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Extending the use of SGLT2 inhibitors from diabetic to non-diabetic kidney disease

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DOI:
[10.33612/diss.136049658](https://doi.org/10.33612/diss.136049658)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2020

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):
Dekkers, C. (2020). *Extending the use of SGLT2 inhibitors from diabetic to non-diabetic kidney disease*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen.
<https://doi.org/10.33612/diss.136049658>

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Extending the use of SGLT2 inhibitors from diabetic to non-diabetic kidney disease

Claire C.J. Dekkers

Colophon

Financial support for the publication of this thesis by the University of Groningen, University Medical Center Groningen, Graduate School of Medical Sciences (GSMS)

Design & Lay-out: Designdays, Nynke Visser, Groningen

Printed by: Van der Eems, Heerenveen

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Extending the use of SGLT2 inhibitors from diabetic to non-diabetic kidney disease

Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. C. Wijmenga
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

maandag 2 november 2020 om 16.15 uur

door

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geboren op 6 mei 1992
te Waalwijk

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Chapter 1

Introduction, aims and outline of this thesis

Published in adapted form

New Diabetes Therapies and Diabetic Kidney Disease Progression:
the Role of SGLT2 Inhibitors.

Curr Diab Rep. 2018;18(5):27

Introduction

The increasing worldwide prevalence of type 2 diabetes mellitus and the associated risks for complications such as cardiovascular disease (CVD) and diabetic kidney disease (DKD) places a heavy burden on individual patients and on national health budgets [1,2]. Targeting multiple risk factors such as high blood pressure, hemoglobin A1c (HbA_{1c}), body weight, albuminuria, and cholesterol reduce the risk of development or progression of cardiovascular and kidney disease in these patients. Nevertheless, still 33 to 49% of patients do not reach their target blood glucose levels, blood pressure or cholesterol levels [1]. Approximately 20 to 40% of all patients with diabetes mellitus will develop DKD, and a substantial number of these patients will progress to end-stage kidney disease [1]. Diabetic kidney disease is also independently associated with an increased risk of cardiovascular disease and a significant reduction in life-expectancy. Continuous efforts are necessary to develop new interventions in order to improve the prognosis of patients with diabetes mellitus. One of these relatively new interventions is the development of new types of promising medicines: sodium-glucose co-transporter 2 (SGLT2) inhibitors.

Working mechanism of SGLT2 inhibitors

Sodium-glucose co-transporters reabsorb glucose that is filtered by the kidney. Together with glucose sodium is co-transported. In healthy individuals with a normal kidney function 180 gram glucose is filtered each day by the kidneys. In these healthy conditions urinary glucose is absent. This is the result of an effective reabsorption system, consisting of two sodium-glucose co-transporters: SGLT1 and SGLT2 [3]. The SGLT2 transporter is located on the luminal side of the first segment of the proximal tubule in the kidney. This is a high-capacity, low-affinity transporter. It is responsible for the reabsorption of approximately 90% of all filtered glucose. The remaining 10% of the filtered glucose is reabsorbed by the low-capacity high-affinity SGLT1 transporter which is located in more distal segments of the proximal tubule [4]. In patients with diabetes mellitus in whom plasma glucose levels exceed 400 mg glucose per 100 ml plasma, SGLT2 transporters become saturated and the maximum capacity threshold to reabsorb glucose is reached resulting in increased urinary glucose loss (Figure 1) [4].

Figure 1: Working mechanism of sodium-glucose co-transporters in patients with diabetes mellitus.

Early experimental studies with phlorizin, an old drug that competitively blocks SGLT1 and SGLT2, showed that SGLT1 and SGLT2 inhibition increases urinary glucose excretion and decreases plasma glucose levels [5,6]. Yet, the drug development program was stopped partly because of gastro-intestinal side effects such as diarrhea and malabsorption caused by SGLT1 inhibition in the small intestine. Later on more selective SGLT2 inhibitors were developed. These SGLT2 inhibitors increase urinary glucose excretion by approximately 70–80 gram per day and decrease hemoglobin A1c (HbA_{1c}) by approximately 0.5 to 0.8% [7]. It is important to note that only approximately 50% of the total filtered glucose is blocked by SGLT2 inhibitors [8]. This probably contributes to the low risk of hypoglycemia. At present, three SGLT2 inhibitors are registered to use in the United States and Europe: empagliflozin, canagliflozin and dapagliflozin.

Natriuresis, blood pressure, and body weight

SGLT2 inhibitors were developed as glucose lowering drugs. However, phase 2 and phase 3 studies already showed positive effects on two other risk factors for cardiovascular and kidney disease, namely: blood pressure and body weight. Reductions of 2 to 4 mmHg systolic blood pressure have been reported in almost all clinical trials with SGLT2 inhibitors in patients with type 2 diabetes mellitus [9]. SGLT2 inhibitors also appear to improve the ability of having a nocturnal fall in blood pressure (dipping) in non-dipping patients with type 2 diabetes mellitus [10]. This is of relevance as blood pressure non-dipping patients are at higher risk of cardiovascular events [11]. The effects on blood pressure occurs on top of concomitant blood pressure lowering medication, such as renin-angiotensin-aldosterone system inhibitors. It is not yet completely known how SGLT2 inhibitors lower blood pressure, but it is thought to be mainly

caused by the natriuretic characteristics of this drug. It has been shown that dapagliflozin at doses of 5, 25, and 100 mg causes a dose dependent increase in 3-days sodium excretion ranging from 55 to 134 mmol/24-hours in patients with type 2 diabetes mellitus in a controlled environment [12,13]. The urinary excretion of sodium leads to natriuresis and the excretion of glucose likely contributes to the diuretic effect of SGLT2 inhibitors by inducing osmotic diuresis. While many anti-hyperglycemic medications are associated with weight gain, SGLT2 inhibitors are associated with a 1 to 3 kg body weight loss [14]. The effect on body weight is observed directly after treatment initiation with a faster decline followed by a more gradual loss or plateau after approximately 6 months [15,16]. The direct body weight reduction (within a few days) is thought to reflect increased diuresis, while the consecutive body weight reduction can be attributed to loss of both visceral and subcutaneous adipose tissue as a consequence of net calorie loss due to increased glucose excretion [15]. The stabilization of body weight loss is likely explained by increased food intake resulting in a new caloric balance and possibly also by changes in gluconeogenesis [16].

Cardiovascular Outcome Trials

Soon after marketing authorization of the first SGLT2 inhibitors large cardiovascular outcome trials were initiated. The outcome trials were launched to investigate the cardiovascular safety of these drugs, as this is required for all new glucose lowering drugs by the United States Food and Drug Administration (FDA). The first cardiovascular outcome trial, the EMPA-REG OUTCOME trial (Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients), was published in 2015 and showed surprising and unexpected beneficial findings [17]. The EMPA-REG OUTCOME trial consisted of 7020 participants with type 2 diabetes mellitus and established cardiovascular disease, who were treated with empagliflozin 10 mg or 25 mg per day versus placebo for 2.6 years (median) and were followed for 3.1 years. The trial showed a 14% relative risk reduction (Hazard ratio (HR) 0.86; 95% confidence interval (CI): 0.74 to 0.99; $p < 0.001$ for non-inferiority and $p = 0.04$ for superiority) for the composite cardiovascular endpoint of death from cardiovascular causes, nonfatal myocardial infarction or nonfatal stroke in the empagliflozin group compared with the placebo group [17]. Empagliflozin also resulted in a 35% relative risk reduction of hospitalization for heart failure (HR 0.65; 95% CI: 0.50 to 0.85; $p = 0.002$) and a 39% relative risk reduction of incident or worsening nephropathy (HR 0.61; 95% CI: 0.53 to 0.70; $p < 0.001$) [17,18]. Two years later similar results were published for canagliflozin in the CANVAS study (Canagliflozin Cardiovascular Assessment Study) (Table 1) [19]. Finally in 2019 the DECLARE-TIMI 58 trial (Multicenter Trial to Evaluate the Effect of Dapagliflozin on the Incidence of Cardiovascular Events) was published [20]. The DECLARE-TIMI 58 trial showed that dapagliflozin was noninferior to placebo with respect to major adverse cardiovascular events, but resulted in a lower rate of cardiovascular death or hospitalization for heart failure and a lower incidence of the kidney composite outcome compared with placebo (Table 1) [20,21].

Table 1: Summary table of EMPA-REG OUTCOME, CANVAS, and DECLARE-TIMI 58 trials.

Study	Population	CV endpoints	CV risk reduction (%)	Kidney endpoints	Kidney risk reduction (%)
Empagliflozin EMPA-REG OUTCOME (n=7020)	Participants with type 2 diabetes, an eGFR > 30 ml/min/1.73m ² , who were at high risk for cardiovascular disease (CVD)	Composite of death from CV-14 causes, nonfatal myocardial infarction, nonfatal stroke	14%	Incident/worsening nephropathy	39%
		Hospitalization for heart failure	35%	Progression to macroalbuminuria	38%
		CV-death	38%	Doubling serum creatinine	44%
Canagliflozin CANVAS (n=10142)	Participants with type 2 diabetes and an eGFR > 30 ml/min/1.73m ² , who were ≥ 30 years of age and had a history of symptomatic atherosclerotic CVD	Composite of death from CV-14 causes, nonfatal myocardial infarction, nonfatal stroke	14%	Renal replacement therapy (RRT)	55%
		Hospitalization for heart failure	33%	Progression of albuminuria	27%
		CV-death	13%	40% reduction in eGFR, RRT, or renal death	40%
Dapagliflozin DECLARE-TIMI 58 (n= 17160)	Participants with type 2 diabetes and a creatinine clearance ≥ 60 ml/min, who were ≥ 40 years of age, and had established atherosclerotic CVD or multiple risk factors	Death from any cause	13%		
		2 risk factors for CV-disease	13%		
		Composite of CV-death, myocardial infarction, or ischemic stroke	7%*	40% or more reduction in eGFR to < 60 ml/min/1.73m ²	46%
		Hospitalization for heart failure	27%	End-stage renal disease or renal death	59%
		CV-death	2%*		
		Death from any cause	7%*		

* not statistically significant

Cardiovascular protective mechanisms

The reductions in HbA_{1c}, blood pressure, and body weight induced by SGLT2 inhibition likely contribute to the lower cardiovascular and heart failure endpoints in the large outcome trials. In addition to these risk factors, it is thought that SGLT2 inhibitors mediate multiple other pathways known to be involved in the development or progression of cardiovascular disease. It is hypothesized that SGLT2 inhibitors improve myocardial efficiency, increase cardiac energetics, reduce fibrosis, inflammation and oxidative stress, and reduce the ventricular workload. SGLT2 inhibitors might improve myocardial efficiency by increasing the production of ketone bodies, which can be used as alternative fuel source for the heart in case of stress [22,23]. Experimental studies also suggest that SGLT2 inhibitors increase cardiac energetics. The cardiac energy production is linked to the concentration of mitochondrial calcium. SGLT2 inhibitors might indirectly increase mitochondrial [Ca²⁺] via inhibition of the sodium-hydrogen exchanger 1 (NHE-1) in the myocardium, and thereby increase cardiac energetics [24,25]. Furthermore, dapagliflozin decreased diabetes-induced cardiac inflammation (via suppression of Nlrp3 inflammasome activation), decreased fibrosis and remodeling, and increased the left ventricular ejection fraction in a mice study, possibly via AMPK activation [26]. Finally, SGLT2 inhibitors can reduce the ventricular workload in patients with type 2 diabetes mellitus. Aortic pulse wave velocity, an established method to determine arterial stiffness, has been shown to decrease significantly in response to empagliflozin treatment in subjects with type 1 diabetes mellitus [27]. A reduction in vascular stiffness can result in reduced cardiac afterload, however, these results were not replicated by others. SGLT2 inhibitors might also reduce the cardiac preload by decreasing plasma volume. Plasma volume reduction can be of benefit in patients with type 2 diabetes mellitus, because they are often volume overloaded and have an increased risk for heart failure [28]. In this thesis we will further examine the effects of SGLT2 inhibitors on (estimated) plasma volume and on several volume markers.

Kidney protective mechanisms

A number of pathways described above can also contribute to the marked protective effects of SGLT2 inhibitors on the kidney. Other kidney protective pathways include restoration of the tubuloglomerular feedback mechanism, improving proximal tubule oxygenation, and suppressing anti-inflammatory and anti-fibrotic pathways. Glomerular hyperfiltration is one of the earliest manifestations of diabetic kidney disease. Older studies, in particular in subjects with type 1 diabetes mellitus, have shown that glomerular hyperfiltration is associated with a higher risk for microalbuminuria and kidney function decline [29]. It is assumed that decreased sodium delivery to the macula densa leads to suppression of the tubuloglomerular feedback mechanism, resulting in increased intraglomerular pressure. In patients with diabetes mellitus the SGLT2 expression is increased, causing more reabsorption of sodium and less NaCl delivery to the macula densa. It is hypothesized that inhibition of SGLT2 increases the delivery of NaCl to the macula densa, which restores the tubuloglomerular

feedback mechanism, and consequently reduces hyperfiltration via pre-glomerular vasoconstriction or via post-glomerular vasodilatation [30–32]. Clinically this is manifested by an acute decrease in estimated glomerular filtration rate (eGFR) of approximately 4 to 6 ml/min/1.73m².

This drop in eGFR is completely reversible after SGLT2 inhibitor cessation. Cardiovascular and kidney outcome trials showed that after the first drop, the eGFR decline is slower with SGLT2 inhibition than with placebo [21,33,34]. SGLT2 inhibitors have also been shown to reduce albuminuria. This effect appears to be independent of concomitant use of angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs), and cannot be explained by reductions in HbA_{1c}, blood pressure or body weight [35].

Another mechanism that could be protective relates to kidney hypoxia [36,37]. Inhibition of SGLT2 reduces the tubular transport workload. In the proximal tubule this might ameliorate kidney cortical oxygenation consumption [38,39]. However, the reduced reabsorption of sodium in the proximal tubule might increase the oxygen consumption associated with sodium reabsorption in the outer medulla [38,39]. The relevance of these proposed effects remain unclear. Furthermore, SGLT2 inhibitors might also increase the production of erythropoietin which can stimulate the formation of red blood cells and improve oxygen delivery [12].

Experimental studies have linked SGLT2 inhibitors with reductions in anti-inflammatory, anti-oxidant and anti-fibrotic markers. For example, MCP-1, NF-κB, levels of 8-OHdG and L-fatty acid binding protein (markers of oxidative stress and macrophages) decreased in experimental studies after treatment with ipragliflozin and empagliflozin [40–42]. Reductions of inflammatory markers and oxidative stress markers were also observed in mice with diabetic nephropathy [43]. In this thesis we will further investigate the effects of SGLT2 inhibitor dapagliflozin on urinary inflammatory and kidney injury markers in humans with type 2 diabetes mellitus and signs of kidney damage.

SGLT2 inhibitors in patients with impaired kidney function

SGLT2 inhibitors were first not allowed to be used in patients with an eGFR lower than 60 ml/min/1.73m², since their effects on glycemic control attenuates at lower eGFR levels [44–47]. Over the last years, we and others performed pooled analysis from phase 3 trials and analyzed patient-subgroups with lower kidney functions in the outcome trials [48,49]. Moreover, in 2019 the results of the canagliflozin outcome trial in patients with type 2 diabetes mellitus and reduced kidney function (the CREDENCE study) came available and showed positive kidney and cardiovascular outcomes [33]. The results of these studies and the subsequent changes in guidelines and labels, namely the broadened indication for the use of SGLT2 inhibitors, will be discussed in more detail this thesis.

SGLT2 inhibitors in patients with diabetic and non-diabetic kidney disease

A clinical trial that compared canagliflozin with glimepiride showed that with canagliflozin

the rate of kidney function decline was significantly lower while glycemic control was similar between the two classes [50]. In addition, data from phase III trials, the CREDENCE study, and several meta-analyses indicate that the protective effects of SGLT2 inhibitors are unlikely to be mediated by improvements in glycemic control [33,51,52]. The restoration of the tubuloglomerular feedback mechanism along with pre-glomerular (afferent) vasoconstriction or post-glomerular (efferent) vasodilatation and a reduction of the intraglomerular pressure is thought to be an important mechanism accounting for the protective effects of SGLT2 inhibitors on kidney function. Based on this evidence we initiated the DIAMOND study to examine the short-term efficacy and safety of dapagliflozin in patients with non-diabetic chronic kidney disease (CKD) that are characterized by glomerular hypertension, hyperfiltration and significant albuminuria.

Aims and outline of this thesis

This thesis consists of two parts. In the first part we aimed to investigate the effects of SGLT2 inhibitor dapagliflozin in patients with type 2 diabetes mellitus and focused on its effects on volume status and albuminuria. Volume status and albuminuria are important parameters to clinically evaluate the development and progression of heart failure and kidney disease. SGLT2 inhibitors might influence, among others, the patient's volume status and level of albuminuria. These non-glycemic effects raise the question whether SGLT2 inhibitors are effective in non-diabetic conditions. Therefore, in the second part of this thesis we performed a meta-analysis and prospective clinical trial to investigate whether SGLT2 inhibitors are beneficial in patients with non-diabetic CKD.

Part I: effects of SGLT2 inhibitors in diabetic kidney disease

SGLT2 inhibitors have emerged as promising drugs for patients with type 2 diabetes mellitus at high risk for cardiovascular events. The EMPA-REG OUTCOME trial showed for the first time that empagliflozin, compared with placebo, reduced the risk for cardiovascular mortality in this patient population [17]. A mediation analysis from the EMPA-REG OUTCOME trial suggested that increased hematocrit and hemoglobin are important mediators of the reduction in cardiovascular mortality [53]. Similar findings were observed in the CANVAS trial underscoring that changes in plasma volume during SGLT2 inhibition contribute to the observed risk reduction for heart failure [54]. Direct measurement of plasma volume requires time-consuming invasive methods and is therefore cumbersome to perform in large epidemiological research. However, estimation equations are available and provide an opportunity to estimate plasma volume changes. In **Chapter 2** we used one of these equations to compare changes in estimated plasma volume with changes in measured plasma volume in patients with type 2

diabetes mellitus, and subsequently examined the effects of dapagliflozin on estimated plasma volume in a large and broad population of patients with type 2 diabetes mellitus.

Changes in plasma volume and extracellular volume may elicit a range of compensatory responses in the kidney to restore fluid homeostasis. Therefore we assessed in **Chapter 3** the effects of dapagliflozin compared to placebo on various markers of volume status, such as urinary osmolality, urine volume, renin, copeptin (a surrogate marker of vasopressin), and free water clearance.

Besides hematocrit and hemoglobin, the benefit of SGLT2 inhibitors on heart failure and kidney outcomes are mediated by changes in albuminuria as documented in the CANVAS trial [54,55].

However, the important question how SGLT2 inhibitors lower albuminuria remains uncertain.

In **Chapter 4** we therefore characterized the effects of dapagliflozin on various biomarkers that are associated with glomerular and tubular structure and function to gain more information about the potential underlying albuminuria lowering mechanisms.

A substantial proportion of patients with type 2 diabetes mellitus develop micro- or macroalbuminuria. Generally, albuminuria levels further rise when kidney function worsens over time. This patient population with very high albuminuria and low eGFR levels (between 15 and 30 ml/min/1.73m²) is systematically excluded from participation in large clinical trials. Nevertheless, these patients may particularly benefit from SGLT2 inhibitors due to their high risk to develop kidney and heart failure [1]. To assess the efficacy and safety of dapagliflozin in these patients with severe CKD (eGFR between 12 and 45 ml/min/1.73m²) we performed a pooled analysis of 11 Phase 3 randomized controlled trials (**Chapter 5**).

Part II: effects of SGLT2 inhibitors in non-diabetic kidney disease

The second part of this thesis (**Chapter 6**) starts with a review and meta-analysis of the cardiovascular and kidney outcome trials with empagliflozin, canagliflozin and dapagliflozin. We examined the heart failure and kidney outcomes per baseline eGFR- and HbA_{1c}-subgroups. Additionally, we summarized the few articles that are available on SGLT2 inhibition in non-diabetic animals and humans with kidney disease or at risk for kidney function decline. The results of the meta-analysis indicated a beneficial influence of SGLT2 inhibitors in patients with lower eGFR-levels and in patients with lower HbA_{1c}-levels. To test this in a prospective manner we designed and performed a multicenter randomized double blind 6-weeks cross-over trial, the DIAMOND study. This study examines the effects of dapagliflozin on proteinuria in non-diabetic patients with CKD. The results of the DIAMOND study are presented in **Chapter 7**.

Finally, in the last chapter of this thesis, we will discuss the future perspectives of SGLT2 inhibitors in patients with and without diabetes mellitus at risk for progression of CVD or CKD.

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Part I

Effects of SGLT2 inhibitors in diabetic kidney disease

Chapter 2

Effects of the sodium–glucose co–transporter 2 inhibitor dapagliflozin on estimated plasma volume in patients with type 2 diabetes

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Diabetes Obes Metab. 2019;21(12):2667–2673

Abstract

Background and aims: To compare the effects of the sodium-glucose co-transporter 2 (SGLT2) inhibitor dapagliflozin on estimated (ePV) and measured plasma volume (mPV) and to characterize the effects of dapagliflozin on ePV in a broad population of patients with type 2 diabetes mellitus.

Methods: The Strauss formula was used to calculate changes in ePV. Change in plasma volume measured with ¹²⁵I-human serum albumin (mPV) was compared with change in ePV in 10 patients with type 2 diabetes mellitus randomized to dapagliflozin 10 mg/day or placebo. Subsequently, changes in ePV were measured in a pooled database of 13 phase 2b/3 placebo-controlled clinical trials involving 4533 patients with type 2 diabetes mellitus who were randomized to dapagliflozin 10 mg daily or matched placebo.

Results: The median change in ePV was similar to the median change in mPV (−9.4% and −9.0%) during dapagliflozin treatment. In the pooled analysis of clinical trials, dapagliflozin decreased ePV by 9.6% (95% CI: 9.0 to 10.2%) compared to placebo after 24 weeks. This effect was consistent in various patient subgroups, including subgroups with or without diuretic use or established cardiovascular disease.

Conclusions: Estimated plasma volume may be used as a proxy to assess changes in plasma volume during dapagliflozin treatment. Dapagliflozin consistently decreased ePV compared to placebo in a broad population of patients with type 2 diabetes mellitus.

Introduction

Sodium-glucose co-transporter 2 (SGLT2) inhibitors induce glycosuria and sodium excretion by inhibiting glucose and sodium reabsorption in the proximal tubule, and several members of this class are approved for the treatment of type 2 diabetes mellitus. The renal inhibition of glucose and sodium reabsorption by SGLT2 inhibitors promotes osmotic/natriuretic diuresis and a reduction in plasma, interstitial, and extravascular volume [1,2]. A previous study assessed plasma volume by ^{125}I -human serum albumin, a gold standard technique, and demonstrated a reduction of 7% after 12 weeks treatment with the SGLT2 inhibitor dapagliflozin [3]. The reduction in plasma volume was accompanied by increases in haematocrit and haemoglobin [3]. A decrease in plasma volume reduces ventricular filling pressure and cardiac workload and may explain some of the beneficial effects regarding hospitalization for heart failure and associated mortality observed in recent cardiovascular outcome trials with SGLT2 inhibitors [4-6]. A *post hoc* analysis of the EMPA-REG cardiovascular outcome trial for the SGLT2 inhibitor empagliflozin suggested that the increase in haematocrit and haemoglobin are important mediators of the reduction in cardiovascular mortality observed in the EMPA-REG OUTCOME trial [7].

To extend the initial findings of the effects of dapagliflozin on plasma volume, we aimed to determine the plasma volume effects in a large and broad population of patients with type 2 diabetes mellitus. The gold standard techniques to measure plasma volume require dilution methods, either with radioactive isotopes or fluorescent dyes. These are cumbersome procedures and challenging to implement in large multicenter clinical trials; therefore, we used a plasma volume estimation equation, the Strauss formula, to define changes in plasma volume during dapagliflozin or control treatment [8].

To our knowledge, the Strauss formula has not been used in patients with type 2 diabetes mellitus. To ensure that the formula could be reliably used to assess changes in plasma volume in this population, we first compared plasma volume measured by ^{125}I -human serum albumin (mPV) with estimated plasma volume (ePV). Secondly, we characterized the effects of dapagliflozin on ePV in a large population of patients with type 2 diabetes mellitus and various relevant subgroups.

Materials and Methods

Study design and study population

We used data from a previous study to assess and compare changes in ePV, calculated with the Strauss formula, with mPV [3]. The original study examined the effects of dapagliflozin versus placebo or hydrochlorothiazide in 75 patients with inadequately controlled levels of glycated haemoglobin ($\text{HbA}_{1c} \geq 6.6\%$ and $\leq 9.5\%$) and blood pressure (systolic blood pressure ≥ 130 and < 165 mmHg, diastolic blood pressure ≥ 80 and < 105 mmHg) [3]. Patients were randomly assigned to a 12-week treatment period of dapagliflozin 10 mg/day, hydrochlorothiazide 25 mg/

day, or matched placebo. The original mechanistic study enrolled, in a sub-study, 30 patients in whom plasma volume was measured at baseline and at week 12. In the present analysis we included subjects in whom plasma volume, haemoglobin and haematocrit were recorded to compare mPV with ePV (N=10).

Subsequently, we performed a pooled analysis of 13 phase 2b/3 placebo-controlled clinical trials in patients with type 2 diabetes mellitus (Supplementary figure 1). These studies examined the glucose-lowering effects of dapagliflozin 10 mg/day as monotherapy or in combination with other glucose-lowering drugs in patients with inadequately controlled HbA_{1c} levels. The core study periods were 12 to 24 weeks in duration. The results of these studies were published previously [9–21].

Measurements

Plasma volume was measured by using ¹²⁵I-labeled human serum albumin, as previously explained [3,22]. Percentage changes from baseline in ePV were calculated by the Strauss formula according the following equation:

$$(((\text{Hb}_{\text{baseline}}/\text{Hb}_{\text{end}}) \times ((100-\text{Ht}_{\text{end}})/(100-\text{Ht}_{\text{baseline}}))) - 1) \times 100$$
, where Hb_{baseline} and Ht_{baseline} are the haemoglobin and haematocrit levels at baseline, and Hb_{end} and Ht_{end} are haemoglobin and haematocrit levels at end of treatment. The Strauss formula was used on the assumption that there was no or limited change in red blood cell production and red blood cell lifespan during 12 weeks dapagliflozin therapy.

In the pooled analysis of phase 2b/3 placebo-controlled trials, only patients with non-missing baseline haemoglobin and haematocrit values and at least one post-baseline value were included. The effects of dapagliflozin on ePV over 24 weeks of follow-up were determined. Various subgroup analyses were performed to assess the consistency in ePV response.

Statistical analyses

Baseline characteristics are presented by descriptive statistics. Mean change from baseline in ePV and its 95% confidence interval were calculated using a longitudinal repeated-measures mixed model with fixed terms for treatment, study, week, and week-by-treatment interaction. The Kenward-Roger method was used. If the model did not converge, the Satterthwaite approximation was used. The effect of dapagliflozin on ePV was assessed in various subgroups including subgroups defined by baseline estimated glomerular filtration rate (eGFR), diuretic use, and cardiovascular disease history. Subgroup analyses were performed by adding the subgroup and the interactions subgroup-by-treatment and subgroup-by-week-by-treatment to the model. Pearson or Spearman correlation analyses were performed to calculate correlations between changes in ePV and changes in HbA_{1c}, fasting plasma glucose, systolic blood pressure, eGFR, body weight, and urinary albumin to creatinine ratio (UACR; for the subgroup with baseline UACR > 30 mg/g) at week 24 of dapagliflozin therapy.

Results

Comparison of mPV and ePV

Plasma volume measurements by ^{125}I -human serum albumin, as well as haemoglobin and haematocrit measurements, were available in ten patients and were included in the present analysis [3]. The median (25th to 75th percentile) baseline mPV was 2539 mL (2535 to 2787 mL) in the patients who received dapagliflozin and 2547 mL (2330 to 2677 mL) in the placebo group. Median haemoglobin levels were 14 g/dL in both the dapagliflozin group and placebo group. Median haematocrit levels were ~40% in both groups, respectively (Supplementary table 1). Median (25th to 75th percentile) changes in mPV and ePV during dapagliflozin treatment were -9.0% (-11.5 to -5.5%) and -9.4% (-9.9 to -7.7%) ($P=0.80$ vs mPV), respectively. The changes in mPV and ePV during placebo treatment were 5.2% (-2.5 to 7.1%) and 0.3% (-2.0 to 5.0%) ($P=0.96$ vs mPV), respectively. Lin's concordance index was 0.6 ($P<0.01$; Figure 1). The similar median changes in mPV and ePV during dapagliflozin treatment and the significant Lin's concordance index supported the use of the Strauss formula to assess effects of dapagliflozin on ePV in the pooled clinical trial database.

Effects of dapagliflozin on ePV in the pooled clinical trial database

The pooled analysis included 4533 patients of whom 2295 received dapagliflozin 10 mg/day and 2238 received placebo. Baseline characteristics are shown in Table 1. HbA_{1c} was 8%, haemoglobin was 14 g/dL, and haematocrit was 42% in both the dapagliflozin group and the placebo group. Changes in haemoglobin and haematocrit over time are shown in Supplementary figure 2.

Figure 1: Correlation between measured plasma volume, assessed by ^{125}I -human serum albumin, and estimated plasma volume, estimated with the Strauss formula, in a sub-study of patients in whom measured plasma volume as well as haemoglobin and haematocrit values were available.

In the placebo group, ePV increased by 1.3% (95% confidence interval (CI): 0.8 to 1.9%) after 12 weeks of treatment, and by 2.2% (95% CI: 1.7 to 2.7%) at week 24. In the dapagliflozin group, ePV decreased after 12 weeks of treatment by 7.1% (95% CI: 6.6 to 7.6%), which remained stable, ending at a decrease of 7.4% (95% CI: 7.0 to 7.9%) at week 24 (Figure 2). Accordingly, relative to placebo, dapagliflozin significantly decreased ePV by 9.6% (95% CI: 9.0 to 10.2%) after the 24-weeks follow-up (Figure 2).

The effects of dapagliflozin versus placebo on ePV observed in the overall population were consistent in various patient subgroups (Figure 3). Specifically, compared to placebo, dapagliflozin reduced ePV by 9.9% (95% CI: 7.7 to 12.2%) in patients receiving diuretics and by 9.6% (95% CI: 8.9 to 10.2%) in patients not using diuretics (P value for treatment by subgroup interaction= 0.37). Among patients with a history of cardiovascular disease or heart failure, dapagliflozin compared to placebo reduced ePV by 9.7% (95% CI: 8.8 to 10.6%). In patients without a history of cardiovascular disease or heart failure ePV was reduced by 9.5% (95% CI: 8.7 to 10.3%), compared to placebo (P value for treatment by subgroup interaction= 0.66). Dapagliflozin decreased ePV by 9.5% in patients with an eGFR < 60 ml/min/1.73m², as well as in patients with an eGFR ≥ 90 ml/min/1.73m² (P value for treatment by subgroup interaction= 0.90).

Table 1: Baseline characteristics.

Subgroups	Placebo (N= 2238)	Dapagliflozin 10mg (N= 2295)
Age, years	58.9 (9.9)	58.4 (10.0)
Gender, n (%)		
Male	1312 (58.6 %)	1320 (57.5 %)
Female	926 (41.4 %)	975 (42.5 %)
BMI, kg/m ²	32 (5.8)	32 (5.7)
HbA _{1c} , %	8.2 (0.9)	8.2 (0.9)
Fasting plasma glucose, mg/dL	165.3 (45.3)	165.2 (46.7)
Systolic blood pressure, mmHg	131.6 (14.9)	131.7 (15.4)
Estimated GFR, mL/min/1.73 m ²	82.3 (20.1)	82.8 (20.2)
UACR, mg/g	10.0 (5.0 to 33.0)	10.0 (5.0 to 33.0)
Hb, g/dL	14.1 (1.3)	14.1 (1.3)
Ht, %	42.4 (4.0)	42.3 (4.0)
Diuretic use, yes, n (%)	261 (11.7 %)	229 (10.0 %)
Insulin use, yes, n (%)	779 (34.8 %)	756 (32.9 %)
History of CVD/HF at baseline, yes, n (%)	1105 (49.4 %)	1115 (48.6 %)
History of PVD/PAD at baseline, yes, n (%)	288 (12.9 %)	287 (12.5 %)

Data are mean (SD) or number (%). UACR values represent median (25th to 75th percentile). Abbreviations: BMI, body mass index; UACR, urinary albumin creatinine ratio; CVD/HF, cardiovascular disease/ heart failure; PVD/PAD, peripheral vascular disease/ peripheral artery disease.

Figure 2: Adjusted mean changes from baseline in estimated plasma volume (%) in placebo- and dapagliflozin-treated patients.

In continuous analyses, there were statistically significant though weak correlations between changes in ePV at week 24 and concurrent changes in HbA_{1c}, fasting plasma glucose, body weight and eGFR during dapagliflozin treatment. ePV did not correlate with systolic blood pressure and UACR (Table 2).

Table 2: Pearson correlations between percentage change from baseline at 24 week in estimated plasma volume (ePV) and change from baseline in various cardiovascular risk markers during dapagliflozin treatment.

Parameter	ePV	P-value
HbA1c	-0.08	<0.01
Fasting plasma glucose	-0.05	0.04
Systolic blood pressure	-0.01	0.62
Estimated GFR	0.15	<0.01
Body weight	0.09	<0.01
UACR ^a .	-0.07	0.10

Abbreviations: ePV, estimated plasma volume; GFR, glomerular filtration rate; HbA1c, glycated haemoglobin; UACR, urinary albumin creatinine ratio.

