

# The renal hemodynamic effects of the SGLT2 inhibitor dapagliflozin are caused by post-glomerular vasodilatation rather than pre-glomerular vasoconstriction in metformin-treated patients with type 2 diabetes in the randomized, double-blind RED trial

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**Sodium-glucose cotransporter 2 inhibitors (SGLT2i) improve hard renal outcomes in type 2 diabetes. This is possibly explained by the fact that SGLT2i normalize the measured glomerular filtration rate (mGFR) by increasing renal vascular resistance, as was shown in young people with type 1 diabetes and glomerular hyperfiltration. Therefore, we compared the renal hemodynamic effects of dapagliflozin with gliclazide in type 2 diabetes. The mGFR and effective renal plasma flow were assessed using inulin and para-aminohippurate clearances in the fasted state, during clamped euglycemia (5 mmol/L) and during clamped hyperglycemia (15 mmol/L). Filtration fraction and renal vascular resistance were calculated. Additionally, factors known to modulate renal hemodynamics were measured. In 44 people with type 2 diabetes on metformin monotherapy (Hemoglobin A1c 7.4%, mGFR 113 mL/min), dapagliflozin versus gliclazide reduced mGFR by 5, 10, and 12 mL/min in the consecutive phases while both agents similarly improved Hemoglobin A1c (-0.48% vs -0.65%). Dapagliflozin also reduced filtration fraction without increasing renal vascular resistance, and increased urinary adenosine and**

**prostaglandin concentrations. Gliclazide did not consistently alter renal hemodynamic parameters. Thus, beyond glucose control, SGLT2i reduce mGFR and filtration fraction in type 2 diabetes. The fact that renal vascular resistance was not increased by dapagliflozin suggests that this is due to post-glomerular vasodilation rather than pre-glomerular vasoconstriction.**

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**D** iabetic kidney disease (DKD) is the leading cause of end-stage kidney disease and predominantly accounts for the increased risk of cardiovascular disease and death in people with type 2 diabetes (T2D).<sup>1</sup> Despite multifactorial risk management focusing on lifestyle factors, hyperglycemia, and hypertension (with blockers of the renin-angiotensin system [RAS]), residual risk remains high,<sup>2</sup> indicating an unmet medical need.

In this light, sodium-glucose cotransporter 2 inhibitors (SGLT2is), a drug class developed to improve glycemic control, have gained much attention since their introduction in 2012. SGLT2is lower blood glucose levels by inhibiting glucose reuptake in the proximal tubule, thereby inducing urinary glucose excretion.<sup>3</sup> SGLT2is also induce a transient increase in urinary sodium excretion.<sup>4</sup>

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In the cardiovascular safety trials with empagliflozin, canagliflozin, and dapagliflozin, all these drugs improved cardiovascular outcome in people with T2D without apparent DKD but with established atherosclerotic cardiovascular disease, while renal outcomes were improved in people with *and* without cardiovascular disease.<sup>5–8</sup> In the Dapagliflozin Effect on Cardiovascular Events–Thrombolysis in Myocardial Infarction 58 (DECLARE-TIMI 58), the largest cardiovascular safety trial that included patients with T2D with (40%) and without (60%) atherosclerotic cardiovascular disease at baseline, dapagliflozin reduced the occurrence of the secondary composite renal end point (40% estimated glomerular filtration rate [eGFR] reduction to <60 ml/min per 1.73 m<sup>2</sup>, new end-stage kidney disease, and renal death) by 47% compared with placebo.<sup>7</sup> In the first dedicated trial studying people with T2D and established DKD, canagliflozin compared with placebo reduced the renal composite end point consisting of end-stage kidney disease, doubling of serum creatinine or renal death by 34%,<sup>9</sup> showing that SGLT2is consistently improve renal outcomes across different stages of renal function.

The mechanisms by which SGLT2is improve renal outcomes are not well understood. However, it is generally accepted that the drug-induced benefits in plasma glucose concentrations, blood pressure, body weight, and uric acid levels cannot fully explain the observed renal benefits.<sup>3,10</sup> Clinically, SGLT2is cause an acute decrease in eGFR of 2 to 5 ml/min per 1.73 m<sup>2</sup>, which remains stable and is reversible after cessation of therapy. This suggests that SGLT2is have direct renal hemodynamic actions. Based on studies in a rodent model of type 1 diabetes (T1D) as well as a mechanistic study in fairly young people with T1D and whole-kidney hyperfiltration (defined as measured glomerular filtration rate [mGFR] > 135 ml/min per 1.73 m<sup>2</sup>), it was postulated that SGLT2i-induced proximal natriuresis activates tubuloglomerular feedback (TGF), leading to preglomerular vasoconstriction, via macula densa–derived adenosine. This induces preglomerular vasoconstriction, increases renal vascular resistance (RVR), and reduces hyperfiltration, which could preserve long-term renal function.<sup>11–14</sup>

To date, it is unclear how SGLT2is affect renal hemodynamics in people with T2D whose renal physiology markedly differs from that of the previously studied people with hyperfiltrating T1D, partly because of concomitant RAS blockade. It is also unknown whether the effects of SGLT2is are independent of glucose lowering. Therefore, we studied the effects of dapagliflozin versus gliclazide on renal hemodynamics in people with T2D, a population that is relevant with respect to available data from the cardiovascular safety trials. We hypothesized that SGLT2is, beyond glucose control, reduce mGFR and effective renal plasma flow (ERPF) in people with T2D by increasing RVR via TGF activation.

## RESULTS

Between February 2016 and March 2018, 75 people were screened, of whom 50 were included and 44 were randomized

to a 12-week treatment with dapagliflozin (n = 24) or gliclazide (n = 20) (Supplementary Figure S1). Four included people withdrew consent before testing because of personal reasons and 1 person was unable to participate because of iodine allergy when iohexol had to be used instead of inulin. One patient was excluded after baseline testing, but before randomization, because of urinary retention. No participants dropped out after randomization, and overall adherence to study medication was 99%. In the dapagliflozin group, we missed 1 measurement of GFR and ERPF in the fasting phase and during hyperglycemia. The data set was complete during euglycemia and in all states in the gliclazide group. Analyses were performed without these missing data.

At baseline, demographic and clinical characteristics, as well as renal risk factors, were generally well balanced between the treatment groups (Table 1). Most participants received other medication in addition to metformin, most commonly RAS inhibitors (73%) and statins (68%); medication remained unchanged during the treatment period.

## Glycemic control

There was no significant difference in glucose lowering between the treatment arms. Compared with dapagliflozin, 12 weeks of gliclazide reduced glycated hemoglobin nonsignificantly by 0.2% (95% confidence interval [CI], –0.1% to 0.4%; *P* = 0.12) and fasting plasma glucose by 0.4 mmol/l (95% CI, –0.3 to 1.1 mmol/l; *P* = 0.23). Both treatments significantly improved glycemic control (Table 2).

**Table 1 | Baseline characteristics**

Characteristic	Dapagliflozin (n = 24)	Gliclazide (n = 20)
Age (yr)	63 ± 7	63 ± 7
Male sex	19 (79)	15 (75)
Education level		
Higher education	4 (17)	8 (40)
Vocational education	11 (45)	5 (25)
Secondary education	9 (38)	7 (35)
Diabetes duration (yr)	9.8 ± 4.1	10.7 ± 7.3
Current smoker	3 (13)	1 (5)
Alcohol intake (units/wk)	5 (2–13)	4 (2–8)
ASCVD	4 (17)	1 (5)
Hypertension	16 (67)	16 (80)
eGFR (CKD-EPI) (ml/min per 1.73 m <sup>2</sup> )	85 ± 13	89 ± 19
UACR (mg/mmol)	11 (6–17)	12 (4–17)
Medication use		
Platelet aggregation inhibitor	4 (17)	2 (10)
Metformin dose (mg)	1556 ± 736	1585 ± 765
Statin	16 (67)	14 (70)
β-blocker	6 (25)	3 (15)
Calcium antagonist	6 (25)	6 (30)
RAS inhibitor	16 (67)	16 (80)
ACE inhibitor	5 (21)	5 (25)
ARB	11 (46)	11 (55)

ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; ASCVD, atherosclerotic cardiovascular disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; RAS, renin-angiotensin system; UACR, urinary albumin-to-creatinine ratio. Data are expressed as mean ± SD, median (interquartile range), or n (%).

