



## Research paper

# Acute effects of dapagliflozin on renal oxygenation and perfusion in type 1 diabetes with albuminuria: A randomised, double-blind, placebo-controlled crossover trial

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## ARTICLE INFO

## Article History:

Received 23 March 2021

Revised 22 April 2021

Accepted 23 April 2021

Available online 28 June 2021

## Keywords:

SGLT2 inhibitors

Diabetic kidney disease

Hypoxia

## ABSTRACT

**Background:** Inhibitors of the sodium-glucose cotransporter 2 (SGLT2) slow the progression of diabetic kidney disease, possibly by reducing the proximal tubule transport workload with subsequent improvement of renal oxygenation. We aimed to test this hypothesis in individuals with type 1 diabetes and albuminuria.

**Methods:** A randomised, double-blind, placebo-controlled, crossover trial with a single 50 mg dose of the SGLT2 inhibitor dapagliflozin and placebo in random order, separated by a two-week washout period. Magnetic resonance imaging (MRI) was used to assess renal  $R_2^*$  (a low value corresponds to a high tissue oxygenation), renal perfusion (arterial spin labelling) and renal artery flow (phase contrast imaging) at baseline, three- and six hours from tablet ingestion. Exploratory outcomes, including baroreflex sensitivity, peripheral blood oxygen saturation, peripheral blood mononuclear cell mitochondrial oxygen consumption rate, and biomarkers of inflammation were evaluated at baseline and 12 h from medication. The study is registered in the EU Clinical Trials Register (EudraCT 2019–004,557–92), on ClinicalTrials.gov (NCT04193566), and is completed.

**Findings:** Between February 3, 2020 and October 23, 2020, 31 individuals were screened, and 19 eligible individuals were randomised. Three dropped out before receiving any of the interventions and one dropped out after receiving only placebo. We included 15 individuals (33% female) in the per-protocol analysis with a mean age of 58 (SD 14) years, median urinary albumin creatinine ratio of 46 [IQR 21–58] mg/g and an eGFR of 73 (32) ml/min/1.73m<sup>2</sup>. The mean changes in renal cortical  $R_2^*$  from baseline to six hours were for dapagliflozin  $-1.1$  (SD 0.7) s<sup>-1</sup> and for placebo  $+1.3$  (0.7) s<sup>-1</sup>, resulting in a difference between interventions of  $-2.3$  s<sup>-1</sup> [95% CI  $-4.0$  to  $-0.6$ ];  $p = 0.012$ . No between-intervention differences were found in any other MRI outcomes, physiological parameters or exploratory outcomes. There were no adverse events.

**Interpretation:** A single dose of 50 mg dapagliflozin acutely improved renal cortical  $R_2^*$  without changing renal perfusion or blood flow. This suggests improved renal cortical oxygenation due to a reduced tubular transport workload in the proximal tubules. Such improved oxygenation may in part explain the long-term beneficial renal effects seen with SGLT2 inhibitors, but it remains to be determined whether the observed effects can be achieved with lower doses, with chronic treatment and if they occur in type 2 diabetes as well.

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**Funding:** The study was funded by the Novo Nordisk Foundation grant PROTON personalising treatment of diabetic nephropathy (NNF14OC0013659).

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## Research in context

### *Evidence before this study*

We searched PubMed for publications in English from between January 1, 1990 and February 2, 2021, using the search terms “SGLT2”, “oxygenation”, “magnetic resonance imaging”, “type 1 diabetes”, and “type 2 diabetes”. Studies in diabetic animals showed increased renal cortical oxygenation with SGLT2 inhibition but reduced renal medullary oxygenation. Human studies were limited but one study showed no effect of SGLT2 inhibition on renal oxygenation in healthy individuals.

### *Added value of this study*

The SGLT2 inhibitors have demonstrated kidney benefits which are not explained. It has been suggested based on animal studies, that an amelioration of renal hypoxia is central, but human studies are lacking. We showed that SGLT2 inhibition improves renal cortical oxygenation within six hours in individuals with type 1 diabetes and albuminuria. This finding improves the understanding of the mechanisms behind the impressive renoprotective effects recently seen with SGLT2 inhibitors in major clinical trials.

### *Implications*

Our findings support an effect of SGLT2 inhibitors on kidney hypoxia. Long term studies should address if this effect is sustained and linked to reduction in inflammation and fibrosis in the kidney in both type 1 and type 2 diabetes. Hypoxia could be a target for other new interventions.

effects have not been investigated in individuals with diabetes [12]. Therefore, we aimed to evaluate the acute effects of SGLT2 inhibition on renal oxygenation assessed by magnetic resonance imaging (MRI) in individuals with type 1 diabetes and albuminuria. We hypothesised that renal oxygenation could be improved within six hours as previous studies have demonstrated a rapid plasma uptake of dapagliflozin and subsequent dose-dependant induction of glucosuria [13].

## 2. Methods

### 2.1. Trial design

This randomised, double-blind, placebo-controlled, crossover, single-centre clinical trial was conducted between February 3, 2020 and October 23, 2020 in Copenhagen, Denmark. A crossover design was chosen because the within-individual variation is typically smaller than the variation between individuals thus fewer participants are required to demonstrate a difference. Participants were randomly assigned in a 1:1 ratio to one of the following sequence-groups: dapagliflozin-placebo: a single dose of 50 mg dapagliflozin for visit 2 and again for visit 3 and placebo for visits 4 and 5; or placebo-dapagliflozin: placebo for visits 2 and 3 and dapagliflozin for visits 4 and 5 (Fig. 2). Washout periods between all intervention visits were two weeks. The study protocol was approved by the Regional Ethical Committee and the Danish Medicines Agency. All participants provided written consent. The study was registered in the EU Clinical Trials Register (EudraCT 2019–004,557–92) and on ClinicalTrials.gov (NCT04193566). The study complied with the Declaration of Helsinki and Good Clinical Practice Guidelines.

### 2.2. Changes from protocol

Treatment with beta-blocking medication was initially a study exclusion criterion due to potential interference with measures of baroreflex sensitivity. The primary outcomes were not affected by this treatment and the criterion was removed. In the three included participants treated with beta-blocking medication, the treatment was phased out at least two days before each study visit to avoid potential influence of betablockade on measures of baroreflex sensitivity. One participant was not abstinent from smoking 24 h before visit 3.

