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Published in:
Journal of Hypertension

DOI:
10.1097/HJH.0000000000002480

Publication date:
2020

Original Article

Effects of dapagliflozin and gliclazide on the cardiorenal axis in people with type 2 diabetes

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Objectives: There is a bidirectional relationship between cardiovascular and renal disease. The drug-class of SGLT2 inhibitors improves outcomes at both ends of this so called cardiorenal axis. We assessed the effects of SGLT2 inhibition and sulfonylurea treatment on systemic hemodynamic function and investigated whether SGLT2 inhibitor-induced changes in systemic hemodynamics correlate with changes in renal hemodynamics.

Methods: Forty-four people with type 2 diabetes were randomized to 12 weeks of dapagliflozin 10 mg/day or gliclazide 30 mg/day treatment. Systemic hemodynamic function, autonomic nervous system activity, and vascular stiffness were measured noninvasively, whereas renal hemodynamics, glomerular filtration rate (GFR) and effective renal plasma flow, were assessed with gold-standard urinary clearances of inulin or iohexol and para-aminohippuric acid, respectively. Correlation analyses were performed to assess relationships between dapagliflozin-induced changes in cardiovascular and renal variables.

Results: Dapagliflozin reduced stroke volume by 4%, cardiac output by 5%, vascular stiffness by 11%, and mean arterial pressure by 5% from baseline, without increasing heart rate or sympathetic activity, while simultaneously lowering glomerular filtration rate by 8%. Despite similar improvements in glycemic control by dapagliflozin and gliclazide (-0.5 ± 0.5 versus -0.7 ± 0.5%; $P = 0.12$), gliclazide did not affect any of these measurements. There was no clear association between the dapagliflozin-induced changes in cardiovascular and renal physiology.

Conclusion: Dapagliflozin seemingly influences systemic and renal hemodynamics independently and beyond glucose lowering in people with type 2 diabetes.
This clinical trial was registered at https://clinicalTrials.gov (ID: NCT02682563).

Keywords: cardiorenal, diabetic complications, hemodynamics, sodium-glucose linked transporter 2 inhibition, type 2 diabetes

Abbreviations: CKD, chronic kidney disease; CO, cardiac output; DKD, diabetic kidney disease; ERPF, effective renal plasma flow; HRV, heart rate variability; PAH, para-aminohippuric acid; PP, pulse pressure; PWA, pulse wave analysis; RBF, renal blood flow; RMSSD, root mean square of successive differences; RPP, rate-pressure product; RVR, renal vascular resistance; SDNN, standard deviation of the R-R intervals; SGLT2, sodium-glucose linked transporter 2; SV, stroke volume; SVR, systemic vascular resistance

INTRODUCTION

Sodium-glucose linked transporter 2 (SGLT2) inhibitors lower plasma glucose by blocking sodium-coupled glucose reabsorption in the proximal tubule of the kidney and are used to treat hyperglycemia in people with type 2 diabetes (T2D) [1]. Increased urinary loss of sodium and glucose additionally reduces body weight, blood pressure and plasma uric acid [1]. The observed reduction in plasma volume (as reflected by increased hematocrit) is thought to be a main determinant of the beneficial effects of SGLT2 inhibitors on major adverse cardiovascular events, in particular heart failure, and mortality in patients with atherosclerotic disease [2]. SGLT2 inhibitors are particularly effective in reducing heart failure hospitalizations [3], and progression of heart failure, irrespective of the presence of diabetes [4]. Reductions in plasma volume and blood pressure occur without a rise in heart rate, which may be because of a reduction in sympathetic nervous system activity. However, this has not yet been studied in people with T2D. It is also unknown if, and to what extent, glucose-lowering by SGLT2 inhibitors contributes to these cardiorenal effects.

