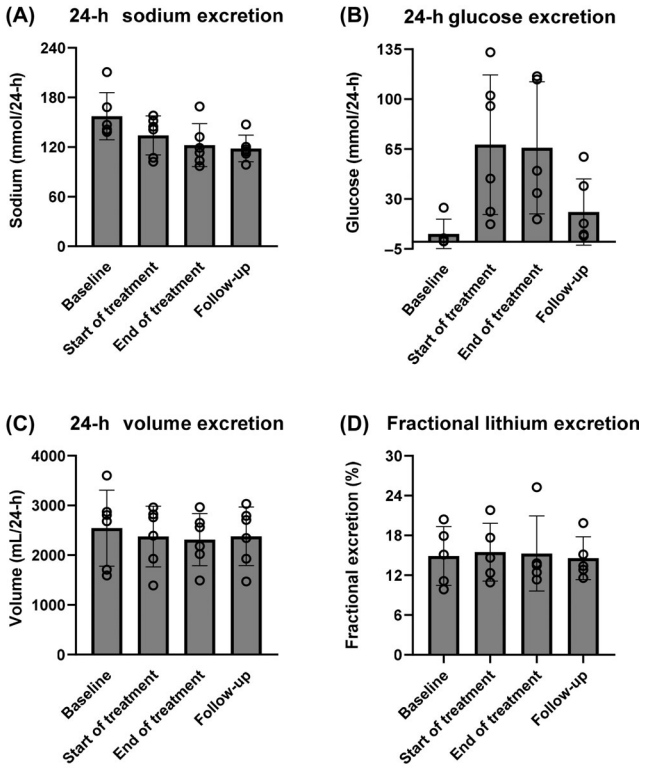

FIGURE 1 Mean (standard deviation) 24-hour sodium (A), glucose (B), volume (C) and fractional lithium excretion (D) at baseline, start of treatment, end of treatment and follow-up of the DAPASALT trial participants. Fractional lithium excretion was >2 times the upper limit of normal in one participant at baseline, which could not be explained by concomitant use of medication or other circumstances. At 4 days a large decrease in fractional lithium excretion was observed. The baseline lithium excretion in this participant was considered to be an outlying value and the patient was removed from the fractional lithium excretion analysis

mL/min/1.73 m^2 and the median (IQR) UACR was 111 (4, 392) mg/g. DIAMOND participants had a mean (SD) eGFR of 59.0 (27.6) mL/min/1.73 m^2 and a median (IQR) UACR of 608 (387, 952) mg/g.

3.2 | Effect of dapagliflozin on urinary sodium, glucose and volume

Following initiation of dapagliflozin, mean (SD) 24-hour urinary sodium excretion in DAPASALT participants decreased from 157 (28) mmol/24 hour to 134 (23) mmol/24-hour on Day 4 and 122 (26) mmol/24-hour on Day 14 (LS mean change from baseline on Day 4 and 14: −23.2 [95% CI −56.3, 9.8] mmol/24-hour and −35.0 [95% CI −74.7, 4.7] mmol/24-hour, respectively). Mean 24-hour sodium did not change during wash-out (Figure 1A). Daily 24-hour sodium excretion is shown in Figure S3. Mean (SD) 24-hour urinary glucose excretion increased during dapagliflozin treatment from 5.5 (10.3) mmol/24-hour to 68.0 (49.0) mmol/24-hour on Day 4 and 65.9 (46.4) mmol/24-hour on Day 14 (LS mean difference on Day 4 and 14: 55.7 [95% CI −4.9, 116.3] mmol/24-hour and 47.1 [95% CI −24.1, 118.3] mmol/24-hour, respectively). The 24-hour glucose excretion value returned towards baseline values during wash-out (Figure 1B). Mean (SD) 24-hour urine volume at baseline and after 4 days of dapagliflozin treatment was 2544.1 (765) mL/24-hour and 2375.2 (610) mL/24-hour and remained stable during follow-up and wash-out (Figure 1C). There was a small increase in fractional lithium excretion after 4 days (LS mean difference from baseline 0.6 [95% CI −2.2, 3.4]%; Figure 1D).