**Table 2 | General measurements performed before the clamps commenced**

Variable	Dapagliflozin (n = 24)			Gliclazide (n = 20)			Baseline corrected mean difference between dapagliflozin and gliclazide treatment (95% CI) and P value	
	Week 0	Week 12	Within group	Week 0	Week 12	Within group		
Body weight (kg)	96.6 ± 17.9	93.7 ± 16.9	<b>P &lt; 0.001</b>	98.5 ± 17.9	99.6 ± 18.3	<b>P = 0.001</b>	−4.03 (−2.81 to −5.24)	<b>P &lt; 0.001</b>
Body mass index (kg/m <sup>2</sup> )	30.8 ± 3.9	29.8 ± 3.7	<b>P &lt; 0.001</b>	31.6 ± 3.9	31.9 ± 4.1	<b>P = 0.001</b>	−1.30 (−0.93 to −1.67)	<b>P &lt; 0.001</b>
Systolic blood pressure (mm Hg)	137.7 ± 13.6	129.2 ± 10.7	<b>P = 0.001</b>	131.6 ± 11.4	131.1 ± 11.8	<i>P = 0.83</i>	−5.3 (−10.9 to 0.2)	<i>P = 0.06</i>
Diastolic blood pressure (mm Hg)	84.4 ± 5.7	80.4 ± 5.6	<b>P &lt; 0.001</b>	81.7 ± 5.4	80.9 ± 6.5	<i>P = 0.51</i>	−2.6 (−5.3 to 0.2)	<i>P = 0.06</i>
Heart rate (beats/min)	65.0 ± 9.9	63.2 ± 7.9	<i>P = 0.27</i>	69.4 ± 11.6	69.2 ± 11.3	<i>P = 0.90</i>	−3.1 (−7.2 to 1.0)	<i>P = 0.14</i>
Hematocrit (%)	40.7 ± 3.3	42.5 ± 2.9	<b>P &lt; 0.001</b>	40.2 ± 2.5	40.2 ± 2.8	<i>P = 0.89</i>	1.8 (0.9–2.7)	<b>P &lt; 0.001</b>
Erythropoietin (IU/l)	11.4 ± 4.2	13.7 ± 6.1	<b>P = 0.01</b>	11.3 ± 4.1	12.1 ± 3.8	<i>P = 0.42</i>	1.5 (−1.0 to 4.0)	<i>P = 0.24</i>
Albumin (g/l)	38.0 ± 2.5	38.3 ± 1.7	<i>P = 0.28</i>	38.0 ± 2.9	37.9 ± 2.7	<i>P = 0.80</i>	0.4 (−0.4 to 1.2)	<i>P = 0.31</i>
HbA1c (%)	7.39 ± 0.66	6.92 ± 0.56	<b>P &lt; 0.001</b>	7.36 ± 0.60	6.71 ± 0.49	<b>P &lt; 0.001</b>	0.19 (−0.05 to 0.43)	<i>P = 0.12</i>
Fasting plasma glucose (mg/dl)	9.2 ± 1.5	8.2 ± 1.5	<b>P &lt; 0.001</b>	8.8 ± 1.6	7.5 ± 1.1	<b>P = 0.001</b>	0.4 (−0.3 to 1.1)	<i>P = 0.23</i>
Fasting insulin (pmol/l)	72.7 ± 58.3	54.3 ± 31.2	<b>P &lt; 0.05</b>	55.5 ± 19.6	58.2 ± 23.1	<i>P = 0.45</i>	−12.8 (−3.3 to −22.3)	<b>P = 0.01</b>
Urinary sodium (mmol/24 h)	178 ± 52	188 ± 63	<i>P = 0.55</i>	178 ± 75	186 ± 67	<i>P = 0.62</i>	1 (−37 to 40)	<i>P = 0.95</i>
Urinary glucose (mmol/24 h) <sup>a</sup>	7.3 (0.7–45.2)	462 (27–712)	<b>P &lt; 0.001</b>	2.1 (1.1–17.0)	3.1 (0.9–14.2)	<i>P = 0.36</i>	488 (365–612) <sup>b</sup>	<b>P &lt; 0.001</b>
Urinary albumin (mg/24 h) <sup>a</sup>	11 (6.3–17.0)	11.5 (4.4–23.3)	<i>P = 0.73</i>	11.7 (4.2–17.0)	7.5 (3.4–19.8)	<i>P = 0.24</i>	3.8 (−9.9 to 17.5) <sup>b</sup>	<i>P = 0.59</i>

CI, confidence interval; HbA1c, glycated hemoglobin.

Multivariable linear regression models were used to examine week 0–corrected dapagliflozin- compared with gliclazide-induced effects. Paired *t* tests or Wilcoxon signed rank tests were used for within-group comparisons. Data are expressed as mean ± SD or median (interquartile range). Significant differences are indicated in boldface.

<sup>a</sup>Analysis performed after log transformation.

<sup>b</sup>Mean difference calculated before log transformation.

### Renal hemodynamics

Compared with gliclazide, dapagliflozin reduced mGFR by 5 ml/min (95% CI, −12 to 1 ml/min; *P* = 0.11) in the fasting phase, by 10 ml/min (95% CI, −21 to 1 ml/min; *P* = 0.06) during euglycemia, and by 12 ml/min (95% CI, −20 to −4; *P* < 0.01) during hyperglycemia. Dapagliflozin versus gliclazide reduced ERPF in all 3 conditions by 17 ml/min (95% CI, −58 to 23 ml/min; *P* = 0.39), 52 ml/min (95% CI, −93 to −11 ml/min; *P* = 0.01), and 68 ml/min (95% CI, −110 to −24 ml/min; *P* < 0.01), respectively (Table 3 and Figure 1). This resulted in reduced filtration fraction (FF) in all 3 phases in the dapagliflozin group without increasing RVR; RVR was even reduced in the fasting phase (Table 3). The effect of dapagliflozin was similar in participants who were treated with RAS blockade compared with those without RAS blockade (Supplementary Table S1). Gliclazide did not consistently affect renal hemodynamics.

### Estimated intrarenal hemodynamics

Dapagliflozin consistently and significantly reduced intraglomerular pressure as well as postglomerular arteriolar resistance in all 3 phases (Table 4). Preglomerular arteriolar resistance was significantly reduced by dapagliflozin during fasting. Gliclazide significantly reduced intraglomerular pressure during fasting and postglomerular arteriolar resistance during hyperglycemia.

### Urinary excretion of glucose, sodium, and albumin

Twenty-four-hour glucose excretion increased from 7.3 to 462 mmol in the dapagliflozin group, while it was unaffected

by gliclazide. Urinary excretion of sodium and albumin was unaffected (Table 2).

### RAS system and other mediators of renal hemodynamics

Dapagliflozin increased renin levels significantly, while they were not affected by gliclazide (Table 5). Dapagliflozin augmented the ratios of urinary adenosine, 6-keto-prostaglandin F-1 $\alpha$ , and prostaglandin E2 to urinary creatinine (Table 5). None of these ratios were altered by gliclazide. Urinary excretion of 8-oxo-7,8-dihydro-2'-deoxyguanosine was reduced by both agents. Urinary excretion of endothelin-1, thromboxane B2, and 8-oxo-7,8-dihydro-guanosine was unaffected by either treatment (Table 5). None of the observed changes were consistently associated with changes in mGFR (data not shown).

### Anthropometrics, systemic hemodynamics, and hematocrit

Dapagliflozin decreased body weight by 2.8 ± 2.4 kg, while gliclazide increased body weight by 1.1 ± 1.3 kg (Table 2). Dapagliflozin reduced systolic blood pressure by 8.5 ± 11.4 mm Hg and diastolic blood pressure by 4.0 ± 4.0 mm Hg, while heart rate was not changed. Gliclazide did not affect any of these parameters (Table 2). Hematocrit increased by 1.8% ± 1.5% after dapagliflozin treatment. The increase in hematocrit with dapagliflozin was paralleled by an increase in erythropoietin, while serum albumin was not altered by either treatment (Table 2). There were no significant associations between changes in mGFR and changes in mean arterial pressure, body weight, or hematocrit (data not shown).