Furthermore, cardiovascular and renal (patho)physiology are often closely linked, which is referred to as the cardiorenal axis. As such, chronic kidney disease (CKD) strongly increases the risk for cardiovascular events to a similar extent in people with and without diabetes [5]. In addition, CKD is the main driver of increased (cardiovascular) mortality in

Journal of Hypertension 2020, 38:000–000

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Received 15 January 2020 **Revised** 21 March 2020 **Accepted** 31 March 2020

DOI:10.1097/HJH.0000000000002480

people with T2D [6]. On the other hand, renal impairment is very common in people with heart failure and increases mortality in this population [7]. Interestingly, SGLT2 inhibitors improve renal as well as cardiovascular outcomes [3]. In the first dedicated renal outcome trial, canaglifozin lowered the risk for end-stage kidney disease by 32% [95% confidence interval (CI) −46 to −14%] in people with established diabetic kidney disease (DKD), possibly by reducing intraglomerular pressure [8]. In light of this bidirectional relationship between cardiovascular and renal disease and the fact that the majority of people with T2D is affected by both, the interactive effects of SGLT2 inhibitors on the cardiorenal axis are of interest. It is believed that SGLT2 inhibitors confer renoprotection by reducing intraglomerular pressure in people with T2D, which is likely driven by tubuloglomerular feedback activation [9]. Despite the fact that the relationship between cardiovascular and renal physiology and disease is often discussed, it is currently unknown whether the effects of SGLT2 inhibition on systemic hemodynamics mediate these renal hemodynamic changes.

Therefore, in this study, we firstly assessed the effects of SGLT2 inhibition and sulfonylurea treatment on systemic hemodynamic function as well as on sympathetic nervous system activity in people with T2D; and secondly correlated SGLT2 inhibitor-induced changes in systemic hemodynamic function with changes in gold-standard measured renal hemodynamics.

METHODS

Trial design

This was a prespecified secondary analysis of the RED (Renoprotective Effects of Dapagliflozin in Type 2 Diabetes) trial: a phase−4, monocenter, randomized, open-label, comparator-controlled, parallel-group, intervention trial, primarily designed to assess the renal hemodynamic effects of dapagliflozin versus gliclazide [9]. The study consisted of a 4-week run-in period, followed by a 12-week intervention period, and was conducted between July 2016 and September 2018 at the Amsterdam University Medical Centers, location VUMC, in Amsterdam, The Netherlands. The study protocol, protocol amendments and any other protocol-specific documents were reviewed and approved by local authorities and the ethics 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 https://clinicalTrials.gov (ID: NCT02682563).

Study population

We included Caucasian men or postmenopausal women, aged 35–75 years, who were diagnosed with T2D and had an HbA1c between 6.5 and 9.0% (48–75 mmol/mol) and a BMI greater than 25 kg/m^2, were treated with metformin monotherapy (stable dose for ≥3 months), and had well controlled blood pressure (i.e. <140/90 mmHg). In case of previously diagnosed hypertension and/or albuminuria, treatment included at least a stable dose of a renin–angiotensin system (RAS) inhibitor for at least 3 months at maximal tolerable dose. People with a history of unstable or rapidly progressing renal or malignant disease (excluding basal cell carcinoma), estimated glomerular filtration rate (eGFR) less than 60 ml/min per 1.73 m^2) (CKD-EPI), macroalbuminuria (i.e. ACR >300 mg/g), urinary retention (bladder ultrasonography at screening visit was performed to objectively assess bladder emptying), (re)current urinary tract or genital infection, diabetic ketoacidosis or cardiovascular events within 6 months prior to inclusion, or use of nonsteroid anti-inflammatory drugs or diuretics that could not be stopped 3 months prior to and during the intervention period, were excluded. Participants were recruited from our study database and by advertisements in local newspapers. Written informed consent was obtained before any trial-related activities.

Randomization and intervention

To blind both participants and investigators until database lock, patients were randomized to identical oral capsules (A15 pharmacy, Gorinchem, The Netherlands; encapsulation did not change pharmacokinetics/pharmacodynamics) containing dapagliflozin 10 mg or gliclazide 30 mg (block-size of 4, performed by an independent trial pharmacist using computer-generated numbers). Patients were instructed to take their study medication once daily at 2000 h. Adherence was monitored by counting the remaining capsules at all visits.