### 2.3. Participants, settings and location

Adults with type 1 diabetes and albuminuria defined as a urine albumin creatinine ratio  $\geq 30$  mg/g in two out of three consecutive samples prior to randomisation assessed from the hospitals laboratory database were eligible for participation. Exclusion criteria were (I) non-diabetic kidney disease as indicated by medical history and/or laboratory findings, (II) renal failure (eGFR < 15 ml/min/1.73m<sup>2</sup>), (III) dialysis or kidney transplantation, (IV) uncontrolled arrhythmia, (V) 2nd or 3rd degree AV-block or sick sinus syndrome (assessed from a standard 12-lead electrocardiogram), (VI) pregnancy or breastfeeding, (VII) systolic blood pressure < 90 or > 200 mmHg, (VIII) known heart disease contraindicating MRI (permanent pacemaker), (IX) known lung disease (asthma, chronic obstructive pulmonary disease), (X) history of surgery in the past six weeks, or (XI) presence of foreign bodies of metal in the body or not able to lie in an MRI scanner for two hours. MRI measurements were performed at

## 1. Introduction

Inhibitors of the sodium-glucose cotransporter 2 (SGLT2) are a new class of drugs initially introduced to lower glucose. In addition, they have been found to reduce the risk of major cardiovascular events, hospitalisation for heart failure, renal events, and to slow the progression of diabetic kidney disease in individuals with type 2 diabetes [1–3]. Furthermore, it was recently demonstrated that the SGLT2 inhibitor dapagliflozin improves hard renal outcomes and reduces mortality in individuals with chronic kidney disease with and without diabetes [4]. The exact mechanisms behind the beneficial effects of SGLT2 inhibition are unknown.

One hypothesis is that SGLT2 inhibition increases the renal cortical oxygenation by reducing the oxygen-consuming tubular reabsorption of sodium and glucose [5]. Directly, by inhibiting the SGLT2 and indirectly, by reducing hyperfiltration [6]. Reduced renal cortical oxygenation may be important in the pathogenesis of diabetic kidney disease as it has been observed in type 1 diabetes [7,8] and shown to predict a progressive decline of renal function in individuals with chronic kidney disease [9]. Amelioration of renal cortical hypoxia has been suggested to preserve kidney function by reducing inflammation and fibrosis [10].

Experimental studies with phlorizin, a dual SGLT2 and SGLT1 inhibitor, have shown a normalisation of renal cortical hypoxia in diabetic rats [11]. In a study by Zanchi and colleagues, SGLT2 inhibition had no effect on renal oxygenation in healthy humans, but the

Rigshospitalet Glostrup, Copenhagen, Denmark, while the remaining assessments and procedures, including screening and randomisation, were performed at the Steno Diabetes center Copenhagen, Copenhagen, Denmark.

#### 2.4. Randomisation and masking

For randomisation we used an allocation schedule with one block of 20 sequences generated from the webpage [www.randomization.com](http://www.randomization.com). The allocation schedule, sealed envelopes with unblinding-details and 20 sequentially numbered sealed opaque plastic bags containing study medication were produced by Glostrup Pharmacy, Copenhagen, Denmark. The sealed envelope with unblinding-details were kept in a locked drawer during the study. The first author recruited, enrolled and assigned all participants for sequence allocations. The first author was blinded to sequence allocations until after the last visit of the last participant (October 23, 2020). All other investigators, lab-technicians, statisticians, outcome assessors and study participants were blinded until the end of data analysis (January 22, 2021).

#### 2.5. Procedures

We chose to use a single dose of 50 mg dapagliflozin as the study intervention to achieve optimal efficacy. Once-per-day doses of dapagliflozin for 12 weeks of 2.5, 5, 10, 20, and 50 mg have been demonstrated to be relatively safe across the mentioned doses [14] and no apparent risk were expected from instituting two single-doses of 50 mg dapagliflozin. The study interventions were the oral intake of five film-coated tablets of 10 mg dapagliflozin and five matching tablets of placebo with an equal composition, except for the omission of the active ingredient. The active drug and placebo were identical in appearance and smell. The dapagliflozin- and placebo tablets were produced by Glostrup Pharmacy, Copenhagen, Denmark.

#### 2.6. Outcomes

The primary outcome was change in renal oxygenation ( $R_2^*$ ) measured with quantitative MRI on a 3-T Philips Achieva scanner.  $R_2^*$  measures were interleaved by measures of renal perfusion using arterial spin labelling and measures of renal artery blood flow using phase contrast.  $R_2^*$  has been validated to have a linear relationship with cortical and medullary tissue oxygen partial pressure and numerous previous works have validated  $R_2^*$  or changes in dynamic  $R_2^*$ -weighted signal (BOLD) as an indirect measure of renal blood oxygenation in both patients and healthy subjects as it is driven by deoxyhaemoglobin concentrations [15–18].  $R_2^*$  and perfusion maps were obtained for a five mm coronal slice covering both kidneys and measures of renal artery blood flow obtained for the right kidney. Reported  $R_2^*$  and perfusion values are the means of regions of interest covering the medulla and cortex of both kidneys. Details regarding the arterial spin labelling, phase contrast and  $R_2^*$  MRI scanning protocols and data processing have been published previously [19,20] and are in accordance with expert consensus on protocol optimisation for quantitative measures of renal hemodynamics [21–24]. Arterial spin labelling and phase contrast MRI data were respiratory gated whereas  $R_2^*$  data were acquired during a single breath-hold. All three measures were repeated three times for each time point. Perfusion calculations were performed using the kinetic model presented by Buxton et al. [25], with a tissue-blood partition coefficient of 0.9, a labelling efficiency of 0.95 and blood T1 value of 1.65 s. The intra-individual coefficient of variation for a given time-point measure was 4.3% for cortical  $R_2^*$ , 5.9% for medullary  $R_2^*$ , 7.3% for cortical perfusion, 15.4% for medullary perfusion, and 3.8% for renal artery blood flow.

Blood pressure, blood oxygen saturation and heart rate were measured using a Veris Monitor System (MEDRAD, Pittsburg, Pennsylvania, USA). Blood glucose was measured using a Contour Next One device (Ascensia Diabetes Care, New Jersey, USA).

Autonomic nerve function was assessed by measuring the baroreflex sensitivity which is defined as the change in heart rate in relation to change in blood pressure. Baroreflex sensitivity was determined from spontaneous fluctuations in the heart rate and systolic blood pressure over 5 min. Exact methods have previously been described [26].

The mitochondrial oxygen consumption rate of peripheral blood mononuclear cells was quantified with the Seahorse XFe24 Analyser (Agilent Technologies, Santa Clara, CA, USA). The cells were isolated from fresh peripheral blood through density gradient centrifugation, within 1 h after collection and seeded onto a Seahorse XFe24 plate coated with poly-D-lysine in Seahorse XF DMEM medium without phenol red supplemented with 5 mM HEPES, 2 mM L-Glutamine and 1 mM Sodium Pyruvate. After 1 h incubation at 37 °C in a CO<sub>2</sub>-free incubator three basal oxygen consumption rate measurements were performed, followed by an injection of 0.5 μM Oligomycin (Sigma O4876) to inhibit ATP synthase or an injection of 3 μM FCCP (Sigma C2920) which uncoupled mitochondrial respiration. Both Oligomycin and FCCP effects were recorded during four oxygen consumption rate measurements, followed by a combined injection of 2 μM Rotenone (Sigma R8875) and 2 μM Antimycin A (Sigma A8674) and two oxygen consumption rate measurements to correct the data for non-mitochondrial oxygen consumption rate. All data was normalised for the number of peripheral blood mononuclear cells isolated.