^a Only subjects with baseline UACR > 30 mg/g were included.

Figure 3: Changes from baseline in estimated plasma volume (%) during 24-week treatment with dapagliflozin relative to placebo in various subgroups.

Abbreviations: CVD, cardiovascular disease; ePV, estimated plasma volume; FPG, fasting plasma glucose; GFR, glomerular filtration rate; HF, heart failure; PAD, peripheral artery disease; PVD, peripheral vascular disease; UACR, urinary albumin creatinine ratio. ePV at baseline was calculated with the Kaplan-hakim formula [24].

Discussion

The present study demonstrated that the Strauss formula might be a useful equation to estimate changes in plasma volume during dapagliflozin treatment in patients with type 2 diabetes mellitus. Using the formula we observed that dapagliflozin 10 mg/day relative to placebo reduced ePV by 9.6% in a broad population of patients with type 2 diabetes mellitus. The reduction in ePV was fully present after 8 to 12 weeks of dapagliflozin therapy and was sustained until 24-week follow-up. The effect of dapagliflozin on ePV was consistent in various patient subgroups, highlighting the consistency of this effect among patients with type 2 diabetes mellitus.

To our knowledge, only two small studies have examined the effects of SGLT2 inhibitors on

plasma volume in people with diabetes mellitus [3,23]. Heerspink *et al.* [3] found a median (interquartile range) plasma volume change from baseline of -7.3% (-12.4 to -4.8%) after 12 weeks of dapagliflozin treatment. Sha *et al.* [23] found a mean plasma volume change from baseline of -5.4%, with a difference compared to placebo of -9.7% (95% CI: -17.8 to -1.6%) after 1 week of treatment with canagliflozin, which was attenuated at week 12. These studies used ¹²⁵I-labeled human serum albumin or indocyanine green to measure plasma volume. These measurements are cumbersome for patients and time-consuming. Estimation equations have therefore been developed. The Strauss formula was originally developed to estimate changes in plasma volume over time in patients with congestive heart failure and has not yet been used to estimate plasma volume changes in patients with type 2 diabetes mellitus [8,24]. The results of the present study indicate that the Strauss formula may be a useful equation to estimate changes in plasma volume in patients with type 2 diabetes mellitus who receive dapagliflozin or placebo.

The effects of dapagliflozin on ePV occurred soon after treatment initiation and were fully present after 8 to 12 weeks. This finding is in keeping with data from the DECLARE TIMI 58 cardiovascular outcome trial for dapagliflozin, demonstrating that the benefits of dapagliflozin on heart failure were also present directly after treatment initiation [6]. Plasma volume contraction effectively reduces circulatory volume and decreases ventricular filling pressure and cardiac workload, which is a relevant mechanism that can explain the reduction in heart failure risk. Similar benefits with regard to heart failure have been reported with traditional diuretics, but differences between SGLT2 inhibitors and diuretics exist [25]. Mathematical modeling analyses of head-to-head studies with dapagliflozin and bumetanide have suggested that dapagliflozin produces a weaker natriuresis and diuresis effect than bumetanide, but the reduction in interstitial fluid as compared to blood volume might be proportionally larger with dapagliflozin. This reduction in interstitial fluid may account for the marked reductions in risk for heart failure observed with SGLT2 inhibitors [2]. A reduction in interstitial fluid may effectively relieve signs and symptoms of peripheral and pulmonary congestion without decreasing effective circulating volume [2,26]. The interstitial fluid reduction is thought to be secondary to SGLT2 inhibitor-induced urinary glucose excretion, leading to osmotic diuresis and a greater electrolyte-free water clearance. Dedicated outcome and mechanistic trials in patients with congestive heart failure are currently ongoing to more definitively assess the effects of SGLT2 inhibitors in patients with congestive heart failure (DAPA-HF [NCT03036124], DELIVER [NCT03619213], EMPEROR-Reduced [NCT03057977], SOLOIST-WHF [NCT03521934], and ERADICATE [NCT03416270]).

Reduction of plasma volume is one of the hypothesized mechanisms underlying the observed reduction in heart failure events in cardiovascular outcome trials with SGLT2 inhibitors [4-6]. Other underlying mechanisms that are hypothesized to contribute to the beneficial heart failure outcomes are: a reduction of the cardiac afterload by reducing blood pressure and arterial stiffness; reducing inflammatory pathways; improved myocardial energy use as a result

of shifts in metabolic substrates from fatty acids to ketone bodies; and activation of energy synthesis and antioxidant pathways in cardiomyocytes by inhibiting the Na^+/H^+ exchanger and consequently decreasing intracellular sodium/calcium while increasing mitochondrial calcium [27,28]. SGLT2 inhibitors also delay the progression of kidney function loss, which may also contribute to heart failure protection [2,29]. According to the prescribing information for SGLT2 inhibitors, they are not recommended for clinical use in patients with impaired kidney function due to reduced glycaemic efficacy; therefore one might expect to observe a smaller effect on ePV in patients with a reduced kidney function. However, non-glycaemic effects, including effects on ePV as observed in the present study, persist among patients with eGFR levels $< 60 \text{ ml/min/1.73m}^2$ [30,31]. Presumably the natriuretic and osmotic diuretic effects persist in patients with a moderate decreased kidney function. Yet the proportion of patients in the present analysis with moderate to severe chronic kidney disease was relatively small; only one patient had an eGFR $< 30 \text{ ml/min/1.73m}^2$. Hence, we cannot extrapolate our findings to this population with severe loss of renal function. In addition, our analysis demonstrated that effects on ePV were consistent regardless of diuretic use or prevalent heart failure. These findings were also observed in recent cardiovascular outcome trials that showed that effects of SGLT2 inhibitors were not modified by baseline diuretics use or presence of congestive heart failure [4-6].

The present study has some limitations. First, the number of patients in whom both ePV and mPV was determined was small. Although changes in ePV corresponded with the changes in mPV, we acknowledge that the Strauss formula should be validated in larger cohorts of patients with diabetes mellitus without heart failure. Second, ePV remains a proxy for the actual plasma volume. The Strauss formula uses changes in haematocrit and haemoglobin, which could have been influenced by dapagliflozin-induced changes in erythropoietin [3]. We cannot exclude the possibility that dapagliflozin has an effect on red blood cell production or turnover. Accordingly, changes in ePV may not only reflect changes in volume status and may be an overestimation of the true change; however, it is interesting that the change in ePV comparing dapagliflozin with placebo in the pooled analysis was similar to the change in mPV in the mechanistic study. The notion that ePV may also be affected by direct effects on haematopoiesis might explain the relatively slow onset of the reduction of ePV in the pooled analysis, which was expected to occur faster. Increased haematocrit can improve the myocardial oxygen delivery, which may also play a beneficial role. Imaging studies such as DAPACARD (NCT03387683) and SIMPLE (NCT03151343) will specifically investigate intra-cardiac oxygen consumption. These studies may provide additional insight into the mechanism behind the cardiovascular and heart failure benefits of SGLT2 inhibitors. In addition, further studies in broader populations with type 2 diabetes are needed, such as those with and without congestive heart failure and with different stages of chronic kidney disease, to confirm and generalize our results.

To conclude, dapagliflozin significantly reduced estimated plasma volume in a broad range of patients with type 2 diabetes mellitus. Ongoing studies such as DAPA-HF and DELIVER

in patients with heart failure with reduced or preserved ejection fraction (NCT03036124 and NCT03619213) as well as mechanistic studies will provide additional insight into the cardioprotective effects of this SGLT2 inhibitor.

Acknowledgments

We acknowledge the supportive role of all subjects, investigators, and support staff to perform the clinical trials with dapagliflozin.

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Supplementary files

Supplementary table 1: Baseline characteristics of patients in whom plasma volume, haemoglobin and haematocrit was measured.

Baseline characteristic	Placebo (N= 5)	Dapagliflozin 10mg (N= 5)
Age, years	65 (3)	51 (11)
Fasting plasma glucose, mg/dL	116 (110 to 140)	183 (143 to 201)
24h systolic blood pressure, mmHg	124 (124 to 125)	141 (127 to 145)
24h diastolic blood pressure, mmHg	71 (69 to 74)	76 (75 to 81)
measured GFR, mL/min/1.73m ²	93 (88 to 93)	92 (88 to 102)
Hb, g/dL	13.7 (13.0 to 13.9)	14.0 (13.3 to 14.2)
Ht, %	39.2 (39.1 to 40.3)	41.3 (39.2 to 41.3)
Measured plasma volume, mL	2547 (2330 to 2677)	2539 (2535 to 2787)

Data are mean (SD) or median (25th to 75th percentile).
Abbreviations: GFR, glomerular filtration rate; Hb, haemoglobin; Ht, haematocrit.

Supplementary figure 1: Listing and duration of the Phase 2b/3 studies included in the 13-study pool.

Supplementary figure 2: Adjusted mean changes from baseline in haemoglobin (A) and haematocrit (B) in placebo and dapagliflozin treated patients.

A

B

Chapter 3

Effects of dapagliflozin on volume status when added to renin-angiotensin system inhibitors

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J Clin Med. 2019;8(6):779

Abstract

Background: Sodium-glucose co-transporter 2 (SGLT2) inhibitors reduce the risk of heart and kidney failure in patients with type 2 diabetes mellitus, possibly due to diuretic effects. Previous non-placebo-controlled studies with SGLT2 inhibitors observed changes in volume markers in healthy individuals and in patients with type 2 diabetes with preserved kidney function. It is unclear whether patients with type 2 diabetes and signs of kidney damage show similar changes.

Methods: A *post hoc* analysis was performed of two randomized controlled trials (N= 69), assessing effects of dapagliflozin 10 mg/day when added to renin-angiotensin system inhibition in patients with type 2 diabetes mellitus and urinary albumin-to-creatinine ratio ≥ 30 mg/g. Blood and 24-hour urine was collected at the start and the end of treatment periods lasting six and 12 weeks. Effects of dapagliflozin compared to placebo on various markers of volume status were determined. Fractional lithium excretion, a marker of proximal tubular sodium reabsorption, was assessed in 33 patients.

Results: Dapagliflozin increased urinary glucose excretion by 217.2 mmol/24h (95% confidence interval (CI): 155.7 to 278.7, $p < 0.01$) and urinary osmolality by 60.4 mOsmol/kg (30.0 to 90.9, $p < 0.01$), compared to placebo. Fractional lithium excretion increased by 19.6% (6.7 to 34.2; $p < 0.01$), suggesting inhibition of sodium reabsorption in the proximal tubule. Renin and copeptin increased by 46.9% (21.6 to 77.4, $p < 0.01$) and 33.0% (23.9 to 42.7, $p < 0.01$), respectively. Free water clearance (FWC) decreased by -885.3 ml/24h (-1156.2 to -614.3, $p < 0.01$).

Conclusions: These changes in markers of volume status suggest that dapagliflozin exerts both osmotic and natriuretic diuretic effects in patients with type 2 diabetes and kidney damage, as reflected by increased urinary osmolality and fractional lithium excretion. As a result, compensating mechanisms are activated to retain sodium and water.

Introduction

Sodium-glucose co-transporter 2 (SGLT2) inhibitors reduce the incidence of heart failure and renal events in type 2 diabetes patients at risk for cardiovascular disease, as well as in patients with diabetes and chronic kidney disease [1–4]. The early separation of the event curves between the intervention and control group and the only modest reduction in hemoglobin A1c (HbA_{1c}) suggest that the long-term benefits conferred by SGLT2 inhibitors are unrelated to improvements in glycemic control [1, 2, 4].

SGLT2 transporters are responsible for glucose and sodium reabsorption in the proximal tubule. Inhibition of SGLT2 promotes the urinary excretion of glucose and sodium, leading to osmotic diuresis and natriuresis. A few studies reported acute increases in urinary volume and sodium levels, supporting the natriuretic/diuretic properties of this drug class [5, 6]. These effects dissipated during prolonged treatment, reflecting a transient natriuretic effect with a subsequent new steady state [5, 7]. Other studies reported a decrease in plasma volume and interstitial fluid volume during SGLT2 inhibition, which would be in line with their natriuretic/diuretic profile [8, 9]. These effects may, at least in part, explain the observed risk reduction of heart failure events in patients with diabetes mellitus [1–3].

Achieving and controlling optimal volume status can be a challenge in patients with diabetic kidney disease, due to the impaired net excretion of sodium. Guideline-recommended treatment for these patients consists of optimizing glucose and blood pressure control, the latter preferably with agents that intervene in the renin-angiotensin-aldosterone system. However, novel drugs that target HbA_{1c} and, at the same time, optimize volume control are a welcome addition to the therapeutic armamentarium for these patients.

Previous studies that assessed the effects of SGLT2 inhibitors on volume markers were performed in either healthy subjects or in patients with type 2 diabetes mellitus with preserved renal function, but not in patients with impaired renal function [5–7]. In addition, the previous studies did not control for placebo effects and included small populations, which limits the precision of the reported effect sizes and preclude subgroup analyses.

Therefore, we examined the effects of six to 12 weeks of treatment with SGLT2 inhibitor dapagliflozin compared to placebo on markers of volume status in patients with type 2 diabetes mellitus with albuminuric kidney disease. In addition, we aimed to characterize the effects of dapagliflozin on specific markers of volume status in relevant subgroups.

Experimental Section

Design and Participants

This was a *post hoc* combined analysis of two similar studies: the IMPROVE study and the DapKid study. Both were prospective, double-blinded, placebo-controlled cross-over clinical trials designed to assess the albuminuria-lowering effects of dapagliflozin 10 mg/day. The study designs and primary outcomes of both clinical trials were published previously [10, 11].

In short, the IMPROVE study enrolled 34 patients with type 2 diabetes, an HbA_{1c} level between 55 and 100 mmol/mol, and a first morning void urinary albumin creatinine ratio (UACR) ≥ 100 mg/g and < 3500 mg/g from the Department of Internal Medicine Ziekenhuisgroep Twente, the Netherlands. Thirty-three patients completed the study and were included in the primary analysis. In the DapKid study, 36 patients completed the study. These were patients with type 2 diabetes, an HbA_{1c} level > 58 mmol/mol, and a first morning void UACR ≥ 30 mg/g from Steno Diabetes Center Copenhagen, Denmark. Both studies included patients between 18 and 75 years old with an estimated glomerular filtration rate (eGFR) ≥ 45 ml/min/1.73m². All patients were on a stable dose of renin-angiotensin-aldosterone system (RAAS) blocking treatment for at least four weeks prior to randomization.

Eligible patients were randomly assigned to two successive treatment periods in which they received dapagliflozin 10 mg/day or matching placebo added to standard treatment (Supplementary figure 1). The IMPROVE study consisted of six-weeks treatment periods with a wash-out period of six weeks in between. In the DapKid study, patients were assigned to two consecutive 12-weeks treatment periods without a wash-out period in between. For both studies, the study medication was provided by Astra Zeneca, Södertälje, Sweden. The two studies were similar in design. The only difference between the studies was the duration of study periods (six vs. 12 weeks). We merged both studies under the assumption that a new steady state of body-fluid homeostasis arises early after start of SGLT2 inhibition (i.e. day two) [5, 7].

Both studies were approved by the regional medical ethics committees (IMPROVE: METC 2014/111; DapKid: H-15006370). All patients gave written consent before any study-related procedures commenced. Both studies complied with the Declaration of Helsinki and Good Clinical Practice Guidelines. The IMPROVE study was registered with the Netherlands Trial Register (NTR 4439) and the DapKid study was registered with ClinicalTrials.gov (identifier NCT02914691).

Measurements

Office blood pressure was measured at the beginning and at the end of each treatment period. In both studies, the average of multiple blood pressure readings was recorded. Blood samples, three consecutive first morning void urine samples, and 24-hour urine samples were obtained at the start and end of the two treatment periods. In these blood samples, routine biochemistry assessments were performed, using the Roche COBAS 6000 analyzer series (Basel, Switzerland) in the IMPROVE study and the Ortho Clinical's VITROS 5600 (Raritan, Somerset, NJ, USA) in the DapKid study. Glomerular filtration rate was estimated (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. In addition, urine and plasma samples were centrifuged, aliquoted, and stored at -80°C for later assessment of the volume markers: copeptin, renin, N-terminal pro b-type natriuretic peptide (NT-proBNP), and osmolality in the blood. Copeptin was measured as a surrogate marker of vasopressin.

Unlike vasopressin, copeptin is easy to measure and is very stable *ex vivo* [12, 13]. Glucose, sodium, and osmolality were also measured in the 24-hour urine samples to calculate the fractional urinary sodium excretion, the urinary glucose excretion, and free water clearance (FWC). Free water clearance, the excreted solute-free water per unit time, was calculated as urine volume minus osmolar clearance. Osmolar clearance was calculated as ((urine osmolality x urine volume)/plasma osmolality) [8]. In the IMPROVE study serum- and 24-hour urine lithium were measured to calculate the fractional lithium excretion, which is a proxy for proximal tubular sodium reabsorption [14]. Lithium analyses were performed by using inductively coupled plasma mass spectrometry (ICP-MS). Automated immunofluorescence assay on a KRYPTOR platform (Thermo Fischer, Waltham, MA, USA) was used to analyze copeptin in the DapKid study and KRYPTOR Compact analyzer (Brahms GMBH, Henningsdorf, Germany) was used to analyze copeptin in the IMPROVE study. Renin concentration was measured using immunoradiometric assay (Cisbio, Codolet, France) [15].

Statistical analysis

Changes in individual outcomes during the intervention period and effects of the treatment were modelled by linear mixed-effects models with a patient-specific random intercept to account for the correlation of repeated measurements within patients. We included sequence, site, treatment, and site-by-treatment interaction as fixed variables in the model. Volume markers measured at the start of the first treatment period were characterized as baseline value. Due to differences in study design, we compared the end of the treatment periods and included the baseline value in the mixed model. Changes in all markers were analyzed in the merged database, as well as in both studies separately. Markers that were not normally distributed were log-transformed before entering the data into the mixed-effects model. The mean percentage changes of these log-transformed volume markers during dapagliflozin therapy versus placebo were derived by $(1 - \exp[\text{mean change}]) \times 100$ and the same was done for the 95% confidence limits. We tested for carry-over effect which was not present in either of the studies.

Secondly, the effects of dapagliflozin on specific volume markers, namely urinary osmolality, NT-proBNP, copeptin, and renin were assessed in the following baseline subgroups: HbA_{1c} level < 63 mmol/mol vs. HbA_{1c} ≥ 63 mmol/mol, eGFR < 82 ml/min/1.73m² vs. ≥ 82 ml/min/1.73m², UACR < 199.7 mg/g vs. ≥ 199.7 mg/g, and use of diuretics at baseline (yes/no). The median was used to stratify the subgroups. Subgroup analyses were performed by adding relevant subgroups and an interaction term between treatment assignment and subgroup to the mixed models. Data processing and analyses were performed using SAS software, version 9.4 of the SAS System for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Baseline characteristics

Baseline characteristics of the IMPROVE study and the DapKid study were similar, except for renin, glucose, and HbA_{1c} levels, as well as urinary glucose excretion (Supplementary table 1). The mean age of the subjects in the merged database was 62.7 years (standard deviation (SD): 8.8). Most of the subjects were male (82.6%) and 44 subjects (63.8%) used diuretics at baseline. The baseline variables related to glucose status, renal function, and volume status, can be found in Table 1.

Changes in HbA_{1c}, renal function, and markers of volume status

Dapagliflozin, compared to placebo, decreased HbA_{1c} by 5.2 mmol/mol (95% confidence interval (CI): 3.2 to 7.2 mmol/mol, $p < 0.01$) (Table 1). Estimated GFR was decreased by 4.1 ml/min/1.73m² (2.4 to 5.9 ml/min/1.73m², $p < 0.01$) and 24-hour urine albumin excretion was reduced by 52.0% (34.0 to 72.3%, $p < 0.01$), relative to placebo.

Dapagliflozin increased urinary glucose excretion by 217.2 mmol/24h (155.7 to 278.7 mmol/24h, $p < 0.01$) and urinary osmolality by 60.4 mOsmol/kg (30.0 to 90.9 mOsmol/kg, $p < 0.01$), relative to placebo (Table 1 and Figure 1). Fractional sodium excretion was increased by 104.2% (19.0 to 189.4, $p = 0.02$), but there was no change in 24-hour urinary sodium excretion (Table 1 and Figure 1). There was a 19.6% (6.7 to 34.2%, $p < 0.01$) increase in fractional lithium excretion relative to placebo, suggesting that, during chronic treatment with dapagliflozin, sodium reabsorption in the proximal tubule is inhibited (Table 1 and Figure 1).

Compared to placebo, dapagliflozin reduced systolic blood pressure by 5.7 mmHg (2.3 to 9.1 mmHg, $p < 0.01$), decreased body weight by 1.3 kg, and increased serum sodium and urea, but did not change NT-proBNP (Table 1). Furthermore, compared to placebo, renin increased by 46.9% (21.6 to 77.4%, $p < 0.01$) and copeptin increased by 33.1% (23.9 to 42.7%, $p < 0.01$; Table 1 and Figure 2). Free water clearance decreased by -885.3 ml/24h (-1156.2 to -614.3 ml/24h, $p < 0.01$), relative to placebo (Table 1 and Figure 1). In general, the changes in volume markers were consistent between both studies (Supplementary table 2).

As shown in Table 2, the effects of dapagliflozin on urinary osmolality, NT-proBNP, copeptin, and renin were consistent in subgroups defined by baseline HbA_{1c}, eGFR, albuminuria, and diuretic use.

Table 1: Volume markers at baseline, at the end of placebo treatment, and at the end of dapagliflozin treatment, and changes in volume markers during dapagliflozin treatment versus placebo in the merged database (IMPROVE study and DapKid study).

Characteristics	At Baseline (N= 69)	End of Placebo Treatment	End of Dapagliflozin Treatment	Change during Dapagliflozin vs. Placebo (95% CI; p-value)
Weight (kg)	99.2 (21.5)	98.9 (21.2)	97.9 (21.2)	-1.3 (-1.8, 0.9; p< 0.01)
BMI (kg./m ²)	31.9 (5.7)	31.8 (5.7)	31.5 (5.8)	-0.39 (-0.6, -0.2; p< 0.01)
Systolic blood pressure (mmHg)	141.2 (15.2)	140.4 (14.5)	134.7 (15.9)	-5.7 (-9.1, -2.3; p< 0.01)
Diastolic blood pressure (mmHg)	79.8 (8.6)	78.1 (9.4)	76.8 (8.3)	-1.2 (-2.9, 0.5; p= 0.2)
Fasting plasma glucose (mmol/l)	9.8 (3.6)	10.0 (3.4)	8.2 (2.8)	-1.8 (-2.6, -0.9; p< 0.01)
HbA _{1c} (mmol/mol)	65.4 (15.0)	66.6	61.3	-5.2 (-7.2, -3.2; p< 0.01)
Sodium (mmol/l)	139.2 (2.7)	139.6 (2.8)	140.5 (2.8)	0.9 (0.4, 1.5; p< 0.01)
Potassium (mmol/l)	4.3 (0.5)	4.3 (0.4)	4.2 (0.4)	-0.02 (-0.1, 0.1; p= 0.61)
Urea (mmol/l)	6.4 (2.2)	6.6 (2.4)	7.1 (2.6)	0.5 (0.1, 0.9; p= 0.02)
Osmolality (mOsmol/kg)	294.8 (14.4)	291.1 (8.6)	291.6 (7.3)	0.5 (-1.5, 2.6; p=0.61)
Copeptin (pmol/l) ‡	8.3 (5.7, 11.2)	8.3 (5.4, 12.6)	11.6 (6.8, 16.6)	33.0% (23.9, 42.7; p< 0.01)
Renin (ng/l) ‡	37.1 (17.1, 85.0)	33.6 (16.0, 70.1)	59.3 (21.1, 101.0)	46.9% (21.6, 77.4; p< 0.01)
NT-proBNP (ng/l) ‡	103.0 (35.0, 205.5)	107.5 (43.8, 227.0)	105.0 (48.0, 185)	-5.2% (-19.6, 8.1; p= 0.4)
Estimated GFR (ml/min/1.73m ²)	79.4 (19.3)	80.1 (18.8)	76.1 (20.8)	-4.1 (-5.9, -2.4; p< 0.01)
UACR (mg/g) ‡	199.7 (102.3, 405.3)	202.3 (106.3, 480.0)	133.7 (75.3, 282.3)	-52.0% (-72.3, -34.0; p< 0.01)
Urinary volume (ml/24h)	2057 (762)	2120 (741)	2394 (804)	266.3 (100.6, 432.0; p< 0.01)
Urine glucose excretion (mmol/24h) ‡	21.5 (2.0, 130.2)	23.0 (2.0, 154.0)	211.3 (121.1, 512.5)	217.2 (155.7, 278.7; p< 0.01)
Urinary osmolality (mOsmol/kg)	560.7 (177.3)	553.4 (175.6)	614.2 (131.7)	60.4 (30.0, 90.9; p< 0.01)
Urinary sodium excretion (mmol/24h)	205.2 (110.6)	200.5 (84.5)	195.9 (98.3)	-4.5 (-27.5, 18.5; p= 0.70)
Fractional sodium excretion (%)	937.8 (321.2)	898.9 (335.1)	1006.3 (384.8)	104.2% (19.0, 189.4; p= 0.02)
Fractional lithium excretion (%##)	11318.7 (8984.9, 17,344.4)	10484.6 (8648.9, 13,734.9)	12437.4 (10,461.9, 16,275.4)	19.6% (6.7, 34.2; p< 0.01)
Free water clearance (ml/24h)	-1727.1 (-1335.4)	-1724.3 (-1230.6)	-2606.1 (-1390.7)	-885.3 (-1156.2, -614.3; p< 0.01)

Data are given as mean (standard deviation) and ‡median (25th - 75th percentile). # Fractional lithium excretion was only measured in the IMPROVE study and not in the DapKid study. Abbreviations: BMI, body mass index; GFR, glomerular filtration rate; HbA_{1c}, hemoglobin A1c; NT-proBNP, N-terminal pro b-type natriuretic peptide; UACR, urinary albumin creatinine ratio.

Figure 1: Volume markers at baseline, at the end of placebo treatment, at the end of dapagliflozin treatment, and changes in volume markers during dapagliflozin treatment versus placebo in the merged database (IMPROVE study and DapKid study).

Abbreviation: FWC, free water clearance.

Figure 2: Changes in plasma volume markers during dapagliflozin treatment versus placebo treatment in the merged database.

Table 2: Changes in urinary osmolality, NT-proBNP, copeptin, and renin during dapagliflozin therapy versus placebo in various subgroups.

Baseline Subgroups	Mean Baseline Urinary Osmolality (mOsmol/kg) (SD)	Change in Urinary Osmolality (mOsmol/kg) (95% CI; p-value)	Median Baseline NT-proBNP (ng/l) (IQR)	Change in NT-proBNP (%) (95% CI; p-value)	Median Baseline Copeptin (pmol/l) (IQR)	Change in Copeptin (%) (95% CI; p-value)	Median Baseline Renin (ng/l) (IQR)	Change in Renin (%) (95% CI; p-value)
Diuretics use								
Yes (N= 44)	554 (168)	63.1 (24.7, 101.5; p< 0.01)	75 (35, 201)	-3.8% (-22.2, 13.5; p= 0.65)	7.3 (5.4, 11.2)	29.6% (18.7, 41.6; p< 0.01)	38 (19, 85)	52.6% (20.4, 93.4; p< 0.01)
No (N= 25)	573 (196)	59.1 (8.2, 110.0; p= 0.02)	113 (38, 206)	7.1% (-33.6, 16.4; p= 0.54)	9.0 (5.9, 10.9)	39.1% (23.9, 56.1; p< 0.01)	29 (17, 72)	38.7% (1.6, 89.3; p= 0.04)
p for interact.		p= 0.19		p= 0.39		p= 0.41		p= 0.08
HbA_{1c}								
<63	532 (175)	71.7 (27.7, 115.8; p< 0.01)	110 (35, 209)	4.1% (-16.2, 25.8; p= 0.68)	7.5 (5.6, 11.2)	32.0% (19.0, 46.5; p< 0.01)	29 (19, 87)	32.2% (1.07, 73.0; p= 0.04)
≥63	589 (177)	51.4 (9.0, 93.8; p= 0.02)	100 (35, 201)	-13.0% (-35.0, 5.8; p= 0.18)	9.0 (5.8, 14.0)	35.3% (19.0, 53.7; p< 0.01)	40 (15, 85)	62.7% (25.3, 111.2; p< 0.01)
p for interact.		p= 0.51		p= 0.76		p= 0.49		p= 0.56
eGFR								
<82	509 (152)	65.4 (21.2, 109.5; p< 0.01)	118 (53, 263)	-4.8% (-26.1, 14.7; p= 0.61)	9.2 (6.3, 16.4)	36.7% (23.6, 51.2; p< 0.01)	29 (20, 113)	48.5% (13.3, 94.7; p< 0.01)
≥82	611 (188)	57.1 (14.6, 99.6; p< 0.01)	66 (35, 150)	-4.6% (-26.2, 15.3; p= 0.63)	7.1 (4.5, 10.0)	29.5% (17.4, 42.8; p< 0.01)	41 (11, 70)	46.0% (12.2, 90.0; p= 0.01)
p for interact.		p= 0.68		p= 0.85		p= 0.46		p= 0.66
UACR								
<199.7	591 (199)	76.8 (33.6, 120.0; p< 0.01)	75 (35, 201)	-10.3% -33.3, 9.5; p= 0.30)	7.7 (5.8, 10.9)	32.5% (19.9, 46.4; p< 0.01)	38 (17, 67)	45.4% (11.3, 89.8; p< 0.01)
≥199.7	532 (151)	46.6 (3.7, 89.5; p= 0.03)	112 (53, 263)	-0.1% (-19.8, 20.0; p= 0.99)	8.9 (5.7, 11.2)	33.5% (20.8, 47.5; p< 0.01)	37 (19, 85)	49.0% (14.1, 94.7; p< 0.01)
p for interact.		p= 0.92		p= 0.71		p= 0.29		p= 0.41

HbA_{1c} (mmol/mol), eGFR (ml/min/1.73m²), and UACR (mg/g) were split by using the median value (merged database).Abbreviations: eGFR, estimated glomerular filtration rate; HbA_{1c}, hemoglobin A1c; p for interact, p for interaction; UACR, urinary albumin to creatinine ratio.

Discussion

The present study examined effects of dapagliflozin on volume markers in type 2 diabetes patients with micro- or macroalbuminuria. Dapagliflozin increased urinary osmolality and fractional lithium excretion, and it decreased blood pressure and body weight after six to 12 weeks, compared to placebo. The observed increases in renin and copeptin suggest activation of compensatory mechanisms to retain sodium and water and restore volume homeostasis. Taken together, these data provide more insight into the natriuretic and diuretic effects of SGLT2 inhibition.

What does this study add to the existing literature? The present data confirm previous studies on the diuretic and natriuretic effects of SGLT2 inhibitors and extend these to a placebo-controlled setting. We also report for the first time the effect of chronic SGLT2 inhibition on fractional lithium excretion as a proxy for tubular sodium reabsorption inhibition, and we demonstrate activation of compensatory mechanisms to restore extracellular volume homeostasis during prolonged SGLT2 inhibition. Finally, prior studies included patients with type 2 diabetes mellitus and preserved kidney function, while our studies enrolled patients with type 2 diabetes mellitus and kidney damage in whom sodium and fluid homeostasis is often impaired.

Our results indicate that the diuretic effect of dapagliflozin can be attributed to both osmotic diuresis and natriuretic diuresis (Figure 3). The increase in urinary glucose excretion supports an osmotic diuretic effect, while increases in fractional lithium excretion suggest changes in sodium handling during dapagliflozin treatment. We note that the natriuretic effects of SGLT2 inhibitors appear to be weaker compared to traditional diuretics, as evidenced by smaller increases in renin and aldosterone in head-to-head studies [6,7,9]. The osmotic diuresis, thus, seems to be the driving component of the diuretic effects of SGLT2 inhibitors. We also note that the current observations were performed after six to 12 weeks of dapagliflozin treatment when patients established a new steady state in extracellular volume and when sodium intake matched sodium excretion. Hence, it is unlikely that the observed increase in fractional sodium excretion is a product of increased dietary sodium intake, since we did not observe changes in 24-hour urinary sodium excretion. Fractional sodium excretion is calculated as the clearance of sodium divided by the creatinine clearance. The increase in fractional sodium excretion might, thus, be a consequence of decreased glomerular filtration.

Inhibition of sodium reabsorption in the proximal tubule and increased sodium delivery in the distal tubule will generally trigger compensatory mechanisms to maintain sodium and fluid homeostasis (Figure 3). Such effects were also observed in our study, even after six to 12 weeks of treatment with dapagliflozin. Firstly, increased sodium delivery in the distal tubule promotes renin secretion by the juxtaglomerular cells [16]. Increased secretion of copeptin, a surrogate marker of vasopressin, is another mechanism activated in the setting of increased diuresis and reduction in body-fluid volumes to promote water retention. The decrease in FWC may be a consequence of increased vasopressin secretion secondary to volume contraction and/or

increased glucosuria. Finally, although not measured in our study, a reduction in plasma volume due to diuretic effects decreases medullary blood flow, which is expected to increase the abstraction of water in the descending limb of Henle (which is impermeable to sodium). This in turn leads to an increased sodium concentration, and an increased passive reabsorption of sodium further down the ascending loop of Henle to maintain fluid homeostasis [17].

Figure 3: Effects of the sodium-glucose co-transporter 2 inhibitor dapagliflozin on volume homeostasis.

Dapagliflozin increases urinary glucose and sodium excretion leading to increased urinary osmolality. Both natriuresis and osmotic diuresis will decrease extracellular volume and decrease body weight and systolic blood pressure. The reduction in extracellular volume activates compensatory mechanisms such as renin and copeptin to restore volume homeostasis.