3.3 | Effect of dapagliflozin on blood pressure and extracellular and intracellular volume

Mean 24-hour systolic blood pressure (SBP) decreased from 125.2 (10) mmHg at baseline to 115.8 (11) mmHg after 4 days dapagliflozin treatment (LS mean change from baseline −9.3 [95% CI −19.1, 0.4] mmHg) and persisted until end of treatment (LS mean change from baseline −8.2 [95% CI −22.0, 5.5] mmHg). SBP did not change during wash-out (Figure 2A). Mean 24-hour diastolic blood pressure (DBP) showed a similar trend. DBP decreased after 4 days from 72.4 (6)

mmHg to 66.0 (5) mmHg (LS mean change from baseline −6.4 [95% CI −11.5, −1.3] mmHg), and persisted at the end of treatment (LS mean change from baseline −5.8 [95% CI −11.2, −0.5]) and wash-out (Figure 2B). In DIAMOND, the difference in SBP and DBP between dapagliflozin and placebo was −3.6 (95% CI −7.7, 0.4; $P = 0.078$) mmHg and −1.4 (95% CI −4.1, 1.3; $P = 0.31$) mmHg, respectively.

Extracellular and intracellular volume, and total body water decreased after 4 days of dapagliflozin treatment by 0.6 (95% CI 0.1, 1.0) L, 0.8 (95% CI −0.2, 1.8) L and 1.4 (95% CI 0.3, 2.5) L, respectively, in DAPASALT. However, at the end of treatment on Day 14 extracellular and intracellular volume and total body water had returned to baseline levels (Figure 3A,B,C).

3.4 | Effect of dapagliflozin on neurohormonal hormones

Plasma renin and angiotensin II increased after 4 days of dapagliflozin treatment. This effect persisted on Day 14 (Table 2). With respect to urinary RAAS markers in DAPASALT, aldosterone, angiotensinogen and renin increased after 4 days of dapagliflozin treatment. This effect persisted on Day 14 (Table 2). During wash-out, urinary aldosterone and urinary angiotensinogen remained stable, whereas urinary renin returned towards baseline (Table 2). In the DIAMOND study, dapagliflozin modestly increased both plasma renin and aldosterone compared to placebo, but these effects were not statistically significant (Table 3).

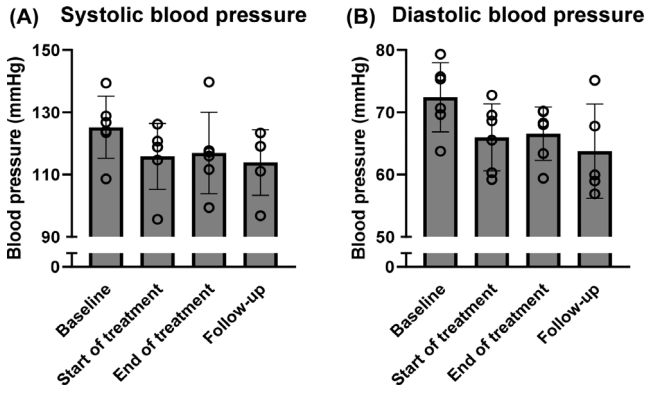

FIGURE 2 Mean (standard deviation) systolic (A) and diastolic (B) blood pressure at baseline, start of treatment, end of treatment and follow-up of the DAPASALT trial participants

3.5 | Effect of dapagliflozin on markers of volume status

In DAPASALT participants, mean plasma copeptin level increased during treatment with dapagliflozin after 4 days, which persisted throughout follow-up and returned towards baseline values during wash-out (Table 2). Mean plasma osmolality was stable during dapagliflozin treatment and wash-out. Mean plasma urea nominally increased after 4 days of treatment but returned to baseline on Day 14 and was stable afterwards during wash-out (Table 2). Mean urinary urea gradually decreased in DAPASALT during dapagliflozin treatment and increased during wash-out (Table 2). Mean 24-hour osmolality showed a modest increase at the start of dapagliflozin treatment and decreased during wash-out. Mean free water clearance did not change during dapagliflozin treatment but decreased during wash-out (Table 2). Mean fractional urea excretion gradually decreased on Day 4 and persisted on Day 14, while a nominal initial increase in fractional potassium excretion was observed after 4 days of dapagliflozin treatment (Table 2).