**Table 3 | Directly measured and calculated measures of renal hemodynamics during the 3 phases of the protocol**

Variable	Dapagliflozin (n = 24)			Gliclazide (n = 20)			Baseline corrected mean difference between dapagliflozin and gliclazide treatment (95% CI) and P value	
	Week 0	Week 12	Within group	Week 0	Week 12	Within group		
mGFR (ml/min)								
Fasting	113 ± 20	104 ± 17	<b>P &lt; 0.05</b>	113 ± 19	109 ± 20	P = 0.12	-5 (-12 to 1)	P = 0.11
Euglycemia	110 ± 27	101 ± 30	<b>P = 0.01</b>	112 ± 25	113 ± 26	P = 0.85	-10 (-21 to 1)	P = 0.06
Hyperglycemia	105 ± 29	93 ± 29	<b>P &lt; 0.001</b>	108 ± 16	106 ± 18	P = 0.62	-12 (-20 to -4)	<b>P &lt; 0.01</b>
ERPF (ml/min)								
Fasting	654 ± 153	639 ± 141	P = 0.12	692 ± 120	678 ± 122	P = 0.31	-17 (-58 to 23)	P = 0.39
Euglycemia	503 ± 124	477 ± 121	P = 0.09	509 ± 107	534 ± 106	P = 0.12	-52 (-93 to -11)	<b>P = 0.01</b>
Hyperglycemia	488 ± 130	460 ± 136	<b>P &lt; 0.05</b>	487 ± 99	525 ± 92	<b>P &lt; 0.05</b>	-68 (-110 to -24)	<b>P &lt; 0.01</b>
FF (%)								
Fasting	17.8 ± 2.9	17.0 ± 2.3	P = 0.08	16.4 ± 1.8	16.3 ± 2.2	P = 0.67	-0.2 (-1.2 to 0.9)	P = 0.77
Euglycemia	22.0 ± 3.0	20.9 ± 3.2	<b>P &lt; 0.05</b>	22.1 ± 2.6	21.4 ± 2.9	P = 0.23	-0.5 (-1.8 to 0.9)	P = 0.48
Hyperglycemia	21.8 ± 2.8	20.2 ± 3.1	<b>P &lt; 0.05</b>	22.5 ± 2.5	21.0 ± 2.6	<b>P &lt; 0.01</b>	-0.4 (-1.9 to 1.0)	P = 0.57
Hematocrit (%)								
Fasting	40.7 ± 3.3	42.5 ± 2.9	<b>P &lt; 0.001</b>	40.2 ± 0.025	40.2 ± 2.8	P = 0.89	1.8 (0.9-2.7)	<b>P &lt; 0.001</b>
Euglycemia	41.1 ± 3.4	42.3 ± 3.2	<b>P &lt; 0.01</b>	40.4 ± 2.8	40.5 ± 3.1	P = 0.82	1.6 (0.2-2.4)	<b>P &lt; 0.05</b>
Hyperglycemia	40.1 ± 3.3	41.3 ± 3.2	<b>P = 0.001</b>	38.9 ± 3.3	38.7 ± 3.1	P = 0.65	1.5 (0.6-2.5)	<b>P = 0.001</b>
RBF (ml/min)								
Fasting	1064 ± 362	1108 ± 272	P = 0.46	1162 ± 227	1140 ± 232	P = 0.37	24 (-90 to 138)	P = 0.67
Euglycemia	859 ± 232	835 ± 273	P = 0.37	858 ± 202	903 ± 208	P = 0.11	-69 (-145 to 6)	P = 0.07
Hyperglycemia	824 ± 235	795 ± 255	P = 0.21	802 ± 183	868 ± 166	<b>P &lt; 0.05</b>	-92 (-165 to -18)	<b>P &lt; 0.05</b>
MAP (mm Hg)								
Fasting	102.2 ± 6.7	96.7 ± 6.5	<b>P &lt; 0.001</b>	98.3 ± 6.6	97.6 ± 7.4	P = 0.64	-3.5 (-7.0 to 0.0)	<b>P = 0.05</b>
Euglycemia	107.9 ± 9.1	102.3 ± 7.3	<b>P &lt; 0.01</b>	100.4 ± 7.0	101 ± 6.8	P = 0.66	-2.1 (-6.2 to 2.1)	P = 0.33
Hyperglycemia	107.1 ± 8.9	100.6 ± 8.5	<b>P &lt; 0.001</b>	99.4 ± 8.6	101.4 ± 6.2	P = 0.26	-4.7 (-8.9 to -0.5)	<b>P &lt; 0.05</b>
RVR (mm Hg/l/min)								
Fasting	99 ± 29	93 ± 24	<b>P &lt; 0.05</b>	88 ± 18	89 ± 21	P = 0.41	-5 (-12 to 1)	P = 0.11
Euglycemia	135 ± 39	133 ± 42	P = 0.68	122 ± 26	118 ± 28	P = 0.30	4 (-9 to 17)	P = 0.53
Hyperglycemia	142 ± 48	140 ± 50	P = 0.65	129 ± 28	121 ± 23	P = 0.08	8 (-4 to 20)	P = 0.16

CI, confidence interval; ERPF, effective renal plasma flow; FF, filtration fraction; MAP, mean arterial pressure; mGFR, measured glomerular filtration rate; RBF, renal blood flow; RVR, renal vascular resistance.

Multivariable linear regression models were used to examine week 0-corrected dapagliflozin- compared with gliclazide-induced effects. Paired t tests or Wilcoxon signed rank tests were used for within-group comparisons. Data are expressed as mean ± SD. Significant differences are indicated in boldface.

### Adverse events

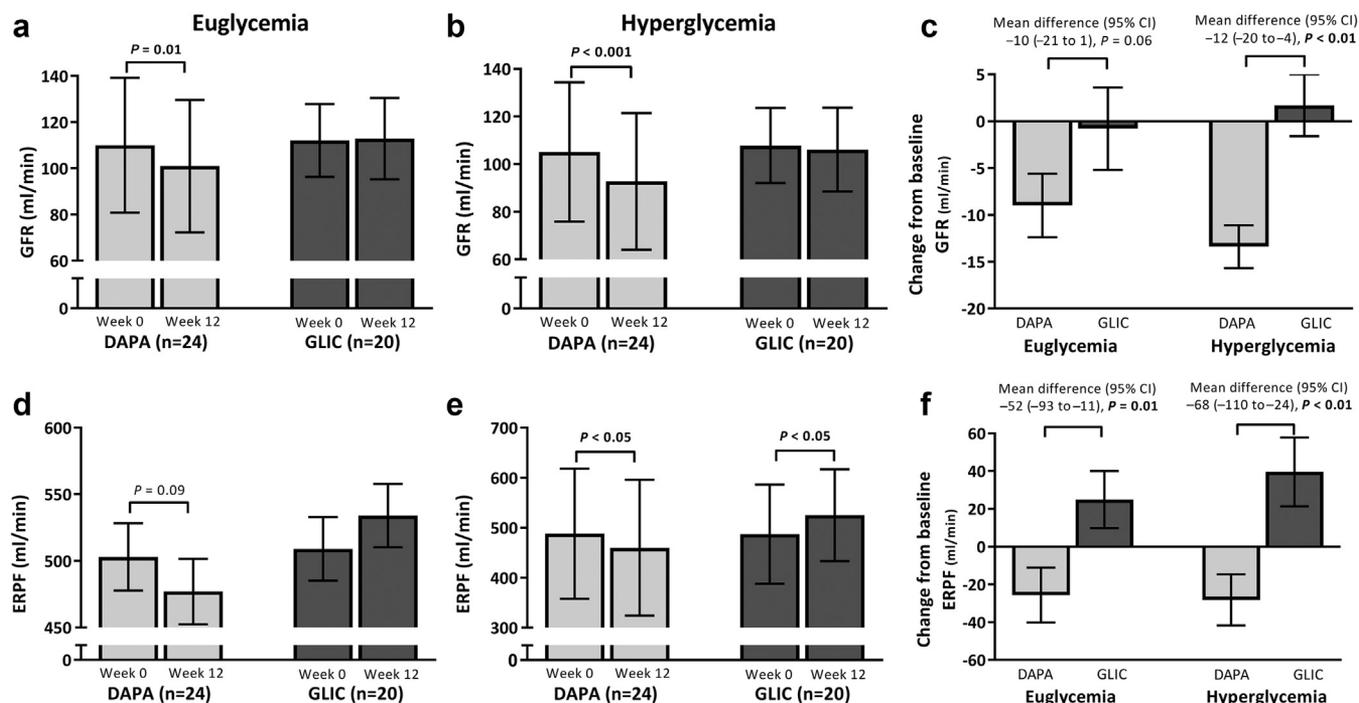
Both treatments were generally well tolerated; there were no serious adverse events, and no participants dropped out after treatment commenced. No hypoglycemic events occurred, and there were 5 genital fungal infections in the dapagliflozin group versus none in the gliclazide group (Table 6; Supplementary Figure S1).