Abbreviations: BP, blood pressure; ECV, extracellular volume; FWC: free water clearance; SGLT2, sodium-glucose co-transporter 2.

The increases in osmotic and natriuretic diuresis, along with reductions in body weight and systolic blood pressure and the activation of compensatory mechanisms, as described above, indicate that dapagliflozin reduces plasma volume. Plasma volume contraction may also explain the modest, yet statistically significant increases in plasma sodium and urea. The finding that NT-proBNP did not change during dapagliflozin treatment was surprising, but was also observed in other dapagliflozin studies [9]. A possible explanation is that, in most studies, subjects were not evidently volume overloaded and had NT-proBNP levels in the normal range. Future studies in subjects with elevated NT-proBNP levels and volume overload are required to study this in more detail.

The effects of dapagliflozin on urinary osmolality, NT-proBNP, copeptin, and renin in the overall study population were consistent irrespective of HbA_{1c}, eGFR, albuminuria, or diuretic use at baseline. This finding adds to a growing body of evidence that the effects of SGLT2 inhibitors on volume states are consistent across a wide range of patients with type 2 diabetes mellitus, regardless of whether subjects used cardiovascular medication (such as diuretics), or whether they had renal impairment.

This study has limitations. Unfortunately, direct measurements of plasma volume were not available for the included studies. However, previous studies already showed that SGLT2 inhibitors decrease extracellular fluid/plasma volume [8,9,18,19]. We recognize that we combined two studies with different durations of treatment periods. The IMPROVE study consisted of six-week treatment periods, while the DapKid study had treatment periods of 12 weeks. Overall, there was generally a consistent trend in the changes of volume markers between both studies, as illustrated in Supplementary table 2. These data support the notion that differences in treatment periods did not influence the observed changes in volume markers, because a new steady sodium and water balance arises after a few days of treatment. There were also differences in baseline glycemic control between patients enrolled in the IMPROVE study (Almelo, Ziekenhuisgroep Twente) and patients enrolled in the DapKid study (Copenhagen, Steno Diabetes Center). We, therefore, adjusted all our analyses for recruitment location. Determining acute changes in volume markers was beyond the scope of these studies. The currently ongoing study “DAPASALT” (ClinicalTrials.gov identifier: NCT03152084) was designed to prospectively assess short-term and long-term changes in several volume-related markers, such as 24-hour urinary sodium, blood pressure, and copeptin.

In conclusion, the present study showed that dapagliflozin increased urinary osmolality, urinary glucose, and fractional lithium excretion, and it decreased blood pressure and body weight. These results suggest that dapagliflozin exerts a diuretic effect through both osmotic and natriuretic diuresis in individuals with type 2 diabetes mellitus and kidney damage. During prolonged treatment with dapagliflozin, compensatory mechanisms are activated to restore body-fluid homeostasis.

Acknowledgments

We thank the participants and the research staff of both studies, the laboratory technician Bettine Haandrikman at the University Medical Center Groningen, and the laboratory technicians Berit R. Jensen, Tina R. Juhl, Jessie A. Hermann and Anne G. Lundgaard at Steno Diabetes Center Copenhagen.

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Supplementary files

Supplementary figure 1: Design of the DapKid study and the IMPROVE study.

Supplementary table 1: Baseline characteristics in the IMPROVE study and DapKid study separately.

Characteristics	IMPROVE (N=33)	DapKid (N=36)
Age (years)	61.4 (9.2)	63.9 (8.3)
Male sex (%)	25 (75.8%)	32 (88.9%)
Diuretic use (%)	26 (78.8%)	17 (50.0%)
Weight (kg)	95.5 (22.3)	103.2 (20.1)
BMI (kg/m ²)	31.0 (5.6)	32.8 (5.7)
Systolic blood pressure (mmHg)	141.8 (14.8)	140.7 (15.8)
Diastolic blood pressure (mmHg)	77.3 (5.9)	82.1 (10.0)
Fasting plasma glucose (mmol/l)	8.6 (3.1)	11.0 (3.6)
HbA _{1c} (mmol/mol)	56.8 (9.6)	73.3 (14.8)
Sodium (mmol/l)	139.9 (3.1)	138.6 (2.2)
Potassium (mmol/l)	4.4 (0.4)	4.3 (0.5)
Urea (mmol/l)	6.6 (2.1)	6.1 (2.2)
Osmolality (mOsmol/kg)	287.9 (6.4)	300.6 (16.6)
Copeptin (pmol/l) ‡	9.0 [5.4, 11.2]	9.0 [5.9, 12.6]
Renin (ng/l) ‡	20.6 [10.5, 52.3]	48.0 [24.5, 106.0]
NT-proBNP (ng/l) ‡	68.9 [30.0, 208.9]	110.5 [42.5, 201.0]
Estimated GFR (ml/min/1.73m ²)	74.4 (18.3)	84.0 (19.3)
UACR (mg/g)‡	255.1 [114.0, 598.3]	153.8 [91.3, 337.5]
Urinary volume (ml/24h)	1892 (706)	2207 (789)
Urine glucose (mmol/24h)	2.2 [0.7, 14.0]	82.0 [23.0, 372]
Urinary osmolality (mOsmol/kg)	533.9 (163.5)	583.7 (187.6)
Urinary sodium excretion (mmol/24h)	187.8 (82.0)	211.1 (130.7)
Fractional sodium excretion (%)	964.1 (343.3)	932.3 (356.2)
Fractional lithium excretion (%)	11318.7 [88984.9, 17344.4]	Not available
Free water clearance (ml/24h)	-1418.7 (858.4)	-1981.0 (1595.7)

Data are given as mean (SD) and ‡median [25th to 75th percentile]. Abbreviations: BMI, body mass index; GFR, glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}; NT-proBNP, N-terminal pro b-type natriuretic peptide; UACR, urinary albumin creatinine ratio.

Supplementary table 2: Changes in characteristics and volume markers during dapagliflozin treatment versus placebo in the IMPROVE study and DapKid study separately.

Volume markers	Change during dapagliflozin vs placebo, IMPROVE (N=33)		Change during dapagliflozin vs placebo, DapKid (N=36)	
	(95% CI)	p-value	(95% CI)	p-value
Weight (kg)	-0.7 (-1.4, -0.1)	0.03	-1.9 (-2.6, -1.3)	< 0.01
Systolic blood pressure (mmHg)	-7.7 (-13.2, -2.3)	< 0.01	-3.7 (-8.0, 0.6)	0.09
Diastolic blood pressure (mmHg)	-3.0 (-5.4, -0.6)	0.02	0.5 (-1.9, 3.0)	0.6
Fasting plasma glucose (mmol/l)	-1.0 (-2.2, 0.3)	0.11	-2.6 (-3.8, -1.3)	< 0.01
HbA _{1c} (mmol/mol)	-3.1 (-5.7, -0.5)	0.02	-7.3 (-10.3, -4.3)	< 0.01
Sodium (mmol/l)	0.3 (-0.5, 1.2)	0.3	1.5 (0.7, 2.2)	< 0.01
Potassium (mmol/l)	0.03 (-0.1, 0.2)	0.69	-0.07 (-0.2, 0.0)	0.18
Urea (mmol/l)	0.9 (0.3, 1.5)	< 0.01	0.07 (-0.5, 0.6)	0.8
Osmolality (mOsmol/kg)	1.2 (-0.8, 3.3)	0.2	-0.2 (-3.7, 3.3)	0.9
Copeptin (%)	33.1% (21.8, 45.5)	< 0.01	33.1% (19.1, 48.9)	< 0.01
Renin (%)	40.3% (11.9, 75.9)	< 0.01	53.5% (13.1, 108.3)	< 0.01
NT-proBNP (%)	5.5% (-14.0, 27.0)	0.6	-15.7% (-39.3, 4.1)	0.12
Estimated GFR (ml/min/1.73m ²)	-6.6 (-9.3, -3.9)	< 0.01	-1.6 (-3.9, 0.7)	0.2
Urinary albumin excretion (%)	-57.4 (-89.8, -30.5)	< 0.01	-46.7 (-74.7, -23.2)	< 0.01
Urinary volume (ml/24h)	-1.2 (-135, 133)	0.98	534 (233, 835)	< 0.01
Urinary glucose excretion (mmol/24h)	113.7 (108.0, 160.8)	< 0.01	320.5 (194.4, 446.5)	< 0.01
Urinary osmolality (mOsmol/kg)	46.8 (9.5, 84.0)	0.02	74.4 (25.5, 123.3)	< 0.01
Urinary sodium excretion (mmol/24h)	-27.7 (-40.6, -14.7)	< 0.01	18.5 (-24.8, 61.8)	0.39
Fractional sodium excretion (%)	51.7 (-29.3, 132.7)	0.20	153.5 (3.7, 303.4)	0.04
Fractional lithium excretion (%)	19.6% (6.7, 34.2)	< 0.01	Not available	
Free water clearance (ml/24h)	-444.1 (-702.8, -185.4)	< 0.01	-1330.4 (-1806.9, -853.9)	< 0.01

Abbreviations: GFR, glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}; NT-proBNP, N-terminal pro b-type natriuretic peptide.

Chapter 4

Effects of SGLT2 inhibitor dapagliflozin on glomerular and tubular injury markers

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Diabetes Obes Metab. 2018;20(8):1988–1993

Abstract

The mechanisms by which sodium-glucose co-transporter 2 (SGLT2) inhibitors lower albuminuria are incompletely understood. We assessed in a *post hoc* analysis of a cross-over trial the effects of the SGLT2 inhibitor dapagliflozin on glomerular markers (IgG to IgG4 and IgG to albumin), tubular markers (urinary KIM-1, NGAL and LFABP) and inflammatory markers (urinary MCP-1 and IL-6) to provide more insight into the kidney protective effects. Dapagliflozin decreased albuminuria by 43.9% (95% confidence interval (CI): 30.3 to 54.8%) and eGFR by 5.1 (2.0 to 8.1) ml/min/1.73m², compared to placebo. Dapagliflozin did not change the glomerular charge or size selectivity index, compared to placebo. Dapagliflozin decreased urinary KIM-1 excretion by 22.6% (0.3 to 39.8%; *p* = 0.05) and IL-6 excretion by 23.5% (1.4 to 40.6%; *p* = 0.04) compared to placebo, whereas no changes in NGAL, LFABP, and MCP-1 were observed. During dapagliflozin treatment, changes in albuminuria correlated with changes in eGFR (*r* = 0.36, *p* = 0.05) and KIM-1 (*r* = 0.39, *p* = 0.05). In conclusion, the albuminuria lowering effect of six weeks dapagliflozin therapy may be the result of decreased intraglomerular pressure or reduced tubular cell injury.

Introduction

Sodium-glucose co-transporter 2 (SGLT2) inhibitors lower glycated haemoglobin (HbA_{1c}), decrease blood pressure, body weight and albuminuria, and, in the long-term, SGLT2 inhibitors appear to slow progression of kidney function decline [1–3]. The mechanisms responsible for the albuminuria-lowering effect are not yet completely understood. A reduction in glomerular hyperfiltration as a result of restoration of tubuloglomerular feedback may be one of the potential mechanisms [4,5]. SGLT2 inhibitors could also hypothetically lower albuminuria by restoring the charge and/or size selectivity of the glomerular basement membrane. Alternatively, improvement in tubular reabsorption of filtered albumin can potentially decrease albuminuria [6]. SGLT2 inhibition reduces the reabsorption of filtered sodium and glucose in the proximal tubule, and thereby it reduces the oxygen-consuming transport workload. Less oxygen stress may help to improve tubular cell integrity and, potentially, tubular albumin reabsorption [7,8]. As hyperglycaemia and albuminuria have proinflammatory influences on renal tubules, reductions in these parameters could, hypothetically, reduce the generation of proinflammatory cytokines [9].

Various biomarkers that are associated with glomerular and tubular structure and function are presently available. The aim of the present study was to characterize the effect of the SGLT2 inhibitor dapagliflozin on glomerular and tubular injury and inflammatory markers. Secondly, we assessed whether changes in albuminuria or estimated glomerular filtration rate (eGFR) during dapagliflozin therapy correlated with kidney injury markers to provide insight into the mechanisms responsible for the lowering of albuminuria with dapagliflozin.

Materials and methods

Study design and patients

This was a *post hoc* analysis of a prospective, randomized, double-blind, placebo-controlled cross-over clinical trial. The study design, patient population and primary results have been described previously [10]. In short, 33 patients with type 2 diabetes mellitus, aged between 18 and 75 years, with a first morning void albumin to creatinine ratio (U_{ACR}) ≥ 100 mg/g and < 3500 mg/g (11.3 – 395.5 mg/mmol) and an eGFR ≥ 45 ml/min/1.73m² were enrolled. Participants were required to use a maximum tolerated stable dose of an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker for more than four weeks. Patients were randomly assigned to two consecutive treatment periods of six weeks, during which they received dapagliflozin 10 mg per day or placebo, with wash-out periods of six weeks in between. The primary outcome was change in 24-hour urinary albumin excretion rate (24-hour UAE). The study was registered with the Netherlands Trial Register (NTR 4439) and complied with the Declaration of Helsinki and Good Clinical Practice Guidelines.

Measurements

Blood and urine samples were obtained at baseline, in the beginning and at the end of the two treatment periods, as well as at the end of the wash-out period. These urine and plasma samples were stored at -80°C for no more than 24 months (range, 6 to 24 months). Blood and urine samples from 31 patients were available for this study.

Urinary IgG and IgG4 were measured as markers of glomerular damage. Urinary kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), and liver-type fatty acid-binding protein (LFABP) were measured as markers of tubular damage. Urinary monocyte chemoattractant protein-1 (MCP-1) and urinary interleukin-6 (IL-6) were measured as markers of inflammation [11]. To assess effects of dapagliflozin on size-selectivity and charge-selectivity of the glomerular basement membrane, plasma IgG and IgG4 were also determined. The ratio of IgG to albumin clearance was calculated as an index of glomerular size selectivity [12]. Glomerular charge selectivity was calculated by the ratio of the clearance of the neutrally charged IgG to the clearance of the negatively charged IgG4 [12]. For quantification of IgG4, KIM-1, NGAL, LFABP, MCP-1 and IL-6, direct sandwich-ELISAs were used according to the manufacturer's specifications (R&D systems, Minneapolis, Minnesota, USA). IgG was quantified with nephelometry.

Statistical analysis

The effect of dapagliflozin compared to placebo on the kidney injury markers was determined with a mixed effects repeated measures analysis. The model included sequence, period, treatment and subject as factors and baseline biomarker of interest as a covariate. All biomarkers were log-transformed before entering the data in the repeated measures model. The between-group geometric mean change in 24-hour excretion of the biomarker of interest was derived by $100 \times (\exp[\text{least square mean change}] - 1)$, and the same transformation was applied to the 95% confidence limits. Pearson's correlation coefficient was used to calculate correlations between eGFR, 24-hour UAE and the kidney injury markers. All variables were log-transformed before calculation of the Pearson's correlation coefficient. SAS software version 8.2 (SAS Institute, Inc., Cary, North Carolina, USA) was used. All statistical tests were two-sided, with a statistical significance level of $p\text{-value} < 0.05$.

Results

Baseline characteristics of the 31 patients with available urine and plasma samples are reported in Supplementary table 1. Dapagliflozin decreased 24-hour UAE by 42.5% (95% confidence interval (CI): 29.9 to 52.9%; $p < 0.01$) and eGFR by $5.2 \text{ ml/min/1.73m}^2$ (2.5 to $7.8 \text{ ml/min/1.73m}^2$; $p < 0.01$) (Figure 1A, B). The decrease in eGFR occurred within the first weeks of dapagliflozin therapy. Six weeks after dapagliflozin discontinuation the mean eGFR was $71 \text{ (SD: 19) ml/min/1.73m}^2$, indicating that the decline in eGFR was reversible after treatment discontinuation.

Effect of dapagliflozin on glomerular, tubular and inflammatory markers

Dapagliflozin significantly reduced urinary glomerular damage markers compared to placebo. Compared to placebo, dapagliflozin decreased the fractional clearance of IgG and IgG4 by 28.4% (95% CI: 7.2 to 44.8%; $p=0.01$) and 34.6% (3.0 to 55.9%; $p=0.04$), respectively. However, the IgG to IgG4 clearance ratio (marker of glomerular charge selectivity) and the IgG to albumin clearance ratio (marker of glomerular size selectivity) did not change significantly during dapagliflozin treatment compared to placebo (Table 1, Figure 1C, D).

At the tubular level, dapagliflozin, compared to placebo, reduced urinary KIM-1 excretion by 22.6% (0.3 to 39.8%; $p=0.05$; Table 1 and Figure 1E), but did not change urinary excretion of NGAL (-13.4% [-35.6 to 16.6%]; $p=0.33$) (Table 1 and Figure 1F) or LFABP (0.9% [-13.2 to 17.2%]; $p=0.91$) (Table 1 and Figure 1G).

Changes in urinary excretion of inflammatory biomarkers with placebo and dapagliflozin therapy are shown in Table 1 and Figure 1H, I. Dapagliflozin, compared to placebo, reduced urinary IL-6 excretion by 23.5% (1.4 to 40.6%; $p=0.04$) and urinary MCP-1 excretion by 14.1% (-32.2 to 8.9%; $p=0.20$).

Table 1: Mean percent changes from baseline in kidney injury markers.

Injury markers	Baseline ^a	Mean change % from baseline placebo (95% CI) ^b	P-value	Mean change % from baseline dapagliflozin (95% CI) ^b	P-value
Glomerular					
IgG	2269 [875-4600]	4.3 (-12.4, 24.2)	0.64	-25.3 (-38.1, -9.9)	0.01
IgG4	4 [1-8]	3.6 (-21.0, 36.0)	0.80	-32.2 (-49.1, -9.7)	0.01
IgG/IgG4	920 [396-1271]	-0.9 (-24.0, 29.2)	0.95	16.7(-11.9, 54.5)	0.29
IgG/Albumin	0.2 [0.2-0.3]	10.9 (1.2, 21.6)	0.04	18.2 (7.0, 30.4)	<0.01
Tubular					
KIM-1	1218 [597-2705]	-0.9 (-20.4, 23.4)	0.94	-23.3 (-39.8, -2.2)	0.04
NGAL	23 [13-65]	9.3 (-9.7, 32.3)	0.37	-5.3 (-22.5, 15.7)	0.60
LFABP	11 [8-17]	21.2 (6.0, 38.5)	0.01	22.2 (6.4, 40.4)	0.01
Inflammatory					
IL-6	3 [2-5]	-0.7 (-18.1, 20.5)	0.95	-24.0 (-37.9, -7.0)	0.01
MCP-1	268 [213-413]	5.6 (-11.1, 25.5)	0.54	-9.3 (-24.3, 8.7)	0.30

^a Baseline data are given as median pg/24h [25th to 75th percentile] for KIM-1, LFABP, IL-6 and MCP-1, and median ng/24h for NGAL.

^b All biomarkers were log-transformed. Mean change in 24-hour excretion of the individual biomarker was derived by $100 \times (\exp[\text{least square mean change}] - 1)$. The same transformation was applied to the 95% confidence limits.

Figure 1: Change in eGFR (ml/min/1.73m²) (A); percent change in 24-hour UAE (B); in IgG/IgG4 (C); in IgG/Albumin (D); in KIM-1 (E); in NGAL (F); LFABP (G); IL-6 (H); and in MCP-1 (I) during placebo and dapagliflozin treatment.

Boxes show mean change within the 25th and 75th percentile. Mean differences and 95% confidence intervals of eGFR, 24-hour UAE, and kidney injury markers, compared to placebo, are shown under each sub-figure. One subject with an IgG/IgG4 change of 4860% is not shown in this figure (C), and one subject with a LFABP change of 857% is not shown in this figure (G).

Correlations between 24-hour UAE, eGFR, and kidney injury markers

At baseline, 24-hour UAE correlated with urinary NGAL, LFABP, and IL-6 excretions (Supplementary table 2). During dapagliflozin treatment there was a significant correlation between 24-hour UAE change and eGFR change ($r = 0.36$, $p = 0.05$) (Supplementary figure 1A). Changes in 24-hour UAE did not correlate with changes in IgG to IgG4 clearance ratio ($r = -0.01$, $p = 0.97$) and IgG to albumin clearance ratio ($r = -0.36$, $p = 0.06$). Changes in KIM-1 excretion significantly correlated with changes in 24-hour UAE ($r = 0.39$, $p = 0.05$) (Supplementary figure 1D–F). There were no correlations between changes in the other tubular biomarkers and changes in 24-hour UAE. Both reductions in IL-6 and MCP-1 excretions correlated with reductions in 24-hour UAE ($r = 0.59$, $p < 0.01$ and $r = 0.44$, $p = 0.02$, respectively) (Supplementary figure 1G, H). Supplementary figure 2 shows that there were no correlations between eGFR changes and changes in any of the kidney injury markers during dapagliflozin therapy. Finally, after six weeks of dapagliflozin treatment, the residual 24-hour UAE level correlated with achieved urinary excretion of KIM-1, LFABP, NGAL, IL-6 and MCP-1 (Supplementary table 2).

Discussion

This study assessed the effects of dapagliflozin on glomerular, tubular, and inflammatory biomarkers in patients with type 2 diabetes mellitus and albuminuria. Dapagliflozin decreased urinary KIM-1 and IL-6 excretion, suggesting that dapagliflozin may beneficially affect renal inflammation and reduces ischemic proximal tubular cell injury. Reductions in urinary albumin excretion can be a consequence of changes in charge and/or size selectivity of the glomerular filtration barrier, decreased intraglomerular pressure, or improved tubular reabsorptive capacity. The lack of effect on IgG to IgG4 clearance ratio and IgG to albumin clearance ratio suggests that changes in albuminuria in response to dapagliflozin treatment are not a result of effects on charge and size selectivity of the glomerular filtration barrier. Therefore, the observed reduction in albuminuria could still be explained by two other mechanisms: reduced intraglomerular pressure and improved tubular reabsorption processes. The reduction in intraglomerular pressure, manifested by the reversible reduction in eGFR, may better explain the reduction in albuminuria as indicated by the positive correlation between eGFR and albuminuria changes. However, it is unlikely that the reduction in intraglomerular pressure completely explains albuminuria lowering with dapagliflozin as the time-course of effects on eGFR and albuminuria are different; eGFR changes occur rapidly, whereas effects on albuminuria probably take longer to be fully manifested. Nevertheless, acute hemodynamic effects may form the basis of gradual structural improvements in the glomerular filtration barrier. The albuminuria reduction observed in the Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME) was only partially reversed after treatment discontinuation, suggesting that SGLT2 inhibitors induce structural improvements in the kidney over time [13]. Dapagliflozin appeared to decrease urinary KIM-1 excretion, but there was no effect on urinary

NGAL and LFAPB excretion, compared to placebo. KIM-1 is a specific marker for hypoxic injury to proximal tubular cells, which would suggest that dapagliflozin reduces proximal tubular cell injury [14]. The reabsorption of electrolyte and organic solutes in the proximal tubule requires much energy [7]. The proximal tubule therefore accounts for the largest amount of oxygen consumption in the kidney. SGLT2 expression is increased in patients with diabetes mellitus [5]. As a result, more glucose and sodium are reabsorbed, which increases the oxygen demand of tubular cells. This, in turn, renders the proximal tubule particularly susceptible to hypoxia in the setting of type 2 diabetes mellitus [7,8]. Hypoxia appears to be one of the major drivers of kidney disease progression, especially in the diabetic kidney [8,15]. SGLT2 inhibition reduces sodium and glucose reabsorption in the proximal tubule, thereby reducing the workload for proximal tubular cells. Reduced workload may mitigate hypoxia-induced proximal tubular damage, which could lead to improved tubular cell structural integrity and, possibly, function [7,8]. This notion is supported by a previous animal study showing that SGLT inhibition with phlorizin in diabetic rats improved renal cortical oxygen tension [16].

We also observed a reduction in the inflammatory marker IL-6 during dapagliflozin treatment. IL-6 is an inflammatory cytokine and has been implicated in the progression of diabetic kidney disease [17]. The presence of cytokines in the urine is thought to reflect local renal production of these cytokines [18,19]. The decrease of IL-6 excretion observed in our study is in line with previous animal and in vitro studies [20,21]. Previous studies showed that increased albumin leakage into the tubular compartment triggers an inflammatory response leading to MCP-1 and IL-6 release [22]. The reduction in albumin leakage or tubular reabsorption may alleviate intra-renal inflammation and release of IL-6 and MCP-1. It is also possible that increasing urinary glucose excretion reduces intra-renal glucotoxicity and inflammation [9]. Unfortunately, urinary glucose excretion was not measured in our study.

To the best of our knowledge, our study is the first to investigate the effects of SGLT2 inhibition on tubular injury markers. Our data demonstrate that dapagliflozin does not increase tubular injury markers. In addition, the absence of correlation between changes in eGFR and changes in kidney injury markers indicates that six-weeks dapagliflozin therapy is not associated with tubular injury, although large studies, such as CREDENCE and DAPA-CKD, are required to confirm this finding.

This study has limitations. It was performed as a *post hoc* analysis of a short-term study. As such, we were unable to investigate the long-term effects of SGLT2 inhibition on glomerular, tubular and inflammatory biomarkers. It is not clear whether the results would apply to the sustained effects of dapagliflozin. In addition, because of the relatively small sample size, the power may have been too low to detect significant changes in, for example, the proxies for glomerular charge and size selectivity. These results should, thus, be regarded as hypothesis generating rather than hypothesis testing.

In conclusion, dapagliflozin therapy for six weeks decreases urinary excretion of proximal tubular marker KIM-1 and inflammatory marker IL-6. The observed reduction in albuminuria

correlated positively with the decrease in eGFR, and also with the reduction in KIM-1 excretion. These findings support the hypothesis that the albuminuria-lowering effect of dapagliflozin could be the result of a reduction in glomerular pressure and improved proximal tubular cell integrity.

Acknowledgements

The authors are grateful to the study participants, whose time and effort are critical to the success of our research program.

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Supplementary files

Supplementary table 1: Baseline characteristics.

Characteristic	Total (N= 31)
Age (years)	62 (8.1)
Sex (female/male) (n)	7/24
Systolic blood pressure (mmHg)	143 (14.5)
Diastolic blood pressure (mmHg)	77 (6.0)
Weight (kg)	96 (21.2)
BMI (kg/m²)	31 (5.4)
Hb _{A1c} (mmol/mol)	56 (8.5)
Estimated GFR (ml/min/1.73m²)	72 (22)
24h UAE‡ (mg/24hr)	521 [191-997]

Data are given as mean (SD) and ‡median [25th to 75th percentile].

Supplementary table 2: Pearson coefficients (and corresponding P-values) of the correlations of the various glomerular and tubular injury markers with 24-hour albuminuria (UAE) and kidney function (eGFR) at baseline and at the end of the dapagliflozin treatment period.

Kidney injury markers	Pearson correlation coefficient at baseline (p-value)		Pearson correlation coefficient after 6 weeks dapagliflozin (p-value)	
	24h UAE	eGFR	24h UAE	eGFR
eGFR	-0.32 (0.09)	1.00	-0.13 (0.47)	1.00
24h UAE	1.00	-0.32 (0.09)	1.00	-0.13 (0.47)
Glomerular				
IgG	0.76 (<0.01)	0.49 (0.01)	0.80 (<0.01)	-0.38 (0.04)
IgG4	0.63 (<0.01)	0.37 (0.05)	0.75 (<0.01)	-0.21 (0.26)
IgG/IgG4	0.10 (0.62)	0.11 (0.57)	-0.26 (0.17)	-0.12 (0.54)
IgG/Albumin	-0.10 (0.60)	0.15 (0.44)	-0.17 (0.37)	-0.24 (0.20)
Tubular				
KIM-1	0.16 (0.42)	0.15 (0.46)	0.39 (0.04)	0.06 (0.77)
NGAL	0.53 (<0.01)	0.44 (0.02)	0.38 (0.04)	-0.46 (0.01)
LFABP	0.51 (<0.01)	0.34 (0.07)	0.42 (0.02)	-0.05 (0.78)
Inflammatory				
IL-6	0.62 (<0.01)	0.18 (0.34)	0.68 (<0.01)	0.03 (0.87)
MCP-1	0.29 (0.12)	0.01 (0.97)	0.57 (<0.01)	0.02 (0.91)

Supplementary figure 1: Correlation between changes in albuminuria and changes in eGFR (A); IgG/IgG4 (B); IgG/Albumin (C); KIM-1 (D); NGAL (E); LFABP (F); IL-6 (G); and MCP-1 (H) during dapagliflozin treatment.

Supplementary figure 2: Correlation between changes in eGFR and changes in IgG/IgG4 (A); IgG/Albumin (B); KIM-1 (C); NGAL (D); LFABP (E); IL-6 (F); and MCP-1 (G) during dapagliflozin treatment.

Chapter 5

Effects of the SGLT2 inhibitor dapagliflozin in patients with type 2 diabetes and stages 3b–4 chronic kidney disease

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Nephrol Dial Transplant 2018;33:2005–2011

Abstract

Background: The sodium-glucose co-transporter 2 inhibitor dapagliflozin decreases haemoglobin A1c (HbA_{1c}), body weight, blood pressure (BP), and urinary albumin to creatinine ratio (UACR) in patients with type 2 diabetes mellitus. The efficacy and safety of this drug have not been properly defined in patients with type 2 diabetes and stages 3b-4 chronic kidney disease (CKD).

Methods: In a pooled analysis of 11 phase 3 randomized controlled clinical trials, we determined least square mean changes in HbA_{1c} , body weight, BP, estimated glomerular filtration rate (eGFR) and UACR over 102 weeks in patients with type 2 diabetes and an eGFR between 12 to less than 45 ml/min/1.73m² receiving placebo ($n = 69$) or dapagliflozin 5 or 10 mg ($n = 151$). Effects on UACR were determined in a subgroup of patients with baseline UACR ≥ 30 mg/g ($n = 136$).

Results: Placebo-corrected changes in HbA_{1c} with dapagliflozin 5 and 10 mg were 0.03% (95% confidence interval (CI): -0.3 to 0.3) and 0.03% (95% CI: -0.2 to 0.3) during the overall 102-week period. Dapagliflozin 5 and 10 mg compared with placebo reduced UACR by -47.1% (95% CI: -64.8 to -20.6) and -38.4% (-57.6 to -10.3), respectively. Additionally, dapagliflozin 5 and 10 mg compared with placebo reduced BP and body weight. eGFR increased with placebo during the first 4 weeks but did not change with dapagliflozin. There were no between-group differences in eGFR at the end of follow-up. Adverse events associated with renal function occurred more frequently in the dapagliflozin 10 mg group. These events were mainly asymptomatic increases in serum creatinine.

Conclusion: Dapagliflozin did not decrease HbA_{1c} in patients with type 2 diabetes and stages 3b-4 CKD, but decreased UACR, BP and body weight to a clinically meaningful extent. These results support a large outcome trial in this population to confirm long-term safety and efficacy in reducing adverse clinical endpoints.

Introduction

Approximately 30% to 40% of all patients with diabetes mellitus also have chronic kidney disease (CKD) [1]. Both diabetes and CKD account for an increased risk of premature mortality, cardiovascular morbidity and end-stage kidney failure [2]. Few effective therapies are available for such patients and novel therapeutic options aimed at improving clinical outcomes in this high-risk population are therefore highly desirable.

Sodium-glucose co-transporter 2 (SGLT2) inhibitors are anti-diabetes drugs that block the reabsorption of glucose and sodium in the S1 segment of the proximal tubule, thereby augmenting urinary glucose and sodium excretion [3]. Inhibition of urinary glucose reabsorption results in a reduction in plasma glucose and haemoglobin A1c (HbA_{1c}). Inhibition of sodium reabsorption, on the other hand, leads to increased delivery of sodium to the macula densa, which stimulates tubuloglomerular feedback and afferent arterial vasoconstriction and reduces glomerular hyperfiltration. This is clinically manifested as an acute reduction in glomerular filtration rate (GFR) and albuminuria. The GFR decline is completely reversible after drug discontinuation [4,5].

Only a few clinical studies have explored the efficacy and safety of SGLT2 inhibitors in patients with impaired kidney function [6–8]. These studies have predominantly included patients with stage 3 CKD (estimated GFR 30–60 ml/min/1.73m²), and have demonstrated that the glucose-lowering efficacy of SGLT2 inhibitors in these patients is diminished compared with patients with preserved kidney function [6,8]. This attenuated hypoglycemic action is most likely a result of reduced glucose filtration. Whether SGLT2 inhibitors are effective and safe in patients with more severely impaired kidney function (i.e. stages 3b and 4 CKD, characterized by an eGFR of 15 to 45 ml/min/1.73m²) is not fully known. We therefore performed a pooled analysis of multiple phase 3 clinical trials to characterize the efficacy and safety of the SGLT2 inhibitor dapagliflozin in patients with type 2 diabetes mellitus with stages 3b and 4 CKD.

Materials and Methods

Study designs and patient populations

Eleven phase 3 randomized placebo-controlled clinical trials in patients with type 2 diabetes mellitus receiving dapagliflozin were included in this pooled analysis. Supplementary table 1 shows the characteristics of the study populations included in this analysis. The study designs, populations and primary results of all these studies have been previously reported [7,9–18]. In short, all studies had a core study period of 24 weeks and the majority had an extension period up to 102 or 104 weeks (Supplementary figure 1).

The effects of dapagliflozin 5 and 10 mg as monotherapy or in combination with metformin, sulfonylurea derivatives, thiazolidinediones, dipeptidyl peptidase 4 inhibitors or insulin versus placebo were examined. The studies characterized the effects of dapagliflozin treatment in patients with a range of eGFR levels whose diabetes was inadequately controlled with diet and exercise alone or with the above-mentioned glucose-lowering therapies. Each study protocol

was approved by independent local/central ethics committees and informed consent was obtained from all patients.