Inflammation biomarkers were measured on peripheral blood with the Olink® Target 96 Inflammation panel (Olink, Uppsala, Sweden) including 92 inflammation-related human protein biomarkers [27]. Biomarker levels are shown as the Normalized Protein Expression (NPX) which is a relative arbitrary unit on a log<sub>2</sub> scale. Although NPX directly correlates with initial protein concentrations, no comparisons of absolute protein levels can be made.

Renal oxygen consumption was originally a study outcome, but we were unable to validate a suitable method for assessing this outcome and it was thus excluded.

#### 2.7. Study visits

The study consisted of an information meeting and study visits 1–5 (Fig. 2). At the information meeting, the participants were informed about the study and upon providing a signed informed consent was screened for eligibility and randomised. At visit 1, data on baseline characteristics, including measurements of baseline baroreflex sensitivity and mitochondrial oxygen consumption rate were collected. At visits 2 and 4, kidney MRI measurements were performed. The participants fasted from food and liquid from midnight the evening before and abstained from medication (except insulin), alcohol, tobacco use and strenuous exercise for 24 h before the visit. If participants experienced a low blood sugar (< 3 mmol/L) during the fasting period they were instructed to take 10 g dextrose or drink 100 ml apple juice. When the participants arrived, a baseline MRI scan was performed. Immediately thereafter, the participant ingested the intervention tablets along with a standardised breakfast and 500 ml water, with one hour to ingest. Three hours (±5 min) after the intervention, a second scan was performed. Immediately thereafter, the participant had a standardised lunch and 500 ml water, with one hour to ingest. Six hours ± five minutes after the intervention, a third scan was performed. Blood glucose was measured before each scan and during scans, blood pressure, heart rate, and blood oxygen saturation were monitored continuously.

At visits 3 and 5, the participants were instructed to take the intervention tablets exactly 12 h before the study visit. The participants fasted from midnight the evening before and abstained from

medication (except insulin), alcohol, tobacco use and strenuous exercise 24 h before the visits. Blood samples for the peripheral blood mononuclear cells mitochondrial oxygen consumption rate and inflammation biomarkers were drawn 12 h  $\pm$  20 min after the intervention and autonomic testing along with continuous measurements of blood oxygen saturation were performed immediately afterwards.

## 2.8. Statistical analysis

The primary goal was to test the hypothesis that SGLT2 inhibition would improve renal oxygenation with a lowering of  $R_2^*$  within six hours. A power calculation was performed, based on data available from a study by Haddock. et al. investigating the effect of furosemide on  $T_2^*$ , which is equal to  $1/R_2^*$  [20]. The mean resting medullary  $T_2^*$  was 23 (SD 4). We hypothesised that SGLT2 inhibition would improve oxygenation by 20% and the sample size required to demonstrate a significant effect with a power of 80% and a type 1 error of 5%, was thus 13 participants per group. The power calculation did not take the crossover design into account. The power calculation was carried out using the power statement implemented in SAS Enterprise Guide (version 7.15) [28].

Clinical characteristics of participants are presented as n (%), mean (standard deviation (SD)) or, if skewed distributions, as medians with interquartile range [interquartile range (IQR)]. As the normality assumption of the distributions of the data for parametric testing was violated, Wilcoxon signed rank tests were used for comparing dapagliflozin and placebo baseline values in outcomes and twelve-hour values of blood oxygen saturation, baroreflex sensitivity, mitochondrial oxygen consumption rate, and inflammation biomarkers. Due to the large number of tests, p-values from analyses on the 92 inflammation biomarkers were adjusted for multiple testing using the Benjamini-Hochberg approach. A linear mixed effects model for repeated measurements was used to analyse differences between changes in the estimated renal  $R_2^*$ , renal perfusion, and renal blood flow. The model was adjusted for treatment (dapagliflozin/placebo), randomisation-sequence, visit day (visit 2/visit 4), time of measurement (baseline, three hours, six hours), blood glucose, and the interaction between time of measurement and treatment, with a participant-specific random intercept to account for the

correlation of repeated measures within participants. The same model was used to analyse differences between changes in estimated blood glucose, blood oxygen saturation, blood pressure, and heart rate, but without adjusting for blood glucose. Changes in outcomes are presented as least-squares mean changes with standard errors from baseline to three hours after intervention and from baseline to six hours after intervention with dapagliflozin and placebo, respectively. Differences between changes are presented as estimates with 95% confidence intervals and corresponding p-values. All the analyses were carried out using SAS Enterprise Guide (version 7.15) [28]. We used PROC MIXED for mixed effects regressions and PROC UNIVARIATE for Wilcoxon signed rank tests.

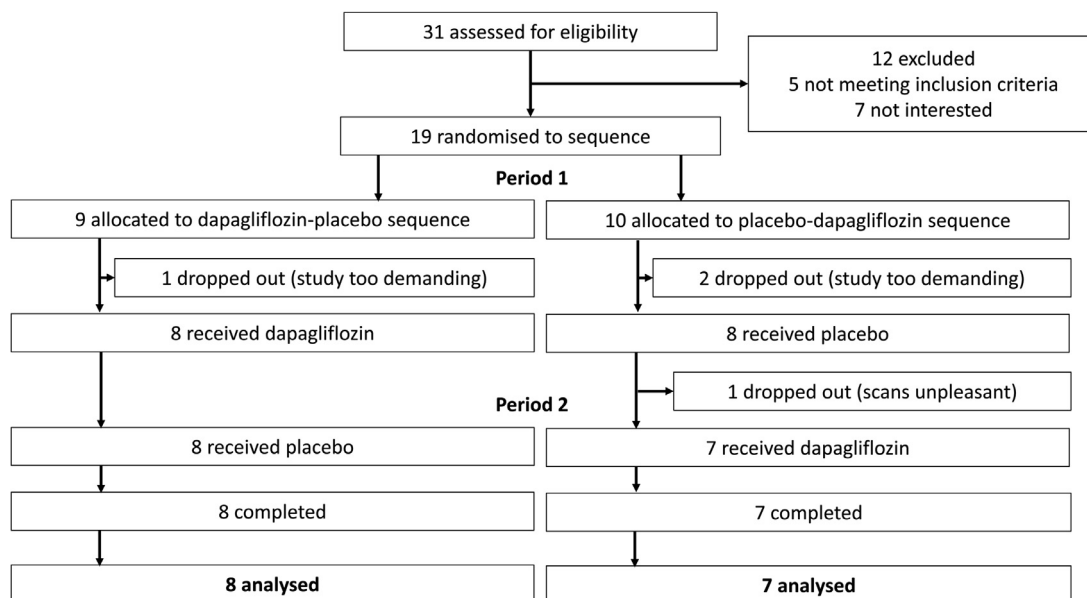
## 3. Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