Outcome measures

The primary endpoints were treatment-induced changes in GFR and effective renal plasma flow (ERPF) from baseline to week 12 of dapagliflozin versus gliclazide, as derived from inulin and para-aminohippuric acid (PAH) clearance methodology, respectively, based on timed blood and urine sampling, as published [9]. Additional cardiovascular measures – including blood pressure, vascular stiffness, stroke volume, cardiac output, and measures of heart rate variability – and tubular handling of sodium and glucose, and albuminuria, at baseline and after 12 weeks, were also collected.

Study protocol

The detailed study protocol was previously published [9]. For this analysis, we used data from the fasting phase. To minimize variation in (renal) physiology because of salt and protein intake, participants were instructed to adhere to 'normal' sodium (9–12 g/day) and protein (1.5–2.0 g/kg per day) diets the week before testing, to abstain from vigorous physical activity and alcohol ingestion for at least 24 h, and to withhold from nicotine or caffeine for more than 12 h. Participants collected urine during a 24-h 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 7.30 a.m. Blood and urine were obtained for fasting outcome variables.

Systemic hemodynamics

SBP, DBP, mean arterial pressure (MAP), and heart rate (HR) were determined by an automated oscillometric device (Dinamap, GE Healthcare, Little Chalfont, UK) at the brachial artery of the nondominant arm. All measurements were performed after a quiet rest period of at least 10 min using appropriate cuff sizes, in supine position, and at the nondominant arm, which was placed comfortably at heart level. Measurements were performed in triplicate at 1–2 min

intervals, using the mean of the last two measurements. Rate-pressure product (RPP) was calculated by multiplying SBP by HR, whereas pulse pressure (PP) was calculated by subtracting DBP from SBP. Stroke volume (SV), cardiac output (CO) and systemic vascular resistance (SVR) were calculated from noninvasive beat-to-beat finger arterial photoplethysmography BP measurements (Nexfin, Amsterdam, The Netherlands). The finger BP-measurements were performed over a period of 30 s, and a mean was derived using dedicated software (Nexfin@PC version 2.0, BM Eye, Amsterdam, the Netherlands). The within-day and between-day variability of these assessments are 9.1% or less [10].

Pulse wave analysis

Pulse wave analysis (PWA) was performed at the level of the radial artery using applanation tonometry with a high-fidelity micromanometer (SPT-301; Millar Instruments, Houston, Texas, USA) coupled to a SphygmoCor apparatus and version 6.31 software (Atcor Medical Pty Ltd, West Ryde, Australia). The average of two measurements of at least 12 s was used, which needed to have adequate pulse wave profiles and a high-quality control, defined as an in-device quality index of more than 80%. The central aortic pressure waveform was derived from the radial artery waveform using the software's mathematical transfer function [11,12]. The augmentation index (an indicator of arterial stiffness) was calculated as the augmentation pressure, that is, the pressure of the second systolic peak minus the pressure at the inflection point, expressed as percentage of the pulse pressure and normalized for a HR of 75 bpm (AIX@HR75).

Heart rate variability

Using an electrocardiogram (ECG)-equipped Nexfin device, 5-min RR-interval recordings were obtained, during which patients were instructed to breath spontaneously (range 10–18 breaths/min) and to refrain from sleeping or speaking. ECG-measurements were visually inspected and artifacts were manually corrected using linear interpolation. ECG-recordings were loaded into Kubios heart rate variability (HRV) analysis software 2.2 (University of Eastern Finland, Biosignal Analysis and Medical Imaging Group, Kuopio, Finland). After additional automated low-level artifact correction and removal of trend components (smoothn priors setting), Fast Fourier spectral analyses were performed to obtain normalized low frequency (0.04–0.15 Hz) and high frequency (0.15–0.5 Hz) bands, from which the low frequency/high frequency ratio, a validated marker for cardiac sympathovagal-balance [13], was calculated. In addition, the root mean square of successive differences (RMSSD) and the standard deviation of the R-R intervals (SDNN) were calculated as measures of heart rate variability.