Measurements and Outcomes

Patients with diabetes and an eGFR between 12 to less than 45 ml/min/1.73m² were included in this pooled analysis. We characterized the effects of dapagliflozin 5 mg/day or dapagliflozin 10 mg/day versus placebo on HbA_{1c}, markers of kidney function (eGFR, urinary albumin to creatinine ratio (UACR), potassium and phosphate) and other cardiovascular parameters (hematocrit, blood pressure (BP), body weight and uric acid) by assessing the changes from baseline to 4 weeks and/or to 102 weeks follow-up. Additionally, the overall least square means were calculated. Spot urine samples were used to measure UACR.

Statistical analyses

Descriptive statistics were used for presenting baseline characteristics and safety data. The mean change from baseline value and 95% confidence interval (CI) were derived using a longitudinal repeated measures mixed model with fixed terms for treatment, study week and study week-by-treatment interaction as well as the fixed covariates of baseline, baseline-by-study, and baseline-by-week interactions. UACR values were log-transformed (using the natural log) and then exponentiated back to the original scale. The Kenward-Roger method was used. If the model did not converge, the Satterthwaite approximation was used. If this model did not converge, the Kenward-Roger method was used, first with the baseline-by-study and then baseline-by-week terms removed. Finally, if this model still did not converge, analysis of covariance (ANCOVA) was done for each week separately.

Subgroup analyses were performed to assess if the effects of dapagliflozin on UACR at week 24 were consistent across subgroups. The population was stratified by median baseline UACR levels and by micro- and macroalbuminuria stages. An ANCOVA model was used with albuminuria subgroup added as a factor and treatment x albuminuria subgroup as interaction term. The week 24 data were chosen since this was the time point used for the primary analysis in the included studies, and data from a large number of patients were available for meaningful analysis.

The influence of other covariates on the effects of dapagliflozin on UACR were also explored. Continuous fixed covariates of change from baseline to week 24 in HbA_{1c}, systolic BP or body weight were added to an ANCOVA model that included treatment as fixed factor. All analyses for both safety and efficacy variables also included data from patients who had received glycaemic rescue therapy. Patients received open-label rescue therapy with an antihyperglycemic agent if predefined rescue criteria were exceeded. Changes in antihypertensive medications were not controlled for in this study. The statistical analyses were performed with both SAS Versions 9.2 and 9.4 (SAS Institute, Cary, NC, USA).

Results

Baseline characteristics

A total of 220 patients with type 2 diabetes mellitus and an eGFR between 12 to less than 45 ml/min/1.73m² at baseline were included in this *post hoc* analysis. The baseline characteristics of this population are reported in Table 1. The mean baseline eGFR in the placebo, dapagliflozin 5 mg and dapagliflozin 10 mg groups was 38.4 (SD: 5.7), 37.6 (4.6), and 38.0 (5.0) ml/min/1.73m², respectively. The median UACR was 52.0, 51.0 and 40.0 mg/g in the placebo, dapagliflozin 5 mg and dapagliflozin 10 mg groups, respectively. A total of 136 patients (62%) had an UACR ≥ 30 mg/g.

Table 1: Baseline characteristics.

	Placebo (N=69)	Dapagliflozin 5mg (N=58)	Dapagliflozin 10mg (N=93)
Age (years)	66.5 (7.7)	66.0 (9.0)	66.3 (7.4)
Female, n (%)	29 (42.0)	26 (44.8)	44 (47.3)
Race, n (%)			
White	60 (87.0)	46 (79.3)	83 (89.2)
Black	1 (1.4)	5 (8.6)	6 (6.5)
Asian	4 (5.8)	2 (3.4)	1 (1.1)
Other	4 (5.8)	5 (8.6)	3 (3.2)
eGFR (ml/min/1.73m ²)	38.4 (5.7)	37.6 (4.6)	38.0 (5.0)
<30	5 (7.1)	4 (6.9)	3 (3.2)
≥30 to < 45	64 (91.4)	54 (93.1)	90 (96.8)
Weight (kg)	97.7 (19.6)	97.1 (21.1)	98.2 (20.4)
BMI (kg/m ²)	34.6 (5.5)	34.7 (5.9)	34.8 (6.3)
Diabetes duration (years)	13.5 (8.3)	17.2 (9.5)	16.7 (10.3)
HbA1c (%)	8.1 (1.0)	8.4 (1.1)	8.2 (0.9)
Systolic BP (mmHg)	129.7 (15.7)	131.3 (18.5)	134.3 (17.0)
Diastolic BP (mmHg)	73.9 (9.7)	74.0 (9.7)	75.0 (8.5)
Pulse pressure (mmHg)	55.8 (13.9)	57.3 (16.4)	59.3 (16.0)
UACR (mg/g)	52.0 (17.0 - 180.0)	51.0 (18.0 - 539.0)	40.0 (9.0 - 285.0)

Data are mean (SD) or number (%). UACR values represent median (25th to 75th percentile). Abbreviations: BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, haemoglobin A1c; UACR, urinary albumin to creatinine ratio.

Effects of dapagliflozin on glycemic parameters

Dapagliflozin compared with placebo did not change HbA_{1c}. The least square mean differences between dapagliflozin and placebo in percent change in HbA_{1c} during the 102-week period were 0.03% (95% CI: -0.3 to 0.3) for the 5 mg group and 0.03% (95% CI: -0.2 to 0.3) for the 10 mg group. The effect of dapagliflozin compared with placebo on HbA_{1c} was similar when all patients receiving rescue therapy were excluded.

Effects of dapagliflozin on parameters of kidney function

From baseline to 4 weeks, the mean eGFR increased in the placebo group, whereas in the dapagliflozin 10 mg treatment group the mean eGFR remained fairly stable (Figure 1A). Compared to placebo, the mean eGFR change from baseline after 4 weeks of dapagliflozin therapy was -3.6 ml/min/1.73m² (95% CI: -6.0 to -1.2) for the 5 mg group and -3.8 ml/min/1.73m² (95% CI: -5.9 to -1.7) for the dapagliflozin 10 mg group. During the subsequent 72-week follow-up, eGFR remained lower in both dapagliflozin groups than the placebo group (Figure 1A). Thereafter, eGFR levels were similar among the three treatment groups. In the subgroup of 136 patients with UACR ≥ 30 mg/g, the difference between dapagliflozin compared to placebo in UACR was -49.7% (95% CI: -66.9 to -23.6) and -30.2% (95% CI: -52.3 to 2.2) in the 5- and 10 mg groups, respectively, after 4 weeks. This effect was sustained throughout the remaining follow-up period. The least square mean differences between placebo and dapagliflozin 5 and 10 mg during the overall period were -47.1% (95% CI: -64.8 to -20.6) and -38.4% (95% CI: -57.6 to -10.3), respectively. The effects of dapagliflozin on UACR 24 weeks post-therapy were consistent in albuminuria subgroups stratified by baseline median albuminuria level (p for interaction= 0.57) and also in subgroups defined by baseline micro- and macroalbuminuria (p of interaction= 0.26).

Compared to placebo, treatment with dapagliflozin resulted in an elevation of the least square mean serum phosphate level by 0.2 mg/dL (95% CI: 0.1 to 0.4) for the 5 mg group and 0.3 mg/dL (95% CI: 0.1 to 0.4) for the 10 mg groups (Figure 1C). No clinically relevant change in potassium was observed for either of the dapagliflozin groups (Figure 1D).

Effects of dapagliflozin on other renal and cardiovascular risk markers

Treatment with both dapagliflozin 5 mg and 10 mg resulted in an increase in the least square mean hematocrit of 2.5% (95% CI: 1.7 to 3.3) and 2.5% (95% CI: 1.7 to 3.2), respectively, compared to placebo, during the overall 102 week period (Figure 2A). Additionally, dapagliflozin compared to placebo caused a reduction in the least square mean systolic BP of -1.4 mmHg (95% CI: -4.8 to 2.0) and -3.8 mmHg (95% CI: -6.9 to -0.7) for the 5 mg and 10 mg groups, respectively (Figure 2B). Body weight decreased progressively in patients receiving dapagliflozin but increased in the placebo group (Figure 2C). The average reduction in body weight during the whole period was -1.7 kg (95% CI: -3.0 to -0.4) for the 5 mg dapagliflozin group and -2.2 kg (95% CI: -3.4 to -1.0) for the 10 mg dapagliflozin group, compared to placebo. The reduction in

UACR during dapagliflozin therapy was modestly attenuated after adjustment for HbA_{1c}, systolic BP and body weight (Table 2). Changes in uric acid were similar among the three treatment groups (Figure 2D).

Figure 1: Changes in parameters of kidney function over time during treatment with placebo or dapagliflozin: (A) eGFR; (B) UACR; (C) phosphate; (D) potassium.

* UACR analysis (UACR $\geq$ 30 mg/g): n= 42 for placebo, n= 37 for dapagliflozin 5 mg, and n= 57 for dapagliflozin 10 mg. Abbreviations: BL, baseline; CI, confidence interval; Dapa, dapagliflozin; LSM, least square mean.

Safety

The average time of follow-up varied due to drop-out rates and different studies in the pool including different dose arms. The average follow up was 384 days in the placebo arm and 527 and 419 days in the dapagliflozin 5 mg and 10 mg arms, respectively. The overall rate of adverse events was similar in the three treatment groups (Table 3). Serious adverse events occurred in 30.4% of patients in the placebo group and 22.4% and 25.8% in the dapagliflozin 5 mg and 10 mg groups, respectively. Three patients receiving placebo (4.3%), one patient receiving dapagliflozin 5 mg (1.7%) and two patients receiving dapagliflozin 10 mg (2.2%) died during follow-up.

Overall, the proportion of hypoglycemic events was similar between the three groups. Three patients receiving placebo (4.3%) experienced a major episode of hypoglycemia, whereas none of the patients receiving dapagliflozin had a major hypoglycemia event. Urinary tract infections occurred more frequently in the placebo group (13%) compared to the dapagliflozin 5 mg and 10 mg groups (10.3% and 9.7%, respectively), whereas genital infections occurred more frequently in the dapagliflozin groups (5.2% and 4.3%, respectively) compared to the placebo group (1.4%). In the dapagliflozin 10 mg group, more adverse events related to kidney function occurred (25.8%), versus 13% in the placebo group and 6.9% in the 5 mg group. Three serious adverse events of acute renal failure were reported, all three in patients receiving placebo. There were no differences in adverse events related to volume depletion (Table 3).

Figure 2: Changes in renal or cardiovascular risk markers over time during treatment with placebo or dapagliflozin: (A) hematocrit; (B) systolic blood pressure; (C) body weight; (D) uric acid.

Abbreviations: BL, baseline; CI, confidence interval; Dapa, dapagliflozin; LSM, least square mean; SBP, systolic blood pressure.

Table 2: Mean percent change (95% CI) from baseline UACR at week 24: unadjusted for covariates and adjusted for changes from baseline in haemoglobin A1c (HbA_{1c}), systolic blood pressure (SBP), and body weight (BW).

	Placebo (N=29)	Dapagliflozin 5mg (N=33)	Dapagliflozin 10mg (N=41)
Change from baseline UACR, unadjusted	-42.8 (-62.2, -13.5)	-71.3 (-81.1, -56.2)	-62.6 (-75.0, -44.0)
Change from baseline compared to placebo		-49.7 (-68.5, -20.0)	-34.6 (-57.6, 0.9)
Change from baseline UACR, adjusted for HbA _{1c}	-33.0 (-58.6, 8.5)	-63.6 (-78.2, -39.4)	-51.8 (-71.1, -19.7)
Change from baseline compared to placebo		-45.8 (-67.3, -10.1)	-28.1 (-55.5, 16.1)
Change from baseline UACR, adjusted for SBP	-33.3 (-56.9, 3.1)	-61.9 (-76.0, -39.4)	-49.6 (-68.2, -20.1)
Change from baseline compared to placebo		-42.8 (-63.9, -9.5)	-24.4 (-50.9, 16.4)
Change from baseline UACR, adjusted for BW	-34.9 (-59.3, 4.0)	-58.8 (-75.5, -30.8)	-46.2 (-67.6, -10.6)
Change from baseline compared to placebo		-36.7 (-62.6, 7.0)	-17.3 (-49.1, 34.3)

Table 3: Adverse events and serious adverse events.

	Placebo (N=69)	Dapagliflozin 5mg (N=58)	Dapagliflozin 10mg (N=93)
≥ 1 AE	58 (84.1)	53 (91.4)	79 (84.9)
≥ 1 SAE	21 (30.4)	13 (22.4)	24 (25.8)
AEs leading to study drug discontinuation	20 (29.0)	13 (22.4)	21 (22.6)
<i>AEs of special interest</i>			
Hypoglycemia*	26 (37.7)	25 (43.1)	29 (31.2)
Urinary tract infection	10 (14.4)	6 (10.3)	9 (9.7)
Genital infection	1 (1.4)	3 (5.2)	4 (4.3)
Renal function	9 (13.0)	4 (6.9)	24 (25.8)
Volume depletion	5 (7.2)	5 (8.6)	7 (7.5)
<i>SAE of special interest</i>			
Urinary tract infection	0	1 (1.7)	0
Genital infection	0	0	0
Renal function	4 (5.8)	1 (1.7)	1 (1.1)
Volume depletion	1 (1.4)	0	1 (1.1)

Number (%) of adverse events (AE) and serious adverse events (SAE). *Total subjects with hypoglycemia.

Discussion

The principal finding of this pooled analysis of a large phase 3 program is that in patients with type 2 diabetes mellitus and stages 3b–4 CKD, dapagliflozin decreases albuminuria, BP and body weight. These beneficial effects were apparent after 4 weeks of treatment with dapagliflozin and generally persisted throughout the 102-week follow-up period. Dapagliflozin did not decrease HbA_{1c} compared to placebo treatment, indicating that the observed effects on albuminuria, BP and body weight are dissociated from hypoglycemic effects and possibly

mediated by natriuretic/diuretic mechanisms. eGFR was relatively stable over time, both with dapagliflozin and placebo treatment. Dapagliflozin was generally well tolerated in the study population. The overall proportion of adverse events was similar among dapagliflozin- and placebo-treated patients.

Improving glycemic control has been proven to be important in reducing the risk of microvascular complications of diabetes [19]. In our population of patients with type 2 diabetes mellitus and stages 3b–4 CKD, dapagliflozin did not improve glycemic control. Based on this lack of efficacy, SGLT2 inhibitors are currently not recommended for the treatment of diabetes mellitus in patients with impaired renal function [20–23]. Dapagliflozin, however, favorably influenced other cardiovascular risk markers, including BP and albuminuria, in the present study. Similar findings have been observed with two other SGLT2 inhibitors, empagliflozin and canagliflozin [6,8]. The magnitude of albuminuria reduction was clinically meaningful and, based on large epidemiological studies, might be expected to translate into an ~40% relative risk reduction for end-stage kidney disease [24]. This finding helps to justify a dedicated clinical outcome trial to investigate the long-term efficacy and safety of SGLT2 inhibitors in patients with type 2 diabetes mellitus and stages 3b–4 CKD.

The mechanisms by which SGLT2 inhibitors reduce body weight, BP and albuminuria in the absence of glycemic effects are unknown. It is plausible that the reduction in glucose filtration resulting from a reduced eGFR decreases the capacity of SGLT2 inhibitors to inhibit glucose reabsorption. Yet, it remains unclear why other effects persist in patients with impaired kidney function. Such patients may have an increased sensitivity to the mild natriuretic/osmotic diuresis induced by dapagliflozin. The DAPASALT trial (NCT03152084), designed to investigate the mechanisms of the natriuretic–diuretic effects of dapagliflozin in patients with preserved and impaired kidney function, will provide more insight into this issue.

The observed reductions in eGFR, albuminuria and BP resulting from treatment with SGLT2 inhibitors are thought to be a clinical manifestation of increased sodium delivery to the macula densa, which in turn reduces tubuloglomerular feedback and decreases intraglomerular pressure. In many primary and secondary kidney diseases, for example, focal segmental glomerulosclerosis (FSGS), immunoglobulin A (IgA) nephropathy, hypertensive nephrosclerosis, and obesity-induced nephropathy, maladaptive functional renal hemodynamic changes are thought to occur, including increases in renal blood flow, GFR and filtration fraction [25]. In these conditions, administration of SGLT2 inhibitors could potentially restore tubuloglomerular feedback, leading to a reduction in the afferent arterial tone and intraglomerular pressure, which can favorably affect long-term renal prognosis. Hence the increase in the prevalence of non-diabetic kidney disease in many parts of the world, together with the lack of glycemic effects of dapagliflozin in patients with CKD stages 3b–4, makes SGLT2 inhibitors an attractive adjunctive therapy to angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers in the broader CKD population.

The overall proportion of adverse events in our study was similar among patients in the

dapagliflozin and placebo groups, but there were more renal adverse events in patients treated with dapagliflozin. However, there was no increase in the overall serious renal adverse events and no increase in serious acute renal failure events in the dapagliflozin group compared to the placebo group. In addition, randomized placebo-controlled trial data also indicate that the risk of acute kidney injury (AKI) is not increased during SGLT2 inhibition [5,26]. Moreover, a recent registry study reported that the use of SGLT2 inhibitors in patients with type 2 diabetes mellitus who were managed in two large health systems was not associated with an increased risk of AKI [27]. These results are in contrast to the observational reports from clinical practice that were sent to the U.S. Food and Drug Administration (FDA), suggesting that SGLT2 inhibitors increase the risk of AKI. In response to these reports, the FDA recently strengthened their AKI warning for SGLT2 inhibitors [28]. Ongoing large randomized placebo-controlled trials in patients with CKD will provide more definitive evidence as to whether SGLT2 inhibitors cause AKI.

Our study showed increased phosphate levels with SGLT2 inhibition. The clinical relevance of this effect is unknown. It has been speculated that this increase in phosphate may precipitate fractures, but recent data with dapagliflozin in patients with type 2 diabetes indicated no increased fracture risk or increased rate of bone turnover as assessed by biomarkers [15,29,30]. Our results extend prior findings demonstrating that the effect of SGLT2 inhibitors on HbA_{1c} levels in patients with type 2 diabetes mellitus and more severe CKD is attenuated, while effects on other renal and cardiovascular risk markers persist [6]. We report for the first time the effects of SGLT2 inhibition in a subgroup of patients with CKD stages 3b–4 over a 102-week follow-up. The previous studies including patients with stage 3 CKD and a small group of patients with stage 4 CKD produced similar reductions in UACR, BP and body weight, but during a shorter follow-up period [6,8]. In contrast to the previous studies, which showed a decline in eGFR during SGLT2 inhibition, we observed no change in eGFR in the dapagliflozin treatment arms and an increase in the placebo arm. This is likely to reflect regression towards the mean, because we specifically selected patients with an eGFR < 45 ml/min/1.73m² at the baseline visit and, as observed in the placebo arm, the eGFR regressed to the mean at the next visit. For that reason, we also reported the mean eGFR change with dapagliflozin relative to placebo. Finally, we observed that a large proportion of the treatment effect of dapagliflozin on UACR remained present after adjustment for changes in HbA_{1c}, systolic BP or body weight. This finding is in line with previous studies with dapagliflozin and empagliflozin [4,31].

A limitation of this study was the relatively small study population. Secondly, the majority of the population was at relatively low risk of renal endpoints, as reflected by the large number of patients with normoalbuminuria. This likely explains the slow rate of eGFR decline both in the placebo and dapagliflozin treatment groups. Not all of the studies in this pooled analysis examined effects of both dapagliflozin 5- and 10 mg. The data on dapagliflozin 10 mg came primarily from two studies in patients at high cardiovascular risk, while the data on dapagliflozin 5 mg came from a clinical trial in patients with predominantly stages 3a–b CKD. This difference

in background characteristics in the populations may have influenced the safety comparison between dapagliflozin 5- and 10 mg.

In conclusion, dapagliflozin did not decrease HbA_{1c} in patients with type 2 diabetes mellitus and stages 3b–4 CKD in this *post hoc* analysis. However, it decreased UACR, BP and body weight without major side effects. These actions of dapagliflozin support the need for a large outcome trial in this population to confirm long-term safety and efficacy in reducing adverse clinical endpoints.

Acknowledgments

We acknowledge the supportive role of all patients, investigators and support staff in performing the clinical trials with dapagliflozin. We thank Alexander Jones, PhD of inScience Communications, who provided proofreading and journal styling prior to submission.

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Supplementary files

Supplementary table 1: Study characteristics of all the included studies.

Study identifier	Study description	Duration (weeks)	Subject population
NCT00528372	Dapagliflozin monotherapy	24 + 78	Drug-naïve patients with HbA1c $\geq 7.0\%$ and $\leq 10.0\%$
NCT00528879	Dapagliflozin added to metformin	24 + 78	Patients on metformin ≥ 1500 mg/day with HbA1c $\geq 7.0\%$ and $\leq 10\%$
NCT00680745	Dapagliflozin added to SU-derivates	24 + 24	Patients on SU with HbA1c $\geq 7.0\%$ and $\leq 10\%$
NCT00663260	Dapagliflozin in patients with moderate renal impairment	24 + 80	Patients with moderate renal impairment (GFR > 30 to < 60 ml/min/1.73m ²) on a stable antidiabetic regimen with HbA1c $\geq 7.0\%$ and $\leq 11\%$
NCT00683878	Dapagliflozin added to thiazolinediones	24 + 24	Patients on pioglitazone with $\geq 7.0\%$ and $\leq 10.5\%$
NCT00673231	Dapagliflozin added to insulin	24 + 80	Patients on insulin ≥ 30 IU/day $\pm$ maximum 2 OAD with HbA1c $\geq 7.5\%$ and $\leq 10.5\%$
NCT00859898	Initial combination of dapagliflozin and metformin $\leq 12.0\%$	24	Treatment-naïve patients with HbA1c $\geq 7.5\%$ and $\leq 12.0\%$
NCT00984867	Dapagliflozin added to DPP-4 inhibitor	24 + 24	Patients on sitagliptin $\pm$ metformin with HbA1c $\geq 7.0\%$ and $\leq 10\%$
NCT00855166	Dapagliflozin added to metformin	24 + 78	Patients on metformin ≥ 1500 mg.day with HbA1c $\geq 6.5\%$ and $\leq 8.5\%$
NCT01031680	Dapagliflozin in patients with high CV risk	24 + 80	Patients on usual care for diabetes with CVD and hypertension and HbA1c $\geq 7.2\%$ and $\leq 10.5\%$
NCT01042977	Dapagliflozin in patients with high CV risk	24 + 80	Patients on usual care for diabetes with CVD and Hb _{A1c} $\geq 7.0\%$ and $\leq 10.0\%$

Supplementary figure 1: Study duration of all the included studies.

Abbreviations: CV, cardiovascular; Dapa, dapagliflozin; DPP-4i, Dipeptidyl peptidase-4 inhibitors; Ins, insulin; Met, metformin; SU, sulphonylurea; pts, patients; TZD, thiazolidinediones.

Part II

Effects of SGLT2 inhibitors in non-diabetic kidney disease

Chapter 6

Sodium-glucose co-transporter 2 inhibitors: extending the indication to non-diabetic kidney disease?

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Nephrol Dial Transplant 2020;35:i33-i42

Abstract

This year the medical community was pleasantly surprised by the results of the first large outcome trial that primarily examined the renal effects of the sodium-glucose co-transporter 2 (SGLT2) inhibitor canagliflozin (CANA) in subjects with diabetes mellitus and impaired kidney function. The Evaluation of the Effects of Canagliflozin on Renal and Cardiovascular Outcomes in Participants With Diabetic Nephropathy (CREDENCE) trial showed that CANA, relative to placebo, reduces the risk for end-stage kidney disease, doubling of creatinine or kidney death by 34% [hazard ratio 0.66 (95% confidence interval: 0.53 to 0.81)]. These effects were consistent across baseline estimated glomerular filtration rate (eGFR) and haemoglobin A1c (HbA_{1c}) subgroups. In this review we combine the results of the CREDENCE trial with those of several cardiovascular outcome trials with SGLT2 inhibitors and show that, unexpectedly, patients with lower eGFR levels may have greater benefit with respect to cardiovascular outcome than patients with normal kidney function. The cardio- and renoprotective effects of SGLT2 inhibitors seem to be independent of their glucose-lowering effects, as shown in several *post hoc* analyses. In this review we discuss the alleged mechanisms of action that explain the beneficial effects of this novel class of drugs. Moreover, we discuss whether these findings indicate that this class of drugs may also be beneficial in non-diabetic chronic kidney diseases.

Introduction

The proximal tubule in the kidney plays an important role in glucose homeostasis by reabsorbing glucose from pre-urine back into the blood. Glucose is co-transported together with sodium by the sodium-glucose co-transporter 1 (SGLT1), located in the S3 segment of the proximal tubule, and by the sodium-glucose co-transporter 2 (SGLT2) located in the S1 segment of the renal proximal tubule. The vast majority of filtered glucose is reabsorbed by SGLT2. These SGLTs were discovered in the late 1970s/early 1980s [1]. By blocking SGLT1 and SGLT2 competitively with phlorizin, an old natural drug obtained from the bark of apple trees, urinary glucose excretion increased and plasma glucose normalized in diabetic rats [2,3]. Yet, phlorizin was not an ideal candidate glucose-lowering drug because of its low oral bioavailability and unselective SGLT1 and SGLT2 inhibition, with intestinal side effects as a result of SGLT1 inhibition, such as diarrhoea and malabsorption [3,4]. Later on, specific SGLT2 inhibitors were developed as glucose-lowering drugs with fewer intestinal side effects. These drugs had an adequate half-life ($T_{1/2}$) to allow oral once-daily administration [dapagliflozin (DAPA) $T_{1/2}$ 12.2h, canagliflozin (CANA) $T_{1/2}$ 11 to 13h and empagliflozin (EMPA) $T_{1/2}$ 12.4h] [5]. In 2012, the first SGLT2 inhibitor, DAPA, was given marketing authorization by the European Medicines Agency as a glucose-lowering drug in patients with type 2 diabetes mellitus, followed by approval from the US Food and Drug Administration in 2014 [3]. These regulatory agencies required the industry to conduct large cardiovascular outcome trials to investigate potential harmful cardiovascular side effects. In 2015, the first outcome trial was published with the SGLT2 inhibitor EMPA [6]. This trial, together with the two cardiovascular outcome trials with CANA and DAPA that were published in the years thereafter, showed unexpected cardiovascular and renal beneficial effects of these drugs in patients with type 2 diabetes mellitus [6-10]. These patients often receive lipid-lowering, antihypertensive, and antiproteinuric treatment with renin-angiotensin-aldosterone system (RAAS) blockade, but despite these interventions, the residual risk for progression of diabetic kidney disease remains high [11]. The development of SGLT2 inhibitors provides new perspectives for these patients.

Six to 10 years ago, Phase 2 and 3 studies already showed that SGLT2 inhibitors not only lowered plasma glucose, but also decreased blood pressure, body weight (BW) and proteinuria [12-14]. The recent large cardiovascular outcome trials Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME) with EMPA, CANagliflozin cardiovascular Assessment Study (CANVAS) with CANA, and Multicenter Trial to Evaluate the Effect of Dapagliflozin on the Incidence of Cardiovascular Events (DECLARE-TIMI 58) with DAPA reproduced these beneficial effects and also showed that SGLT2 inhibitors lowered the risk for renal events by 34 to 47%, the risk for cardiovascular events by 7 to 14% and the risk for hospitalization for heart failure by ~30% (Table 1). Renal events were defined differently in these three trials as a composite of incidence of kidney replacement therapy/end-stage kidney disease or renal- or cardiovascular death, combined in the EMPA-REG OUTCOME and CANVAS

trials with progression to macroalbuminuria and/or doubling of serum creatinine, and in the DECLARE-TIMI 58 trial with 40% reduction in estimated glomerular filtration rate (eGFR) (Table 1). The results obtained in these trials were very promising. However, they were not designed to examine changes in renal outcomes, but to test cardiovascular safety in a non-inferiority design compared with placebo. Therefore specific, well-powered renal outcome trials were launched and small-scale mechanistic studies were initiated to obtain more insight in the underlying renoprotective mechanisms.

This review will focus on the effects and the use of SGLT2 inhibitors in patients with chronic kidney disease (CKD). We will elaborate on the mechanisms underlying the renoprotective effects and question whether, based on these mechanisms, SGLT2 inhibitors might also be indicated for non-diabetic patients with CKD.

Table 1: Summary of outcome trials with SGLT2 inhibitors.

Trial and design	Main inclusion criteria	Main cardiovascular outcomes	Main renal outcomes
DECLARE-TIMI 58 DAPA 10 mg or placebo once daily N= 17160 eGFR= 85.2 ml/min/1.73m ² Median follow-up: 4.2 years	Type 2 diabetes HbA1c 6.5% – 12.0% Established atherosclerotic CVD or multiple risk factors for atherosclerotic CVD Creatinine clearance ≥ 60 ml/min	17% reduction [HR 0.83 (95% CI 0.73 – 0.95), p= 0.005] of the composite of CV-death or HHF. No effect [HR 0.93 (95% CI 0.84 – 1.03), p= 0.17] on MACEs	47% reduction [HR 0.53 (95% CI 0.43 – 0.66), p< 0.0001] of renal-specific composite outcome
EMPA-REG OUTCOME EMPA 10 mg, EMPA 25 mg or placebo once daily N= 7020 eGFR=74.1 ml/min/1.73m ² Median follow-up: 3.1 years	Type 2 diabetes HbA1c 7.0% – 9.0% without glucose-lowering therapy or HbA1c 7.0% – 10.0% with stable glucose-lowering therapy BMI ≤ 45 kg/m ² Established CVD eGFR ≥ 30 ml/min/1.73m ²	14% reduction [HR 0.86 (95% CI 0.74 – 0.99), p= 0.04] of composite of CV-death, non-fatal myocardial infarction or non-fatal stroke	39% reduction [HR 0.61 (95% CI 0.53 – 0.70), p< 0.001] of renal-specific composite outcome
CANVAS CANA 300 mg, CANA 100 mg or placebo once daily N= 10142 eGFR=76.5 ml/min/1.73m ² Median follow-up: 2.4 years	Type 2 diabetes HbA1c 7.0% – 10.5% Established CVD or two or more risk factors for CVD eGFR ≥ 30 ml/min/1.73m ²	14% reduction [HR 0.86 (95% CI 0.75 – 0.97), p= 0.02] of composite of CV-death, non-fatal myocardial infarction or non-fatal stroke	40% reduction [HR 0.60 (95% CI 0.47 – 0.77)] of renal-specific composite outcome
CREDENCE CANA 100 mg or placebo once daily N= 4401 eGFR= 85.2 ml/min/1.73m ² Median follow-up: 2.6 years	Type 2 diabetes ≥ 30 years of age HbA1c 6.5% – 12.0% Established CKD: eGFR 30 – 90 ml/min/1.73m ² and UACR 300 – 5000 mg/g	31% reduction [HR 0.69 (95% CI 0.57 – 0.83), p< 0.001] of composite of CV-death or HHF	34% reduction [HR 0.66 (95% CI 0.53 – 0.81), p< 0.001] of renal-specific composite outcome

Abbreviations: BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; CV-death, cardiovascular death; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HbA1c, haemoglobin A_{1c}; HHF, hospitalization for heart failure; HR, hazard ratio; MACEs, major adverse cardiovascular events; UACR, urinary albumin to creatinine ratio.

Could SGLT2 inhibitors also be beneficial in patients with reduced kidney function?

SGLT2 inhibitors were officially indicated as an adjunct to diet and exercise to lower blood glucose levels in adults with type 2 diabetes mellitus. The labels do not allow the use of these drugs in subjects with impaired kidney function. Likewise, until recently, regulatory agencies and guidelines advised against prescribing SGLT2 inhibitors to patients with an eGFR < 60 ml/min/1.73m² [15–18]. This recommendation was based on studies illustrating that the glucose-lowering effect of SGLT2 inhibitors is less in people with lower kidney functions [6,9,12].

A smaller mean haemoglobin A1c (HbA_{1c}) difference was also found in the Evaluation of the Effects of Canagliflozin on Renal and Cardiovascular Outcomes in Participants With Diabetic Nephropathy (CREDENCE) trial, a large outcome trial with CANA that specifically included subjects with lower kidney functions when compared with the CANVAS trial (mean baseline eGFR 56 versus 77 ml/min/1.73m², respectively). The effects on HbA_{1c} throughout the trial were –0.25% (95% confidence interval (CI): –0.20 to –0.31%) and –0.58% (95% CI: –0.61 to –0.56%), respectively [10,19]. This is not surprising, since the blood glucose-lowering effect of this class of drugs is dependent on the number of intact nephrons [20]. However, multiple studies have suggested that reductions in blood pressure, BW and proteinuria are independent of glucose control and may persist in people with lower kidney functions [21–24]. For instance, Heerspink *et al.* showed that two years treatment with CANA compared to glimepiride resulted in a smaller annual eGFR decline and a relative larger urinary albumin to creatinine ratio (UACR) reduction in subjects with a higher baseline UACR, while the differences in HbA_{1c} between the groups were modest. Adjusting the analysis for these modest differences in HbA_{1c} did not alter the results [24]. Three other groups examined the use of SGLT2 inhibitors in subjects with lower kidney functions and also found attenuated effects on HbA_{1c} but persistent beneficial effects on blood pressure, BW and proteinuria [21–23]. The effects on cardiovascular and renal endpoints might therefore be different from the effects on HbA_{1c} in patients with a reduced kidney function. These findings also suggest that SGLT2 inhibitors may have potential benefits in subjects with CKD, perhaps even in non-diabetic subjects.