In DIAMOND study participants, dapagliflozin compared to placebo significantly increased plasma copeptin and urea levels, while no between-group difference was observed in plasma osmolality. Dapagliflozin compared to placebo significantly increased urine osmolality. Similarly, as in DAPASALT, in the dapagliflozin group fractional potassium excretion increased and fractional urea excretion decreased

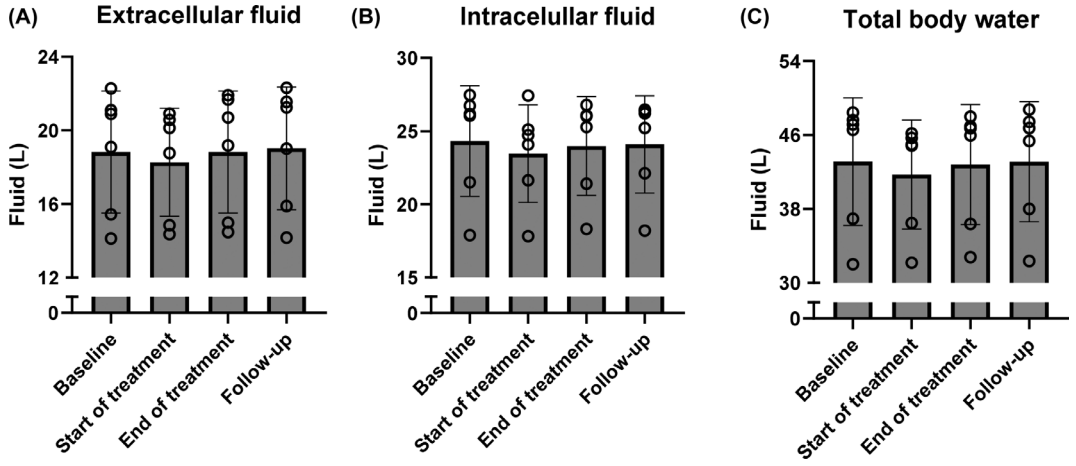

FIGURE 3 Mean (standard deviation) extracellular (A) and intracellular (B) fluid and total body water (C) at baseline, start of treatment, end of treatment and follow-up of the DAPASALT trial participants

TABLE 2 Adaptive responses of the kidney to SGLT2 inhibition in participants of the DAPASALT trial