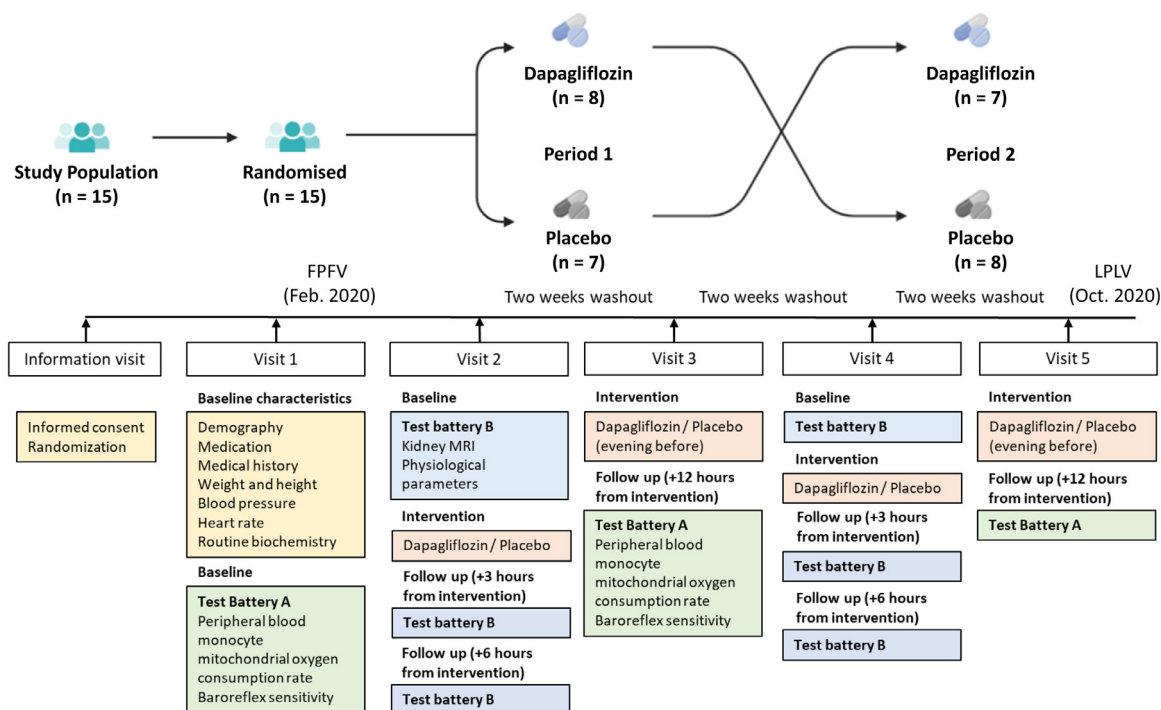
## 4. Results

Participants were recruited between February 3, 2020 and October 23, 2020. 31 individuals were assessed for eligibility and 12 were excluded due to either not meeting the inclusion criteria or because they were not interested in participating. Thus, 19 were included and randomised. Four dropped out during the study, three because the study was too demanding and one because the MRI scans were unpleasant (Fig. 1). The trial was stopped when 15 individuals had completed.

In the per-protocol analyses, we included 15 individuals with type 1 diabetes (33% females) with a mean age of 58 (SD 14) years and a diabetes duration of 39 (16) years. They had a median urinary albumin creatinine ratio of 43 [IQR 21–58] mg/g and an eGFR of 73 (32) ml/min/1.73m<sup>2</sup>. Three (20%) had macroalbuminuria and 12 (80%) had microalbuminuria. Two (13%) were smokers, 13 (87%) were treated with blockers of the renin-angiotensin-aldosterone system and seven (47%) were treated with loop diuretics (Table 1). Participants were on stable doses of medication throughout the study.



**Fig. 1.** Study participants. Flowchart for study participants. “Study too demanding” means that participants dropped out because the study was more demanding than they initially anticipated. “Scans unpleasant” means that one participant dropped out because they had a back pain and did not wish to lie still during a second scan.



**Fig. 2.** Study design and study visits. Study design and study visits. MRI = Magnetic Resonance Imaging. FPFV = First Patient First Visit. LPLV = Last Patient Last Visit. Dapagliflozin = a single dose of 50 mg.

Baseline renal  $R_2^*$ , renal perfusion, and renal blood flow were not significantly different between the dapagliflozin and placebo days. Neither were the baseline physiological parameters of blood glucose, blood oxygen saturation, blood pressure and heart rate (Table 2).

**Table 1**

Baseline Characteristics by Intervention Sequence Group and by Total. Shown are mean (SD), n (%) and median [quartile 1-quartile 3]. Microalbuminuria was defined as a urine albumin creatinine ratio  $\geq 30$  mg/g and  $< 300$  mg/g in 2 out of 3 consecutive urine samples prior to randomisation. Macroalbuminuria was similarly defined but with a urine albumin creatinine ratio  $\geq 300$  mg/g. \*One measurement of LDL was missing from the Placebo-Dapagliflozin sequence group due to an analysis error.