Post hoc analyses of the cardiovascular safety trials evaluated whether these short-term beneficial effects can be translated into risk reductions for cardiovascular and renal events in patients with type 2 diabetes mellitus and established CKD [8,25–27]. The number of subjects with a baseline eGFR < 60 ml/min/1.73m² were 1819 (25.9%), 1265 (7.4%), and 2039 (20.1%), in the EMPA-REG OUTCOME, DECLARE-TIMI 58 and CANVAS trials, respectively. When looking at the EMPA-REG OUTCOME trial, it was concluded that with EMPA in the subjects with a baseline eGFR < 60 ml/min/1.73m², a similar risk reduction for the primary cardiovascular outcome was obtained as in subjects with an eGFR ≥ 60 ml/min/1.73m² [26]. The relative risk reductions for the primary cardiovascular outcome [3-point major adverse cardiovascular events (MACEs)], cardiovascular death and hospitalization for heart failure were also consistent across baseline eGFR subgroups in the CANVAS program [25].

In contrast to the cardiovascular outcome trials, the CREDENCE trial was specifically powered

to assess cardio-renal outcomes in people with type 2 diabetes mellitus and CKD. People with an eGFR between 30 and 90 ml/min/1.73m² and an UACR between 300 and 5000 mg/g were included and randomized to receive treatment with CANA 100 mg/day or placebo. The baseline mean eGFR level was 56 ml/min/1.73m² and the median UACR was 927 mg/g. The trial was stopped early because of overwhelming efficacy. The pre-specified efficacy criteria for early cessation of the trial were achieved at the interim analysis that was conducted after the occurrence of the primary composite renal outcome in 405 patients. Relative to placebo, CANA reduced the risk for end-stage renal disease, doubling of creatinine, or renal death by 34% [hazard ratio (HR) 0.66, 95%CI: 0.53 to 0.81]. Also in this trial, the effects were reported to be consistent across baseline eGFR categories [19]. Nearly all patients included in the CREDENCE trial were on a stable dose of RAAS blockade. Adding CANA slowed the progression of eGFR decline by 1.52 ml/min/1.73m² per year compared with placebo and did not result in an increase in the risk for acute kidney failure [19]. Other renal outcome trials with EMPA (EMPA-KIDNEY) and DAPA (DAPA-CKD) are ongoing.

Taken together, the data of the outcome trials in patients with type 2 diabetes mellitus suggest that SGLT2 inhibitors reduce cardiovascular and renal endpoints regardless of baseline renal function [8,19,25,26]. Surprisingly, a different picture is obtained when data of these trials are combined as shown in Figure 1. This figure shows the primary cardiovascular and renal outcomes of the trials with DAPA, EMPA, and CANA per baseline eGFR subgroup (< 45, 45 to 60, 60 to 90, and ≥ 90 ml/min/1.73m²). Subjects with a lower kidney function seem to have greater beneficial effects on cardiovascular outcomes than subjects with a better kidney function with respect to relative as well as to absolute risk reduction. In line with this, a recent meta-analysis also showed that patients with a lower baseline eGFR have greater reductions of the risk for hospitalization for heart failure than patients with a higher baseline eGFR (P for interaction= 0.007) [28]. These results suggest that from a cardiovascular perspective, especially patients with an impaired kidney function benefit from SGLT2 inhibition. Looking at the renal outcomes in Figure 1, one can observe a beneficial effect of SGLT2 inhibitors in all eGFR subgroups. However, the trend seems opposite to the trend for cardiovascular outcomes. The magnitude of the benefit of SGLT2 inhibition appears to be smaller in people with lower eGFR levels. A similar pattern was observed in the meta-analysis of Neuen *et al.* (P_{trend} for eGFR subgroup 0.073) [27]. Yet, when looking at absolute benefit in Figure 1, expressed as the estimated number needed to treat during five years to prevent one event, it shows that still a better treatment efficacy is found in the lower eGFR subgroups. For example, the average number needed to treat to prevent a renal event is 21 in the subgroup with eGFR < 45 ml/min/1.73m², while it is 30, 62 and 79 in the subgroups of patients with an eGFR 45 to 60, 60 to 90 and ≥ 90 ml/min/1.73m², respectively (Figure 1). Data in Figure 1 suggest that, despite the fact that SGLT2 inhibitors originally were thought to have less efficacy in subjects with lower kidney function, this class of drugs actually has better treatment efficacy in subjects with lower kidney function,

especially when looking at cardiovascular events, but possibly also with respect to the absolute number of renal events to be prevented.

Regarding safety, it can be stated that in the cardiovascular outcome trials in patients with type 2 diabetes mellitus, the SGLT2 inhibitors were generally well tolerated. Overall, there was no increased risk for hyperkalaemia or acute kidney injury [28]. Only the risk for mycotic genital infections appeared to be increased, which is related to the urinary excretion of glucose [29,30]. In the CREDENCE trial, no surprising or unknown adverse events were detected. Only the rates of ketoacidosis were higher in the CANA group than the placebo group (2.2 versus 0.2/1000 patient rates), but the total event rate was low [19]. Subgroup analyses according to baseline kidney function are yet not available.

Based on the new outcome and safety trials, both the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) published in 2018 a consensus statement on the management of hyperglycaemia in patients with type 2 diabetes. They now advise to consider the use of SGLT2 inhibitors in patients with type 2 diabetes mellitus and CKD with or without cardiovascular disease (CVD) (if eGFR is adequate) [17,31]. The revised ADA guideline also included that DAPA is approved for use in patients with type 2 diabetes mellitus and an eGFR ≥ 45 ml/min/1.73m², instead of ≥ 60 ml/min/1.73m² [17]. Given the data that are presented in this review we propose that SGLT2 inhibitors can also be used in subjects with CKD and even lower kidney function.

Figure 1: The primary cardiovascular and renal outcomes of the SGLT2 inhibitor outcome trials according to baseline eGFR subgroups.

The primary cardiovascular outcome was defined as 3-point MACEs. Renal outcomes were defined as sustained 40% decrease of eGFR, renal replacement therapy or end-stage kidney disease or renal death. Only for the CREDENCE trial, the renal outcome was different, namely, doubling of serum creatinine, end-stage kidney disease, or death from renal or cardiovascular causes. The outcomes of subgroup 'eGFR < 60 ml/min/1.73m²' of the DECLARE trial were used in our analysis and were depicted as subgroup 'eGFR 45 to 60 ml/min/1.73m²', on the assumption that there were no subjects with a baseline eGFR below 45 ml/min/1.73m². A detailed description of the methods can be found in the Supplementary material. Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; NNT/5yr, estimated number needed to treat during 5 years to prevent one event; SGLT2i, sodium-glucose co-transporter 2 inhibitor.

Mechanisms underlying the renoprotective effects of SGLT2 inhibitors

After it became clear that the beneficial renal effects of SGLT2 inhibitors were largely independent of the blood glucose-lowering effect of these drugs, intensive research focused on disclosing what mechanism may underlie their renoprotective effect. Multiple mechanisms have been hypothesized to be responsible [32]. Several recent reviews addressed the various

alleged mechanisms leading to renal protection in detail [33]. Therefore this issue will be only briefly discussed here.

Systemic mechanisms involve a decrease in HbA_{1c}, BW, inflammation and blood pressure that are caused by SGLT2 inhibition, which are known risk factors for the development and progression of CKD [32]. SGLT2 inhibition might also contribute to reverse systemic inflammatory and fibrotic processes, as indicated by the decreases in plasma tumor necrosis factor receptor 1 (TNFR1), interleukin-6 (IL-6), matrix metalloproteinase 7 (MMP7) and fibronectin 1 (FN1) during CANA therapy [34]. The effects on blood pressure are not restricted to daytime, as nocturnal blood pressure is also decreased, which is a predictor for cardiovascular and renal disease progression [35,36]. A few articles have also reported that SGLT2 inhibitors reduce aortic stiffness, but others were not able to replicate this [37–39]. Decreases in blood pressure and arterial stiffness might decrease the cardiac afterload. Furthermore, SGLT2 inhibitors might improve the cardiac preload by lowering plasma volume as a result of osmotic and natriuretic diuresis, secondary to urinary sodium and glucose excretion [40,41]. These systemic haemodynamic mechanisms can beneficially influence the heart as well as the kidneys. SGLT2 inhibitors also promote specific intra-renal haemodynamic changes that may protect glomeruli from high-pressure damage. In 2014, Cherney *et al.* [42] showed that EMPA attenuates glomerular hyperfiltration in subjects with type 1 diabetes mellitus. Subsequently he and others speculated about a causal mechanism responsible for the attenuation of glomerular hyperfiltration and for the typical ‘dip’ in GFR that is observed directly after initiation of SGLT2 inhibitors [32]. It was hypothesized that inhibition of SGLT2 decreased the SGLT2-mediated reabsorption of sodium and glucose in the proximal tubule, leading to increased delivery of glucose and sodium chloride to the macula densa. The macula densa interprets this as circulating volume expansion and via tubuloglomerular feedback either dilates the post-glomerular arteriole or constricts the pre-glomerular arteriole. The latter was considered more likely given the absence of changes in vasodilators in the urine. Recently, Kidokoro *et al.* [43] explored the glomerular haemodynamic effects of EMPA in a type 1 diabetic mouse model by visualizing the afferent and efferent arteriole with *in vivo* multiphoton microscopy imaging techniques. They also measured the single-nephron glomerular filtration. EMPA significantly constricted the afferent arteriole within 30 minutes after administration and consequently suppressed glomerular pressure and single-nephron GFR. These effects were abolished by A1 adenosine receptor blockade [43], suggesting that increased adenosine generation following a restored tubuloglomerular feedback mechanism is the key pathway for suppression of hyperfiltration during SGLT2 inhibition.

Inhibition of glucose and sodium reabsorption in the proximal tubule can also lead to other potential beneficial processes in the kidney. For instance, it may improve mitochondrial mechanisms, decrease hypoxic damage to proximal tubular cells, and reduce intra-renal inflammation. A recently published *post hoc* analysis of a short-term clinical trial in subjects with type 2 diabetes mellitus showed that DAPA, compared to placebo, increased the excretion

of urinary ketone bodies and urinary metabolites that are linked to mitochondrial working mechanisms, suggesting a beneficial effect on mitochondria [44]. Plasma metabolites were not changed and there was no correlation with (change in) eGFR, suggesting that the effects were kidney-specific [44]. The authors hypothesized that their results could be explained by increased availability of alternative fuel sources and/or a reduced workload for proximal tubular cells [44]. SGLT2 inhibition might increase the level of ketone bodies as a result of enhanced lipolysis and reduced insulin levels [45]. SGLT2 inhibition might also stimulate tubular ketone body reabsorption by delivering sodium to the sodium-monocarboxylate transporters (SMCT2 and SMCT1) that are dependent on the sodium gradient to reabsorb ketones from the lumen to the proximal tubular cells [45]. Ketone bodies are involved in signalling functions and can act as alternative energy substrate for tubular cells along with glucose and free fatty acids [45]. Furthermore, SGLT2 inhibition might reduce the workload for proximal tubular cells and decrease hypoxia-induced proximal tubular damage, adenosine triphosphate (ATP) consumption and mitochondrial fragmentation [44]. The decrease of hypoxic cell damage is illustrated by a reduction in proximal tubular injury marker kidney injury molecule-1 during SGLT2 inhibitor therapy [46]. Some articles also reported reductions in other kidney injury markers, such as liver fatty acid-binding protein and N-acetyl β -d-glucosaminidase and of inflammatory markers such as IL-6 [34,46,47]. Inflammation is associated with the development and decline of CKD, hence inhibition of inflammatory pathways may also contribute to kidney protection [48].

It is not yet clear whether the above-described mechanisms contribute equally to the favourable kidney outcomes or if certain mechanisms are more important than others. Future experimental studies will have to provide more information and clarification.

Extending to non-diabetic kidney disease

An important question is whether more people can benefit from SGLT2 inhibitors in addition to subjects with diabetes mellitus. At this moment it is unknown whether SGLT2 inhibitors affect cardiovascular and renal outcome in non-diabetic subjects since long-term clinical trials that investigate effects of SGLT2 inhibitors solely in the non-diabetic population are not yet available. An indication may be obtained from studying subgroups of the cardiovascular and renal outcome trials defined by level of glucose control. When meta-analysed, no large differences are observed between subjects with baseline HbA_{1c} levels greater than or less than 8% (Figure 2) [8]. Recently the DAPA-HF trial was published that examined the effects of DAPA in diabetic and non-diabetic subjects with heart failure and reduced ejection fraction. Fifty-five percent of the subjects in each treatment group were non-diabetic at screening [49]. This trial also found a consistent beneficial effect on the primary composite endpoint, i.e. cardiovascular death, hospitalization for heart failure or urgent heart failure visit, between subjects with and without type 2 diabetes mellitus [HR 0.75 (95% CI: 0.63 to 0.90) and HR 0.73 (95% CI: 0.60 to 0.88), respectively] [49]. There was no significant reduction of the secondary composite renal

endpoint, i.e. sustained reduction in eGFR of $\geq 50\%$, end-stage kidney disease or death from renal causes, but the total event numbers were very low (28 subjects in the DAPA group and 39 subjects in the placebo group), and the number of serious renal events was significantly lower in the DAPA group [49]. Hence it might be that SGLT2 inhibitors also reduce eGFR decline in subjects with well-regulated diabetes mellitus, pre-diabetes or even non-diabetic CKD. CKD can lead to nephron loss, resulting in an increased single-nephron glomerular GFR in the remaining glomeruli. This causes intraglomerular hypertension, which in turn can damage the remaining glomeruli, resulting in proteinuria and glomerulosclerosis. Looking at the renoprotective mechanisms of SGLT2 inhibitors, one can think of several kidney diseases that might benefit specifically from SGLT2 inhibitor therapy; for example, obesity-induced CKD, hypertensive nephrosclerosis, or focal segmental glomerulosclerosis (FSGS). Obesity-induced CKD is characterized by renal haemodynamic changes resulting in increased renal plasma flow, GFR, and filtration fraction, possibly due to afferent arterial vasodilation [50]. SGLT2 inhibitors reduce glomerular hyperfiltration and promote BW loss. Both are highly desirable effects in patients with obesity-induced CKD. In patients with hypertensive nephrosclerosis, increased renal plasma flow and glomerular hypertension are a result of arterial stiffening [51]. SGLT2 inhibition can potentially decrease intraglomerular pressure and reduce arterial stiffness in these patients, which could help to slow disease progression. Patients with FSGS or with immunoglobulin A nephropathy might also benefit from SGLT2 inhibition. These glomerular-based diseases often result in proteinuria and hypertension [50]. Most patients respond to RAAS blockade, but not all. Of note, RAAS inhibitors have different mechanisms of action than SGLT2 inhibitors. RAAS inhibitors reduce the intraglomerular pressure by limiting angiotensin II-induced vasoconstriction of the efferent arteriole, while SGLT2 inhibitors activate the tubuloglomerular feedback mechanism, as explained in the previous section. SGLT2 inhibition can therefore be used as an alternative treatment or in addition to RAAS inhibition to further delay the decline of kidney function. However, it is still unclear whether SGLT2 inhibitors are truly effective in non-diabetic kidney diseases.

Currently, several preclinical studies have been published that examined renal effects of SGLT2 inhibitors in non-diabetic animals (Table 2). These studies show contradictory results. Two studies did not find renoprotective effects of SGLT2 inhibitors. The other studies found a reduction of proteinuria, kidney damage, inflammation and fibrosis after SGLT2 inhibition (Table 2). The heterogeneity of the models that were used makes it difficult to compare the results. Summarizing, Zhang *et al.* [52] used a non-diabetic subtotaly nephrectomized rat model representing glomerular hyperfiltration and found no effects on proteinuria, GFR, glomerulosclerosis or tubulointerstitial fibrosis. Ma *et al.* [53] used a mice model of CKD with tubulointerstitial injury and also did not find beneficial effects on GFR, markers of fibrosis and tubular injury and inflammation. Cassis *et al.* [54] used a proteinuric mouse model of CKD and found that DAPA reduced the number of glomerular lesions, proteinuria and podocyte damage. Another group examined the effects of SGLT2 inhibitor luseogliflozin (LUSEO) in an

acute renal injury model and found that LUSEO attenuated endothelial rarefaction, interstitial fibrosis and renal hypoxia. These effects were observed together with an increase in vascular endothelial growth factor (VEGF), suggesting the influence of a VEGF-dependent pathway [55]. Finally, Jaikumkao *et al.* [56] examined the effects of SGLT2 inhibition in a pre-diabetic obese rat model and found that DAPA reduces hyperfiltration, microalbuminuria, inflammation and tubulointerstitial fibrosis. All together the preclinical studies show contrary results. This is possibly due to differences in designs and methods, such as differences in group sizes, interspecies differences (mice versus rats), differences in types of experimental kidney diseases, differences in researcher-induced levels of kidney damage or potential differences in baseline eGFR and HbA_{1c} levels.

Figure 2: The primary cardiovascular and renal outcomes of the SGLT2 inhibitor outcome trials per baseline HbA_{1c} subgroup.

The primary cardiovascular outcome was defined as 3-point MACEs. Renal outcomes were defined as a sustained 40% decrease of eGFR, renal replacement therapy or renal death for the EMPA-REG OUTCOME trial; a composite of doubling of serum creatinine, end-stage kidney disease or renal death for the CANVAS trial; and a composite of doubling of serum creatinine, end-stage kidney disease and renal or cardiovascular death for the CREDENCE trial. A description of the methods can be found in the Supplementary material. Abbreviations: CI, confidence interval; HbA_{1c}, haemoglobin A1c; NNT/5yr, estimated number needed to treat during 5 years to prevent one event; SGLT2i, sodium-glucose co-transporter 2 inhibitor.

Table 2: SGLT2 inhibitors in non-diabetic animals with kidney disease or risk factors for kidney function decline.

References	Design	Main outcomes	Conclusion
Zhang et al.[52]	53 Sprague-Dawley rats were assigned to sham surgery + vehicle, sham surgery + DAPA or subtotal nephrectomy (SNx) + vehicle, SNx + DAPA Treatment period: 12 weeks	DAPA vs vehicle: no change in SBP, 24-h proteinuria excretion, and GFR; no effect on glomerulosclerosis, tubulointerstitial fibrosis and TGF- β 1 mRNA overexpression.	No renoprotective effects in a non-diabetic rat model, representing glomerular hyperfiltration
Ma et al.[53]	20 C57BL/6N mice were assigned to high oxalate diet + vehicle or high oxalate diet + EMPA Treatment period: 7 or 14 days	EMPA vs vehicle: no effect on calcium oxalate crystal deposition; no effect on GFR decline, plasma creatinine, and BUN; no effect on tubular injury, inflammation and fibrosis markers.	No renoprotective effects in a non-diabetic mouse model with progressive CKD due to tubule-interstitial disease
Zhang et al.[55]	C57BL/6J mice were assigned to Model 1: nephrectomy of the right kidney and 11 days later sham surgery or ischemia-reperfusion (IR)-injury left + vehicle or LUSEO; Model 2: contralateral kidney was used as a control and sham surgery or IR-injury left + vehicle or LUSEO Treatment period: 7 days	LUSEO vs vehicle: no effect on creatinine clearance week 1 post-IR. Preserved creatinine clearance at week 4; attenuated TGF- β expression, peritubular capillary congestion and haemorrhage, tissue hypoxia, and CD31-positive cell loss at week 1 and reduced renal interstitial fibrosis at week 4; increased VEGF-A mRNA expression at week 1. Inhibition of VEGF by sunitinib inhibited LUSEO induced renoprotective effects.	LUSEO attenuated endothelial rarefaction, renal hypoxia, and renal interstitial fibrosis after IR-injury in non-diabetic mice, possibly via a VEGF-dependent pathway
Cassis et al.[54]	Unilateral nephrectomy was performed and C57BL/6N mice were assigned to control group (n=12), bovine serum albumin (BSA) injections + vehicle (n=9), BSA + DAPA (n=8), BSA + lisinopril (n=8) Treatment period: 23 days	DAPA and lisinopril reduced SBP; no effects on BW and mGFR decline; DAPA and lisinopril reduced UACR by 63% and 72%; DAPA attenuated glomerular lesions, macrophage infiltration and podocyte loss; DAPA limited cytoskeletal remodeling in vitro.	DAPA reduced proteinuria, glomerular lesions and limited podocyte loss in non-diabetic proteinuric mice.
Jaikumkao et al.[56]	Obese Wistar rats were assigned to control group (n=6), high fat diet (HFD) (n=6), HFD + metformin (n=6) or HFD + DAPA (n=6) Treatment period: 4 weeks	DAPA reduced renal hyperfiltration, microalbuminuria and expression of antioxidant enzyme superoxide dismutase; increased antioxidant glutathione; suppressed markers of inflammation and fibrosis and suppressed the expression of endoplasmic reticulum stress and renal pro-apoptotic proteins.	DAPA decreased renal hyperfiltration, microalbuminuria and markers for renal inflammation, tubulointerstitial fibrosis and apoptosis in a pre-diabetic rat model.

Abbreviations: IR, ischaemia-reperfusion; SBP, systolic blood pressure; TGF, transforming growth factor; BUN, blood urea nitrogen; CD31, an endothelial marker.

Table 3: SGLT2 inhibitors in non-diabetic patients with kidney disease or risk factors for kidney function decline.

References	Design	Main outcomes	Conclusion
Rajasekaran et al.[57]	10 participants with biopsy proven FSGS, eGFR ≥ 45 ml/min/1.73m ² , proteinuria of 30 mg - 6g/day and no history of diabetes were treated with DAPA 10 mg/day for 8 weeks as add-on to RAAS blockade therapy	DAPA increased 24-h urinary glucose excretion and plasma haematocrit, had no effect on BW, aldosterone, renin, 24-h urinary protein, mGFR, ERPF, renal vascular resistance, efferent or afferent resistance, glomerular pressure, renal blood flow or filtration fraction; reduced proteinuria in subjects with urinary proteinuria less than the median (post hoc sensitivity analysis). SGLT2 mRNA levels were reduced in FSGS kidney tissue versus healthy controls.	DAPA on top of RAAS blocking treatment had neutral renal hemodynamic and antiproteinuric effects in non-diabetic patients with FSGS
Bays et al.[58]	376 non-diabetic obese subjects were randomized to receive placebo, CANA 50 mg/day, CANA 100 mg/day or CANA 300 mg/day Treatment period: 12 weeks	CANA 50, 100 and 300 mg/day versus placebo: decreased BW by -0.8, -1.6 and -1.3kg and BMI by -0.3, -0.6 and -0.5 kg/m ² , respectively; no change in waist circumference and SBP; increased haemoglobin, haematocrit and urinary glucose to creatinine ratio; decreased eGFR by -1.0, -1.8 and 0.3 ml/min/1.73m ² , respectively.	CANA reduced BW but had no beneficial renal effects in non-diabetic obese subjects

Abbreviations: BMI, body mass index; BW, body weight; CANA, canagliflozin; DAPA, dapagliflozin; eGFR, estimated glomerular filtration rate; ERPF, effective renal plasma flow; FSGS, focal segmental glomerulosclerosis; mGFR, measured glomerular filtration rate; RAAS blockade, renin-angiotensin-aldosterone system blockade; SBP, systolic blood pressure.

To our knowledge, only two studies have examined the effects of SGLT2 inhibitors in humans with non-diabetic CKD or risk factors for CKD (Table 3). The first is a pilot study of Rajasekaran *et al.* [57] examining the effects of DAPA on renal haemodynamics and proteinuria in patients with FSGS. Ten subjects with an eGFR ≥ 45 ml/min/1.73m² and urinary protein excretion between 30 mg and 6 g/day were treated with DAPA 10 mg/day for eight weeks on top of RAAS blocking therapy. DAPA increased 24-h urinary glucose excretion and plasma haematocrit but, remarkably, had no effect on BW, measured GFR, effective renal plasma flow (ERPF) and proteinuria [57]. A *post hoc* sensitivity analysis did show an effect on proteinuria, but only in subjects with a proteinuria level below the median [57]. The second study is a trial from Bays *et al.* [58] examining the effects of CANA in 376 non-diabetic obese subjects. They found a significant loss of BW, a small decrease in eGFR and an increase of haemoglobin, haematocrit, and urinary glucose to creatinine ratio [58]. Other mechanistic, small-scale clinical trials in non-diabetic CKD patients are still ongoing. Two of these trials are planned to be published in 2020, DIAMOND (ClinicalTrials.gov: NCT03190694) and DAPASALT (NCT03152084). In 2020 and 2022, respectively, the results of large-scale long-term outcome trials DAPA-CKD (NCT03036150) and EMPA-KIDNEY (NCT03594110) are expected. These outcome trials and mechanistic trials will add new information to the existing data of the CREDENCE trial because these trials include patients without diabetes mellitus, patients with an eGFR < 30 ml/min/1.73m² and patients with non-proteinuric CKD.

Conclusion

The data from recent trials show a pattern that suggests that SGLT2 inhibitors are cardiovascular and renoprotective in patients with lower kidney functions, in patients with lower HbA_{1c} levels and in patients with non-diabetic kidney disease. However, the available data are limited, especially in the non-diabetic CKD population. Moreover, the current literature in the non-diabetic population sometimes shows inconsistent results. Based on these data, we can only hypothesize, but not yet conclude, that SGLT2 inhibitors have renoprotective effects in non-diabetic patients with CKD. Ongoing and future trials will have to prove whether SGLT2 inhibitors are indeed effective in non-diabetic patients with kidney diseases.

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Supplementary files

Figure 1 and Figure 2, methods:

A random-effects meta-analysis was performed using STATA version 14.2 to visualize the trends in treatment effects between eGFR-subgroups. The I-squared values depicted in the figures describe the percentage of variation across the studies that is due to heterogeneity. Heterogeneity was considered to be low if I-squared was lower than 25%, moderate if I-squared was between 25% and 75%, or high if I-squared was higher than 75%. The number needed to treat to prevent 1 event (NNT) were calculated for each study by dividing the average number of participants (in placebo + SGLT2 inhibitor groups divided by 2) by the difference in events between the two treatment groups, corrected for the randomization ratio (for example 1:1 or 2:1) $((N_{\text{placebo}} + N_{\text{intervention}})/2) / (n_{\text{placebo}} - n_{\text{intervention}})$. The estimated NNT during 5 years (NNT5) was calculated by multiplying the NNT with trial follow-up time (FU) divided by 5 $((\text{NNT} * \text{FU})/5)$. The weighed mean for NNT during 5 years was calculated as the sum of (NNT per 5 years times total number of participants times follow-up time) for each trial divided by the sum of (total number of participants times follow-up time) for each trial $(\sum(\text{NNT5} * N_{\text{total}} * \text{FU}) / \sum(N_{\text{total}} * \text{FU}))$.

References to articles that were used to make Figure 1:

DECLARE: data from the supplementary material of the DECLARE trial (Figure S6) was used for the primary cardiovascular outcome and for the renal outcome. Primary cardiovascular outcome was defined as cardiovascular death, myocardial infarction or ischemic stroke (3-point MACEs). Renal outcome was defined as 40% decrease in eGFR, end-stage kidney disease (ESKD), or renal death. The outcomes of subgroup 'eGFR < 60 mL/min/1.73m²' of the DECLARE trial were used in our analysis and were depicted as subgroup 'eGFR 45 to 60 mL/min/1.73m²', on the assumption that there were no subjects with a baseline eGFR below 45 mL/min/1.73m². This assumption was based on the exclusion criteria of the DECLARE-trial.

EMPA-REG: numbers were calculated and provided by Boehringer Ingelheim using the original database of the EMPA-REG OUTCOME trial whereby 3-point MACEs was used as primary cardiovascular outcome and sustained eGFR ≥40% decline, renal replacement therapy (dialysis or renal transplantation) or renal death as renal outcome.

CANVAS: data from the *post hoc* analysis of Nueun *et al.* (Circulation 2018, Figure 2) was used for the primary cardiovascular outcome and for the renal outcome. Primary cardiovascular outcome was defined as 3-point MACEs. Renal outcome was defined as 40% decrease in eGFR, ESKD, or renal death.

CREDENCE: data from the recently published analysis from Mahaffey *et al.* (Circulation 2019, Figure 4) was used for the primary cardiovascular outcome, which was defined as cardiovascular death, nonfatal myocardial infarction or nonfatal stroke. Data from the supplementary material of the original CREDENCE trial (Figure S3) was used for the renal

outcome, which was defined as doubling of serum creatinine, ESKD, or death from renal or cardiovascular disease.

References to articles that were used to make Figure 2:

EMPA-REG: numbers were calculated and provided by Boehringer Ingelheim using the original database of the EMPA-REG OUTCOME trial whereby 3-point MACEs was used as primary cardiovascular outcome and sustained eGFR $\geq 40\%$ decline, renal replacement therapy (dialysis or renal transplantation) or renal death as renal outcome.

CANVAS: data from the original CANVAS trial (NEJM 2017, Figure 4) was used for the primary cardiovascular outcome. Primary cardiovascular outcome was defined as a composite of death from cardiovascular causes, nonfatal myocardial infarction and nonfatal stroke. Data from the *post hoc* study from Perkovic *et al.* (Lancet 2018, Figure 3) was used for the renal outcome, which was defined as composite of doubling of serum creatinine, ESKD or death from renal causes.

CREDENCE: data from the recently published analysis from Mahaffey *et al.* (Circulation 2019, Figure 4) was used for the primary cardiovascular outcome, which was defined as cardiovascular death, nonfatal myocardial infarction or nonfatal stroke. Data from the supplementary material of the original CREDENCE trial (Figure S3) was used for the renal outcome, which was defined as doubling of serum creatinine, ESKD, or death from renal or cardiovascular disease.

Chapter 7

Effects of the SGLT2 inhibitor dapagliflozin on proteinuria in non-diabetic patients with chronic kidney disease (DIAMOND): a randomised, double-blind, crossover trial

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Lancet Diabetes Endocrinol. 2020 Jul; 8(7): 582–593

Abstract

Background: SGLT2 inhibition decreases albuminuria and reduces the risk of kidney disease progression in patients with type 2 diabetes. These benefits are unlikely to be mediated by improvements in glycaemic control alone. Therefore, we aimed to examine the kidney effects of the SGLT2 inhibitor dapagliflozin in patients with proteinuric kidney disease without diabetes.

Methods: DIAMOND was a randomised, double-blind, placebo-controlled crossover trial done at six hospitals in Canada, Malaysia, and the Netherlands. Eligible participants were adult patients (aged 18–75 years) with chronic kidney disease, without a diagnosis of diabetes, with a 24-h urinary protein excretion greater than 500 mg and less than or equal to 3500 mg and an estimated glomerular filtration rate (eGFR) of at least 25 mL/min per 1.73 m², and who were on stable renin–angiotensin system blockade. Participants were randomly assigned (1:1) to receive placebo and then dapagliflozin 10 mg per day or vice versa. Each treatment period lasted 6 weeks with a 6-week washout period in between. Participants, investigators, and study personnel were masked to assignment throughout the trial and analysis. The primary outcome was percentage change from baseline in 24-h proteinuria during dapagliflozin treatment relative to placebo. Secondary outcomes were changes in measured GFR (mGFR; via iohexol clearance), bodyweight, blood pressure, and concentrations of neurohormonal biomarkers. Analyses were done in accordance with the intention-to-treat principle. This study is registered with ClinicalTrials.gov, NCT03190694.

Findings: Between Nov 22, 2017, and April 5, 2019, 58 patients were screened, of whom 53 (mean age 51 years [SD 13]; 32% women) were randomly assigned (27 received dapagliflozin then placebo and 26 received placebo then dapagliflozin). One patient discontinued during the first treatment period. All patients were included in the analysis. Mean baseline mGFR was 58.3 mL/min per 1.73 m² (SD 23), median proteinuria was 1110 mg per 24 h (IQR 730–1560), and mean HbA_{1c} was 5.6% (SD 0.4). The difference in mean proteinuria change from baseline between dapagliflozin and placebo was 0.9% (95% CI –16.6 to 22.1; *p*=0.93). Compared with placebo, mGFR was changed with dapagliflozin treatment by –6.6 mL/min per 1.73 m² (–9.0 to –4.2; *p*<0.0001) at week 6. This reduction was fully reversible within 6 weeks after dapagliflozin discontinuation. Compared with placebo, bodyweight was reduced by 1.5 kg (0.03 to 3.0; *p*=0.046) with dapagliflozin; changes in systolic and diastolic blood pressure and concentrations of neurohormonal biomarkers did not differ significantly between dapagliflozin and placebo treatment. The numbers of patients who had one or more adverse events during dapagliflozin treatment (17 [32%] of 53) and during placebo treatment (13 [25%] of 52) were similar. No hypoglycaemic events were reported and no deaths occurred.

Interpretation: 6-week treatment with dapagliflozin did not affect proteinuria in patients with chronic kidney disease without diabetes, but did induce an acute and reversible decline in mGFR and a reduction in bodyweight. Long-term clinical trials are underway to determine whether SGLT2 inhibitors can safely reduce the rate of major clinical kidney outcomes in

patients with chronic kidney disease with and without diabetes.

Funding: AstraZeneca.

Research in context

Evidence before this study

We searched PubMed for publications in English from Jan 1, 1990, to Jan 31, 2020, using the search terms “SGLT2”, “SGLT2 inhibitor”, “albuminuria”, “proteinuria”, “kidney disease”, “nephropathy”, and “HbA_{1c}”. SGLT2 inhibitors have cardiovascular and kidney benefits in people with type 2 diabetes, as shown in large cardiovascular and kidney outcome trials and analyses of data from clinical practice registries. Analyses from these studies suggest that the benefits of SGLT2 inhibitors on kidney function are largely independent of their glucose-lowering effects. In the CREDENCE trial, the SGLT2 inhibitor canagliflozin was shown to reduce the risk for cardiorenal outcomes in people with type 2 diabetes and chronic kidney disease irrespective of baseline HbA_{1c}. Whether kidney protection with SGLT2 inhibitors can similarly be achieved in non-diabetic proteinuric kidney disease is unknown.