	Baseline	Start of treatment (Days 2-4)	End of treatment (Days 12-14)	Follow-up (Days 15-17)	Change from baseline at start of treatment	Change from baseline at end of treatment	Change from end of treatment at follow-up
	Mean (SD) or geometric mean (95% CI)	Mean (SD) or geometric mean (95% CI)	Mean (SD) or geometric mean (95% CI)	Mean (SD) or geometric mean (95% CI)	Mean (95% CI) or percentage change (95% CI)	Mean (95% CI) or percentage change (95% CI)	Mean (95% CI) or percentage change (95% CI)
RAAS markers							
Plasma							
Renin, pg/mL	39.0 (12.8, 118.6)	65.2 (24.8, 171.4)	69.1 (19.3, 247.5)	61.5 (16.4, 230.9)	67.3 (39.6, 100.4)[a]	77.3 (−3.9, 227.3)[a]	−11.1 (−57.5, 86.0)[a]
Angiotensin II, pmol/L	12.4 (5.2, 29.5)	15.4 (6.3, 37.6)	15.0 (3.8, 59.6)	10.9 (3.8, 31.1)	24.3 (−48.8, 201.8)[a]	21.6 (−71.4, 418.0)[a]	−27.6 (−74.9, 108.5)[a]
Urine							
Aldosterone, µg/24-hour	6.5 (4.0)	10.1 (5.8)	7.8 (4.1)	8.2 (4.2)	3.6 (−1.7, 8.9)	1.3 (−2.4, 5.0)	0.4 (−1.8, 2.6)
Angiotensinogen, µg/24-hour	7.4 (0.3, 164.3)	10.9 (0.7, 160.2)	9.8 (0.7, 134.2)	8.6 (0.7, 105.0)	62.3 (−65.8, 670.6)[a]	61.3 (−84.7, 1595.9)[a]	−27.1 (−76.3, 124.2)[a]
Renin, ng/24-hour	4.3 (1.1, 17.6)	11.1 (1.9, 63.8)	14.5 (4.4, 47.9)	5.7 (1.4, 23.4)	177.9 (−42.7, 1248.0)[a]	298.7 (−2.6, 1532.0)[a]	−67.7 (−85.5, −28.3)[a]
Volume-related biomarkers							
Plasma							
Copeptin, pmol/L	94.8 (17.0)	122.3 (17.3)	195.2 (65.3)	98.8 (NA)	27.5 (NA)	100.4 (NA)	−142.6 (NA)
Osmolality, mOsm/kg	310.0 (4.0)	310.2 (8.2)	307.0 (3.1)	310.1 (3.5)	2.6 (−5.3, 10.4)	−2.3 (−6.2, 1.5)	3.1 (0.3, 6.0)
Urea, mmol/L	12.8 (4.6)	14.1 (5.8)	12.1 (2.8)	12.2 (4.1)	1.4 (−0.3, 3.0)	−0.6 (−1.3, 0.1)	0.1 (−0.9, 1.0)
Urine							
Urea, mmol/24-hour	455.2 (110.9)	416.8 (116.2)	372.0 (124.8)	395.4 (112.6)	−38.5 (−84.3, 7.4)	−83.2 (−140.4, −26.0)	23.4 (−41.8, 88.5)
Osmolality, mOsm/24-hour	377.8 (133.2)	394.7 (112.7)	369.6 (104.3)	348.7 (120.3)	8.5 (−79.8, 96.9)	−36.3 (−281.3, 208.7)	0.0 (−97.1, 97.1)
FWC, mL/min	−0.4 (0.5)	−0.3 (0.5)	−0.3 (0.5)	−0.1 (0.6)	−0.04 (−0.79, 0.71)	0.14 (−1.12, 1.39)	0.09 (−0.35, 0.52)
Fractional excretion							
Sodium, %	1.3 (0.2)	1.2 (0.1)	1.1 (0.2)	1.1 (0.3)	−0.1 (−0.2, −0.0)	−0.2 (−0.5, 0.1)	0.0 (−0.3, 0.3)
Potassium, %	18.6 (5.2)	20.4 (6.8)	18.4 (3.7)	18.1 (4.7)	1.8 (−0.6, 4.1)	−0.2 (−2.1, 1.6)	−0.3 (−2.6, 2.0)
Urea, %	19.1 (6.8)	17.6 (3.8)	17.8 (5.3)	20.1 (7.5)	−1.5 (−4.2, 1.2)	−1.3 (−3.1, 0.6)	2.2 (−2.1, 6.6)

Note: Urea nitrogen was measured and reported as urea. All changes are reported as absolute change unless otherwise indicated.

[a]Change reported as percentages.

Abbreviations: CI, confidence interval; RAAS, renin-angiotensin-aldosterone system; SD, standard deviation.

TABLE 3 Effects of dapagliflozin compared to placebo on markers of volume status in the DIAMOND trial

	Baseline	Change from baseline dapagliflozin group	Change from baseline placebo group	Placebo corrected change from baseline	
	Mean (SD) or geometric mean (95% CI)	Mean (95% CI) or percentage change (95% CI)[a]	Mean (95% CI) or percentage change (95% CI)[a]	Mean (95% CI) or percentage change (95% CI)[a]	*P* value
Plasma					
Renin, pg/mL	43.8 (30.9, 62.0)	27.2 (6.3, 52.2)[a]	−8.2 (−23.4, 9.9)[a]	38.6 (7.4, 78.8)[a]	0.012
Aldosterone, pmol/L	158.9 (123.9, 203.8)	21.9 (3.2, 43.9)[a]	2.3 (−13.4, 20.9)[a]	19.1 (−5.9, 50.8)[a]	0.144
Copeptin, pmol/L	21.9 (26.5)	2.6 (−2.5, 7.7)	−4.7 (−9.8, 0.3)	7.3 (0.1, 14.5)	0.046
Osmolality, mOsm/kg	306.6 (23.5)	1.2 (−6.7, 9.1)	0.5 (−7.3, 8.4)	0.7 (−10.5, 11.8)	0.906
Urea, mmol/L	9.6 (4.4)	0.3 (−0.2, 0.8)	−0.2 (−0.7, 0.3)	0.5 (−0.2, 1.2)	0.164
Urine					
Volume, mL	2135 (738)	121 (−37, 279)	34 (−125, 193)	87 (−137, 311)	0.443
Osmolality, mOsm/kg	442.1 (155.8)	40.1 (9.0, 71.2)	−32.7 (−63.8, −1.7)	72.8 (28.9, 116.8)	0.001
Urea, mmol/24-hour	401.4 (151.1)	−24.6 (−58.9, 9.7)	−25.5 (−60.2, 9.2)	0.9 (−47.8, 49.7)	0.970
pH	6.0 (0.8)	−0.3 (−0.4, −0.1)	0.1 (−0.1, 0.3)	−0.4 (−0.6, −0.1)	0.009
Fractional excretion					
Sodium, %	1.1 (0.5)	0.0 (−0.2, 0.1)	0.0 (−0.1, 0.2)	−0.1 (−0.2, 0.1)	0.547
Potassium, %	14.4 (8.1)	1.2 (0.1, 2.4)	0.4 (−0.8, 1.5)	0.9 (−0.7, 2.5)	0.276
Urea, %	42.0 (8.1)	−2.0 (−4.5, 0.4)	0.1 (−2.5, 2.6)	−2.1 (−5.6, 1.4)	0.242