Renal hemodynamics

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 PAH (4-Aminohippuric Acid Solution 20%; Bachem Distribution Services GmbH, Weil am Rhein, Germany) in 10 min after which infusion continued at a lower rate (675 and 320 mg/h, respectively) for the remainder of the day. As previously discussed [9], we switched from inulin to iohexol in our protocol to measure GFR in the remaining 11 participants (bolus of 36 mg/kg in 10 min, followed by 906 mg/h). All measurements within each patient were done with the same agent. Plasma inulin/iohexol and PAH concentrations were measured after 100 and 115 min of tracer infusion in steady-state fasting conditions. An average of the two measurements was used to calculate GFR and effective renal plasma flow (ERPF) from plasma clearances. Filtration fraction, renal blood flow (RBF), and renal vascular resistance (RVR) were calculated as described [9].

Assays

Fasting plasma glucose, HbA1c, plasma creatinine, and urinary albumin excretion were measured by our in-house clinical chemistry department using conventional methods. Renin was determined with a radioimmunoassay (Cisbio, Codolet, France). Aldosterone was measured by radioimmunoassay (Demeditec Diagnostics, Kiel, Germany). Inulin, iohexol, and PAH were analyzed as described [9].

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. Statistical analyses were performed in the per protocol population using SPSS 24.0 (IBM SPSS, Chicago, Illinois, USA). The cardiovascular measures were analyzed by within-group comparisons using paired t tests (Gaussian distributed data) or Wilcoxon signed rank tests (only when data demonstrated a non-Gaussian distributed after log or square root transformation). Multivariable linear regression models were used to examine dapagliflozin–versusgliclazide-induced effects. Posttreatment measurements were entered as dependent variables, and treatment was entered as independent variable, as well as corresponding baseline values to correct for potential between-group baseline differences. To assess whether the dapagliflozin-induced changes in renal and systemic hemodynamics were related, we performed correlational analyses between the changes in SBP, DBP, MAP, CO, SV, and SVR, and the changes in GFR, RBF, hematocrit, urine volume, glucosuria, and natriuresis. These correlational analyses were carried out using Pearson (Gaussian distributed data) or Spearman correlations (non-Gaussian distributed data). When data points were missing, these analysis was performed without the concerning participant. Statistical significance was set at a two-sided α-level of less than 0.05. Data are presented as mean $\pm$ SD, median (IQR), or baseline corrected mean difference with a two-sided 95% confidence interval (CI), as specified.

RESULTS

After telephone and on-site screening, 50 people were found to be eligible to participate. Due to withdrawal of consent ($n = 4$), iodine allergy ($n = 1$), and urinary bladder retention ($n = 1$), 44 people were randomized to 12-weeks of dapagliflozin ($n = 24$) or gliclazide ($n = 20$), after which no participants dropped out [9]. All participants from the primary analysis were used for the secondary analysis presented here. Overall adherence to study medication was 99%. At baseline, demographic and clinical characteristics, as well as renal risk factors, were generally well balanced between