Added value of this study

To our knowledge, this is the first randomised, placebo-controlled, multicentre study examining the effects of an SGLT2 inhibitor on proteinuria in patients with non-diabetic chronic kidney disease and residual proteinuria. 6 weeks of treatment with dapagliflozin did not reduce proteinuria compared with placebo. Dapagliflozin treatment caused an anticipated acute dip in iohexol-measured glomerular filtration rate (mGFR), which was completely reversible after drug discontinuation. Furthermore, dapagliflozin decreased bodyweight and increased haematocrit and haemoglobin (suggesting haemoconcentration); although systolic and diastolic blood pressure were not significantly different between dapagliflozin and placebo treatment, they were numerically lower with dapagliflozin treatment. Dapagliflozin did not reduce HbA_{1c} or fasting plasma glucose in these patients without diabetes and did not affect urine adenosine or other vasoactive mediators, which are factors that have been linked with changes in kidney haemodynamics in previous SGLT2 inhibitor studies in patients with diabetes.

Implications of all the available evidence

Dapagliflozin treatment affects mGFR, bodyweight, and markers of haemoconcentration in patients with and without diabetes, suggesting ubiquitous effects on kidney function irrespective of glycaemic effects. The absence of an effect on proteinuria during 6 weeks of treatment suggests that reduced glomerular pressure via SGLT2 inhibition does not affect proteinuria in this short timeframe in patients without diabetes. The effects of long-term

treatment with dapagliflozin on clinical kidney outcomes in patients with chronic kidney disease with and without diabetes are being evaluated in the ongoing DAPA-CKD and EMPA-KIDNEY trials.

Introduction

Inhibition of proximal tubular SGLT2 reduces plasma glucose and HbA_{1c} in patients with type 2 diabetes by augmenting glucosuria. SGLT2 inhibitors were therefore originally developed as glucose-lowering drugs, but have subsequently been shown to have broader applications as cardiovascular and kidney protective therapies independent of glucose lowering in large cardiovascular and kidney outcome trials [1–4]. Post-hoc analyses and meta-analyses of these trials have shown that favourable effects of SGLT2 inhibitors on cardiorenal outcomes are consistent across baseline estimated glomerular filtration rate (eGFR) and HbA_{1c} subgroups, as well as in patients with and without albuminuria [5–7]. Specifically, in the CREDENCE trial, canagliflozin reduced the risk for the major kidney composite outcome by about a third in participants with type 2 diabetes and chronic kidney disease [4]. This reduction was also apparent in subgroups of patients in whom diabetes was well controlled, suggesting that the effect of SGLT2 inhibitors on kidney function is unlikely to be mediated by further improvement of glycaemic effects [8].

Although the mechanisms of kidney protection by SGLT2 inhibitors are not yet fully understood, kidney benefits might be mediated by natriuresis, leading to systemic and intrarenal haemodynamic changes. Specifically, SGLT2 inhibition increases distal tubular sodium and chloride delivery to the macula densa, which augments tubuloglomerular feedback, causing afferent arteriolar vasoconstriction. As a result, SGLT2 inhibitors cause an acute decrease in intraglomerular pressure and GFR in hyperfiltering animals and humans, and also acutely lower GFR in non-hyperfiltering patients with type 2 diabetes [9,10]. This reduction in intraglomerular pressure could lead to protection against loss of kidney function in patients with various causes of chronic kidney disease, but has so far been studied mainly in the setting of diabetic kidney disease.

In view of the potential role of hyperglycaemia-independent kidney protective pathways, we hypothesised that kidney protection with SGLT2 inhibitors can be similarly applied to other causes of non-diabetic proteinuric kidney disease that are characterised by single-nephron hyperfiltration, such as obesity-induced chronic kidney disease, focal segmental glomerular sclerosis, and IgA nephropathy [11]. The few existing preclinical and clinical pilot studies of SGLT2 inhibitors in non-diabetic chronic kidney disease were non-randomised and did not include control groups, and results have been inconsistent [12–15]. Therefore, in the DIAMOND study, we aimed to examine the effects of an SGLT2 inhibitor on surrogate measures of kidney protection, such as proteinuria and measured GFR (mGFR), in an exclusively non-diabetic population at risk of progressive kidney function loss.

Materials and Methods

Study design and participants

DIAMOND was an investigator-initiated, prospective, randomised, double-blind, placebo-controlled crossover trial done at six hospitals in Canada (University Health Network Toronto, Toronto, and University of British Columbia, Vancouver), Malaysia (Hospital Canselor Tuanku Muhriz and University of Malaya, Kuala Lumpur), and the Netherlands (University Medical Center Groningen, Groningen, and Amsterdam University Medical Center, Amsterdam).

Adult patients (aged 18–75 years) with chronic kidney disease, with a 24-h urinary protein excretion greater than 500 mg and less than or equal to 3500 mg and an eGFR of at least 25 mL/min per 1.73 m², and who had used a stable dose of angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers for at least 4 weeks before randomisation were recruited from the outpatient clinics of the participating hospitals. The main exclusion criteria were a diagnosis of type 1 or type 2 diabetes; a diagnosis of chronic kidney diseases considered unresponsive to SGLT2 inhibition, such as autosomal dominant or recessive polycystic kidney disease, lupus nephritis, or antineutrophil cytoplasmic antibody-associated vasculitis; an indication for immunosuppressants or previous use of immunosuppressants for kidney disease within 6 months before enrolment; peripheral vascular disease; or being at risk of dehydration or volume depletion. A full list of the inclusion and exclusion criteria is provided in the supplement.

The study was approved by the medical ethics committees of each centre and was registered with ClinicalTrials.gov, NCT03190694. The study was done in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and in line with the study protocol and statistical analysis plan. Written informed consent was provided by all participants before any study-specific procedures were initiated.

Randomisation and masking

Participants were randomly assigned (1:1) to one of two treatment groups, in which patients either first received placebo then received dapagliflozin 10 mg per day, or vice versa. Randomisation was done via an interactive web response system (IBM Clinical Development Merge with block size of four). The randomisation codes were provided by an unmasked pharmacist employed by the study sponsor (University Medical Center Groningen, Groningen, Netherlands). The study medication was labelled on the basis of the generated codes. The generated codes were used by the unmasked data manager to set up the randomisation module. The investigational drug or placebo was dispensed by local pharmacists, who were masked to treatment group assignment, on the basis of the randomisation number generated in the interactive web response system. Study medication was dispensed at the beginning of the two treatment periods. Patients, investigators, and all study personnel were masked to treatment group assignment throughout the trial and analysis. There were no medical

emergencies that required activation of the unblinding procedure. The dapagliflozin and placebo tablets appeared identical and were supplied in identical bottles.

Procedures

Patients that met the inclusion criteria and received a maximum tolerable stable dose of an ACE inhibitor or angiotensin receptor blocker for at least 4 weeks proceeded directly to the randomisation visit. Participants for whom ACE inhibitor or angiotensin receptor blocker medication was changed during the preceding 4 weeks of the screening visit entered a run-in phase during which the type and dose of these drugs were stabilised. Thereafter, all patients were randomly assigned to one of the two consecutive double-blind treatment periods in which they were first treated with placebo and then with dapagliflozin 10 mg per day or vice versa. Dapagliflozin and placebo tablets were supplied by AstraZeneca (Gothenburg, Sweden). Each treatment period lasted 6 weeks with a 6-week washout period in between to avoid carryover effects. Both treatment periods consisted of three study visits to the outpatient clinic at the start, week 3, and week 6 of each period. A telephone call was made to participants 1 week after the start of each treatment period to assess tolerability. A follow-up visit was scheduled 6 weeks after the last treatment period to assess off-treatment effects. Participants were instructed to take their study medication in the morning; on study visit days, medication was taken after the visit.

At each study visit, a physical examination was done and the patient's bodyweight, heart rate, and blood pressure were measured. Blood pressure (systolic and diastolic) was measured three times with at least 1 minute between measurements. The mean of the three measurements was used in the analysis. 24-h urine samples were collected and blood samples were taken in fasted condition for measurement of a clinical chemistry panel and biomarkers related to kidney function changes. eGFR was calculated with the CKD-EPI equation [16]. During the first and last visit of each treatment period and at the last follow-up visit, GFR was measured by plasma disappearance of iohexol (mGFR). After a 5 mL bolus infusion of iohexol (300 mg iodine per mL), blood samples were taken at 120 min, 150 min, 180 min, 210 min, and 240 min. Iohexol was measured in a central laboratory by use of a high-performance liquid chromatography tandem mass spectrometry technique with an intra- and interassay coefficient of variation of less than 15%. Iohexol clearance was calculated from the total injected dose of iohexol and the estimated area under the curve by use of a one-compartment model to obtain GFR, after correction for the early part of the plasma concentration-time curve according to Bröchner-Mortensen and body surface area [17,18]. During the last visit of each treatment period, blood samples were taken for assessment of treatment adherence. Additionally, treatment adherence was assessed via a pill count done by the researcher and later by the independent study monitor.

Outcomes

The primary outcome was the percentage change from baseline in 24-h proteinuria during

dapagliflozin treatment relative to placebo. Secondary outcomes were changes in mGFR, bodyweight, systolic and diastolic blood pressure, and neurohormonal biomarkers (N-terminal pro B-type natriuretic peptide and prostaglandin markers).

Prespecified exploratory outcomes were 24-h albuminuria, 24-h protein-to-creatinine ratio, and 24-h albumin-to-creatinine ratio. Post-hoc exploratory outcomes were changes in fasting plasma glucose, HbA_{1c}, haemoglobin, haematocrit, potassium, calcium, phosphate, total protein, and HDL and LDL cholesterol. The numbers of adverse events of special interest (including hypoglycaemia [determined by clinical judgment], acute kidney injury, lower-limb amputation, and fractures) and serious adverse events were also recorded and compared between dapagliflozin and placebo treatments.

Statistical analysis

We calculated that 50 patients completing the study would provide 80% power (at $\alpha=0.05$) to detect a 25% reduction in proteinuria between dapagliflozin and placebo, assuming an SD of 0.7 in log-transformed proteinuria (within-subject SD of 0.475). To account for early treatment discontinuation and early study discontinuations, we enrolled 53 participants.

Descriptive statistics were used for baseline characteristics, which were summarised as means and SDs or medians and IQRs. The analysis was performed according to the intention-to-treat principle, including all available proteinuria measurements. A mixed-effects linear regression model was used to analyse repeated measures and estimate mean differences between dapagliflozin and placebo. The same model was used for the primary, secondary, and exploratory outcomes. The model included treatment and categorical time period as fixed factors and patients as a random factor. Period-specific treatment effects were also explored by adding the interaction between treatment and period covariates in the model.

Furthermore, in a prespecified analysis, the effects of dapagliflozin on the primary outcome of geometric mean change in proteinuria and the key secondary outcome of mean change in mGFR were further assessed in various subgroups, including subgroups defined by sex, region (Europe, North America, or Asia), baseline kidney diagnosis, proteinuria level, mGFR, systolic blood pressure, BMI, and diuretic use. The subgroup analyses were done in two stages, first for each subgroup separately and then comparing the estimated treatment effects and their SEs within each group by a χ^2 test with degrees of freedom equal to the number of subgroups being compared minus one.

Prespecified sensitivity analyses were done to account for changes in medication use during the study that could affect proteinuria. Prespecified sensitivity analyses were also done to examine potential differences in the primary outcome when proteinuria data collected at week 3 of each treatment period was included, when the average of proteinuria at screening and at randomisation was used as baseline proteinuria, and when proteinuria was indexed for mGFR. No formal adjustments for multiplicity were done; a nominal (two-sided) level α was set at 0.05. All statistical analyses were done with SAS version 9.4.

Role of the funding source

The funder of the study had no role in the data collection or data analysis. The funder reviewed the study protocol and a coauthor employed by the funder (PJG) was involved in the data interpretation and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

58 patients were screened between Nov 22, 2017, and April 5, 2019, of whom 53 were enrolled and randomly assigned to receive placebo for 6 weeks then dapagliflozin 10 mg per day for 6 weeks (n=26) or vice versa (n=27; figure 1). All participants except one completed both treatment periods; one participant discontinued during the first treatment period (in which the treatment was dapagliflozin) and did not cross over to the second treatment period. Two participants discontinued during the wash-out after period 2 (in which they received placebo), and had no follow-up visit 6 weeks after the period 2. Baseline data and results from at least one proteinuria assessment during the treatment period were recorded for all randomly assigned participants, so all randomly assigned participants were included in the efficacy analyses. Baseline demographic, clinical, and biochemical characteristics are shown in table 1. The most common kidney disease diagnoses among participants were IgA nephropathy (n=25), focal segmental glomerulosclerosis (n=11), and hypertensive nephropathy (n=7); baseline characteristics for these subgroups are provided in the supplement (supplementary table 1). Treatment adherence, defined as proportion of patients who used between 80% and 120% of their medication (based on pill counts) was excellent. 52 (98%) of 53 patients were adherent to study medication in period 1 (one patient taking dapagliflozin was non-adherent) and 52 (100%) of 52 were adherent in period 2.

Figure 1: Trial profile.

Table 1: Patient characteristics at baseline.

	Overall (N=53)	First Placebo then Dapagliflozin (N=26)	First Dapagliflozin then Placebo (N=27)
Age (years)	51 (13)	51 (16)	52 (10)
Sex (n, %)			
Male	36 (68 %)	18 (69 %)	18 (67 %)
Female	17 (32 %)	8 (31 %)	9 (33 %)
Ethnicity (n, %)			
Asia	17 (32 %)	6 (23 %)	11 (41 %)
Hispanic	2 (4 %)	0 (0 %)	2 (7 %)
Caucasian	29 (55 %)	17 (65 %)	12 (44 %)
Other	5 (9 %)	3 (12 %)	2 (7 %)
CKD diagnosis (n, %)			
IgA nephropathy	25 (47 %)	14 (54 %)	11 (41 %)
FSGS	11 (21 %)	3 (12 %)	8 (30 %)
Hypertensive Nephropathy	7 (13 %)	4 (15 %)	3 (11 %)
Other ^a	10 (19 %)	5 (19 %)	5 (19 %)
Body weight (kg)	83.0 (20.3)	79.6 (15.5)	86.2 (24.0)
BMI (kg/m ²)	28.0 (5.1)	27.2 (4.1)	28.8 (5.8)
Heart rate (bpm)	68.5 (13.7)	69.0 (15.6)	68.0 (12.0)
Systolic blood pressure (mmHg)	126.0 (14.8)	124.6 (13.1)	127.4 (16.4)
Diastolic blood pressure (mmHg)	76.2 (8.2)	75.2 (7.8)	77.2 (8.7)
HbA _{1c} (%)	5.6 (0.4)	5.6 (0.4)	5.6 (0.5)
HDL-cholesterol (mmol/L)	1.3 (0.5)	1.2 (0.3)	1.4 (0.7)
LDL-cholesterol (mmol/L)	2.8 (0.9)	2.7 (1.0)	2.8 (0.8)
Haemoglobin (g/L)	134.6 (20.4)	134.4 (20.8)	134.8 (20.4)
mGFR (ml/min/1.73m ²) ^b	58.3 (23.0)	57.8 (25.5)	58.9 (20.7)
≤60 (n, %)	33 (62%)	17 (65%)	16 (59%)
>60 (n, %)	20 (38 %)	9 (35%)	11 (41%)
Proteinuria (mg/24h) ^b	1110.0 (730.0, 1560.0)	1105.0 (720.0, 1530.0)	1170.0 (730.0, 1690.0)
<1000 (n, %)	20 (38%)	11 (42%)	9 (33%)
≥1000 (n, %)	33 (62%)	15 (58%)	18 (67%)
Albuminuria (mg/24h)	856.5 (559.5, 1225.0)	844.0 (538.0, 1142.0)	891.0 (599.0, 1338.0)
ACE-inhibitor (n, %)	31 (58 %)	16 (62 %)	15 (56 %)
Angiotensin Receptor Blocker (n, %)	22 (42 %)	10 (38 %)	12 (44 %)
Diuretic (n, %)	14 (26 %)	8 (31 %)	6 (22 %)
NSAID (n, %)	2 (4%)	1 (4%)	1 (4%)
Vitamin D analogues (n, %)	12 (23%)	9 (35%)	3 (11%)
Corticosteroids (n, %)	4 (8%)	2 (8%)	2 (7%)

Data are n (%), mean (SD), median (IQR). ACE-inhibitor= angiotensin converting enzyme inhibitor. BMI= body-mass index. bpm= beats per minute. CKD= chronic kidney disease. FSGS = Focal Segmental Glomerulosclerosis. HbA_{1c} = haemoglobin A1c. HDL= high-density lipoprotein. LDL= low-density lipoprotein. IQR= interquartile range. mGFR= measured glomerular filtration rate. SD= standard deviation.

^a 'Other' contains the following causes of CKD: Alport syndrome (n=2) Vater syndrome (n=1); Membranous Nephropathy (n=1), Collagen 4 mutation (n=1) Unknown (n=6).

^b Finer categories of mGFR and proteinuria are presented in supplementary table 2.

Geometric mean 24-h proteinuria at the start of placebo treatment was 1088.1 mg per 24 h (95% CI 904.6–1308.6), which decreased by 15.8% (3.5 to 26.4) after 6 weeks. At the start of dapagliflozin treatment, geometric mean 24-h proteinuria was 1029.8 mg per 24 h (866.1–1224.3), which decreased by 15.0% (2.8 to 25.7) after 6 weeks. Accordingly, the difference in mean percentage change in 24-h proteinuria from baseline between dapagliflozin and placebo treatment was 0.9% (–16.6 to 22.1; $p=0.93$; figure 2A). The difference in mean percentage change in 24-h albuminuria between dapagliflozin and placebo treatment was –11.8% (–30.4 to 12.0; $p=0.30$; figure 2B). The difference in mean percentage change in protein-to-creatinine ratio between dapagliflozin and placebo treatment was –6.1% (–21.7 to 12.5; $p=0.49$; figure 2C). The difference in mean percentage change in albumin-to-creatinine ratio between dapagliflozin and placebo treatment was –17.0% (–33.2 to 3.4; $p=0.095$; figure 2D).

Mean mGFR was 58.0 mL/min per 1.73 m² (SD 23.6) at the start of placebo treatment and 59.3 mL/min per 1.73 m² (23.8) at the start of dapagliflozin treatment. mGFR increased during placebo treatment by 0.3 mL/min per 1.73 m² (95% CI –1.4 to 2.0) and decreased by 6.3 mL/min per 1.73 m² (4.6 to 8.0) during dapagliflozin treatment. After 6 weeks, the difference in mean mGFR change from baseline for dapagliflozin versus placebo was –6.6 mL/min per 1.73 m² (–9.0 to –4.2; $p<0.0001$; figure 3A). The reduction in mGFR with dapagliflozin was completely reversible within 6 weeks after discontinuation, with mGFR values in the dapagliflozin period showing no difference from placebo or from their respective baseline values (figure 3A).

Dapagliflozin treatment for 6 weeks resulted in a significant placebo-corrected change in mean bodyweight of –1.5 kg (95% CI –3.0 to –0.03; $p=0.046$; figure 3C). There were no changes from baseline in systolic blood pressure and HbA_{1c} with dapagliflozin treatment compared with placebo (figure 3B, 3D). There was also no change in heart rate (–0.13 beats per min [–3.1 to –2.8; $p=0.93$]) or diastolic blood pressure (–1.38 mm Hg [–4.1 to 1.3; $p=0.31$]) during dapagliflozin treatment compared with placebo. Additionally, plasma concentrations of biomarkers, including N-terminal pro B-type natriuretic peptide, and prostaglandin markers were unchanged after dapagliflozin treatment (table 2).

Figure 2: Changes in proteinuria, albuminuria, in protein to creatinine ratio during dapagliflozin and placebo treatment.

Absolute values are geometric means. Error bars are 95% CIs. Data are from a total of 53 participants, 27 of whom received dapagliflozin then placebo and 26 of whom received placebo then dapagliflozin.

Figure 3: Changes in measured glomerular filtration rate (mGFR), systolic blood pressure, body weight and haemoglobin A1c (HbA1C) during dapagliflozin and placebo treatment.

Absolute values are means. Error bars are 95% CIs. Data are from a total of 53 participants, 27 of whom received dapagliflozin then placebo and 26 of whom received placebo then dapagliflozin. mGFR=measured glomerular filtration rate.

Table 2: Changes in neurohormonal biomarkers during placebo and dapagliflozin treatment at 6 weeks.

	Placebo	Dapagliflozin
NT-proBNP		
N	50	52
Baseline mean, ng/L (SD)	125.0 (161.1)	118.8 (151.2)
Week 6 mean, ng/L (SD)	152.6 (277.7)	108.7 (148.7)
Adjusted mean percentage change from baseline, % (95% CI) ^a	1.1 % (-13.3, 17.9)	-14.9 % (-26.9, -1.0)
Difference in mean percentage change vs placebo, % (95% CI) ^a		-15.8 % (-32.2, 4.5)
p-value		0.1170
Urine Adenosine^b		
N	50	51
Baseline mean, µmol/mmol (SD)	0.19 (0.15)	0.20 (0.16)
Week 6 mean, µmol/mmol (SD)	0.24 (0.25)	0.22 (0.17)
Adjusted mean change from baseline, µmol/mmol (95% CI)	0.04 (-0.02, 0.10)	0.02 (-0.04, 0.08)
Difference in adjusted mean change vs placebo, µmol/mmol (95% CI)		-0.02 (-0.11, 0.06)
p-value		0.5735
Urine Thromboxane B2^b		
N	50	51
Baseline mean, pg/mmol (SD)	0.096 (0.047)	0.092 (0.057)
Week 6 mean, pg/mmol (SD)	0.093 (0.050)	0.106 (0.101)
Adjusted mean change from baseline, pg/mmol (95% CI)	-0.003 (-0.023, 0.018)	0.015 (-0.006, 0.035)
Difference in adjusted mean change vs placebo, pg/mmol (95% CI)		0.017 (-0.012, 0.047)
p-value		0.2405
Urine 6-keto prostaglandin F1α^b		
N	50	51
Baseline mean, pg/mmol (SD)	0.117 (0.040)	0.117 (0.042)
Week 6 mean, pg/mmol (SD)	0.120 (0.055)	0.128 (0.045)
Adjusted mean change from baseline, pg/mmol (95% CI)	0.005 (-0.007, 0.017)	0.011 (-0.001, 0.023)
Difference in adjusted mean change vs placebo, pg/mmol (95% CI)		0.006 (-0.011, 0.023)
p-value		0.5046
Urine Prostaglandin E2^b		
N	50	51
Baseline mean, pg/mmol (SD)	0.098 (0.103)	0.113 (0.150)
Week 6 mean, pg/mmol (SD)	0.095 (0.143)	0.170 (0.266)
Adjusted mean change from baseline, pg/mmol (95% CI)	-0.002 (-0.051, 0.047)	0.057 (0.008, 0.105)
Difference in adjusted mean change vs placebo, pg/mmol (95% CI)		0.059 (-0.010, 0.128)
p-value		0.0924
Urine PGEM^b		
N	50	51
Baseline mean, pg/mmol (SD)	0.095 (0.055)	0.100 (0.056)
Week 6 mean, pg/mmol (SD)	0.096 (0.057)	0.097 (0.054)
Adjusted mean change from baseline, pg/mmol (95% CI)	0.002 (-0.006, 0.011)	-0.002 (-0.010, 0.007)
Difference in adjusted mean change vs placebo, pg/mmol (95% CI)		-0.004 (-0.016, 0.008)
p-value		0.4978

N is the number with data available. CI= confidence interval. NT-proBNP= N-terminal pro B-type Natriuretic Peptide.

PGEM= prostaglandin E2 metabolite. SD= standard deviation.

^a Log transformed data was back transformed using the formula $1 - \text{Exponent}(\text{"log value"}) * -100$.

^b Biomarkers were corrected for urine creatinine.

Overall, dapagliflozin was well tolerated in these patients with chronic kidney disease without diabetes. 30 patients had one or more adverse events; 17 (32%) of 53 participants had one or more adverse events during dapagliflozin treatment and 13 (25%) of 52 had one or more adverse events during placebo treatment (table 3). Adverse events of special interest were uncommon, with two occurring during placebo treatment and four during dapagliflozin treatment. No participants had a hypoglycaemic event during the study. One participant had a kidney-related adverse event (acute kidney injury) during dapagliflozin treatment. Urinary tract infections and genital infections occurred in one patient each during dapagliflozin treatment. There were two serious adverse events, with one (cellulitis) occurring during placebo treatment and the other (colon cancer) during dapagliflozin treatment.

Table 3: Summary of adverse events.

	Placebo (N=52)*	Dapagliflozin (N=53)
Any adverse event	13 (25.0%)	17 (32.1%)
Adverse event leading to study drug discontinuation†	0	1 (1.9%)
Any serious adverse event‡	1 (1.9%)	1 (1.9%)
Serious adverse event leading to study drug discontinuation	1 (1.9%)	0 (0%)
Death	0 (0%)	0 (0%)
<i>AE of special interest</i>		
Kidney related adverse event§	0 (0%)	1 (1.9%)
Urinary tract infection	0 (0%)	1 (1.9%)
Genital infection	0 (0%)	1 (1.9%)
Volume depletion		
Hypotension	1 (1.9%)	0 (0%)
Dizziness	1 (1.9%)	0 (0%)
Amputations	0 (0%)	0 (0%)
Fractures	0 (0%)	1 (1.9%)
Diabetic ketoacidosis	0 (0%)	0 (0%)
Hypoglycaemia	0 (0%)	0 (0%)

Data are number of patients (%) with one or more adverse event of the specified type. *One patient discontinued the study during the first treatment period with dapagliflozin; this patient did not start the placebo treatment period and was therefore not included in the safety assessments for the placebo group. †The adverse event leading to discontinuation was swelling of the left foot. ‡The two serious adverse events were cellulitis (during placebo treatment) and colon cancer (during dapagliflozin treatment). §The kidney-related adverse event was investigator-reported acute kidney injury.

Post-hoc analysis of exploratory biochemical parameters showed a significant increase in haemoglobin (increase of 5.3 g/L [95% CI 2.7 to 7.9; $p < 0.0001$]) and haematocrit (increase of 0.02 L/L [0.01 to 0.03; $p < 0.0001$]) with dapagliflozin treatment versus placebo (table 4). Other exploratory biochemical parameters, including fasting plasma glucose, potassium, calcium, and phosphate, were unchanged during dapagliflozin treatment. The effects of dapagliflozin on 24-h proteinuria were consistent in subgroups defined by sex, region, kidney diagnosis, proteinuria level, systolic blood pressure, BMI, and diuretic use. However, the effect of dapagliflozin on 24-h proteinuria was modified by baseline mGFR, with a significant between-group difference in proteinuria in those with an mGFR of more than 60 mL/min per 1.73 m² (–27.2% [95% CI –44.6 to –4.2; $p = 0.0040$]; figure 4). The effect of dapagliflozin on mGFR was generally consistent across the examined subgroups, apart from in the subgroup by age (supplementary figure 1).

Table 4: Changes in exploratory biochemical parameters during placebo and dapagliflozin treatment at 6 weeks.

	Placebo	Dapagliflozin
Fasting plasma glucose		
N	52	52
Baseline mean, mmol/L (SD)	5.5 (0.7)	5.6 (1.2)
Week 6 mean, mmol/L (SD)	5.4 (0.7)	5.3 (0.7)
Adjusted mean change from baseline, mmol/L (95% CI)	-0.1 (-0.3, 0.1)	-0.3 (-0.5, -0.1)
Difference in adjusted mean change vs placebo, mmol/L (95% CI; p-value)		-0.2 (-0.5, 0.1; p=0.16)
Haemoglobin		
N	51	53
Baseline mean, g/L (SD)	134.5 (20.2)	133.3 (19.4)
Week 6 mean, g/L (SD)	131.8 (21.0)	136.7 (18.2)
Adjusted mean change from baseline, g/L (95% CI)	-2.3 (-4.1, -0.4)	3.0 (1.2, 4.8)
Difference in adjusted mean change vs placebo, g/L (95% CI; p-value)		5.3 (2.7, 7.9; p<0.0001)
Haematocrit		
N	52	53
Baseline mean, L/L (SD)	0.40 (0.05)	0.40 (0.06)
Week 6 mean, L/L (SD)	0.39 (0.06)	0.41 (0.05)
Adjusted mean change from baseline, L/L (95% CI)	-0.01 (-0.01, -0.002)	0.01 (0.01, 0.02)
Difference in adjusted mean change vs placebo, L/L (95% CI; p-value)		0.02 (0.01, 0.03; p<0.0001)
eGFR		
N	52	52
Baseline mean, ml/min/1.73m ² (SD)	59.9 (28.0)	57.4 (26.7)
Week 6 mean, ml/min/1.73m ² (SD)	59.2 (28.8)	57.2 (27.9)
Adjusted mean change from baseline, ml/min/1.73m ² (95% CI)	-0.8 (-2.5, 0.9)	-1.8 (-3.5, -0.1)
Difference in adjusted mean change vs placebo, ml/min/1.73m ² (95% CI; p-value)		-1.0 (-3.4, 1.4; p=0.42)
Potassium		
N	52	53
Baseline mean, mmol/L (SD)	4.2 (0.4)	4.1 (0.4)
Week 6 mean, mmol/L (SD)	4.2 (0.4)	4.1 (0.4)
Adjusted mean change from baseline, mmol/L (95% CI)	0.02 (-0.06, 0.10)	0.00 (-0.08, 0.08)
Difference in adjusted mean change vs placebo, mmol/L (95% CI; p-value)		-0.02 (-0.13, 0.1; p=0.78)
Calcium		
N	52	52
Baseline mean, mmol/L (SD)	2.3 (0.1)	2.3 (0.1)
Week 6 mean, mmol/L (SD)	2.3 (0.1)	2.3 (0.1)
Adjusted mean change from baseline, mmol/L (95% CI)	-0.01 (-0.03, 0.02)	0.01 (-0.02, 0.03)
Difference in adjusted mean change vs placebo, mmol/L (95% CI; p-value)		0.01 (-0.02, 0.04; p=0.55)

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	Placebo	Dapagliflozin
Phosphate		
N	51	51
Baseline mean, mmol/L (SD)	1.0 (0.2)	1.0 (0.2)
Week 6 mean, mmol/L (SD)	1.1 (0.2)	1.1 (0.2)
Adjusted mean change from baseline, mmol/L (95% CI)	0.01 (-0.04, 0.06)	0.05 (-0.002, 0.09)
Difference in adjusted mean change vs placebo, mmol/L (95% CI; p-value)		0.03 (-0.03, 0.01; p=0.30)
Total protein		
N	49	51
Baseline mean, g/L (SD)	68.7 (5.7)	68.8 (5.5)
Week 6 mean, g/L (SD)	68.9 (6.0)	70.3 (6.2)
Adjusted mean change from baseline, g/L (95% CI)	0.05 (-0.98, 1.09)	1.13 (0.12, 2.14)
Difference in adjusted mean change vs placebo, g/L (95% CI; p-value)		1.08 (-0.37, 2.53; p=0.14)
HDL cholesterol		
N	52	53
Baseline mean, mmol/L (SD)	1.3 (0.3)	1.3 (0.5)
Week 6 mean, mmol/L (SD)	1.2 (0.3)	1.2 (0.3)
Adjusted mean change from baseline, mmol/L (95% CI)	-0.03 (-0.13, 0.08)	-0.10 (-0.20, 0.00)
Difference in adjusted mean change vs placebo, mmol/L (95% CI; p-value)		-0.07 (-0.22, 0.07; p=0.31)
LDL cholesterol		
N	52	53
Baseline mean, mmol/L (SD)	2.7 (0.9)	2.6 (0.8)
Week 6 mean, mmol/L (SD)	2.7 (0.9)	2.6 (0.8)
Adjusted mean change from baseline, mmol/L (95% CI)	-0.07 (-0.21, 0.07)	-0.06 (-0.20, 0.08)
Difference in adjusted mean change vs placebo, mmol/L (95% CI; p-value)		0.02 (-0.18, 0.21; p=0.88)

n is the number of patients with available measurements. CI= confidence interval. eGFR= estimated glomerular filtration rate. HDL= high-density lipoprotein. LDL= low-density lipoprotein. SD= standard deviation.

Figure 4: Effects of dapagliflozin on 24-hour proteinuria in patient subgroups defined by characteristics at baseline.

BMI= body-mass index. CKD= chronic kidney disease. Dapa= dapagliflozin. FSGS= focal segmental glomerulosclerosis. IgA= immunoglobulin A. mGFR= measured glomerular filtration rate. SBP= systolic blood pressure.

Prespecified sensitivity analyses were done to assess the robustness of the results for the primary outcome of proteinuria. The results did not change when proteinuria data collected at week 3 of each treatment period were included in the analysis (percentage difference between dapagliflozin and placebo 2.5% [95% CI -12.3 to 19.7; $p=0.76$]; supplementary table 3). There was also no difference in 24-h proteinuria between dapagliflozin and placebo when we indexed proteinuria for mGFR (data not shown). Additionally, similar results were obtained when eight patients in whom treatments that could modify proteinuria were changed during the trial were excluded from the analysis (difference between dapagliflozin and placebo in 24-h proteinuria -2.5% [95% CI -20.9 to 20.3; $p=0.81$]) or when the average of screening and randomisation proteinuria was used as baseline (1.2% [95% CI -16.4 to 22.6; $p=0.90$]; supplementary table 3).