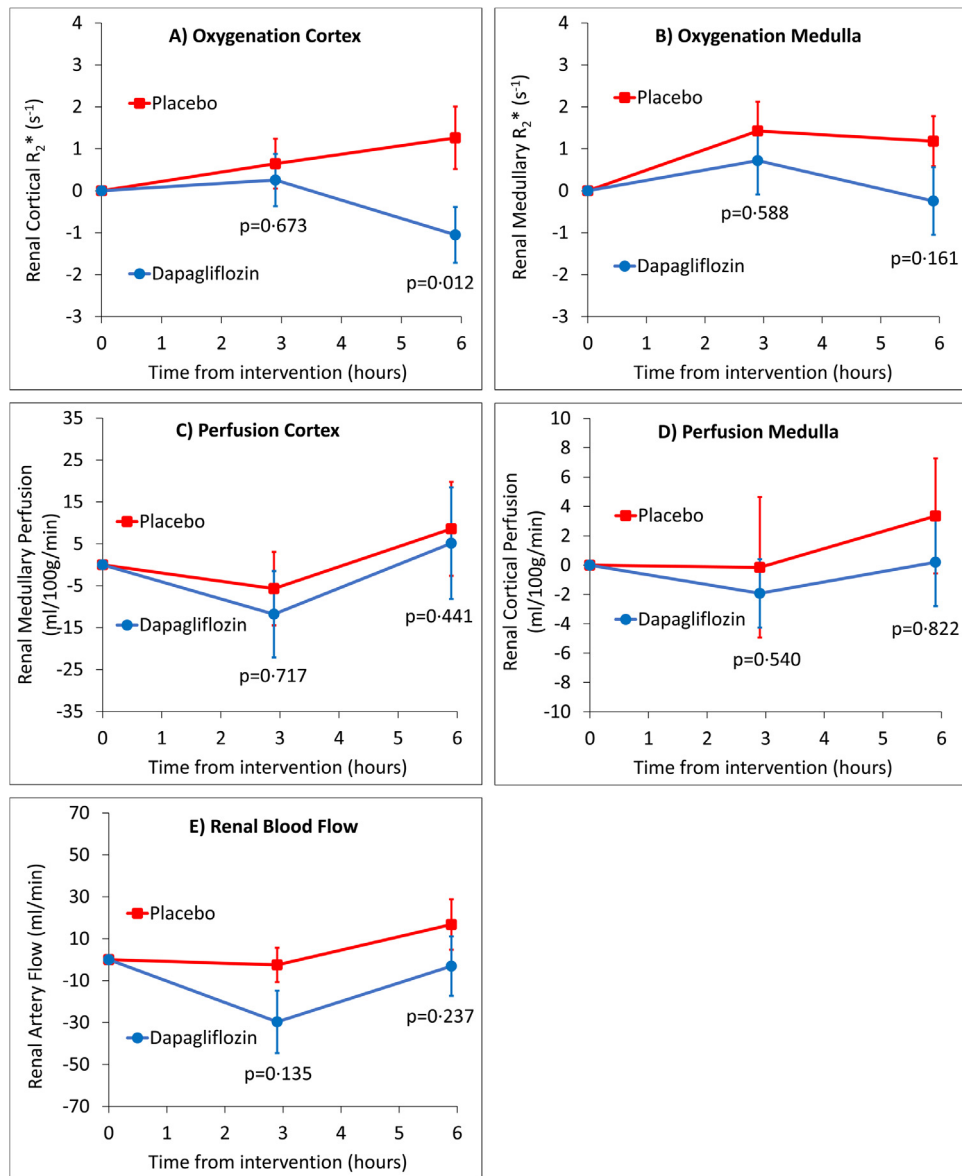
	Dapagliflozin-Placebo	Placebo-Dapagliflozin	Total
n	8	7	15
Age, years	62 (9)	53 (17)	58 (14)
Women	2 (25%)	3 (42%)	5 (33%)
Duration of diabetes, years	39 (16)	38 (18)	39 (16)
Microalbuminuria	8 (100%)	4 (57%)	12 (80%)
Macroalbuminuria	0 (0%)	3 (42%)	3 (20%)
Body-mass index, kg/m <sup>2</sup>	28.4 (2.7)	25.0 (2.6)	26.8 (3.1)
Chronic ischaemic heart disease	2 (25%)	0 (0%)	2 (13%)
Smokers	1 (13%)	1 (14%)	2 (13%)
Hba1c,%	8.0 (0.6)	7.4 (0.7)	7.7 (0.6)
Hba1c, mmol/mol	64 (6)	58 (7)	61 (7)
Estimated Glomerular Filtration Rate, ml/min/1.73m <sup>2</sup>	77 (28)	68 (39)	73 (32)
Urine Albumin Creatinine Ratio, mg/g	43 [23–49]	58 [21–312]	43 [21–58]
Creatinine, $\mu$ mol/L	93 (45)	125 (65)	108 (55)
LDL, mmol/L	2.0 (1.1)	2.2 (1.2)*	2.1 (1.1)
Renin-Angiotensin-Aldosterone System Blockade	8 (100%)	5 (71%)	13 (87%)
Beta blockade	2 (25%)	1 (14%)	3 (20%)
Insulin	8 (100%)	7 (100%)	15 (100%)
Lipid lowering medication	7 (88%)	4 (57%)	11 (73%)
Acetylsalicylic Acid	6 (75%)	3 (43%)	9 (60%)
Loop diuretic	2 (25%)	3 (43%)	5 (33%)

Dapagliflozin significantly lowered the renal cortical  $R_2^*$  (corresponding to an improved oxygenation) compared to placebo after six hours: The least squares mean changes in renal cortical  $R_2^*$  from baseline to six hours were for dapagliflozin  $-1.1$  (SEM 0.7)  $s^{-1}$  and for placebo  $+1.3$  (0.7)  $s^{-1}$  resulting in a difference between interventions of  $-2.3$   $s^{-1}$  (95% CI  $-4.0$  to  $-0.6$ );  $p = 0.012$  (Fig. 3A). Medullary  $R_2^*$  was lowered by dapagliflozin compared to placebo after six hours, but not significantly ( $-1.4$   $s^{-1}$  (95% CI  $-3.5$  to  $0.6$ );  $p = 0.162$ ) (Fig. 3B). Three-hour changes in  $R_2^*$  were similar between interventions in both the renal cortex and the renal medulla (Fig. 3A,B). There were no differences between dapagliflozin and placebo in the three- and six-hour changes in cortical- or medullary perfusion, although there was a tendency towards an increase from three to six hours for both interventions (Fig. 3C,D). Renal blood flow decreased with dapagliflozin after three hours compared to placebo but not significantly ( $-27$  ml/min (95% CI  $-64$  to  $10$ );  $p = 0.135$ ) and then returned to the baseline level at six hours. There was no significant difference between the six-hour changes in renal blood flow between interventions (Fig. 3E).

**Table 2**

Baseline values in study outcomes. Shown are means (SD) at baseline on the dapagliflozin day and the placebo day. A low  $R_2^*$  corresponds to a high tissue oxygenation. P-values from Wilcoxon signed rank sum test.

	Dapagliflozin (n = 15)	Placebo (n = 15)	p
<b>Magnetic Resonance Imaging Outcomes</b>			
$R_2^*$ cortex, $s^{-1}$	23 (4)	22 (5)	0.421
$R_2^*$ medulla, $s^{-1}$	33 (7)	31 (5)	0.095
Perfusion cortex, ml/100 g/min	169 (37)	160 (51)	0.421
Perfusion medulla, ml/100 g/min	43 (11)	42 (14)	0.934
Renal blood flow, ml/min	368 (125)	348 (129)	0.229
<b>Physiological Parameters</b>			
Blood glucose, mmol/L	8 (3)	8 (3)	0.988
Blood oxygen saturation,%	95 (2)	96 (2)	0.217
Systolic blood pressure, mmHg	143 (16)	145 (15)	0.952
Diastolic blood pressure, mmHg	83 (10)	83 (12)	0.761
Heart rate, beats min <sup>-1</sup>	68 (12)	67 (11)	0.890



**Fig. 3.** Changes from baseline in estimated least squares means in magnetic resonance imaging outcomes. shown are the least-squares mean changes from baseline in magnetic resonance imaging outcomes renal cortical oxygenation (A), renal medullary oxygenation (B), renal cortical perfusion (C), renal medullary perfusion (D), and renal blood flow (E), calculated with the use of a repeated-measures analysis including terms for treatment, randomisation-sequence, day of visit, blood glucose, time of measurement and interaction between time of measurement and treatment. A low  $R_2^*$  corresponds to a high oxygenation. P-values for comparisons of changes from baseline between dapagliflozin and placebo.