TABLE 1. Baseline characteristics

	Dapagliflozin ($n=24$)	Gliclazide ($n=20$)
Age (years)	63 ± 7	63 ± 7
Male [*n* (%)]	19 (79)	15 (75)
BMI (kg/m^2)	30.8 ± 3.9	31.6 ± 3.9
Diabetes duration (years)	9.8 ± 4.1	10.7 ± 7.3
HbA1c (%)	7.4 ± 0.7	7.4 ± 0.6
Fasting plasma glucose (mg/dl)	165 ± 27	158 ± 28
Current smoker [*n* (%)]	3 (13)	1 (5)
Alcohol intake, units/week	5 (IQR 2–13)	4 (IQR 2–8)
ASCVD [*n* (%)]	4 (17)	1 (5)
Hypertension [*n* (%)]	16 (67)	16 (80)
Cardiac autonomic neuropathy [*n* (%)]	2 (8)	3 (15)
eGFR (CKD-EPI) (ml/min per 1.73 m^2)	85 ± 13	89 ± 19
UACR (mg/mmol)	11 (IQR 6–17)	12 (IQR 4–17)
Medication use		
Platelet aggregation inhibitor [*n* (%)]	4 (17)	2 (10)
Metformin dose (mg)	1556 ± 736	1585 ± 765
Statin [*n* (%)]	16 (67)	14 (70)
Beta blocker [*n* (%)]	6 (25)	3 (15)
Calcium antagonist [*n* (%)]	6 (25)	6 (30)
RAS inhibitor [*n* (%)]	16 (67)	16 (80)
ACE inhibitor [*n* (%)]	5 (21)	5 (25)
ARB [*n* (%)]	11 (46)	11 (55)

Data are represented as mean ± SD, median (IQR) or frequency. 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–creatine ratio.

treatment groups (Table 1). There was one current smoker and one person with a history of atherosclerotic cardiovascular disease in the gliclazide group, compared with respectively three and four people in the dapagliflozin group. Most participants received other medication in addition to metformin, most commonly RAS inhibitors (73%) and statins (68%); as additional antihypertensive medication, 27% of the participants used a calcium antagonist and 20% a beta-blocker (Table 1). No medication changes occurred during the treatment period. Both treatments were well tolerated without serious adverse events (no hypoglycemic episodes, no cardiovascular or renal events). Five genital fungal infections occurred in the dapagliflozin group versus none in the gliclazide group.

Glycemic control

Both interventions similarly improved glycemic control, thus achieving glycemic equipoise. Dapagliflozin reduced HbA1c by 0.5 ± 0.5% and gliclazide by 0.7 ± 0.5% by gliclazide (between-group $P=0.12$), and fasting plasma glucose (FPG) by 18 ± 22 versus 22 ± 25 mg/dl by gliclazide (between-group $P=0.23$).

Systemic hemodynamics and heart rate

Dapagliflozin reduced SBP by 8.5 ± 11.4 mmHg ($P=0.001$) and DBP and 4.0 ± 4.0 mmHg ($P<0.001$) from baseline, which resulted in a MAP reduction of 5.5 ± 5.7 mmHg ($P<0.001$). Heart rate remained unchanged (Table 2 and