### DISCUSSION

The Renoprotective Effects of Dapagliflozin in Type 2 Diabetes trial is the first study to investigate the renal hemodynamic effects of SGLT2is in people with T2D predominantly receiving RAS blockade, a relevant population given the included people in the cardiovascular safety trials. In contrast to the prevailing view that SGLT2is lower GFR by increased preglomerular arteriolar resistance, we observed that lowering of mGFR was accompanied by a stable or even lowered RVR, suggesting that postglomerular vasodilation explains the acute eGFR decline. By using an active comparator design and by measuring the effects at controlled glucose levels, we were able to demonstrate that the renal hemodynamic effects of SGLT2is are fully independent of their glucose-lowering effects.

Glomerular hyperfiltration is a recognized risk factor for the development and progression of DKD.<sup>15</sup> Although hyperfiltration is defined by elevated GFR in people with early T1D, this condition is more difficult to diagnose in people with T2D, who may maintain a normal or even have a reduced GFR because of functional nephron loss, but can still hyperfiltrate at the single nephron level. It has been proposed that increased FF (mGFR divided by ERPF) is useful to assess hyperfiltration at the single nephron level.<sup>15</sup> Evidence for the importance of (single nephron) hyperfiltration as a risk factor for progression of DKD is derived from pharmacological interventions that modulate renal hemodynamics. As an example, RAS blockers have been described to improve DKD progression by lowering glomerular pressure and hyperfiltration through postglomerular vasodilation. Nevertheless, evidence for the effect of RAS blockers on arteriolar tone in people with T2D is scant.<sup>16</sup> SGLT2is improve renal outcomes in patients with T2D while inducing an early GFR decline, denoting altered renal hemodynamics.<sup>5-7,9</sup> This suggests that the renal hemodynamic effect may contribute to renoprotection in T2D.

Other effects of SGLT2is have also been hypothesized to explain improved renal outcome, including improved glucose



**Figure 1 | Renal hemodynamic responses during clamped euglycemia and hyperglycemia.** Glomerular filtration rate (GFR) during (a) euglycemia and (b) hyperglycemia and (c) the difference between baseline and after treatment in both conditions. Effective renal plasma flow (ERPF) during (d) euglycemia and (e) hyperglycemia and (f) the difference between baseline and after treatment in both conditions. Paired *t* tests were used for within-group comparisons. Multivariable linear regression models were used to examine week 0–corrected dapagliflozin (DAPA)– compared with gliclazide (GLIC)–induced effects. See Table 3 for all renal hemodynamic measurements/calculations. Data are mean  $\pm$  SD. Significant differences are indicated in boldface.

metabolism, weight reduction, reduced blood pressure and arterial stiffness, amelioration of renal hypoxia, less cardiovascular events, a reduction in urinary albumin excretion, and the inhibition of proinflammatory and profibrotic pathways.<sup>17</sup> Furthermore, recent murine data indicate that SGLT2is, like caloric restriction, directly reverse detrimental hyperglycemia-induced metabolic shifts in the renal cortex,

preventing the accumulation of tricarboxylic acid cycle intermediates and oxidative stress. This was accompanied by a reduction in albuminuria, glomerular hyperfiltration, and mesangial expansion.<sup>18</sup>

The regulation of GFR is a complex process that is influenced by an interplay of metabolic and vasoactive factors and TGF.<sup>15</sup> In people with diabetes, SGLT2 expression and activity

**Table 4 | Estimated intraglomerular hemodynamics using the Gomez equations**

Variable	Dapagliflozin (n = 24)			Gliclazide (n = 20)			Baseline corrected mean difference between dapagliflozin and gliclazide treatment (95% CI) and <i>P</i> value	
	Week 0	Week 12	Within group	Week 0	Week 12	Within group		
<b>Intraglomerular pressure (mm Hg)</b>								
Fasting	60.6 $\pm$ 4.3	59.1 $\pm$ 2.8	<b><i>P</i> &lt; 0.05</b>	60.1 $\pm$ 6.1	59.1 $\pm$ 5.6	<b><i>P</i> &lt; 0.05</b>	-0.3 (-1.7 to 1.0)	<i>P</i> = 0.62
Euglycemia	59.3 $\pm$ 4.9	57.3 $\pm$ 5.8	<b><i>P</i> &lt; 0.05</b>	60.0 $\pm$ 7.3	59.2 $\pm$ 5.9	<i>P</i> = 0.49	-1.4 (-4.0 to 1.1)	<i>P</i> = 0.27
Hyperglycemia	57.3 $\pm$ 4.7	54.3 $\pm$ 5.3	<b><i>P</i> &lt; 0.001</b>	58.1 $\pm$ 4.2	56.8 $\pm$ 4.5	<i>P</i> = 0.11	-1.9 (-3.9 to 0.2)	<i>P</i> = 0.07
<b>Preglomerular arteriolar resistance ((dyn·s)/cm<sup>5</sup>)</b>								
Fasting	3925 $\pm$ 1197	3497 $\pm$ 911	<b><i>P</i> &lt; 0.01</b>	3453 $\pm$ 1171	3584 $\pm$ 1202	<i>P</i> = 0.37	-434 (-811 to -57)	<b><i>P</i> &lt; 0.05</b>
Euglycemia	6056 $\pm$ 2063	5777 $\pm$ 2209	<i>P</i> = 0.37	5034 $\pm$ 1777	4926 $\pm$ 1534	<i>P</i> = 0.75	130 (-746 to 1006)	<i>P</i> = 0.77
Hyperglycemia	6499 $\pm$ 2324	6215 $\pm$ 2184	<i>P</i> = 0.30	5358 $\pm$ 1386	5353 $\pm$ 1271	<i>P</i> = 0.99	51 (-721 to 824)	<i>P</i> = 0.89
<b>Postglomerular arteriolar resistance ((dyn·s)/cm<sup>5</sup>)</b>								
Fasting	2626 $\pm$ 550	2398 $\pm$ 418	<b><i>P</i> &lt; 0.01</b>	3454 $\pm$ 1171	3584 $\pm$ 1202	<i>P</i> = 0.37	-130 (-308 to 48)	<i>P</i> = 0.15
Euglycemia	3331 $\pm$ 635	3078 $\pm$ 460	<b><i>P</i> &lt; 0.05</b>	3345 $\pm$ 312	3210 $\pm$ 521	<i>P</i> = 0.20	-125 (-379 to 129)	<i>P</i> = 0.33
Hyperglycemia	3311 $\pm$ 432	3005 $\pm$ 587	<b><i>P</i> &lt; 0.01</b>	3548 $\pm$ 698	3127 $\pm$ 467	<b><i>P</i> &lt; 0.01</b>	1 (-284 to 286)	<i>P</i> = 0.99

CI, confidence interval.

Multivariable linear regression models were used to examine week 0–corrected dapagliflozin– compared with gliclazide–induced effects. Paired *t* tests were used for within-group comparisons. Data are expressed as mean  $\pm$  SD. Significant differences are indicated in boldface.