Three-hour blood glucose tended to increase after the morning meal (+1.9 mmol/L (95% CI -0.5 to 4.4);  $p = 0.117$ ) and six-hour blood glucose was increased after lunch (+3.4 mmol/L (95% CI 1.2 to 5.7);  $p = 0.005$ ). The increases tended to be smaller with dapagliflozin compared to placebo (difference between dapagliflozin and placebo in three-hour change: -1.4 mmol/L (95% CI -3.9 to 1.2);  $p = 0.282$ ; and in six-hour change: -1.7 mmol/L (95% CI -4.3 to 1.0);  $p = 0.192$  (Fig. 4A). There were no significant differences between dapagliflozin and placebo in the changes in blood oxygenation after three hours: +1.1% (95% CI -0.5 to 2.6);  $p = 0.154$ ) or six hours: +1.0% (95% CI -0.2 to 2.2);  $p = 0.092$  (Fig. 4B). Three- and six-hour changes in blood pressure and heart rate were not different between dapagliflozin and placebo (Fig. 4C–E).

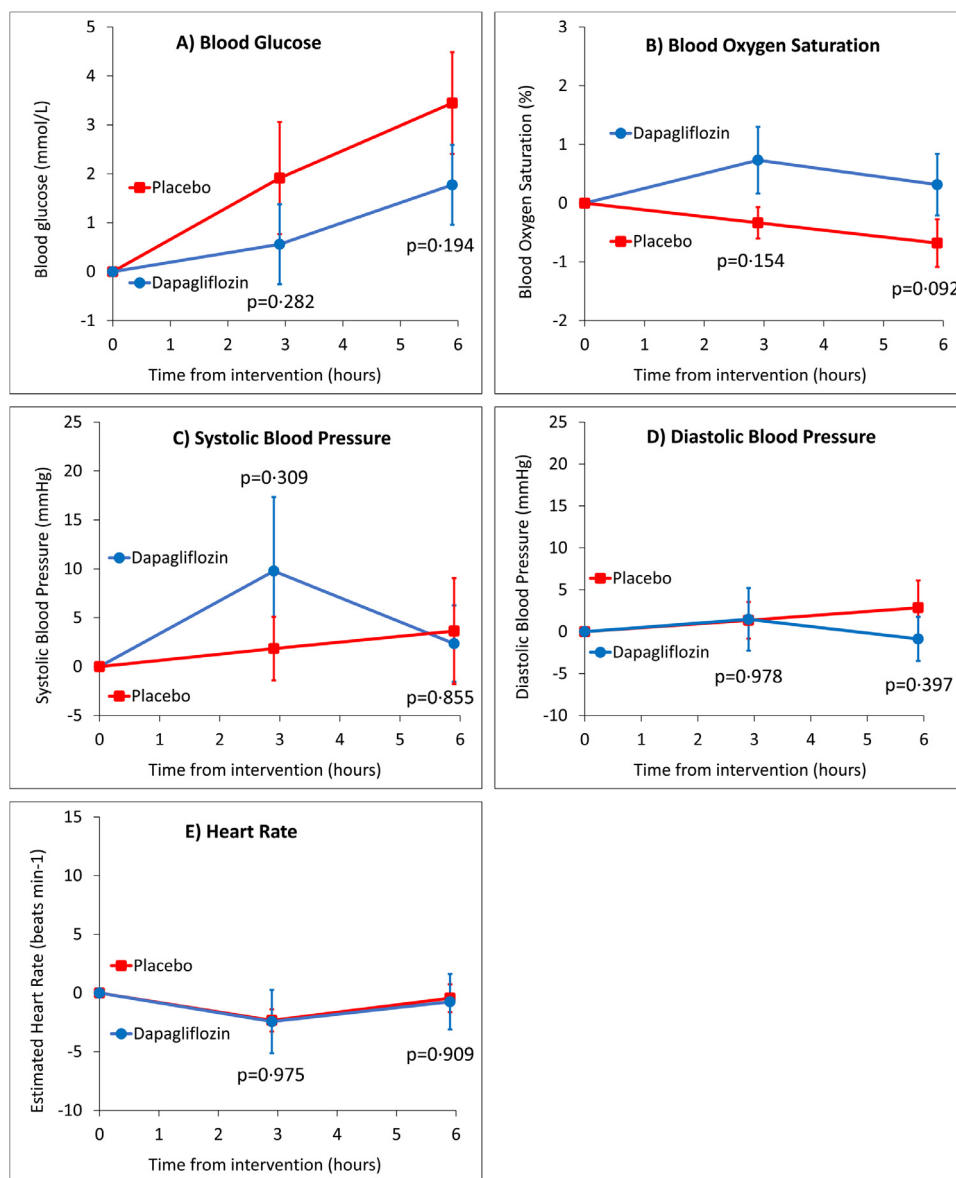
There were no differences between dapagliflozin and placebo twelve hours after intervention in measurements of baroreflex sensitivity, blood oxygen saturation or peripheral blood mononuclear cell mitochondrial oxygen consumption rate in presence of glucose (Table 3) or in inflammation biomarkers (Table S1).

To test if albuminuria interacted with the observed effect on oxygenation, we performed sensitivity analyses including the

urinary albumin creatinine ratio in the models with  $R_2^*$  as the outcome. No significant interaction was observed with either cortical or medullary  $R_2^*$ . We also tried including albuminuria as a categorical variable (macroalbuminuria/microalbuminuria) and again, observed no interaction with either cortical or medullary  $R_2^*$ . (Data not shown).

However, the baseline cortical  $R_2^*$  was higher in the macroalbuminuria group (microalbuminuria: 20.5 (1.0)  $s^{-1}$ ; macroalbuminuria: 29.2 (1.8)  $s^{-1}$ ; difference: +8.8  $s^{-1}$  (95% CI 4.4 to 13.2);  $p < 0.001$ ). This was not the case in the medulla (microalbuminuria: 31.1 (2.1)  $s^{-1}$ ; macroalbuminuria: 36.2 (4.8)  $s^{-1}$ ; difference: +5.7  $s^{-1}$  (95% CI -7.3 to 17.5);  $p = 0.388$ ).

We conducted a sensitivity analysis where we removed the participant that had been smoking before one of the study days and repeated the analyses on MRI variables. The results were similar in magnitude and statistical significance, except the analysis on medullary  $R_2^*$  which was now significantly lowered by dapagliflozin compared to placebo after six hours (-2.1  $s^{-1}$  (95% CI -3.7 to -0.3);  $p = 0.025$ ). We also repeated the analyses after removing the three



**Fig. 4.** Changes from baseline in estimated least squares means in physiological parameter outcomes. shown are the least-squares mean changes from baseline in physiological parameter outcomes blood glucose (A), blood oxygen saturation (B), systolic blood pressure (C), diastolic blood pressure (D), and heart rate (E), calculated with the use of a repeated-measures analysis including terms for treatment, randomisation-sequence, day of visit, time of measurement and interaction between time of measurement and treatment. P-values for comparisons between dapagliflozin and placebo in the changes from baseline.