Discussion

In this first-in-human randomised crossover trial involving patients with proteinuric chronic kidney disease without diabetes, proteinuria did not decrease over a 6-week treatment period with dapagliflozin compared with placebo.

The beneficial effects of SGLT2 inhibitors on kidney function are potentially mediated through non-glycaemic pathways, suggesting that these drugs might also confer clinical benefits in patients without diabetes. The findings of this trial were consistent with the concept that SGLT2 inhibitors exert haemodynamic effects in normoglycaemic individuals, because mGFR decreased acutely and returned rapidly back to baseline after treatment discontinuation, consistent with a reversible decrease in intraglomerular hypertension. Moreover, haemoconcentration occurred (ie, haematocrit and haemoglobin increased), with significant decreases in bodyweight, suggesting a significant natriuretic response occurred even in the absence of ambient hyperglycaemia. Despite these physiological changes that are considered to confer kidney and cardiovascular protection, proteinuria did not decrease significantly.

Previous studies have shown that in patients with type 2 diabetes and microalbuminuria or macroalbuminuria, SGLT2 inhibition reduces albuminuria by 30–50% after 4–8 weeks of treatment [6,19–21]. Furthermore, these studies showed that the antiproteinuric effects are completely reversible after cessation of treatment, suggesting that they have a haemodynamic basis. The results of the DIAMOND trial suggest that, although 6-week SGLT2 inhibition does exert haemodynamic effects in individuals without diabetes, as reflected by changes in mGFR, significant proteinuria lowering does not occur. However, these findings do not rule out the possibility of longer-term kidney protection. The DAPA-CKD trial, which assessed the long-term effects of dapagliflozin on major kidney outcomes in patients with chronic kidney disease, was stopped early due to overwhelming efficacy in March, 2020 [22,23]. Although the detailed results are not yet available, the early trial termination suggests that dapagliflozin reduces the risks of clinical outcomes in a cohort of patients with kidney disease of diverse causes, including the causes of non-diabetic chronic kidney disease of the patients enrolled in the DIAMOND trial [22]. The reason why proteinuria was unaffected by dapagliflozin, despite a dip in mGFR suggesting

reduced intraglomerular pressure, is not known. One possibility is that the treatment period was too short to observe significant changes in proteinuria, although the duration was expected to be sufficient on the basis of studies in people with type 2 diabetes. By contrast with previous studies that included only individuals with diabetic kidney disease, our study included a broader chronic kidney disease population without diabetes. Although most patients had one of three types of non-diabetic kidney disease (IgA nephropathy, focal segmental glomerulosclerosis, and hypertensive nephropathy), and the effects on proteinuria and mGFR were consistent in all subgroups, differences in underlying disease pathophysiology could have contributed to the inability to detect an antiproteinuric response. For example, patients in our cohort might have had a significant tubulointerstitial source of proteinuria that is less responsive to changes in glomerular pressure. Finally, SGLT2 inhibitors might simply not reduce proteinuria in the absence of diabetes, although mathematical modelling simulations of SGLT2 blockade in a non-diabetic kidney predicted an attenuated response rather than an absence of response [24]. Although proteinuria did not change in our overall study population, it decreased significantly in the subgroup of patients with mGFR greater than 60 mL/min per 1.73 m². We must be cautious in interpreting this finding due to the small sample size; however, if a decrease in proteinuria only occurs in the setting of non-diabetic chronic kidney disease in patients with higher kidney function, this would suggest that a sufficient increase in solute delivery to the macula densa in normoglycaemic patients occurs only with higher levels of GFR. By contrast, in patients without diabetes, whose glucose filtration is already lower compared with those with diabetes, a reduction in GFR leads to a further lowering of glucose and sodium delivery to the macula densa. Hence, tubuloglomerular feedback and other autoregulatory systems might not be sufficiently activated by SGLT2 inhibition to reduce proteinuria in individuals without diabetes and with a GFR of less than 60 mL/min per 1.73 m². Finally, we identified slightly larger effects when the 24-h protein and albumin excretion rates were indexed for 24-h creatinine excretion, suggesting that urine collection errors were made during the 24-h collection period. Nevertheless, despite the potential collection errors, the study was well powered to detect a 25% reduction in proteinuria because the observed variability in 24-h proteinuria was 0.68, consistent with our sample size assumption.

Although dapagliflozin did not reduce proteinuria, mGFR dipped significantly. The mechanisms responsible for changes in GFR and kidney haemodynamics are not yet firmly established, although both afferent and efferent kidney arteriolar pathways have been suggested [9,10]. Irrespective of the mechanisms involved, GFR decreases acutely in response to SGLT2 inhibition in patients with type 2 diabetes and, as shown in this study, similar effects occur in patients with non-diabetic proteinuric kidney disease and mean mGFR in the chronic kidney disease stage 3 range. Notably, in our previous pilot study with dapagliflozin in patients with proteinuria caused by focal segmental glomerulosclerosis, inulin-measured GFR tended to decrease by a similar magnitude to that seen in the present study, although the changes in the pilot study were not statistically significant [12]. These acute, reversible changes in kidney

function have been closely linked with reduced intra-glomerular pressure and might thereby reduce clinical kidney risk over time [12]. None of the secondary biomarkers associated with control of kidney haemodynamics, such as adenosine and prostaglandin markers, changed in response to dapagliflozin in the DIAMOND trial, emphasising the importance of completing ongoing mechanistic and outcome trials with SGLT2 inhibitors to better understand the relevance of these drugs in normoglycaemic individuals [9,10]. Moreover, our results suggest that other vasoactive mediators linked with SGLT2 inhibition-related vascular function effects could be involved as regulators of GFR in individuals without diabetes, including endothelin, nitric oxide, and oxidative stress pathways. These possibilities merit dedicated mechanistic investigations [25,26].

In people with type 2 diabetes, SGLT2 inhibitors reduce systolic blood pressure by 3–5 mmHg and diastolic blood pressure by 1–2 mmHg [27]. Although blood pressure changes in our cohort were not significant, the magnitude of the change was similar. Additionally, SGLT2 inhibitors reduce bodyweight in people with type 2 diabetes by 1–3 kg, which is thought to mostly occur via reducing body fat and water [27]. In our non-diabetic cohort, bodyweight changed by a similar, statistically significant amount. Furthermore, haematocrit and haemoglobin increased significantly, which could reflect natriuresis and effective circulating volume contraction, although we cannot exclude potential effects of erythropoietin production. This haemoconcentration might be of additional importance beyond natriuresis, because it has been linked with improved cardiovascular prognosis in cardiovascular outcome trials involving SGLT2 inhibitors for patients with type 2 diabetes with established cardiovascular disease [28]. Changes in haemoconcentration are aligned with anticipated effects in patients with type 2 diabetes and could suggest that these drugs have broader cardiovascular benefits outside of known indications.

Our study was initiated on the basis of growing evidence suggesting that the effect of SGLT2 inhibition on kidney protection is independent of glucose lowering, raising the possibility of benefit of these drugs in patients without diabetes. First, in patients with type 2 diabetes, SGLT2 inhibitors reduce blood pressure in patients who have stage 3 chronic kidney disease, even though HbA_{1c} levels remain unaffected at this level of kidney impairment due to diminished urinary glucose excretion [29]. Second, in the CREDENCE trial, canagliflozin significantly reduced the rate of eGFR decline and reduced the risks of major kidney and cardiovascular outcomes, even in patients with near-normal HbA_{1c} values [8]. Finally, in the DAPA-HF trial, which included patients with and without type 2 diabetes, benefits on heart failure and kidney outcomes were consistent in patients irrespective of their diabetes status [30]. Despite these compelling lines of evidence, 24-h proteinuria did not decrease in our study. Nevertheless, the overall haemodynamic profile of dapagliflozin was consistent with effects in people with type 2 diabetes: mGFR dipped acutely and in a reversible manner, bodyweight decreased, and haematocrit increased. Additionally, systolic blood pressure and urinary albumin-to-creatinine ratio tended to numerically decrease, although these latter changes were not statistically

significant. In this study, we included patients with proteinuric chronic kidney diseases without diabetes. Similar patients (ie, patients with chronic kidney disease without diabetes) are now included in the ongoing EMPA-KIDNEY (NCT03594110; empagliflozin) and DAPA-CKD (NCT03036150; dapagliflozin) clinical outcome trials [22].

The overall pattern of physiological effects in the DIAMOND trial emphasises the importance of kidney protection trials involving patients with chronic kidney disease with and without diabetes to provide more detailed insight into the long-term kidney protective effects of SGLT2 inhibitors in a broader cohort of patients.

The strengths of our study include carefully controlled study procedures, such as precise methods to capture kidney function and the use of 24-h urine samples rather than spot urine collections. However, the study also had some limitations. First, the sample size was small and the primary outcome was based on single 24-h urine collections. Second, the study follow-up was too short to fully characterise the safety of dapagliflozin in this patient population, although the number of adverse events was small and serious adverse events were rare, which is consistent with the good tolerability and safety record of dapagliflozin in patients with type 2 diabetes. Finally, we recognise that our primary outcome, proteinuria, does not necessarily reflect whether or not dapagliflozin has kidney-protective effects in patients with non-diabetic kidney disease. Similarly, we recognise that the reductions in mGFR identified in the DIAMOND trial, as a reflection of reduced glomerular hypertension, might take longer to translate to antiproteinuric effects in patients with proteinuria without diabetes. Hence, longer-term kidney outcome trials are needed to better characterise long-term efficacy and safety.

Acknowledgements

We thank all investigators, trial teams, and patients for their participation in the trial.

We acknowledge Jasper Stevens, Jan Roggeveld, and Bettine Haandrikman (University of Groningen, Groningen, Netherlands) for their contributions to iohexol analysis and glomerular filtration rate calculations. MGW is supported by the Diabetes Australian Research Trust Millennium Grant.

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Supplementary files

Supplementary methods

Inclusion and exclusion criteria

Inclusion criteria

1. Age ≥ 18 and ≤ 75 years
2. Urinary protein excretion > 500 mg/24hr and ≤ 3500 mg/24hr in a 24-hr urine collection
3. eGFR ≥ 25 ml/min/1.73m²
4. On a stable dose of an ACEi or ARB for at least 4 weeks prior to randomisation
5. Willing to sign informed consent
6. Women of Child-Bearing Potential (WOCBP):
 - a. WOCBP must be using an acceptable method of contraception to avoid pregnancy throughout the study and for up to 4 weeks after the last dose of study drug in such a manner that the risk of pregnancy is minimized
 - b. WOCBP must have a negative serum or urine pregnancy test result (minimum sensitivity 25 IU/L or equivalent units of HCG) within 0 to 72 hours before the first dose of study drug
 - c. Women must not be breast-feeding

Exclusion criteria

1. Diagnosis of type 1 or type 2 diabetes mellitus
2. Urinary protein excretion > 3500 mg/day
3. Peripheral Vascular Disease
4. Autosomal dominant polycystic kidney disease or autosomal recessive polycystic kidney disease, lupus nephritis, or ANCA-associated vasculitis
5. Indication for immunosuppressants as per the treating physician's judgment
6. Receiving cytotoxic therapy, immunosuppressive therapy, or other immunotherapy for primary or secondary renal disease within 6 months prior to enrolment.
7. Active malignancy aside from treated squamous cell or basal cell carcinoma of the skin.
8. Use of the co-interventional treatments (outlined in section 4.2) within 6 weeks of screening will not be allowed.
9. Any medication, surgical or medical condition which might significantly alter the absorption, distribution, metabolism, or excretion of medications including, but not limited to any of the following:
 - a. History of active inflammatory bowel disease within the last six months;
 - b. Major gastrointestinal tract surgery such as gastrectomy, gastroenterostomy, or bowel resection;
 - c. Gastro-intestinal ulcers and/or gastrointestinal or rectal bleeding within last six months;

- d. Pancreatic injury or pancreatitis within the last six months;
 - e. Evidence of hepatic disease as determined by any one of the following: ALT or AST values exceeding 3x ULN at the screening visit, a history of hepatic encephalopathy, a history of esophageal varices, or a history of portocaval shunt;
 - f. Evidence of urinary obstruction or difficulty in voiding at screening
10. History of severe hypersensitivity or contraindications to dapagliflozin
 11. History of hypersensitivity or contraindications to iodinated contrast media
 12. Subject who, in the assessment of the investigator, may be at risk for dehydration or volume depletion that may affect the interpretation of efficacy or safety data
 13. Participation in any clinical investigation within 3 months prior to initial dosing
 14. Donation or loss of 400 ml or more of blood within 8 weeks prior to initial dosing
 15. History of drug or alcohol abuse within the 12 months prior to dosing, or evidence of such abuse as indicated by the laboratory assays conducted during the screening
 16. History of noncompliance to medical regimens or unwillingness to comply with the study protocol
 17. Any surgical or medical condition, which in the opinion of the investigator, may place the patient at higher risk from his/her participation in the study, or is likely to prevent the patient from complying with the requirements of the study or completing the study.
 18. Pregnancy or breastfeeding
 19. WOCBP who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period and up to 4 weeks after the last dose of study drug

Supplementary table 1: Patient characteristics at baseline of patients with IgA nephropathy, focal segmental glomerulosclerosis (FSGS) and hypertensive chronic kidney disease.

	IgA nephropathy (N=25)	FSGS (N=11)	Hypertensive CKD (N=7)
Age (years)	48 (14)	55 (8)	65 (5)
Sex (n, %)			
Male	17 (68%)	7 (64%)	6 (86%)
Female	8 (32%)	4 (36%)	1 (14%)
Ethnicity (n, %)			
Asia	10 (40%)	3 (27%)	2 (29%)
Hispanic	1 (4%)	1 (9%)	0 (0%)
Caucasian	11 (44%)	7 (64%)	4 (57%)
Other	3 (12%)	0 (0%)	1 (14%)
Body weight (kg)	81.1 (22.3)	83.7 (19.0)	81.1 (16.1)
BMI (kg/m ²)	27.3 (5.3)	28.2 (5.7)	29.4 (3.4)
Heart rate (bpm)	67.7 (11.7)	63.0 (9.0)	72.1 (17.3)
Systolic blood pressure (mmHg)	126.7 (13.1)	123.4 (17.4)	134.1 (18.4)
Diastolic blood pressure (mmHg)	77.7 (7.3)	73.2 (9.3)	78.6 (7.7)
HbA _{1c} (%)	5.5 (0.5)	5.7 (0.4)	5.9 (0.4)
HDL-cholesterol (mmol/L)	1.3 (0.3)	1.4 (0.4)	1.2 (0.2)
LDL-cholesterol (mmol/L)	2.6 (1.0)	3.2 (0.5)	2.9 (0.8)
Haemoglobin (g/L)	135.3 (17.7)	137.5 (23.8)	137.0 (22.6)
mGFR (ml/min/1.73m ²)	59.5 (23.8)	61.9 (24.7)	48.3 (18.0)
Proteinuria (mg/24h)	970.0 (690.0, 1170.0)	1496.4 (936.5)	1262.9 (324.6)
Albuminuria (mg/24h)	634.0 (498.0, 1089.5)	1140.3 (704.2)	929.7 (264.7)
ACEi (n, %)	13 (52%)	8 (73%)	2 (29%)
ARB (n, %)	11 (44%)	4 (36%)	4 (57%)
Diuretic (n, %)	7 (28%)	1 (9%)	3 (43%)

Data are n (%), mean (SD), median (IQR). ACE-inhibitor= angiotensin converting enzyme inhibitor.

BMI= body-mass index. bpm= beats per minute. CKD= chronic kidney disease. FSGS = Focal

Segmental Glomerulosclerosis. HbA_{1c} = haemoglobin A1c. HDL= high-density lipoprotein. LDL= low-density lipoprotein.

IQR = interquartile range. mGFR= measured glomerular filtration rate. SD = standard deviation.

Supplementary table 2: Number and proportion of patients at baseline in more granular mGFR and proteinuria categories.

	Overall (N=53)	First Placebo then Dapagliflozin (N=26)	First Dapagliflozin then Placebo (N=27)
mGFR (ml/min/1.73m ²)	58.3 (23.0)	57.8 (25.5)	58.9 (20.7)
<45 (n, %)	18 (34%)	10 (39%)	8 (30%)
45–60 (n, %)	15 (28%)	7 (27%)	8 (30%)
60–90 (n, %)	15 (28%)	6 (23%)	9 (33%)
>90 (n, %)	5 (9%)	3 (12%)	2 (7%)
Proteinuria (mg/24h)	1110.0 (730.0, 1560.0)	1105.0 (720.0, 1530.0)	1170.0 (730.0, 1690.0)
<1000 (n, %)	20 (38%)	11 (42%)	9 (33%)
1000–3000 (n, %)	31 (59%)	14 (54%)	17 (63%)
≥3000 (n, %)	2 (4%)	1 (4%)	1 (4%)

Data are n (%), mean (SD), median (IQR). mGFR= measured glomerular filtration rate.

Supplementary table 3: Sensitivity analysis on the primary outcome.

	Dapagliflozin (N = 53)	Placebo (N = 52)	Difference (Dapa – Placebo)	P-value
Using all proteinuria values during the trial	-11.0% (-20.2 to -0.6)	-13.1% (-22.2 to -2.9)	2.5% (-12.3 to 19.7)	0.7566
Using average proteinuria at screening and randomisation as baseline	-15.1% (-25.8 to -2.9)	-16.1% (-26.8 to -3.9)	1.2% (-16.4 to 22.6)	0.9002

	Dapagliflozin (N = 45)	Placebo (N = 44)	Difference (Dapa – Placebo)	P-value
Excluding 8 subjects who changed treatments during the trial that could modify proteinuria ^a	-12.7% (-24.7 to 1.2)	-10.5% (-22.9 to 3.9)	-2.5% (-20.9 to 20.3)	0.8138

^a Medications that were changed during the trial that could modify proteinuria: diuretics N=7, ACEi N=7, ARBs N=1, NSAID N=4, Corticosteroids N=3, Vitamin D N=2.

Supplementary figure 1: Effects of dapagliflozin on mGFR per baseline patient subgroups.

BMI= body-mass index; CKD= chronic kidney disease; Dapa= dapagliflozin; FSGS= focal segmental glomerulosclerosis; IgA= immunoglobulin A; mGFR= measured glomerular filtration rate; SBP= systolic blood pressure.

Summary

The overall aim of this thesis was to explore the non-glycemic effects of sodium-glucose co-transporter 2 (SGLT2) inhibitors in patients with and without diabetes mellitus. Prior studies showed that SGLT2 inhibitors lower hemoglobin A1c (HbA_{1c}), blood pressure, body weight, and albuminuria in patients with type 2 diabetes mellitus [1]. Changes in these risk markers likely contribute to the observed risk reduction in cardiovascular-, heart failure-, and kidney endpoints in large cardiovascular outcome trials with empagliflozin, canagliflozin and dapagliflozin [2-7]. However, the exact mechanisms by which SGLT2 inhibitors exert their salutary effects are incompletely understood. Therefore, in the first part of this thesis, we aimed to gain more insight into the underlying mechanisms of the protective effects of SGLT2 inhibitors. First, we evaluated the effects of SGLT2 inhibitor dapagliflozin on plasma volume in a broad population of patients with type 2 diabetes mellitus. Subsequently, we investigated how the kidney responds to fluid changes induced by the SGLT2 inhibitor dapagliflozin. Thereafter, we focused specifically on the effect of SGLT2 inhibitors on the kidney and characterized the effects of dapagliflozin on glomerular and tubular structure and function. Finally, we explored the effects of dapagliflozin on albuminuria and other risk markers for chronic kidney disease (CKD) progression in patients with type 2 diabetes mellitus and Stage 3b and 4 CKD, a population that is often excluded from SGLT2 inhibitor studies.

Part I: effects of SGLT2 inhibitors in diabetic kidney disease

In **Chapter 2** we examined the effects of dapagliflozin on plasma volume. The gold standard method to measure plasma volume requires dilution methods, such as radioactive isotopes (^{125}I -human serum albumin) or fluorescent dyes (indocyanine green). These procedures are expensive and cumbersome, which may explain why only two small studies examined the effects of SGLT2 inhibitors on plasma volume [8,9]. We first examined whether the Strauss formula, a plasma volume estimation equation, can be used to estimate changes in measured plasma volume during dapagliflozin treatment. To this end, we used data from a prior study that included patients with type 2 diabetes mellitus who were randomized to receive dapagliflozin 10 mg/day, placebo, or hydrochlorothiazide 25 mg/day [8]. Plasma volume was measured by using ^{125}I -human serum albumin in a sub-study. The median change in estimated plasma volume (ePV) calculated by using the Strauss formula was similar to the median change of measured plasma volume (-9.4% and -9.0%) during dapagliflozin treatment. Subsequently, the Strauss formula was used to define ePV-changes in a pooled database of phase 2b/3 placebo controlled dapagliflozin trials involving 4533 patients with type 2 diabetes mellitus. We showed that dapagliflozin 10 mg/day compared to placebo for 12 weeks reduced ePV by 9.6%. We demonstrated that these effects were consistent regardless of baseline diuretic use or the presence of heart failure. The effects were also consistent among patients with eGFR-levels above or below 60 ml/min/1.73m², which suggest that the natriuretic and osmotic diuretic

effect of this drug persist in patients with moderate kidney function loss. In **Chapter 3** of this thesis we further investigate the diuretic effects of dapagliflozin in patients with moderate kidney function loss. We performed a *post hoc* analysis of two randomized placebo-controlled clinical trials (N= 69) in which we measured various markers of volume status at start and at the end of each 6- or 12 week treatment period. We found that dapagliflozin, compared to placebo, increased urinary glucose excretion, urinary osmolality, and fractional lithium excretion, a proxy for proximal tubular sodium handling. The increase in fractional lithium excretion suggests inhibition of sodium reabsorption in the proximal tubule. Dapagliflozin also decreased blood pressure and body weight, compared to placebo. These results indicate that the diuretic effects of dapagliflozin can be attributed to both osmotic diuresis and natriuresis. The kidney seems to respond to these changes by activating compensatory mechanisms to restore fluid homeostasis. This was reflected by the observed increase in renin and copeptin, a surrogate marker of vasopressin, and the decrease in free water clearance. Taken together, this study provided more insight into the effects of dapagliflozin on fluid homeostasis in patients with diabetes mellitus and elevated albuminuria.

In **Chapter 4** of this thesis we aimed to learn more about the mechanisms of albuminuria lowering with dapagliflozin in patients with type 2 diabetes mellitus and elevated albuminuria. We assessed whether the reduction in albuminuria can be explained by restoration of the charge and/or size selectivity of the glomerular basement membrane, by improvement of tubular cell integrity (which might increase tubular albumin reabsorption), or by reduction of inflammatory mediators (which may affect endothelial dysfunction and albumin leakage into the urine). We found that dapagliflozin, compared to placebo, did not change markers of the glomerular charge or size selectivity, but it decreased markers of tubular injury and inflammation, such as KIM-1 and IL-6. Changes in urinary KIM-1 excretion were correlated with changes in albuminuria. Changes in eGFR were also correlated with changes in albuminuria. These results imply that reduced tubular cell injury and inflammation and decreased intraglomerular pressure may contribute to the albuminuria lowering effect of 6-weeks dapagliflozin therapy.

In the last Chapter of the first part (**Chapter 5**) we performed a pooled analysis of multiple phase 3 clinical trials to investigate the efficacy and safety of dapagliflozin in patients with type 2 diabetes mellitus and more severe chronic kidney disease (CKD stages 3b and 4). A total of 220 patients with type 2 diabetes mellitus and an eGFR between 12 and 45 ml/min/1.73m² were included in this *post hoc* analysis. We found that dapagliflozin treatment for 102 weeks, compared with placebo, decreased urine albumin to creatinine ratio (UACR), systolic blood pressure, and body weight. These effects were present after approximately 4 weeks of treatment and generally persisted over the follow-up period. Dapagliflozin did not change eGFR or HbA_{1c}. Based on this study and other studies we hypothesized that the effects of SGLT2 inhibitors on kidney function and on cardiovascular risk factors were glucose independent, supporting the notion that SGLT2 inhibitors may also be efficacious in patients with CKD

without diabetes mellitus. We further investigated this hypothesis in the second part of this thesis.

Part II: effects of SGLT2 inhibitors in non-diabetic kidney disease

The second part of this thesis focused on the use of SGLT2 inhibitors in patients with CKD and the effects of these agents in the non-diabetic CKD population. **Chapter 6** consists of an opinion review and meta-analysis of the large cardiovascular and kidney outcome trials. We investigated the effects on cardiovascular and kidney outcomes per baseline eGFR and HbA_{1c} subgroups, and found that patients in the lower eGFR subgroup seem to have greater cardiovascular benefit than patients in the higher eGFR subgroup. However, the effect on kidney function appeared to be opposite, with a smaller beneficial effect in subjects with lower eGFR levels, compared to subjects with higher eGFR levels. Yet, patients in the lower eGFR subgroup still had a higher absolute benefit with an average estimated number needed to treat to prevent 1 kidney event during 5 years of 21 versus 79 in the highest eGFR subgroup. Furthermore there were no large differences in cardiovascular and kidney outcomes between subjects with baseline HbA_{1c} levels higher or lower than 8%. Overall, these data suggested that SGLT2 inhibitors might be effective in subjects with lower kidney function and potentially also in subjects with pre-diabetes or non-diabetic CKD. The currently available data in non-diabetic animals or humans with CKD is limited and shows inconsistent results. To resolve these inconsistencies and limitations, we designed a dedicated trial, the DIAMOND trial, to assess the effects of the SGLT2 inhibitor dapagliflozin in patients with CKD without type 2 diabetes mellitus. The primary and main secondary outcomes of this trial were changes in proteinuria and measured GFR (**Chapter 7**). DIAMOND was designed as a double-blind placebo-controlled six week cross-over study and this study was performed in six hospitals in the Netherlands, Canada and Malaysia. Fifty-three patients were randomized and received placebo and dapagliflozin 10 mg/day. Treatment with dapagliflozin for six weeks did not affect proteinuria, but it did induce an acute and reversible decline in mGFR, a reduction in body weight of -1.5 kg (95% CI: -3.0 to -0.03) and a small increase in hemoglobin and hematocrit of 5.3 g/L (95% CI: 2.7 to 7.9) and 0.02 L/L (95% CI: 0.01 to 0.03), compared to placebo. The number of adverse events was small and did not differ during dapagliflozin or placebo treatment. These results suggest that dapagliflozin has hemodynamic properties in patients with non-diabetic CKD, but does not affect proteinuria, at least not in short-term. The reason why proteinuria was unaffected is unknown. The treatment duration might have been too short to detect changes in proteinuria, patients might have had a significant tubulointerstitial source of proteinuria that responds less to changes in intraglomerular pressure, or SGLT2 inhibitors might simply not reduce proteinuria in the absence of diabetes mellitus. Nevertheless, we did observe a proteinuria reduction in the subgroup of patients with a baseline measured GFR > 60 ml/min/1.73m². Potentially, significant declines in proteinuria only occur in non-diabetic patients with higher kidney function. Two ongoing trials, DAPA-CKD (Dapagliflozin And Prevention

of Adverse outcomes in Chronic Kidney Disease [10]) and EMPA-KIDNEY (The study of Heart and Kidney protection with Empagliflozin) will provide definitive evidence.

Future perspectives

In this thesis we investigated non-glycemic effects of SGLT2 inhibitors in patients with a reduced kidney function with and without diabetes mellitus. The performed studies provided more information about underlying mechanisms of effects of SGLT2 inhibitors in patients with a reduced kidney function but also raised questions and suggestions for future research (Text box 1).

Box 1: Research recommendations for future studies

Effects of SGLT2 inhibitors on volume status

Investigate the diuretic effects of SGLT2 inhibitors in more detail, focusing on plasma volume and interstitial fluid volume changes in patients with CKD with and without diabetes mellitus.

Effects of SGLT2 inhibitors on tubular metabolic stress and hypoxia

Investigate effects of SGLT2 inhibitors on urine metabolomics and on kidney cortex and medulla oxygenation using imaging approaches such as ¹⁵O positron emission tomography and Blood Oxygen Level-Dependent Magnetic Resonance Imaging (BOLD-MRI).

Effects of SGLT2 inhibitors on inflammation and fibrosis

Perform kidney biopsies in CKD patients with and without type 2 diabetes mellitus and examine anti-inflammatory and anti-fibrotic effects of SGLT2 inhibitors.

These studies should include an active control group when performed in patients with diabetes mellitus to distinguish direct and indirect glycemic pathways.

Effects of SGLT2 inhibitors on glomerular hemodynamics

Investigate changes in intraglomerular pressure and changes in vasoconstriction or vasodilatation of the pre- and post-glomerular arteriole in patients with type 1 and type 2 diabetes mellitus, as well as in patients with CKD.

Identify kidney responders and non-responders

Perform imaging studies with radiolabeled SGLT2 inhibitors to quantify tissue drug distribution and SGLT2 density in order to identify if these factors determine the inter-individual variation in response.

The first two chapters of this thesis focus on changes in plasma volume and volume biomarkers. Reductions in plasma or interstitial volume may account for the heart failure and kidney protective effects of SGLT2 inhibitors. Currently, there is limited data on volume effects of SGLT2 inhibitors. So far only two small studies have investigated the effects of SGLT2 inhibitors

on measured plasma volume in a prospective manner [8,9]. Larger clinical trials are necessary to examine if and how SGLT2 inhibitors influence body fluid composition. Future studies should measure both extracellular fluid volume and plasma volume to obtain better understanding of how SGLT2 inhibitors change body fluid composition. This can be done simultaneously by using gold standard techniques with dilution methods. Subsequently, changes in interstitial fluid volume can be calculated as the difference between extracellular fluid volume and plasma volume. These mechanistic studies should not only be performed in subjects with type 2 diabetes mellitus, but also in subjects with CKD or heart failure without diabetes mellitus. In addition, hematocrit, hemoglobin, and body weight should be measured. Hematocrit and hemoglobin are markers used in the estimation equations to estimate changes in plasma volume. By measuring plasma volume, hematocrit and hemoglobin we might be able to validate plasma volume estimation equations such as the Strauss formula in this patient population. The validation of these equations in this patient population can be helpful for future large epidemiological studies, because the gold standard dilution methods are time-consuming, invasive, and expensive.

Before plasma volume estimation equations can be used on large scale we first need to fully understand the mechanisms behind the elevation of hemoglobin and hematocrit during SGLT2 inhibition. It is hypothesized that the increase in hemoglobin and hematocrit together with the decrease in body weight and blood pressure reflects a decrease in plasma volume. However, SGLT2 inhibition is also associated with an increased production of erythropoietin (EPO) and reticulocytes. Increased EPO-production can be another explanation of elevated hemoglobin and hematocrit levels. The mechanisms underlying the increased production of EPO are incompletely understood, but it might be a result of reduced metabolic stress on the proximal tubules [11,12]. In Chapter 4 we performed a *post hoc* analysis and showed that dapagliflozin decreases proximal tubular injury marker KIM-1. We hypothesized that the reduction of urinary KIM-1 excretion may reflect reduced metabolic stress of proximal tubular cells. Future studies that investigate the effects of SGLT2 inhibitors on urine metabolomics and the oxygenation of the kidney cortex and medulla are necessary to provide more insight into these mechanisms. In this thesis we also observed a reduction of the inflammatory marker IL-6 in 24-hour urine. SGLT2 inhibitors might reduce local inflammation as result of reduced albuminuria or increased urinary glucose excretion, since glucose and albumin leakage can trigger kidney inflammation and glucotoxicity [13,14]. Moreover, it is possible that the proposed anti-inflammatory and anti-fibrotic effects are indirectly caused by reduced plasma glucose. Mechanistic studies in CKD patients might help to clarify this. These studies should have an active control group if they are performed in patients with CKD and diabetes mellitus.

Additional studies are also required to clarify the effects of SGLT2 inhibitors on glomerular hemodynamics. Activation of the tubuloglomerular feedback mechanism in response to SGLT2 inhibition is a widely discussed kidney-protective mechanism in patients with reduced kidney function and glomerular hyperfiltration. Cherney *et al.* [15] found that SGLT2 inhibitor

empagliflozin reduced intraglomerular pressure in patients with type 1 diabetes mellitus, most likely via pre-glomerular vasoconstriction. Van Bommel *et al.* [16] also observed a reduction in intraglomerular pressure in patients with type 2 diabetes mellitus, but they found changes in prostaglandin markers that may indicate a post-glomerular vasodilatory effect. These studies indicate that the effects on glomerular hemodynamics might not be entirely the same in different patient populations. Preclinical and clinical studies are required to unravel the exact mechanism in patients with type 1 and type 2 diabetes mellitus, and in patients with non-diabetic chronic kidney diseases.

In Chapter 7 we examined the effects of dapagliflozin on proteinuria in patients with CKD without diabetes mellitus. Dapagliflozin did not affect proteinuria in these patients, although we did observe an effect in patients with higher baseline GFR levels. A previous study indicated a large variation in the proteinuria response between individuals [17]. It is clinically relevant to identify responders and non-responders in order to provide the optimal treatment. Therefore we need the results of large kidney outcome trials in patients without diabetes mellitus.

In addition, imaging studies with radiolabeled SGLT2 inhibitors can help to understand the variation in response by quantifying tissue drug distribution and SGLT2 density. Kidney biopsies may be required to understand the structural basis of the response. Finally, prospective studies can be performed in patients who most likely benefit from SGLT2 inhibition (a precision medicine approach).

In conclusion, short-term (pre-)clinical studies are necessary to fully understand the mechanisms of SGLT2 inhibitors and to examine if these drugs are applicable and clinically relevant in new patient populations, for example in patients with CKD without diabetes mellitus. These studies may help to guide best use of SGLT2 inhibitors in clinical practice and may inform design of clinical trials to characterize the long-term efficacy and safety of SGLT2 inhibitors in new patient populations.