**Table 5 | Potential mediators of renal hemodynamics**

Variable	Dapagliflozin (n = 24)			Gliclazide (n = 20)			Baseline corrected mean difference between dapagliflozin and gliclazide treatment (95% CI) and P value	
	Week 0	Week 12	Within group	Week 0	Week 12	Within group		
Plasma renin <sup>a</sup>								
Fasting (pg/ml)	9.1 (4.8–16.5)	15.2 (5.8–20.2)	<b>P &lt; 0.05</b>	12.5 (6.3–16.2)	9.6 (6.0–22.2)	P = 0.51	5.7 (–1.7 to 13.1) <sup>b</sup>	P = 0.22
Euglycemia (pg/ml)	10.0 (4.6–16.9)	16.2 (8.2–21.4)	<b>P &lt; 0.05</b>	12.3 (6.9–22.6)	11.7 (6.7–25.3)	P = 0.92	4.2 (–0.4 to 8.8) <sup>b</sup>	<b>P &lt; 0.05</b>
Hyperglycemia (pg/ml)	8.2 (4.1–12.2)	11.6 (7.6–17.2)	<b>P &lt; 0.05</b>	10.4 (6.3–15.3)	10.9 (6.9–18.5)	P = 0.61	3.1 (–1.3 to 7.3) <sup>b</sup>	<b>P &lt; 0.05</b>
Fasting urinary measurements (creatinine corrected)								
Adenosine (μmol/mmol)	0.30 ± 0.11	0.36 ± 0.12	<b>P = 0.001</b>	0.37 ± 0.17	0.38 ± 0.21	P = 0.39	0.05 (–0.01 to 0.10)	P = 0.08
Endothelin-1 (pg/kmol)	8.8 (5.8–20.2)	25.8 (6.6–42.3)	P = 0.28	8.1 (5.0–31.8)	7.5 (5.7–31.8)	P = 0.88	7.7 (–4.0 to 19.4)	P = 0.19
Thromboxane B2 (pg/mmol)	0.112 ± 0.056	0.115 ± 0.072	P = 0.74	0.107 ± 0.056	0.114 ± 0.045	P = 0.41	–0.002 (–0.028 to 0.023)	P = 0.84
8-oxoGuo (nmol/mmol)	2.24 ± 0.14	2.21 ± 0.19	P = 0.86	2.10 ± 0.51	1.97 ± 0.48	P = 0.15	0.13 (–0.19 to 0.46)	P = 0.40
8-oxodG (nmol/mmol)	1.68 ± 0.49	1.46 ± 0.49	<b>P = 0.001</b>	1.65 ± 0.67	1.27 ± 0.44	<b>P = 0.001</b>	0.16 (–0.02 to 0.34)	P = 0.07
6kPGF <sub>1α</sub> (pg/mmol) <sup>a</sup>	0.084 (0.058–0.163)	0.105 (0.086–0.220)	<b>P &lt; 0.001</b>	0.088 (0.068–0.129)	0.099 (0.060–0.141)	P = 0.99	0.048 (0.006–0.090) <sup>b</sup>	<b>P &lt; 0.01</b>
PGE2 (pg/mmol) <sup>a</sup>	0.023 (0.017–0.038)	0.027 (0.020–0.037)	<b>P &lt; 0.05</b>	0.027 (0.018–0.037)	0.028 (0.019–0.038)	P = 0.20	0.011 (–0.12 to 0.034) <sup>b</sup>	P = 0.41
PGEM (pg/mmol)	0.041 ± 0.027	0.059 ± 0.046	P = 0.08	0.041 ± 0.022	0.042 ± 0.029	P = 0.75	0.016 (–0.006 to 0.039)	P = 0.15

CI, confidence interval; 6KPGF<sub>1α</sub>, 6-keto-prostaglandin F1-α; 8-oxoGuo, 8-oxo-7,8-dihydroguanosine; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; PGE2, prostaglandin E2; PGEM, prostaglandin E metabolite.

Multivariable linear regression models were used to examine week 0–corrected dapagliflozin- compared with gliclazide-induced effects. Paired *t* tests or Wilcoxon signed rank tests were used for within-group comparisons. Data are expressed as mean ± SD or median (interquartile range). Significant differences are indicated in boldface.

<sup>a</sup>Analysis performed after log transformation.

<sup>b</sup>Mean difference calculated before log transformation.

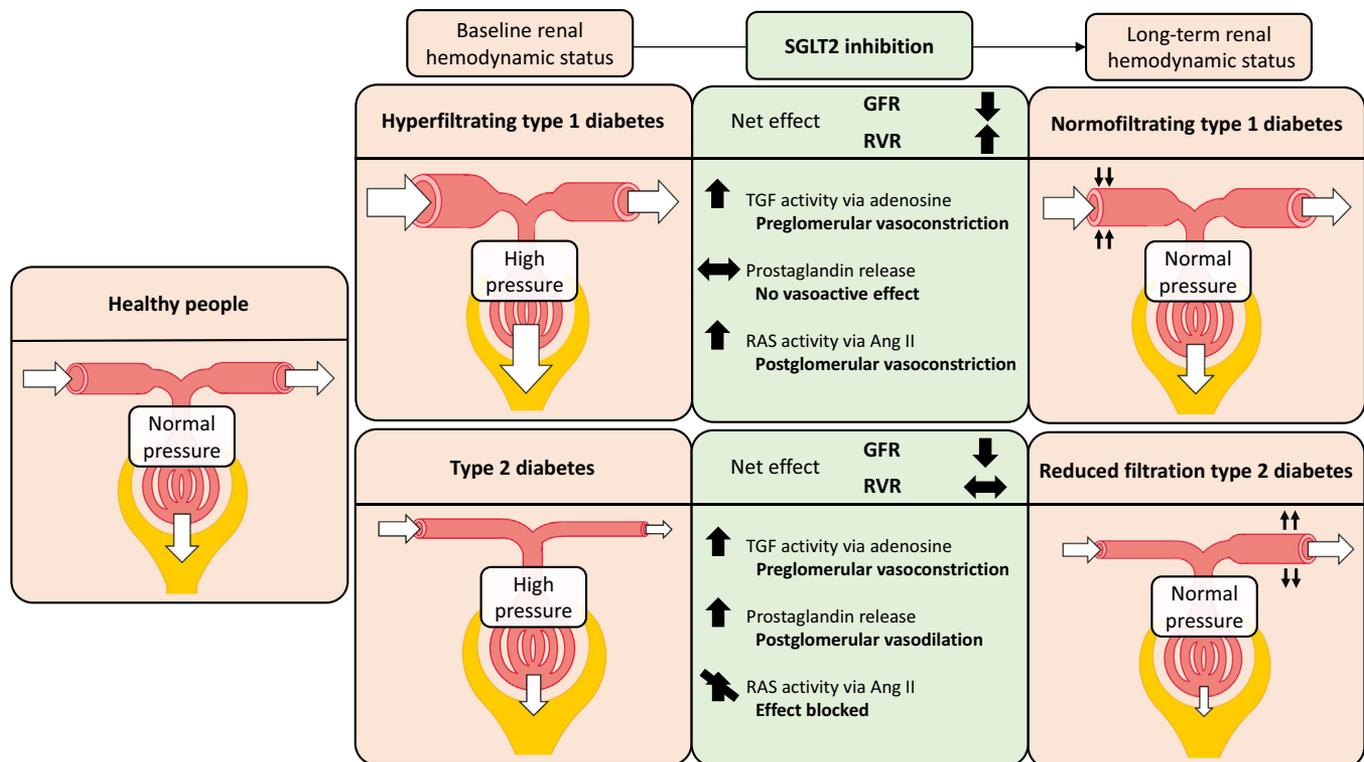
are increased because of chronic hyperglycemia,<sup>19</sup> resulting in augmented proximal sodium reabsorption and decreased sodium (and chloride) delivery to the macula densa. This blunts TGF, leads to dilation of the preglomerular arteriole, and causes glomerular hyperfiltration. In young and otherwise healthy people with T1D receiving only insulin treatment and whole-kidney hyperfiltration (mGFR > 135 ml/min per 1.73 m<sup>2</sup>), who are characterized by low preglomerular

resistance and high renal perfusion, empagliflozin was shown to reduce hyperfiltration by increasing preglomerular renal resistance, probably via adenosine release.<sup>11–14</sup> Although iohexol-measured GFR has been found to be reduced after dapagliflozin treatment in patients with T2D,<sup>20</sup> the involved renal hemodynamic mechanisms remained unknown because ERPF was not measured. In the present study, we confirm that SGLT2i treatment reduces mGFR. However, in contrast to what was found in patients with T1D and hyperfiltration,<sup>12</sup> dapagliflozin did not increase RVR. This fact, in combination with reduced FF and mGFR, points toward postglomerular vasodilation, as confirmed by using estimates of arteriolar resistances. Surprisingly as this may seem, it is important to emphasize that our study population greatly differs from young patients with T1D and hyperfiltration. Baseline mGFR and ERPF were both much lower in our population, while RVR was much higher (Figure 2). This indicates that the preglomerular arteriolar diameter was much narrower, thus limiting the possibility to constrict the preglomerular arteriole. Also, the majority of our study population was treated with RAS blockers. This may have a great impact on the renal hemodynamic effects of SGLT2is, because this drug class is known to activate RAS in both T1D<sup>12</sup> and T2D.<sup>20</sup> Indeed,

**Table 6 | Adverse events**

Variable	Dapagliflozin (n = 24)	Gliclazide (n = 20)
Participants with adverse events	9 (37.5)	10 (50)
Genital fungal infections	5 (20.8)	0 (0)
Hypoglycemia	0 (0)	0 (0)
Dizziness	3 (12.5)	2 (10)
Polyuria/thirst	1 (4.2)	1 (5)
Infection	1 (4.2)	3 (15)
Musculoskeletal	3 (12.5)	2 (10)
Headache	0 (0)	3 (15)
Gastrointestinal	3 (12.5)	2 (10)
Other	2 (8.3)	3 (15)

Data are expressed as n (%).