**Table 3**

Twelve-hour values in exploratory outcomes. shown are mean ± SD in exploratory outcomes at baseline and twelve hours from intervention. P-values from Wilcoxon signed rank sum test. \*One participant was excluded from analysis of baroreflex sensitivity because there were too many ectopic beats in the continuous ECG.

	Baseline	Dapagliflozin	Placebo	p
<b>Autonomic Nerve Function and Blood Oxygenation</b>				
Baroreflex Sensitivity (ms/mmHg)*	8 ± 7	7 ± 5	6 ± 4	0.903
Blood Oxygen Saturation (%)	96 ± 2	95 ± 3	96 ± 1	0.252
Peripheral Blood Mononuclear Cells Mitochondrial Oxygen Consumption Rate ((pmol/min)/cell number) × 10 <sup>-5</sup>				
Basal Respiration	8 ± 3	8 ± 3	8 ± 3	0.561
ATP Production	6 ± 2	7 ± 2	7 ± 2	0.679
Maximal Respiration	23 ± 10	24 ± 12	24 ± 8	0.421
Spare Respiratory Capacity	15 ± 8	16 ± 9	16 ± 6	0.330
Non-mitochondrial Respiration	1.8 ± 0.7	1.7 ± 0.9	2.0 ± 0.9	0.489
Proton Leak	1.7 ± 0.6	1.6 ± 0.6	1.6 ± 0.6	0.489

participants on beta blockade and the results were similar in magnitude and statistical significance. (Data not shown).

In the repeated-measures analyses of renal  $R_2^*$ , renal perfusion, and renal blood flow there were no sign of a carryover effect as there were no significant differences in outcomes between randomisation-sequence groups. There was a period effect on the renal medullary  $R_2^*$  (least squares mean for visit 2:  $34.1 (1.8) s^{-1}$ ; visit 4:  $29.5 (1.8) s^{-1}$ ; difference:  $-5.5 s^{-1}$  (95% CI  $-7.4$  to  $-3.6$ );  $p < 0.001$ ). There were no period effects on renal cortical  $R_2^*$ , renal perfusion, or renal blood flow. (Data not shown)

There were no adverse events.

## Discussion

In this randomised, double-blind, crossover study in individuals with type 1 diabetes and albuminuria, a single dose of 50 mg dapagliflozin improved renal cortical  $R_2^*$  compared to placebo after six hours, suggesting improved cortical tissue oxygenation. This occurred without differences between dapagliflozin and placebo in blood oxygenation, renal tissue perfusion, renal blood flow, autonomic nerve function, or mitochondrial energy production. Our findings are therefore consistent with the hypothesis that acute SGLT2 inhibition improves renal cortical oxygenation by reducing energy expenditure by inhibition of the oxygen demanding glucose- and sodium cotransport in the renal proximal tubule.

Improved oxygenation in the kidneys can result from a reduced oxygen demand or an increased supply of oxygen as discussed by Vallon and Verma [5]. As we found no difference between dapagliflozin and placebo in the changes in renal perfusion, renal blood flow, blood pressure, heart rate, or baroreflex sensitivity, an increased supply of blood does not explain the improved renal cortical oxygenation. Furthermore, the blood supply did not seem to carry more oxygen either, as there were no differences between dapagliflozin and placebo in blood oxygen saturation or the mitochondrial oxygen consumption rate in circulating mononuclear cells. Our findings thus suggest that a reduced renal cortical oxygen demand drives the improved oxygenation observed. In the renal cortex, the oxygen demand is primarily determined by the activity of reabsorbing glucose and sodium from the glomerular filtrate [29]. The reduced apical reabsorption of glucose by the SGLT2 and the subsequent basolateral activity of  $Na^+/K^+$  pump might both contribute to this reduced oxygen consumption. Previous studies have demonstrated peak plasma concentrations of dapagliflozin within one-two h and an increase in glucosuria after two-four h [13,30]. The dose of dapagliflozin was five times the normal daily dose. We speculated that a higher dose might provide a stronger signal in the acute setting, which may be seen with lower doses in the chronic setting, where clinical studies have not seen dose dependant differences in outcomes [1,31]. Our findings support the hypothesis that the beneficial effects of SGLT2 inhibition on renal outcomes are in part caused by an increase in the cortical oxygen tension, which in turn may reduce inflammation and fibrosis [10]. Our findings of an absent or insignificant reduction of renal blood flow lends no support to the hypothesis that an acute reduction of hyperfiltration contributes to the improved oxygenation, even though we did not measure changes in the glomerular filtration rate in this study.

Renal oxygenation tended to increase throughout the day with placebo, but renal perfusion and blood flow tended to decrease after three hours and increase again after six hours. Eckerbom et al. used MRI to study circadian variations in these parameters in 16 healthy individuals and found no circadian variation in oxygenation or perfusion. They did observe a circadian variation in renal blood flow similar to what we observed in the present study, with a decrease from morning to noon and an increase from noon to afternoon [32].

Our findings agree well with previous animal studies. Early studies in diabetic rats with the non-selective sodium-glucose

cotransporter 1 + 2 inhibitor phlorizin led to a normalisation of the renal oxygen consumption [33,34]. This finding has since been confirmed by O'Neill and colleagues in a study showing an acute normalisation of reduced renal cortical oxygen tension in diabetic rats by phlorizin [11]. Of note, they also observed a decrease in the renal medullary oxygen tension, whereas our study shows a tendency towards an increased medullary oxygenation. Human studies investigating the effects of SGLT2 inhibition on renal oxygenation are limited. Two *in vitro* studies performed in human proximal tubular cells both showed possible beneficial effects of SGLT2 inhibition: (1) Empagliflozin decreases markers of inflammation [35] and (2) Luseogliflozin inhibits hypoxia-induced HIF-1 $\alpha$  accumulation, possibly by suppressing mitochondrial oxygen consumption [36]. An MRI study by Zanchi et al. in healthy individuals observed no acute- (three hours) or chronic (one month) effects of SGLT2 inhibition (empagliflozin 10 mg daily) on renal tissue oxygenation ( $R_2^*$ ) in a parallel, randomised, placebo-controlled trial with 45 healthy volunteers assessed by MRI [12]. This is in contrast to our study in individuals with type 1 diabetes and albuminuria, and underlines that the action of SGLT2 inhibitors appears to be an amelioration of the pathological process in nephropathy (hypoxia) rather than induction of a simple physiological change. This is further supported by our sensitivity analysis showing a much higher  $R_2^*$  in the macroalbuminuria group compared with the microalbuminuria group, indicating severe tissue hypoxia. Previous studies have demonstrated anti-inflammatory effects of dapagliflozin after 12 weeks of treatment in individuals with type 2 diabetes [37,38]. In our study, we observed no difference 12 h from treatment in inflammation biomarkers with dapagliflozin vs. placebo. This could be due to lack of power or because it takes more time than 12 h to achieve these effects.