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Inleiding

De wereldwijde toename van het aantal patiënten met suikerziekte (diabetes mellitus) en het daarmee gepaard gaande risico op het ontstaan en verergeren van hart- en vaatziekten en diabetische nierziekte legt een zware last op individuele patiënten en leidt tot hoge nationale zorguitgaven [1,2]. Om het risico op chronische nierschade en hart- en vaatziekten te verlagen, richten behandelingen zich op meerdere risicofactoren zoals een verhoogde bloeddruk, een hoog hemoglobine A1c (HbA_{1c} , een maat voor de gemiddelde glucosewaarden over enkele maanden), overgewicht, een verhoogd cholesterolgehalte en op de hoeveelheid eiwitten in de urine (albuminurie). Helaas bereikt nog circa 33 tot 49% van de patiënten met diabetes mellitus type 2 niet de beoogde streefwaarden voor HbA_{1c} , bloeddruk of cholesterol [1]. Ongeveer 20 tot 40% van de patiënten met diabetes mellitus ontwikkelt een diabetische nierziekte (diabetische nefropathie), wat bij een aanzienlijk aantal patiënten uiteindelijk zal leiden tot eindstadium nierfalen [1]. Diabetische nefropathie is geassocieerd met een significante verlaging van de levensverwachting [1]. Er wordt veel onderzoek gedaan om nieuwe interventies te ontwikkelen om de prognose van patiënten met diabetes mellitus te verbeteren. Een voorbeeld daarvan is de ontwikkeling van een relatief nieuwe groep medicijnen, genaamd sodium-glucose co-transporter 2 (SGLT2) remmers.

Werkingsmechanisme van SGLT2 remmers

Glucose (suiker) wordt door de nieren gefilterd en voor een groot deel door middel van SGLT2 transporters vanuit de 'voorurine' heropgenomen in het bloed. Samen met glucose wordt ook zout heropgenomen. Bij gezonde personen met een normale nierfunctie wordt ongeveer 180 gram glucose per dag door de nieren gefilterd. Er is bij gezonde personen echter geen glucose in de urine aanwezig. Dat is het resultaat van een effectief systeem van twee sodium-glucose co-transporters: SGLT1 en SGLT2 [3]. De SGLT2 transporter bevindt zich in het eerste deel van de proximale tubulus van de nier en neemt ongeveer 90% van al het gefilterde glucose op (Figuur 1). De overige 10% van het gefilterde glucose wordt heropgenomen door de SGLT1 transporter [4]. Bij patiënten met diabetes mellitus, bij wie de plasma glucosespiegel 400 mg glucose per 100 ml plasma overschrijdt, raken SGLT2 transporters verzadigd en is de maximale capaciteit bereikt om glucose te heropnemen. Dit resulteert in verlies van glucose in de urine (Figuur 1) [4].

Figuur 1: Werkingsmechanisme van sodium-glucose co-transporters bij personen met diabetes mellitus.

Door SGLT2 te remmen met een SGLT2 remmer wordt er minder glucose (en zout) heropgenomen in het bloed. Hierdoor wordt er ongeveer 70 tot 80 gram meer glucose per dag uitgeplast, wat resulteert in een daling van het plasma glucose en een daling in HbA_{1c} van ongeveer 0,5 tot 0,8% [5].

Eerdere onderzoeken met SGLT2 remmers

Momenteel zijn er drie SGLT2 remmers geregistreerd voor gebruik in de Verenigde Staten en Europa: empagliflozine, canagliflozine en dapagliflozine. Klinische studies hebben aangetoond dat behandeling met SGLT2 remmers bij patiënten met diabetes mellitus type 2 leidt tot een daling in HbA_{1c} , een daling in bloeddruk, lichaamsgewicht en een afname van albuminurie. Deze veranderingen dragen waarschijnlijk bij aan de positieve resultaten die zijn waargenomen in grotere klinische studies. In deze grote studies werden de langetermijneffecten van SGLT2 remmers onderzocht bij patiënten met diabetes mellitus type 2. SGLT2 remming resulteerde op langere termijn in een daling van het risico op nierfalen, een lager risico op een ziekenhuisopname als gevolg van hartfalen en een daling van het risico op overlijden aan hart- en vaatziekten [6-10]. De exacte mechanismen die hieraan ten grondslag liggen, zijn nog niet volledig bekend.

Dit proefschrift

Dit proefschrift bestaat uit twee delen. In het eerste deel van dit proefschrift onderzochten we enkele onderliggende mechanismen die ten grondslag liggen aan de beschermende effecten van SGLT2 remmers op het hart en de nieren. In het tweede deel van dit proefschrift hebben

we een meta-analyse en een prospectieve klinische studie uitgevoerd om de effecten van SGLT2 remmers te onderzoeken bij patiënten met een chronische nierziekte, zonder diabetes mellitus.

Deel I: effecten van SGLT2 remmers bij patiënten met een diabetische nierziekte

Patiënten met hartfalen en patiënten met diabetes mellitus type 2 hebben vaak een te hoog plasmavolume (het vloeibare gedeelte van het bloed waarin zich de bloedcellen bevinden). Een daling in plasmavolume zou een beschermend effect kunnen hebben op de ontwikkeling en progressie van hartfalen. In **hoofdstuk 2** hebben we onderzocht of een behandeling met de SGLT2 remmer dapagliflozine leidt tot een verandering in plasmavolume in een brede populatie van patiënten met diabetes mellitus type 2. De gouden standaard om veranderingen in plasmavolume te meten vereist het gebruik van radioactieve isotopen (^{125}I -humaan serum albumine) of fluorescerende kleurstof (indocyanine groen). Deze methoden zijn duur en omslachtig. Dit verklaart mogelijk waarom, tot op heden, slechts twee kleine studies onderzoek hebben gedaan naar de effecten van SGLT2 remming op plasmavolume [11,12]. In hoofdstuk 2 hebben we daarom eerst onderzocht of we een bestaande formule, de Strauss formule, konden gebruiken om een schatting te maken van de veranderingen in plasmavolume. Om dit te kunnen doen hebben we gegevens gebruikt van een eerdere studie. Patiënten met diabetes mellitus type 2 werden gerandomiseerd en ontvingen dapagliflozine 10 mg/dag, placebo, of hydrochloorthiazide 25 mg/dag. Plasmavolume werd gemeten met ^{125}I -humaan serum albumine in een sub-studie. De mediane verandering in het geschatte plasmavolume (ePV) gedurende dapagliflozine therapie, door ons berekend met de Strauss formule, was vergelijkbaar met de verandering in het gemeten plasmavolume in deze eerdere studie (-9,4% en -9,0%). Vervolgens hebben we de Strauss formule gebruikt om veranderingen in ePV te bepalen met gegevens uit een database bestaande uit 4533 patiënten met diabetes mellitus type 2. We vonden dat 12 weken dapagliflozine therapie, in vergelijking met placebo, leidt tot een daling in ePV van 9,6%. Een dergelijke daling in ePV was consistent aanwezig, ongeacht of patiënten wel of geen diuretica gebruikten en ongeacht of ze wel of geen hartfalen hadden. Eenzelfde effect werd gezien bij patiënten met een eGFR (glomerulaire filtratiesnelheid, een maat voor nierfunctie) boven de 60 ml/min/1,73m², maar ook bij patiënten met een eGFR onder de 60 ml/min/1,73m², wat suggereert dat het vocht afdrijvende (diuretische) effect van dit medicijn aanhoudt bij patiënten met een matige nierfunctie.

In **hoofdstuk 3** hebben we onderzocht hoe de nieren reageren op veranderingen in de vochtbalans veroorzaakt door dapagliflozine. Dit hebben we onderzocht bij patiënten met diabetes mellitus type 2 met matig nierfunctieverlies (eGFR $\geq$ 45 ml/min/1,73m² en een urine albumine/creatinine ratio $\geq$ 30 mg/g). We hebben gebruik gemaakt van twee eerder uitgevoerde placebogecontroleerde studies (N=69) waarin verschillende volume markers zijn gemeten aan het begin en aan het eind van elke behandelperiode van 6- of 12 weken.

De resultaten laten zien dat dapagliflozine, vergeleken met placebo, leidt tot een toename van glucose excretie via de urine, een toegenomen urine osmolaliteit en een toename in fractionele lithiumexcretie. De toename in fractionele lithiumexcretie suggereert een remming van de reabsorptie (heropname) van natrium in de proximale tubulus. Daarnaast werd een daling in bloeddruk en lichaamsgewicht gezien. Deze resultaten impliceren dat de diuretische effecten van dapagliflozine toegeschreven kunnen worden aan zowel osmotische diurese als natriurese. De nieren lijken hierop te reageren door de activatie van compensatoire mechanismen om de vochtbalans te herstellen, zoals te zien is aan de toename van renine en copeptine (een surrogaat marker voor vasopressine) en een afname in vrije waterklaring. Concluderend gaf hoofdstuk 3 meer inzicht in de effecten van dapagliflozine op de vochtbalans bij patiënten met diabetes mellitus type 2 en albuminurie.

Het doel van **hoofdstuk 4** was om beter te begrijpen wat de mechanismen zijn achter de verlaging van albuminurie gedurende SGLT2 remming. Hiervoor hebben we gebruik gemaakt van een eerdere studie met dapagliflozine in patiënten met diabetes mellitus type 2 en albuminurie. We onderzochten of de daling in albuminurie gedurende 6 weken SGLT2 remming verklaard kon worden door herstel van het glomerulaire basaalmembraan voor ladingsafhankelijke permeabiliteit en/of grootte afhankelijke permeabiliteit, door verbetering van de integriteit van tubulaire cellen (wat zou kunnen leiden tot een toename van de reabsorptie van albumine via de tubulus) of door een afname van inflammatoire mediators (wat invloed kan hebben op endotheel-dysfunctie en lekkage van albumine in de urine). We vonden dat dapagliflozine, vergeleken met placebo, geen invloed had op de ladings- en grootte selectiviteit van het glomerulaire basaalmembraan, maar wel zorgde voor een afname van tubulaire schade- en inflammatie markers in de urine, zoals KIM-1 en IL-6. Deze afname van KIM-1 correleerde met de afname in albuminurie. Daarnaast was er een correlatie tussen verandering in eGFR en verandering in albuminurie. Deze resultaten suggereren dat een afname van tubulaire schade, een afname van inflammatie en een afname van de intra-glomerulaire druk mogelijk kan bijdragen aan het albuminurie-verlagende effect van dapagliflozine.

In het laatste hoofdstuk van het eerste deel van dit proefschrift (**hoofdstuk 5**) onderzochten we de werkzaamheid en veiligheid van dapagliflozine bij patiënten met diabetes mellitus type 2 met een matig/vergevoorderde chronische nierziekte (CKD-stadium 3b en 4). Data uit meerdere fase 3 onderzoeken werd samengevoegd, waardoor we data van 220 patiënten met diabetes mellitus type 2 met een eGFR tussen de 12 en 45 ml/min/1,73m² konden analyseren. We vonden dat behandeling met dapagliflozine voor 102 weken, vergeleken met placebo, ook in deze patiëntengroep leidde tot een afname van de urine albumine tot creatinine ratio (UACR), een afname in systolische bloeddruk en een afname in lichaamsgewicht. Deze effecten werden na ongeveer 4 weken therapie zichtbaar en hielden aan gedurende de follow-up periode. Er was geen verandering in eGFR en HbA_{1c} gedurende de behandeling met dapagliflozine.

Eerdere studies suggereren dat de effecten van SGLT2 remmers op cardiovasculaire risicofactoren en op de nierfunctie grotendeels glucose-onafhankelijk zijn. Mogelijk zouden SGLT2 remmers ook werkzaam kunnen zijn bij patiënten met een chronische nierziekte maar zonder diabetes mellitus. Dit hebben we verder onderzocht in het tweede deel van dit proefschrift.

Deel II: effecten van SGLT2 remmers bij patiënten met een niet-diabetische chronische nierziekte

In het tweede deel van dit proefschrift hebben we ons gefocust op het gebruik van SGLT2 remmers in patiënten met een chronische nierziekte en in patiënten met een niet-diabetische chronische nierziekte. **Hoofdstuk 6** bestaat uit een opinie review en een meta-analyse van grote cardiovasculaire en nier-specifieke uitkomststudies met SGLT2 remmers. In dit hoofdstuk onderzochten we de effecten van SGLT2 remmers op cardiovasculaire- en nier-specifieke eindpunten in subgroepen ingedeeld op basis van baseline (aan de start van het onderzoek) eGFR- en baseline HbA_{1c}-waarden. Patiënten met een lagere eGFR-waarde (< 45 ml/min/1,73m²) leken een groter positief effect te hebben op het cardiovasculaire eindpunt dan patiënten met een hogere eGFR-waarde (≥ 90 ml/min/1,73m²). Echter, dit was andersom voor de nier-specifieke eindpunten, waarbij een kleiner effect werd gezien bij patiënten met een lagere eGFR-waarde vergeleken met patiënten met een hogere eGFR-waarde. Daarentegen hadden patiënten in de subgroepen met lagere eGFR-waarden nog steeds een hoger absoluut voordeel. Het gemiddelde geschatte aantal mensen wat behandeld moet worden om één nier-specifiek eindpunt te voorkomen (number needed to treat) gedurende 5 jaar was 21 in de subgroep met een lagere eGFR-waarde, vergeleken met 79 in de subgroep met een hogere eGFR-waarde. Tussen de HbA_{1c} subgroepen (patiënten met een baseline HbA_{1c} hoger of lager dan 8%) was er geen groot verschil in cardiovasculaire- en in nier-specifieke eindpunten. Samenvattend suggereren deze gegevens dat SGLT2 remmers effectief zouden kunnen zijn bij patiënten met een slechte nierfunctie en eventueel ook bij patiënten met pre-diabetes of bij patiënten met een niet-diabetische chronische nierziekte. Om nader te onderzoeken of SGLT2 remmers werkzaam zijn bij patiënten met een niet-diabetische chronische nierziekte, hebben we een prospectieve klinische studie opgezet. De DIAMOND studie (**hoofdstuk 7**) was een dubbelblinde, placebogecontroleerde studie met een cross-over design. Deze studie werd uitgevoerd in zes ziekenhuizen in Nederland, Canada en Maleisië. Drieënvijftig patiënten werden gerandomiseerd en ontvingen 6 weken dapagliflozine 10 mg/dag, dan 6 weken geen therapie (wash-out), gevolgd door 6 weken placebo therapie, óf eerst 6 weken placebo therapie, dan 6 weken wash-out, gevolgd door 6 weken dapagliflozine 10 mg/dag. De behandeling met dapagliflozine, vergeleken met placebo, had geen effect op de hoeveelheid eiwitten in de urine (proteïnurie). Wel werd er een effect gezien op de gemeten GFR. Daarnaast zagen we een daling in lichaamsgewicht van -1,5 kg en werd er een lichte stijging gezien van hemoglobine (5,3 g/L) en hematocriet (0,02 L/L). Deze resultaten suggereren dat

dapagliflozine leidt tot hemodynamische veranderingen in deze patiëntenpopulatie. Het is nog onduidelijk waarom er geen effect op proteïnurie werd gezien in de gehele groep. Wel zagen we een daling van de proteïnurie in de subgroep van patiënten met een GFR-waarde groter dan 60 ml/min/1,73m². Mogelijk treden er alleen significante dalingen in proteïnurie op bij niet-diabetische patiënten met een betere nierfunctie. Twee onderzoeken die op dit moment nog lopen, DAPA-CKD (Dapagliflozin And Prevention of Adverse outcomes in Chronic Kidney Disease) en EMPA-KIDNEY (The study of Heart and Kidney protection with Empagliflozin) zullen hier meer duidelijkheid over geven.

Toekomstperspectieven

In dit proefschrift hebben we de niet-glycemische effecten van SGLT2 remmers onderzocht bij patiënten met een verminderde nierfunctie, met en zonder diabetes mellitus. De verschillende studies gaven meer informatie over de onderliggende werkingsmechanismen van SGLT2 remmers, maar riepen ook vragen op en suggesties voor toekomstig onderzoek (Tekst box 1).

Box 1: Aanbevelingen voor toekomstige studies

Effecten van SGLT2 remmers op volume status

Onderzoek de diuretische effecten van SGLT2 remmers in meer detail, waarbij de nadruk gelegd moet worden op eventuele veranderingen in plasmavolume en interstitieel volume bij patiënten met een chronische nierziekte met- of zonder diabetes mellitus.

Effecten van SGLT2 remmers op tubulaire metabolische stress en hypoxie

Onderzoek naar de effecten van SGLT2 remmers op metabolieten in de urine en op de oxygenatie van de cortex en medulla van de nier door gebruik te maken van beeldvorming, zoals 15O positron emissie tomografie (PET) en Blood Oxygen Level-Dependent Magnetic Resonance Imaging (BOLD-MRI).

Effecten van SGLT2 remmers op inflammatie en fibrose

Onderzoek naar anti-inflammatoire of anti-fibrotische effecten van SGLT2 remmers bij patiënten met een chronische nierziekte met- of zonder diabetes mellitus type 2 door het nemen en onderzoeken van nierbiopsen. Indien een dergelijke studie wordt uitgevoerd bij patiënten met diabetes mellitus dient er een actieve controlegroep te worden toegevoegd om directe en indirecte glycemische pathways te kunnen onderscheiden.

Glomerulaire hemodynamische effecten van SGLT2 remmers

Onderzoek naar veranderingen in intra-glomerulaire druk en veranderingen in vasoconstrictie of vasodilatatie van de pre- en post-glomerulaire arteriole bij patiënten met diabetes mellitus type 1 en type 2 en bij patiënten met een chronische nierziekte.

Identificeer responders en non-responders

Beeldvormende onderzoeken met radioactief gelabelde SGLT2 remmers om de distributie van het geneesmiddel in het weefsel te onderzoeken en om de SGLT2 densiteit te kwantificeren. Mogelijk kunnen deze factoren helpen om het verschil tussen responders en non-responders te verklaren.

Aanbevelingen voor toekomstige studies

De eerste twee hoofdstukken van dit proefschrift zijn gericht op plasmavolume en volume biomarkers. Een afname van plasmavolume of van de interstitiële vloeistof (het vloeibare gedeelte tussen de cellen) zou kunnen bijdragen aan de beschermende effecten van SGLT2 remmers op hartfalen en nierziekten. Slechts twee kleine studies hebben op een prospectieve manier veranderingen in plasmavolume onderzocht [11,12]. Grotere klinische studies zijn noodzakelijk om te onderzoeken *of* en *hoe* SGLT2 remmers de volumehuishouding beïnvloeden. Toekomstige studies zouden zowel het extracellulaire volume (het lichaamsvocht dat zich buiten de cellen bevindt) als het plasmavolume moeten bepalen. Het extracellulaire volume en het plasmavolume kunnen tegelijkertijd bepaald worden met behulp van verdunningstechnieken. Vervolgens kan de verandering in de interstitiële vloeistof berekend worden door het plasmavolume van het extracellulaire volume af te trekken. Deze studies zouden uitgevoerd moeten worden bij patiënten met diabetes mellitus type 2, maar ook bij patiënten met een chronische nierziekte of met hartfalen zonder diabetes mellitus. Daarnaast zou het hemoglobine, hematocriet en het lichaamsgewicht gemeten moeten worden. Door zowel plasmavolume, hemoglobine als hematocriet te meten, zouden we mogelijk de Strauss formule (de formule om veranderingen in plasmavolume te schatten) kunnen valideren in deze patiëntenpopulatie. Dit kan nuttig zijn voor toekomstige grote epidemiologische studies. Echter, vóórdat deze formules op grote schaal kunnen worden gebruikt, is het van belang om eerst te begrijpen waarom er een toename van hemoglobine en hematocriet wordt gezien gedurende SGLT2 remming. Er wordt verondersteld dat de toename van hemoglobine en hematocriet, samen met de afname in lichaamsgewicht en bloeddruk, een afname van het plasmavolume weerspiegelt. Echter, SGLT2 remming is ook geassocieerd met een toegenomen productie van erytropoëetine (EPO). EPO stimuleert de productie van rode bloedcellen, wat de toename van hemoglobine en hematocriet gedurende SGLT2 remming ook zou kunnen verklaren. De mechanismen die ten grondslag liggen aan de verhoogde productie van EPO zijn nog onduidelijk, maar mogelijk speelt metabole stress in de proximale tubuli een rol [13,14]. In hoofdstuk 4 zagen we dat dapagliflozine zorgde voor een daling van de proximale tubulaire schademarker KIM-1. We suggereerden dat de afname van de uitscheiding van KIM-1 in de urine mogelijk een reflectie is van de afname in proximale tubulaire metabole stress. In toekomstige studies kunnen de effecten van SGLT2 remmers op metaboliëten in de urine worden onderzocht. Daarnaast kan er onderzoek gedaan worden naar de oxygenatie van de cortex en medulla van de nier om meer inzicht te krijgen in de onderliggende mechanismen.

In dit proefschrift vonden we dat SGLT2 remming leidt tot een afname van inflammatoire marker IL-6 in de 24-uurs urine. SGLT2 remmers lijken te zorgen voor een afname van lokale inflammatie, mogelijk als gevolg van afname in albuminurie of als gevolg van een toename van glucose excretie. Zowel lekkage van albumine als lekkage van glucose kan een inflammatoire reactie en glucotoxiciteit uitlokken in de nier [15,16]. Daarnaast is het ook mogelijk dat de

veronderstelde anti-inflammatoire en anti-fibrotische effecten indirect veroorzaakt worden door een daling van het plasma glucose. Toekomstige studies naar deze effecten bij patiënten met een chronische nierziekte kunnen helpen om dit mechanisme te verduidelijken. Deze studies zouden dan een actieve controlegroep moeten hebben indien ze worden uitgevoerd bij patiënten met een chronische nierziekte en diabetes mellitus.

Een ander mechanisme dat beschermend zou werken voor de nieren is de activatie van het tubulo-glomerulaire feedback mechanisme als gevolg van SGLT2 remming. Activatie van het tubulo-glomerulaire feedback mechanisme leidt tot een daling van de intra-glomerulaire druk, wat wenselijk is bij patiënten met glomerulaire hyperfiltratie. Cherney *et al.* [17] vonden dat de SGLT2 remmer empagliflozine de intra-glomerulaire druk verlaagt als gevolg van pre-glomerulaire vasoconstrictie (het samenknijpen van het bloedvat dat naar de glomerulus toe loopt) bij patiënten met diabetes mellitus type 1. Van Bommel *et al.* [18] zagen ook een afname van de intra-glomerulaire druk bij patiënten met diabetes mellitus type 2. Echter, zij zagen een toename van prostaglandine (vaatverwijdende-) markers, wat op een post-glomerulair vasodilatoir effect duidt. Deze twee studies laten zien dat de glomerulaire hemodynamische effecten van SGLT2 remmers mogelijk verschillend zijn in verschillende populaties. Preklinische en klinische studies zijn daarom nodig om de exacte mechanismen te ontrafelen in patiënten met diabetes mellitus type 1 en type 2 en in patiënten met een niet-diabetische nierziekte.

In hoofdstuk 7 van dit proefschrift onderzochten we de effecten van dapagliflozine op proteïnurie bij patiënten met een niet-diabetische chronische nierziekte. Behandeling met dapagliflozine zorgde niet voor een daling van de proteïnurie in de gehele populatie, maar er werd wel een daling van proteïnurie gezien bij patiënten met een hogere baseline glomerulaire filtratiesnelheid. In een eerdere studie met dapagliflozine werd een grote variatie gevonden in de proteïnurie-response tussen verschillende individuen [19]. Klinisch is het relevant om de individuen die wél reageren op het geneesmiddel (responders) en de individuen die niet reageren (non-responders) te identificeren. Om hier meer duidelijkheid over te krijgen, is het noodzakelijk om de resultaten van grote studies met nierpatiënten zonder diabetes mellitus af te wachten. Daarnaast kunnen beeldvormende onderzoeken uitgevoerd worden met radioactief gelabelde SGLT2 remmers om de distributie van het geneesmiddel in het weefsel te onderzoeken en om de SGLT2 densiteit te kwantificeren. Ten slotte zouden prospectieve studies uitgevoerd kunnen worden met enkel de patiënten die meest waarschijnlijk positief zouden reageren op SGLT2 remmers, de waarschijnlijke 'responders' (een 'precision medicine' benadering).

Concluderend zijn er kortdurende (pre-)klinische studies vereist om verschillende werkingsmechanismen van SGLT2 remmers beter te begrijpen en om te onderzoeken of deze medicijnen toepasbaar zijn op nieuwe patiëntenpopulaties. De informatie uit deze kortdurende

studies kan vervolgens gebruikt worden voor de ontwikkeling van langdurige studies waarin de werkzaamheid en veiligheid van SGLT2 remmers wordt onderzocht in verschillende nieuwe patiëntenpopulaties.

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Dankwoord

Ik moet eerlijk bekennen dat ik heb getwijfeld of ik aan een promotietraject zou beginnen. De ervaring die ik had met wetenschap was nihil. Ik vroeg me af of ik dit wel zou kunnen. Trynke, een medestudent die me begeleidde met mijn stage wetenschap, moedigde me aan om contact op te nemen met Hiddo Lambers Heerspink. Ze had gehoord dat hij op zoek was naar een arts onderzoeker die interesse had in een promotieonderzoek. Tijdens het eerste gesprek met Hiddo was ik direct overtuigd. Wat een gaaf project had hij voor ogen! En wat een enthousiasme!

Gedurende dit promotietraject heb ik enorm veel geleerd. Daar ben ik veel mensen dankbaar voor! Ik wil dit dankwoord dan ook gebruiken om iedereen te bedanken die een rol heeft gespeeld in het tot stand komen van dit proefschrift.

Prof. dr. H.J. Lambers Heerspink, beste Hiddo, ik wil je ontzettend bedanken voor alle mogelijkheden die je me hebt aangeboden tijdens dit promotietraject, zoals de cursussen, presentaties op (internationale) congressen, de contacten met (buitenlandse) onderzoekers en met de farmacie. Ik vind het nog steeds heel gaaf dat ik mee mocht helpen met het opzetten en uitvoeren van een internationale, multicenter klinische studie. Bedankt dat je me de vrijheid hebt gegeven om mijn rol hierin te vinden. Dat ik aan het einde van de studie ook nog naar Australië mocht om daar samen aan het artikel te werken was helemaal top! Ik vond het prettig om met je samen te werken. Je bent makkelijk benaderbaar, waardoor ik me snel thuis voelde op de afdeling. Je ziet waar iemands kwaliteiten liggen en motiveert vervolgens om het maximale eruit te halen. Dit, tezamen met je kritische blik en je (aanstekelijke) enthousiasme waardeer ik enorm.

Prof. dr. R.T. Gansevoort, beste Ron, man wat ben ik onder de indruk van jouw ervaring, kennis en kunde! In het begin werd ik er zelfs een beetje zenuwachtig van. Maar door je prettige, rustige houding en je openheid voelde ik me al snel op mijn gemak. Ik vond het leuk om met je samen te werken, zowel als het ging om mijn promotieonderzoek (de brainstorm sessies over veranderingen in vochthuishouding, samen een review schrijven), maar ook rondom de organisatie van de Kidney Center Meetings. Ik heb veel van je geleerd en hoop in de toekomst ook nog veel van je te mogen leren.

Lieve Michelle, Jasper, Job en Sieta. Normaal gesproken volgt hier het stukje over de co-promotors. Nu had ik die officieel niet, maar ik noem jullie niet voor niets. Want ondanks dat jullie niet direct betrokken waren bij mijn promotietraject, kon ik wel altijd bij jullie terecht met al mijn vragen en onzekerheden. Dank daarvoor!

Many thanks to all the collaborators of the DIAMOND study, the study sites, George Clinical, and AstraZeneca. It was a pleasant cooperation. Together we completed a nice trial, within the given time period, and published it in a highly ranked journal. I appreciate all your work! In het bijzonder wil ik hier Bart (Bartje), Bettine, Jan, Roelie, Marian en Dirkina noemen. Roelie, Marian en Dirkina, ofwel 'de nierfunctiedames', jullie hebben me uit de brand geholpen tijdens het opstarten van de DIAMOND studie. Bedankt voor jullie behulpzaamheid, gezelligheid, maar vooral ook voor jullie prik-skills. Bart, bedankt voor jouw bereidheid om in te vallen als ik op vakantie was en voor het meedenken rondom mijn onderzoek. Leuk dat we binnenkort weer collega's worden! Bettine en Jan, jullie hebben ervoor gezorgd dat alle samples netjes in Groningen werden verzameld en verwerkt. Jan, bedankt voor al je werk ten aanzien van de iohexol samples. Bettine, bedankt voor je labtechnische- en logistieke kunsten. En daarnaast ook bedankt voor de (sportieve) gezelligheid!

De DIAMOND studie was natuurlijk nooit een succes geworden als er geen proefpersonen aan deelgenomen hadden. Heel veel dank aan alle deelnemers die bereid zijn geweest om naar het ziekenhuis te komen voor de studievizites, iedereen die van te voren 24uurs urine wilden verzamelen, en zich daarna vrijwillig lieten prikken voor het nierfunctieonderzoek. Jullie inzet is niet voor niets geweest!

Regarding all publications: thanks to all co-authors for their contribution to this thesis! In particular I would like to mention Mie and her supervisors from the Steno Diabetes Center in Copenhagen. I enjoyed working together! A pity that I can no longer attend the group dinners during EDNSG and ASN.

I would also like to thank the assessors of my PhD thesis, prof. dr. J. Vora, prof. dr. PH. Groop and prof. dr. A. Voors. Thank you for your time and effort to read my thesis.

Collega's en supervisors van de Kidney Center Meeting: bedankt voor jullie actieve deelname en voor de hulp met het organiseren van de KCM-bijeenkomsten. Ook dank aan de meiden waarmee ik deze bijeenkomsten georganiseerd heb, het was leuk om met jullie samen te werken!

Lieve collega's/Dear colleagues, thank you for all the fun you brought to my PhD-life! I really appreciate your willingness to join me for crazy activities, sport events, cooking experiences, Sinterklaas, vrijmibo's, etc.etc. Kamergenoten, jullie in het bijzonder bedankt voor de hilarische momenten op kantoor, voor het meezingen met oude hits, het uitvoeren van rek- en strekoefeningen, het samen squashen en sporten, en voor het delen van persoonlijke verhalen en blunders. Ik heb het echt ontzettend naar mijn zin gehad met jullie op kantoor.

Lieve paranimfen Vivian en Nienke, bedankt dat jullie voor me klaarstaan en mijn paranimfen willen zijn. Nienke: Op mijn eerste congres, een paar weken nadat ik gestart was, kwamen we op dezelfde kamer terecht. Dat beviel goed, want sindsdien waren we vaste kamergenoten en soms zelfs bedgenoten (haha)! Bedankt voor jouw gezelschap op kantoor. Ik heb regelmatig kunnen genieten van de wijze waarop jij situaties nuchter en ongecompliceerd tegemoet kan treden. Vivian: buiten werk ben jij als een van mijn beste vriendinnen mijn vaste kamergenoot op vakanties of weekendjes weg. Je kwam me bezoeken in Madrid, je wandelde mee in Duitsland voor de Nierstichting, en je hebt het zelfs aangedurfd om met mij naar Malawi te gaan voor een stage. Ik waardeer jouw betrokkenheid, zorgzaamheid en je eerlijkheid en ik hoop nog veel mooie reisjes samen te gaan maken!

Vriendinnen van thuisthuis, van de studie, oud straat- en oud-huisgenootjes: ik ben superblij met jullie als vriendinnen! Bij jullie kan ik altijd terecht voor leuke en minder leuke dingen. Dank daarvoor.

Familie Dekkers/Suermondt, familie Lambert/Meijering, Gijs, en lieve opa, ik wil jullie bedanken voor jullie interesse, voor alle gezellige etentjes, BBQ's, de (verhalen over-) reizen en voor jullie support.

Lieve pap en mam, ik ben dankbaar dat ik heb mogen opgroeien in jullie warme nest. Jullie hebben mij altijd aangemoedigd om het beste uit mezelf te halen. Bedankt voor jullie onvoorwaardelijke steun en vertrouwen. Lieve Judith (Juut), toen we klein waren trokken we de haren uit elkaars hoofd, maar inmiddels zijn we echte zussen geworden. Ik heb respect voor jouw zorgzaamheid, je creativiteit en je doorzettingsvermogen.

Lieve Bart, je laat me lachen, nadenken, je laat me gek doen, geeft me vrijheid, helpt me te relativeren, en je accepteert (soms) monkey-cuddles. Kortom: je maakt me gelukkig, DANK daarvoor!!

About the author

Claire C.J. Dekkers was born on May 6, 1992 in Waalwijk, the Netherlands. After graduating secondary school (VWO, Willem van Oranje College in Waalwijk) in 2010, she started studying Medicine at the University of Groningen. During her studies she was member of several student committees and she conducted an internship Global Health in Malawi. She performed her master thesis at the department of endocrinology, University Medical Center Groningen, and conducted her final internship at the internal medicine ward at the Deventer Hospital. In 2017 she started her PhD project at the department of clinical pharmacy and pharmacology of the University Medical Center Groningen under supervision of prof. dr. H.J. Lambers-Heerspink and prof. dr. R.T. Gansevoort. Her research focused on the non-glycemic effects of SGLT2 inhibitors, a relatively new class of medications normally used for treating type 2 diabetes mellitus. She presented her work at several conferences, such as the American Society of Nephrology Kidney Week and the European Diabetic Nephropathy Study Group. In May 2020 Claire started to work as a physician at the department of internal medicine in the Medical Center Leeuwarden. She will soon start training to become an internist.