[a]Change reported as percentages.
Abbreviations: CI, confidence interval; SD, standard deviation.

although these differences were not statistically significant when compared with placebo (Table 3).

4 | DISCUSSION

In two mechanistic studies designed to study the effect of the SGLT2 inhibitor dapagliflozin in patients with CKD without T2D, we demonstrate that during strictly controlled sodium intake, dapagliflozin increased glucosuria but not natriuresis or diuresis. However, dapagliflozin was associated with a reduction in 24-hour blood pressure along with an acute reduction in extracellular fluid and total body water, which dissipated during 14 days of treatment. We also demonstrate that multiple compensatory mechanisms in the kidney are activated during SGLT2 inhibition that may attenuate a natriuretic/osmotic-induced diuresis. These mechanisms include activation of the RAAS, stimulation of anti-diuretic hormone (ADH) release, and decreasing urea excretion and free water clearance that may collectively facilitate maintenance of volume homeostasis.

We previously reported that in patients with T2D without kidney disease dapagliflozin increased fractional lithium excretion, a proxy for sodium reabsorption at the proximal tubule, but did not enhance natriuresis or 24-hour volume excretion during standardized sodium intake. These data suggest that sodium delivery to the macula densa may persist to activate tubule-glomerular feedback but does not lead to increased natriuresis due to more pronounced compensatory sodium reabsorption further downstream in the nephron.[14] Despite the lack of increased natriuresis and diuresis in these patients, SBP and DBP significantly decreased. In the present study, we extend these findings to patients with CKD without T2D. Despite the small sample size, which warrants cautious interpretation of results, 24-hour sodium excretion and urinary volume did not increase in any participant, whereas blood pressure decreased in all but one participant. The finding that both 24-hour glucosuria and fractional lithium nominally increased indicates that SGLT2 was effectively inhibited. These findings suggest that mechanisms other than natriuresis and diuresis are probably involved in the blood pressure-lowering capacity of dapagliflozin and suggest that regulatory mechanisms likely counteract the continuous dapagliflozin-induced osmotic diuresis.

Various compensatory mechanisms in the kidney were activated to maintain appropriate sodium and water balance. Firstly, both systemic and intra-renal parameters of the RAAS, which regulates sodium balance, were acutely increased. However, at follow-up, most RAAS parameters had returned to baseline values whereas the reduction in blood pressure persisted. This suggests that changes in blood pressure cannot only be attributed to RAAS effects and may be explained by other systemic effects, as described below. It is noteworthy that we observed a numerical increase in fractional potassium excretion which may be attributed to the acute increase in urinary aldosterone.[7] Secondly, ADH, which mainly regulates water as opposed to sodium homeostasis, is also likely involved in fluid conservation during SGLT2 inhibition. ADH regulates water and urea reabsorption in the medulla

and thereby contributes to water conservation. In our studies we observed an increase in copeptin, an established proxy for ADH. The onset of this effect is instantaneous and present after 4 days in the DAPASALT study and persisted at 6 weeks in the DIAMOND study. In addition to RAAS and ADH-regulated water conservation, urea-driven water reabsorption is another mechanism by which the kidney prevents excessive water loss. Urea is a well-known osmolyte and in the setting of diuresis urea transporters in the inner medulla actively reabsorb urea to generate an osmotic gradient required for water reabsorption.[15] Indeed, in both the DAPASALT and DIAMOND studies we observed a numerical decrease in fractional urea excretion supporting a possible role for urea-driven water preservation during SGLT2 inhibition.