Initially SGLT2 inhibitors were introduced to lower glucose in type 2 diabetes. Subsequent cardiovascular and kidney outcome studies demonstrated cardiovascular-, heart failure-, and kidney benefits in patients with type 2 diabetes [1–3]. There have been multiple reviews trying to explain these benefits which do not seem to relate to the blood glucose lowering effect [39,40]. Suggested mechanisms include weight loss, reduction in the systemic or intraglomerular pressure, lowering of uric acid, decreased inflammation, as well as changes in cell metabolism [41,42]. Recently, increasing focus has been on a unifying mechanism related to the potential impact on hypoxia as demonstrated experimentally and reviewed by Packer [10]. Diabetes leads to kidney hypoxia and oxidative stress which changes the balance between HIF1 $\alpha$  and HIF2 $\alpha$  causing inflammation and impaired erythropoiesis. Treatment with SGLT2 inhibitors reverse these unfavourable effects in experimental settings, but human data have been lacking until now.

Patients with type 2 diabetes have a high prevalence of comorbidities and a heterogenous background for kidney disease [43]. In our mechanistic, mode-of-action study we chose to study patients with type 1 diabetes, as a more direct model of diabetic kidney disease. This is in line with previous studies investigating the effect of SGLT2 inhibitors on hyperfiltration [6]. Thus, our results may not be generalised to patients with type 2 diabetes, but are important for the general understanding of diabetic kidney disease and support future studies to confirm our findings with chronic SGLT2 inhibition in type 2 diabetes and chronic kidney disease. Additionally, our findings emphasise the need for studies with interventions improving renal tissue oxygenation to prevent or treat diabetic kidney disease as already suggested by Hesp and colleagues [44]. Furthermore, our study can serve as a proof-of-concept study for the consideration of initiating major clinical trials with SGLT2 inhibitors in individuals with type 1 diabetes to prevent or reverse diabetic kidney disease. Dapagliflozin is approved as an adjunctive glucose lowering treatment for individuals with type 1 diabetes with a body mass index  $> 27 kg/m^2$ , but no major cardiovascular- or renal outcome trials in type 1 diabetes have been conducted so far. The main reason is the



risk of diabetic ketoacidosis [45]. However, preliminary post hoc analyses from the studies in type 1 diabetes do support kidney benefits [46] and our results might contribute additionally to the debate on the potential risks and benefits with SGLT2 inhibition in type 1 diabetes [45].

Our study has limitations. The sample size was in accordance with our power calculation but precludes any sub-analyses. The sample-size was adequate for showing a difference in renal cortical oxygenation, but this was not the case for renal medullary oxygenation due to a lower signal to noise ratio. It is a weakness that our power calculation did not take the crossover design into account. Regarding renal perfusion, there was no significant difference, but it tended to be lower with dapagliflozin. With the small sample-size studied, we cannot rule out entirely an acute effect on renal perfusion which might have had an effect on renal oxygenation. Renal oxygen tension estimated with MRI is not a direct measure per se as it is dependant on the concentration of deoxyhaemoglobin and a possible source of error when using  $R_2^*$  to estimate tissue oxygenation is that changes in blood volume can also change the amount of deoxyhaemoglobin in the tissue. However, we observed a difference between dapagliflozin and placebo in the change in cortical  $R_2^*$  without significant differences in perfusion. We used five-times the normal daily dose of dapagliflozin and only studied the acute effects, thus we do not know if the same effects are present with for example 10 mg dapagliflozin which is the standard dose or with chronic treatment. Glucose reabsorption is particularly important for the proximal tubule workload [47,48] which is why we adjusted our repeated measures analyses for blood glucose. Hydration and salt intake could have altered renal oxygenation [49,50] and we tried to account for this with overnight fasting before the study visits and with standardised food and water intake during study the visits. Potential limitations of the crossover design are the risks of carry over effects and period effects. To avoid carry over, we incorporated a long washout period of two weeks, corresponding to 19 half-lives of dapagliflozin. In addition, the repeated measures analyses were adjusted for the randomisation-sequence. We observed a period effect on  $R_2^*$  showing an increase over time in the medulla. This observation emphasises the need for adjusting analyses for a time-variable as we have done. The primary strength of our study design is that effects of both dapagliflozin and placebo could be evaluated in the same participant, allowing comparison at the individual-level instead of the group-level. Thus, confounding was limited [51]. Another strength is that the study was performed under daily living conditions with participants sitting and eating between scans rather than under fixed experimental conditions e.g. with patients lying supine.

In conclusion, a high dose of dapagliflozin improved renal  $R_2^*$  within six hours and with no indication of changed renal blood or oxygen supply. This suggests an improved renal cortical oxygenation due to a reduced tubular transport workload in the proximal tubules. Such improved oxygenation may contribute to the long-term renal beneficial effects of SGLT2 inhibitors, but it remains to be determined whether the observed effects are sustained with chronic treatment and if they occur in type 2 diabetes as well.

### Declaration of Competing Interest

P.R. has received honoraria to Steno Diabetes center Copenhagen for consultancy from AstraZeneca, Astellas, Bayer, Boehringer Ingelheim, Gilead, Novo Nordisk, Merck, Mundipharma, Sanofi, Vifor, and research support from Astra Zeneca and Novo Nordisk.

P-H.G. has received lecture honoraria from Astellas, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Elo Water, Genzyme, Medscape, MSD, Mundipharma, Novartis, Novo Nordisk, PeerVoice, Sanofi and Sciar, and is an advisory board member of AbbVie, Astellas, AstraZeneca, Bayer, Boehringer Ingelheim, Eli Lilly, Medscape, MSD, Mundipharma, Novo Nordisk and Sanofi. N.S-H. reports having stock equity

in Novo Nordisk A/S and Akcea Therapeutics Inc. C.S.H. has received lecture honoraria to Steno Diabetes center Copenhagen from Novo Nordisk.

J.C.L., J.M.L.M., B.H., I.K.B.R., F.S., J.S., H.B.W.L., M.F-M., and U.B.A. declare no competing interests.

### Acknowledgments

We thank all study participants and the laboratory technicians Maja Lis Dybdahl Halkjaer, Tina Nielsen, Dorthe Riis, Tina Ragnholm Juhl and Jessie Armand Hermann from Steno Diabetes centre Copenhagen. Also thank you for the valued contribution from research assistants Mathilde Overgaard Lauen, Gidega Vijayakumar, Linnéa Haugen and Büsra Köylü from Rigshospitalet Glostrup.

### Funding

The study was funded by the Novo Nordisk Foundation grant PRO-TON personalising treatment of diabetic nephropathy (NNF140C0013659).

### Data sharing

Individual, de-identified participant data are not freely available because of the risk of patient re-identification, but interested parties can request access to de-identified participant data or anonymised clinical study reports through submission of a request for access to the corresponding author, provided that the necessary data protection agency and ethical committee approvals are provided in compliance with relevant legislation.

### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.eclinm.2021.100895.

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