TABLE 2. Measures of renal and cardiovascular function

	Dapagliflozin ($n=24$)			Gliclazide ($n=20$)				
	Week 0	Week 12	Within-group	Week 0	Week 12	Within-group	Corrected mean difference dapagliflozin – gliclazide (95% CI) and *P*-value	
Renal hemodynamics								
GFR (ml/min)	113 ± 20	104 ± 17	$P<0.01$	113 ± 19	109 ± 20	$P=0.12$	−5 (−12 to 1)	$P=0.11$
ERPF (ml/min)	654 ± 153	639 ± 141	$P=0.12$	692 ± 120	678 ± 122	$P=0.31$	−17 (−58 to 23)	$P=0.39$
FF (%)	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)	$P=0.77$
RBF (ml/min)	1064 ± 362	1108 ± 272	$P=0.46$	1162 ± 227	1140 ± 232	$P=0.37$	24 (−90 to 138)	$P=0.67$
RVR (mmHg/l)	99 ± 29	93 ± 24	$P<0.05$	88 ± 18	89 ± 21	$P=0.41$	−5 (−12 to 1)	$P=0.11$
Cardiovascular hemodynamics								
SBP (mmHg)	137.7 ± 13.6	129.2 ± 10.7	$P=0.001$	131.6 ± 11.4	131.1 ± 11.8	$P=0.83$	−5.3 (−10.9 to 0.2)	$P=0.06$
DBP (mmHg)	84.4 ± 5.7	80.4 ± 5.6	$P<0.001$	81.7 ± 5.4	80.9 ± 6.5	$P=0.51$	−2.6 (−5.3 to 0.2)	$P=0.06$
MAP (mmHg)	102.2 ± 6.7	96.7 ± 6.5	$P<0.001$	98.3 ± 6.6	97.6 ± 7.4	$P=0.64$	−3.5 (−7.0 to 0.0)	$P=0.05$
Heart rate (bpm)	65.0 ± 9.9	63.2 ± 7.9	$P=0.27$	69.4 ± 11.6	69.2 ± 11.3	$P=0.90$	−3.1 (−7.2 to 1.0)	$P=0.14$
Stroke volume (ml)	99.8 ± 19.7	96.1 ± 17.5	$P<0.05$	45.2 ± 7.1	46.0 ± 6.6	$P=0.46$	−1.49 (−3.8 to 0.83)	$P=0.20$
Cardiac output (l/min)	6.4 ± 1.5	6.1 ± 1.3	$P<0.05$	6.5 ± 1.3	6.6 ± 0.9	$P=0.68$	−0.4 (−0.8 to −0.1)	$P<0.05$
Systemic vascular resistance (dyn/s/cm)	1277 ± 417	1258 ± 360	$P=0.55$	1155 ± 321	1143 ± 182	$P=0.80$	31 (−55 to 117)	$P=0.47$
AIX@HR75	23.8 ± 7.7	21.1 ± 7.6	$P<0.05$	24.5 ± 11.4	26.2 ± 12.7	$P=0.39$	−4.5 (−8.7 to −0.3)	$P<0.05$
Pulse pressure (mmHg)	53.3 ± 13.1	48.8 ± 8.7	$P<0.05$	49.9 ± 9.1	50.2 ± 9.3	$P=0.80$	−3.3 (−7.3 to 0.7)	$P=0.10$
Rate-pressure product (mmHg*bpm)	8899 ± 1211	8157 ± 1130	$P=0.001$	9181 ± 2007	9103 ± 1864	$P=0.76$	−741 (−1320 to −161)	$P=0.01$
LF/HF ratio	2.11 ± 2.35	1.87 ± 1.58	$P=0.72$	1.53 ± 1.29	1.70 ± 1.13	$P=0.53$	0.12 (−0.85 to 1.08)	$P=0.81$
SDNN	17.9 ± 6.5	18.3 ± 8.0	$P=0.82$	18.8 ± 8.3	21.2 ± 10.0	$P<0.05$	−2.1 (−5.8 to 1.6)	$P=0.25$
RMSSD	16.2 ± 4.7	16.7 ± 8.2	$P=0.77$	17.1 ± 6.9	19.6 ± 10.8	$P=0.13$	−1.9 (−6.2 to 2.4)	$P=0.38$
Body weight, hematocrit, RAAS, and urinary sodium/glucose excretion								
Body weight (kg)	96.6 ± 17.9	93.7 ± 16.9	$P<0.001$	98.5 ± 17.9	99.6 ± 18.3	$P=0.001$	−4.03 (−2.81 to −5.24)	$P<0.001$
Hematocrit (%)	40.7 ± 3.3	42.5 ± 2.9	$P<0.001$	40.2 ± 2.5	40.2 ± 2.8	$P=0.89$	1.8 (0.9 to 2.7)	$P<0.001$
Plasma renin (pg/ml)[a]	9.1 (4.8–16.5)	15.2 (5.8–20.2)	$P<0.05$	12.5 (6.3–16.2)	9.6 (6.0–22.2)	$P=0.51$	5.7 (−1.7 to 13.1)	$P=0.22$
Plasma aldosterone (pg/ml)[a]	90 (64–150)	116 (86–169)	$P<0.01$	98 (63–123)	107 (71–146)	$P=0.20$	−0.2 (−23.7 to 23.2)	$P=0.42$
Natriuresis (mmol/24 h)	178 ± 52	188 ± 63	$P=0.55$	178 ± 75	186 ± 67	$P=0.62$	−1 (−40 to 37)	$P=0.95$
Urinary glucose excretion (mmol/24 h)[a]	7 (1–45)	462 (327–711)	$P<0.001$	2 (1–17)	3 (1–14)	$P=0.36$	488 (365 to 612)	$P<0.01$
Urine volume (l/24 h)	1.9 ± 0.8	2.2 ± 0.9	$P<0.05$	2.2 ± 0.7	2.2 ± 0.9	$P=0.74$	0.2 (−0.2 to 0.6)	$P=0.27$