How can we explain the marked reduction in 24-hour blood pressure if it is unlikely to be attributed to enhanced osmotic or natriuretic diuresis? SGLT2 inhibitors have been shown to improve endothelial function, arterial stiffness, and pulse wave velocity.[16-22] In addition, amelioration of the abnormal endothelial glycocalyx as observed during SGLT2 inhibition in experimental and clinical studies may contribute to improved blood pressure control.[23,24] A mechanistic study reported that dapagliflozin causes an increase in the vasodilators cyclic guanosine monophosphate and atrial natriuretic peptide, supporting a direct effect of dapagliflozin on smooth muscle cells resulting in blood pressure reduction.[25]

We had expected an increase in sodium excretion during dapagliflozin treatment in keeping with prior studies which enrolled patients with T2D.[9,10,26-28] These contrasting findings may be explained by differences in patient cohorts and study designs. Whilst prior studies enrolled healthy volunteers or patients with T2D without CKD,[12-16] we recruited patients with CKD and without diabetes. In addition, in contrast to the other studies, in our study patients adhered to a strict sodium diet targeted at 150 mmol sodium intake per 24 hours. Our findings on 24-hour sodium excretion are in line with the strata of patients with T2D and a preserved kidney function published previously, although in these patients we noted a small increase in sodium excretion after one day treatment with dapagliflozin which we did not observe in the present study.[14] As expected, and previously predicted in a simulation and modelling study, effects on glucosuria were much smaller in the stratum of patients without T2D and CKD compared to patients with T2D without CKD.[29]

Extracellular volume decreased after 4 days of treatment with dapagliflozin. This effect on extracellular volume is smaller compared to what has been published with conventional diuretics,[30] but was of similar magnitude as compared to patients with T2D and preserved kidney function.[14]

This study has limitations, the most obvious being the small sample size of the DAPASALT study. Due to the demanding design of the DAPASALT study, we were unable to recruit the prespecified 15 participants. The small sample size resulted in wide CIs and low precision of the effect estimates. Another limitation is the open-label design of the DAPASALT study. The findings of the DAPASALT study should thus be interpreted with caution and can only be considered hypothesis-generating. We note however that the directionality of the effects in DAPASALT are consistent with the DIAMOND study, which was a larger study with a double-blind design, although patient characteristics varied between the two studies.

In summary, the SGLT2 inhibitor dapagliflozin decreased blood pressure despite no clear changes in 24-hour sodium and volume excretion in patients with CKD without T2D. Furthermore, we demonstrate that dapagliflozin activates kidney-specific adaptive mechanisms to maintain body fluid and sodium balance to prevent volume depletion including activation of RAAS, and stimulation of ADH release in patients with CKD without T2D.

AUTHOR CONTRIBUTIONS

All authors contributed to collection of the data and data interpretation. **Qiang Li** and **Hiddo J. L. Heerspink** performed the statistical analysis. **Taha Sen** and **Hiddo J. L. Heerspink** wrote the first draft of the manuscript. **Peter J. Greasley**, **David Cherney**, **Claire C. J. Dekkers**, **Marc Vervloet**, **A. H. Jan Danser**, **Sean Barbour**, **Cecilia Karlsson**, **Ann Hammarstedt**, **Qiang Li**, **Gozewijn D. Laverman**, **Petter Bjornstad**, **Daniel H. van Raalte** and **Hiddo J. L. Heerspink** were involved in the design. All authors provided critical revision for important intellectual content and approved the final version of the manuscript for submission. The corresponding author (**Hiddo J. L. Heerspink**) takes full responsibility for the work and/or the conduct of the study, had access to the data and controlled the decision to publish.

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CONFLICT OF INTERESTS

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DATA AVAILABILITY STATEMENT

Deidentified participant data will be made available on reasonable request 2 years after the date of publication. Requests should be directed to the senior author (Hiddo J.L. Heerspink). Requestors will be required to send a protocol, statistical analysis plan and sign a data access agreement to ensure the appropriate use of the study data.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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