**Figure 2 | Hypothesized renal hemodynamic effects of sodium-glucose cotransporter 2 (SGLT2) inhibition in hyperfiltrating type 1 diabetes (defined as glomerular filtration rate [GFR] > 135 ml/min per 1.73 m<sup>2</sup>) and metformin-treated type 2 diabetes without renal disease.** Renal hemodynamic status in healthy people is added as a reference. In hyperfiltrating type 1 diabetes, renal vascular resistance (RVR) is low and GFR is high compared to healthy people. In type 2 diabetes, the opposite is true. After SGLT2 inhibition, tubuloglomerular feedback (TGF) activation causes preglomerular arteriolar constriction in type 1 diabetes via adenosine, resulting in increased vascular resistance and reduced GFR. Preglomerular arteriolar constriction is prevented in type 2 diabetes by prostaglandin release, which also causes postglomerular arteriolar dilation, leading to a reduced GFR while simultaneously reducing vascular resistance. The effect of renin-angiotensin system (RAS) activation, with the effect of angiotensin II, causing postglomerular vasoconstriction, is pharmacologically blocked in our study population and thus has not prevented postglomerular vasodilation.

dapagliflozin increased renin in our study, but these changes are unlikely to be of biological importance, given the simultaneous use of RAS blockers. In T1D, empagliflozin was recently shown to cause an increase in the favorable alternative RAS component angiotensin(1-7) in combination with RAS blockade.<sup>21</sup> However, angiotensin(1-7) is presumed to cause preglomerular vasodilation, indicating that the interaction between RAS, SGLT2is, and renal hemodynamics is likely to be complex. We observed a clear effect of the clamps on renal hemodynamic function: GFR, ERPF, and renal blood flow were reduced, while RVR and FF were increased. This was not the case in young people with T1D.<sup>12</sup> An in-depth discussion of these effects is beyond the scope of this study, but it might be explained by differences in insulin and glucose infusion or patient characteristics.

To gain more insight into mechanisms of dapagliflozin-induced changes in renal hemodynamics, we measured several potential mediators of arteriolar tone.<sup>15</sup> Adenosine, which was recently demonstrated to play a pivotal role in the amelioration of preglomerular arteriolar dilation and hyperfiltration by empagliflozin via the adenosine A1 receptor,<sup>11</sup> was significantly increased after dapagliflozin, as was also observed in people with T1D.<sup>12,13</sup> The increment in

adenosine was however not related to changes in mGFR in our study. Adenosine is known to increase preglomerular arteriolar resistance. However, it can also induce postglomerular vasodilation via adenosine A1 receptor activation in the presence of RAS blockade.<sup>22</sup> Interestingly, increasing sodium chloride concentrations at the macula densa reduced the postglomerular arteriolar diameter via increasing adenosine release in *ex vivo* rabbit arterioles that were pre-constricted with norepinephrine.<sup>23</sup> This clearly indicates that when the potential for preglomerular vasoconstriction is limited, this predisposes adenosine to cause postglomerular vasodilation during TGF activation.<sup>24</sup> Furthermore, we found a significant reduction in the urinary excretion of 8-oxo-dG, a marker of DNA oxidation, but no change in the urinary excretion of 8-oxo-7,8-dihydro-guanosine, a marker of RNA oxidation. Reactive oxygen species can cause postglomerular constriction, and a reduction might therefore support postglomerular dilation.<sup>25</sup> However, because the reduction in 8-oxo-dG excretion was evident after both gliclazide and dapagliflozin treatment, and mGFR was reduced only with dapagliflozin, this reduction in DNA oxidation does not seem to explain the renal hemodynamic effects. The excretion of prostaglandins 6-keto-prostaglandin F-1 $\alpha$ , the major

metabolite of prostaglandin I<sub>2</sub>, and prostaglandin E<sub>2</sub> was also increased with dapagliflozin, not with gliclazide, while prostaglandin E metabolite, the major urinary metabolite of prostaglandin E<sub>2</sub>, showed a similar trend. These substances are known to cause vasodilation and may therefore have contributed to a stable or mildly reduced RVR with dapagliflozin. The source of increased prostaglandin production is unknown.

Given that the regulation of arteriolar tone and GFR depends on a complex interplay of different mediators, which are not easily disentangled in clinical studies, we can only speculate how dapagliflozin exerted its effects. Hypothetically, dapagliflozin may have activated TGF and increased adenosine production, but the potential vasoconstrictive action of adenosine on the preglomerular arteriole was counteracted by an increase in prostaglandin synthesis or concomitant RAS blockade. Subsequently, the increase in prostaglandins may have induced postglomerular vasodilation, which could not be prevented by angiotensin II, because this was pharmacologically blocked. This resulted in a net reduction in postglomerular tone, while preglomerular tone remained unchanged, and thus effectively reduced mGFR (Figure 2). Additionally, the increase in adenosine might have directly contributed to postglomerular vasodilation,<sup>23,24</sup> especially in the presence of RAS blockade.<sup>22</sup>

We aimed to study the effect of dapagliflozin on renal hemodynamics beyond glucose control, as lowering glucose as such may also impact renal hemodynamics. Therefore, we chose to use sulfonylurea gliclazide as an active comparator and incorporated clamps in the test protocol. This allowed us to delineate the effects of SGLT2is on renal hemodynamics beyond glucose control, especially because gliclazide had no consistent impact on renal hemodynamics. Dapagliflozin did improve several renal risk factors in contrast to gliclazide. We observed a reduction in body weight as well as reductions in blood pressure. These risk factors may also impact on mGFR, but changes in these variables were not related to changes in mGFR or ERPF in correlation analyses.

Our findings have clinical implications. When SGLT2is would induce preglomerular vasoconstriction in T2D, especially when combined with postglomerular vasodilating RAS blockers, increased acute kidney injury would be expected. However, the fact that preglomerular perfusion is relatively maintained in people with T2D because of unchanged or even reduced RVR might explain why SGLT2is do not induce acute kidney injury in large T2D trials. We furthermore report increases in both hematocrit and erythropoietin, as shown in other studies,<sup>17</sup> which could improve renal oxygenation, contributing to improved outcome and further reducing acute kidney injury risk. Interestingly, luseogliflozin indeed prevented hypoxic damage in a nondiabetic murine model of ischemia-reperfusion injury mediated by increased vascular endothelial growth factor A levels, thereby reducing tubular injury.<sup>26</sup> This study confirms previous findings and provides important information for the use of SGLT2is in people with CKD.<sup>27</sup>

If both RAS blockers and SGLT2is have postglomerular effects, it could explain why nonresponders to RAS blocker therapy do not respond to SGLT2is<sup>28</sup>—a hypothesis that merits further investigation.

Because of the retraction of inulin from the market, we were forced to use iohexol instead of inulin to measure GFR in 11 participants. Importantly, all measurements within each participant were done with the same substance. Despite the high correlation between measurements with both substances, this inconsistency forms a potential limitation. In addition, it is not possible to perform direct intrarenal measurements in clinical research and we therefore relied on measurements on whole-kidney level and estimates of intrarenal hemodynamics. Our study was furthermore limited by the relatively small sample size, with low power for between-group testing. Also, we did not include patients with T2D and (advanced) chronic kidney disease. Nevertheless, patient characteristics were similar to those studied in the DECLARE-TIMI 58 trial, with age, body mass index, diabetes duration, eGFR, blood pressure, statin use, and cholesterol concentrations being almost identical. This implies that despite the relatively small sample size, our results can be extrapolated to a general population with T2D without established renal disease when using dapagliflozin as a second-line agent on top of standard of care.