Paired *t*-tests were used for within-group comparisons. Multivariable linear regression models were used to examine week 0-corrected dapagliflozin-induced effects compared with gliclazide-induced effects. Data are represented as mean ± SD or median (IQR). Significant differences indicated in bold font. AIX@HR75, augmentation index standardized for a heart rate of 75 bpm; ERPF, effective renal plasma flow; FF, filtration fraction; GFR, measured glomerular filtration rate; LF/HF, low frequence/high frequency; MAP, mean arterial pressure; RAAS, renin–angiotensin–aldosterone system; RBF, renal blood flow; RMSSD, root mean square of successive differences; RVR, renal vascular resistance; SDNN, standard deviation of N-N (R-R) intervals.
[a]Analysis performed after log transformation, mean difference calculated before log transformation.

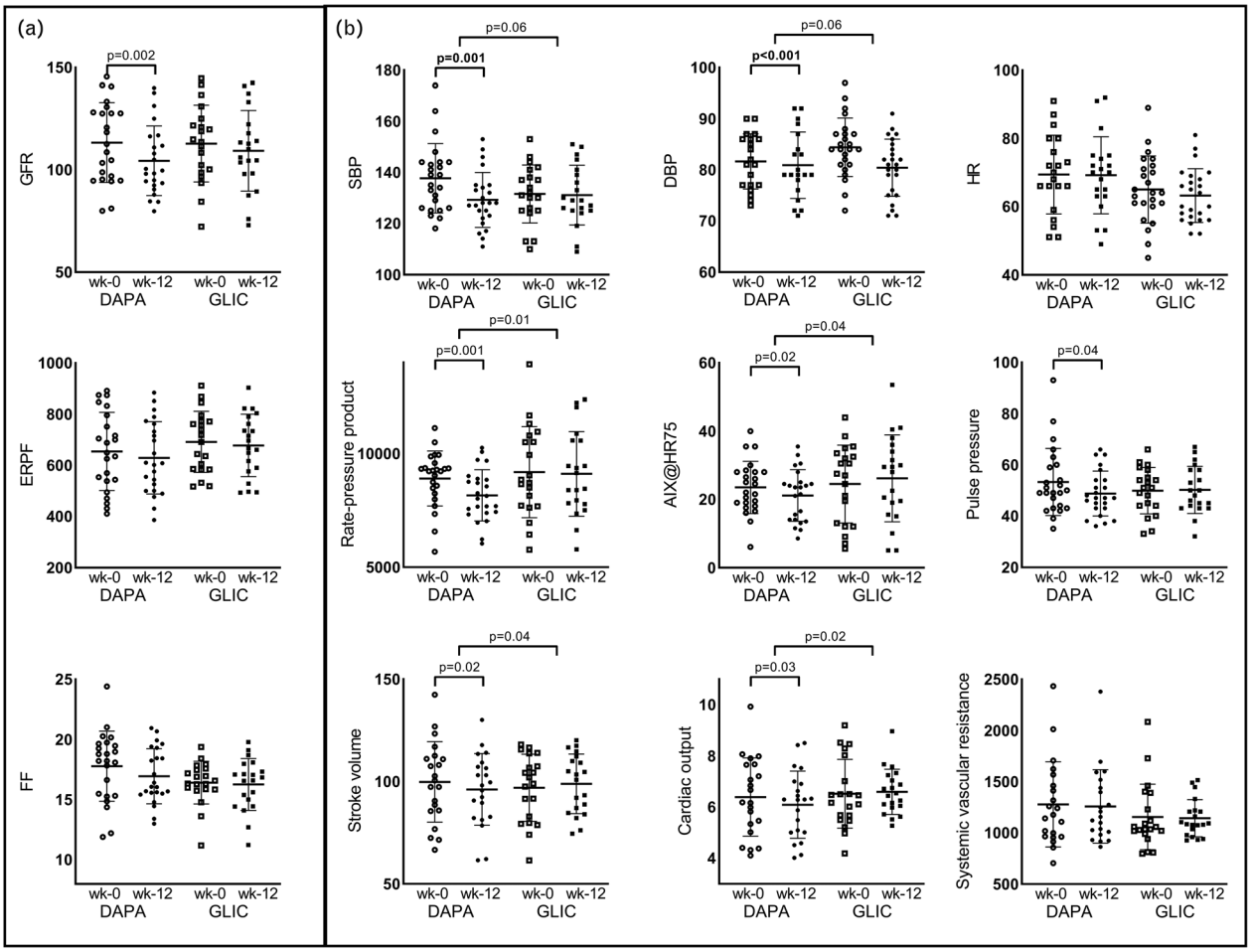

FIGURE 1 (a) Renal and (b) cardiovascular effects of dapagliflozin and gliclazide. Paired *t*-tests were used for within-group comparisons. Multivariable linear regression models were used to examine week 0-corrected dapagliflozin-induced effects compared with gliclazide-induced effects. Significant differences indicated in bold-faced font. Data are mean ± SD. ERPF, effective renal plasma flow; FF, filtration fraction; GFR, measured glomerular filtration rate; HR, heart rate; AIX@HR75, augmentation index standardized for a heart rate of 75 bpm.

Fig. 1b). As a consequence, RPP was reduced by 742 ± 944 mmHg*bpm ($P = 0.001$). Lastly, from baseline, SV was reduced by 3.7 ± 6.4 ml ($P = 0.02$) and CO by 0.3 ± 0.6 l/min ($P = 0.03$), whereas SVR remained unchanged. None of these variables were significantly affected by gliclazide.