In conclusion, we demonstrate that the SGLT2i dapagliflozin lowers mGFR beyond glucose control on top of standard of care, including RAS blockade, in metformin-treated patients with T2D without overt nephropathy. In contrast to people with T1D and hyperfiltration, in T1D animal models, this mGFR reduction occurs without an increase in RVR. Together with the increase in urinary prostaglandin excretion, and supported by estimates of arteriolar resistances, this suggests that the renal hemodynamic effects of SGLT2i in T2D are caused by postglomerular vasodilation rather than preglomerular vasoconstriction.

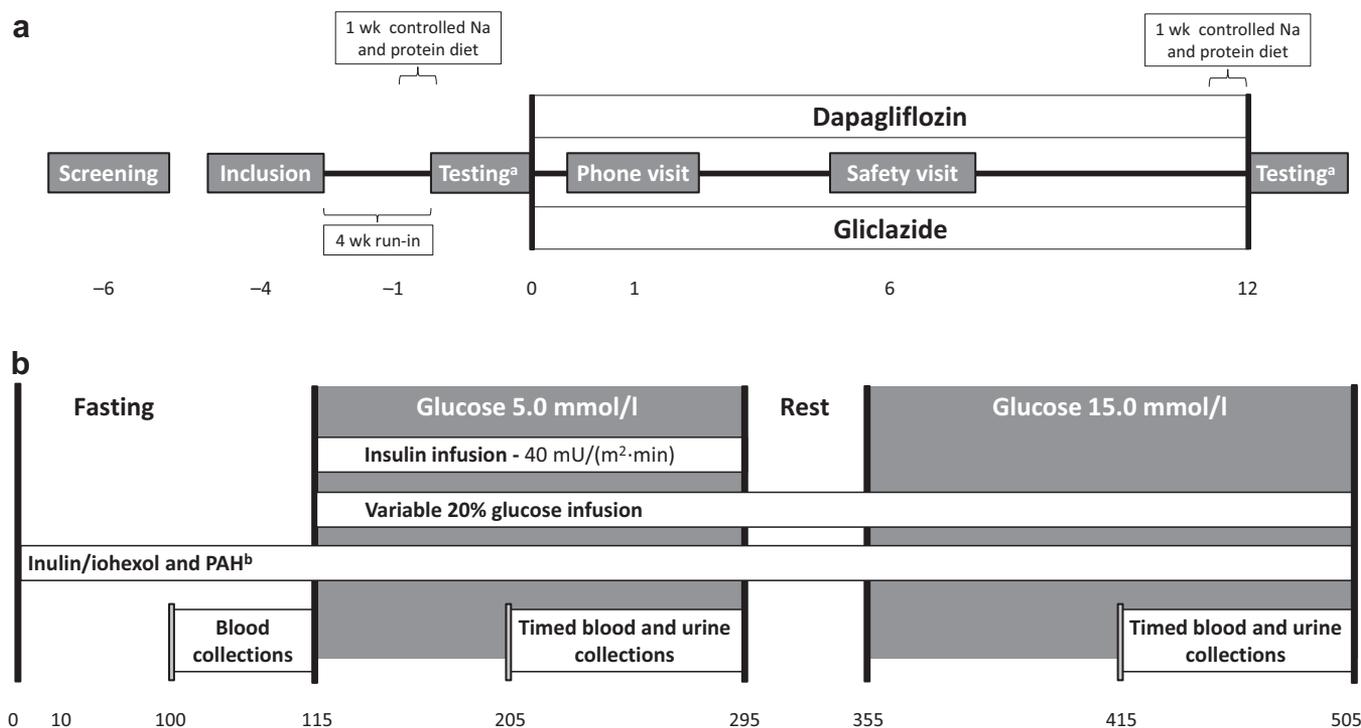
## METHODS

### Trial design

The Renoprotective Effects of Dapagliflozin in Type 2 Diabetes trial was a phase 4, monocenter, randomized, double-blind, comparator-controlled, parallel group, intervention trial conducted between July 2016 and September 2018 at the Amsterdam University Medical Centers, location VUMC, Amsterdam, The Netherlands. The study consisted of a 4-week run-in period, followed by a 12-week intervention period (Figure 3a).

### Study population

Participants were recruited from our study database and by advertisements in local newspapers. Eligible people were white, men or postmenopausal women, aged 35 to 75 years, who were diagnosed with T2D and had glycated hemoglobin from 6.5% to 9.0% (48–75 mmol/mol) and body mass index > 25 kg/m<sup>2</sup>, were treated with metformin monotherapy (stable dose for ≥3 months), and had a well-controlled blood pressure (i.e., <140/90 mm Hg). In the case of previously diagnosed hypertension and/or albuminuria, treatment included at least a stable dose of a RAS inhibitor for ≥3 months at a



**Figure 3 | (a) Study design with time in weeks.** <sup>a</sup>Including 24-hour urine collections. **(b)** Schematic overview of the test protocol with time in minutes. <sup>b</sup>Bolus infusion in the first 10 minutes. PAH, *para*-aminohippuric acid.

maximum tolerable dose. Exclusion criteria included a history of unstable or rapidly progressing renal or malignant disease (excluding basal cell carcinoma), eGFR < 60 ml/min per 1.73 m<sup>2</sup>, macroalbuminuria (i.e., albumin-to-creatinine ratio > 300 mg/g), urinary retention (bladder ultrasonography at the screening visit was performed to objectively assess bladder emptying), (re)current urinary tract or genital infection, diabetic ketoacidosis or cardiovascular events within 6 months before inclusion, or use of nonsteroid anti-inflammatory drugs or diuretics that could not be discontinued 3 months before and during the intervention period. Written informed consent was obtained from all participants before any trial-related activities. The study protocol, protocol amendments, and any other protocol-specific documents were reviewed and approved by local authorities and the medical ethical review board of the VU University Medical Center (Amsterdam, The Netherlands). The study complied with the Declaration of Helsinki and Good Clinical Practice guidelines and was registered at [ClinicalTrials.gov](https://www.clinicaltrials.gov) (ID: NCT02682563).

### Randomization and intervention

Participants were randomized to dapagliflozin 10 mg or gliclazide 30 mg (block size of 4, performed by an independent trial pharmacist using computer-generated numbers). The tablets were encapsulated, producing identical oral capsules (A15 Pharmacy, Gorinchem, The Netherlands; encapsulation did not change pharmacokinetics or pharmacodynamics); participants and investigators remained blinded to treatment status until database lock. Patients were instructed to take their study medication once daily at 8 PM during the 12-week treatment period. Adherence was followed up by counting the remaining capsules at all visits.

### Outcome measures

The primary end points were treatment-induced changes in mGFR and ERPF from baseline to week 12 of dapagliflozin versus gliclazide, as derived from inulin and *para*-aminohippuric acid (PAH) clearance methodology, respectively, with timed blood and urine sampling. In addition to the fasting phase, we performed the renal measurements during clamped euglycemia and hyperglycemia to rule out any potential differences in glycemic control between the treatment arms. All other renal hemodynamic measures, tubular handling of sodium and glucose, albuminuria, and other measurements obtained at baseline and after 12 weeks of treatment were considered secondary end points and were analyzed within groups.

### Study protocol

The week before renal testing, participants adhered to “normal” sodium (9–12 g/d) and protein (1.5–2.0 g/kg/d) diets to minimize variation in renal physiology due to salt and protein intake (Figure 3b). Participants collected urine during a 24-hour period that ended on the night before renal testing. After an overnight fast, participants drank 500 ml of tap water (to stimulate diuresis) before arriving at the clinical research unit at 07:30 AM; at that time, blood and urine were obtained for fasting outcome variables. Then, the renal tests commenced by a weight-calculated bolus infusion of 22.5 mg/kg inulin (Inutest, Fresenius Kabi Austria GmbH, Graz, Austria) and 3 mg/kg of PAH (4-aminohippuric acid solution 20%, Bachem Distribution Services GmbH, Weil am Rhein, Germany) in 10 minutes after which infusion continued at a lower rate (675 and 320 mg/h, respectively) for the remainder of the day. After 33 participants completed the trial, inulin was retracted from the market because of anaphylactic reactions in another center. Because iohexol

and inulin have a similar pharmacokinetic profile and clearances correlate almost perfectly ( $r = 0.986$ ),<sup>29</sup> we subsequently switched to iohexol in our protocol to measure GFR in the remaining 11 participants (bolus of 36 mg/kg in 10 minutes, followed by 906 mg/h). All measurements within each patient were done with the same agent. Separate analysis in participants tested with inulin or iohexol gave the same results.

We drew blood 100 and 115 minutes after starting bolus infusion of inulin/iohexol and PAH to measure plasma concentrations in steady-state fasting conditions (Figure 3b). This was followed by the initiation of a hyperinsulinemic-euglycemic clamp, with insulin (NovoRapid, Novo Nordisk Farma B.V., Alphen aan den Rijn, The Netherlands) infusion at 40 mU/(min·m<sup>2</sup>) while maintaining plasma glucose at 5 mmol/l by adjusting the rate of glucose 20% infusion. After 90 minutes of equilibration, urine was collected by spontaneous voiding for two 45-minute periods. Diuresis was prompted by oral intake of 10 ml/kg (maximum 1000 ml) of tap water up to minute 90, followed by a standardized schedule of water intake throughout the day. All participants were seated while voiding, were instructed to use a double voiding technique, and reached a subjective feeling of total bladder emptying. The hyperinsulinemic-euglycemic clamp was followed by a 60-minute rest period to clear exogenous insulin. Then, a hyperglycemic clamp was initiated. Bolus infusion of glucose 20% (depending on body weight and plasma glucose concentration) was followed by adjusting the rate of glucose 20% infusion to maintain plasma glucose at 15 mmol/l. After 60 minutes of equilibration, urine was collected by spontaneous voiding for two 45-minute periods. Systolic blood pressure, diastolic blood pressure, mean arterial pressure, and heart rate were determined during all 3 phases by using an automated oscillometric device (Dinamap, GE Healthcare, Little Chalfont, UK) at the brachial artery of the nondominant arm. Measurements were performed in triplicate at 1- to 2-minute intervals by using the mean of the last 2 measurements.

### Renal calculations

mGFR and ERPF were calculated from inulin/iohexol and PAH clearances, respectively, with timed urine sampling and averaged from consecutive urine collection periods. Calculations for mGFR, ERPF, FF, effective renal blood flow, and RVR have been described previously.<sup>30,31</sup> FF was calculated by dividing mGFR by ERPF, ERBF by dividing ERPF by (1 – hematocrit), and RVR by dividing mean arterial pressure by renal blood flow. We used the Gomez equations to estimate intrarenal hemodynamics, namely, intraglomerular pressure, preglomerular arteriolar resistance, and postglomerular arteriolar resistance, as described previously.<sup>30–32</sup> We assumed a gross filtration coefficient of 0.075 ml/s/mm Hg given the mean GFR of 113 ml/min in the present population. For eGFR we used the Chronic Kidney Disease Epidemiology Collaboration 2009 equations.<sup>33</sup> Urinary excretion of sodium, glucose, and albumin was measured in 24-hour urine collections and calculated as absolute excretion.

### Assays

Inulin and PAH were analyzed as described previously.<sup>30</sup> Iohexol was measured using liquid chromatography tandem mass spectrometry (TSQ Quantiva with UHPLC Vanquish, Thermo Fisher Scientific, Waltham, MA). Insulin was measured using a chemiluminescence immunoassay (Atellica IM, Siemens Healthcare Diagnostics, Breda, The Netherlands). Serum erythropoietin levels were measured using a sandwich chemiluminescent immunoassay on an IMMULITE 2000 platform (Siemens Healthcare Diagnostics, Breda, The Netherlands). Aldosterone was measured using a radioimmunoassay (Demeditec

Diagnostics, Kiel, Germany). Renin was determined with a radioimmunoassay (Cisbio, Codolet, France). Oxidative modifications of DNA and RNA were determined by urinary excretion of 8-oxo-7,8-dihydro-2'-deoxyguanosine and 8-oxo-7,8-dihydro-guanosine, respectively, using ultra-performance liquid chromatography tandem mass spectrometry, as described previously.<sup>34</sup> Prostaglandin E<sub>2</sub>, prostaglandin E metabolite, and 6-keto-prostaglandin F-1 $\alpha$  were measured using commercial competitive enzyme-linked immunosorbent assay kits (Cayman Chemical, Ann Arbor, MI). Thromboxane B<sub>2</sub> was measured using a competitive enzyme-linked immunosorbent assay (Enzo Life Sciences, Farmingdale, NY). Endothelin-1 was measured using a sandwich immunoassay (R&D Systems, Minneapolis, MN). Urinary adenosine was measured using liquid chromatography tandem mass spectrometry with a modified method as described previously.<sup>13</sup>

### Sample size calculation

We based our sample size on the expected between-group difference in mGFR using Stata version 11 (Breda, The Netherlands). Assuming an SD of 15.0 ml/min and considering  $\alpha = 0.05$  as significant, we calculated to need 19 participants per treatment arm to achieve a power (1 –  $\beta$ ) of 80% to detect a between-group mGFR difference of 14 ml/min (89 ml/min in the gliclazide group vs. 75 ml/min in the dapagliflozin group and thus 16% difference).<sup>12,35</sup> We calculated to need 10 subjects to achieve a power (1 –  $\beta$ ) of 80% to detect a dapagliflozin-induced mGFR reduction (i.e., within group) from 89 to 75 ml/min (16% difference) with an SD of 15.0 ml/min, assuming  $\alpha = 0.05$  (2-sided testing) is significant.<sup>12,35</sup> We increased the number of subjects per group to 22 to allow a maximum dropout percentage of 15%.

### Data management and statistics

Data were entered in an electronic data management system (Castor EDC, version 1.4, Amsterdam, The Netherlands) and transferred to the final study database. Before unblinding, inulin/iohexol and PAH extraction ratios were inspected, and urine collection periods characterized by profound collection errors were discarded from the analyses. This resulted in the use of plasma concentrations instead of urinary excretion to calculate clearances for 6 participants during euglycemia. Statistical analyses were performed in the per protocol population using SPSS 24.0 (IBM Corporation, Chicago, IL). Multivariable linear regression models were used to examine dapagliflozin- versus gliclazide-induced effects. The corresponding baseline values were added as independent variables to correct for potential between-group baseline differences. Within-group comparisons were analyzed using paired *t* tests (Gaussian distributed data) or Wilcoxon signed rank tests (non-Gaussian distributed data, even after log or square root transformation). Statistical significance was set at a 2-sided  $\alpha$  level of <0.05. Data are expressed as mean  $\pm$  SD, median (interquartile range), or baseline corrected mean difference with a 2-sided 95% confidence interval unless otherwise specified.

### DISCLOSURE

MHAM is a consultant and speaker for Eli Lilly, Sanofi, and Novo Nordisk; all honoraria are paid to his employer (Amsterdam University Medical Centers, location VU University Medical Center). DHvR has served as a consultant and received honoraria from Boehringer Ingelheim and Lilly, Merck, Novo Nordisk, Sanofi, and AstraZeneca and has received research operating funds from Boehringer Ingelheim and Lilly Diabetes Alliance, AstraZeneca, and Novo Nordisk; all honoraria are paid to his employer (Amsterdam University Medical Centers, location VU University Medical Center). DJT reports grants from

ZonMw and Chiesi Pharmaceuticals. ELL and HEP received an unrestricted research grant for an investigator-initiated study from Boehringer Ingelheim not related to this study. MN received an unrestricted investigator-initiated grant on sodium-glucose cotransporter 2 inhibitors and lipid fluxes from AstraZeneca. All the other authors declared no competing interests.

#### DATA STATEMENT

Data cannot be shared due to patient privacy.

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#### AUTHOR CONTRIBUTIONS

EJMvB, MHAM, LT, MN, MHHK, JAJ, and DHvR designed and set up the trial. EJMvB, MHAM, LT, MJBvB, ALE, AB, AHJD, EJH, DJT, EJH, ELL, FG, and HEP were involved in sample collection and/or analysis. EJMvB and MMS performed statistical analysis. EJMvB and DHvR wrote the first draft of the paper. The submitted version was approved by all authors.

#### SUPPLEMENTARY MATERIAL

[Supplementary File \(PDF\)](#)

**Table S1.** The effect of dapagliflozin with or without renin-angiotensin system (RAS) blockade on directly measured and calculated measures of renal hemodynamics during the 3 phases of the protocol.

**Figure S1.** Flow diagram of study participants.

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