Vascular stiffness and heart rate variability

Vascular stiffness was reduced by dapagliflozin, both when measured as AIX@HR75 (−2.7 ± 5.2, $P = 0.02$) and when measured as PP (−4.5 ± 9.8, $P = 0.04$) (Fig. 1b). Gliclazide did not influence vascular stiffness, but did slightly increase SDNN by 2.4 ± 4.3 ($P = 0.03$). HRV, measured as SDNN, RMSSD, and low frequency/high frequency ratio, was otherwise unaffected by either treatment (Table 2).

Renal hemodynamics

Although gliclazide did not significantly affect renal hemodynamic measures, dapagliflozin reduced GFR by 9 ± 12 ml/min ($P < 0.01$) from baseline and RVR by 6 ± 12 mmHg/l ($P < 0.05$). ERPF (−25 ± 74, $P = 0.12$), filtration fraction (−0.8 ± 2.2%; $P = 0.08$), and RBF (+44 ± 288; $P = 0.46$) were not significantly changed by dapagliflozin (Table 2).

Urinary sodium/glucose excretion and hematocrit

Dapagliflozin increased plasma renin and aldosterone levels significantly, whereas they were not affected by gliclazide (Table 2). Neither treatment had an effect on natriuresis after 12 weeks. As expected, dapagliflozin increased 24 h urinary glucose excretion by 466 ± 243 mmol, mmol, urine volume by 335 ± 729 ml and hematocrit by 1.8 ± 1.5% (Table 2).

Relationship between renal and cardiovascular effects of dapagliflozin

When not correcting for multiple testing, the reductions in PP ($r = 0.50$, $P = 0.02$) and SBP ($r = 0.45$, $P = 0.03$) were associated with - and statistically explained respectively 25 and 21% (R^2) of – the reduction in GFR (Fig. 2). No other measures of renal hemodynamics were significantly associated with measures of systemic hemodynamics.

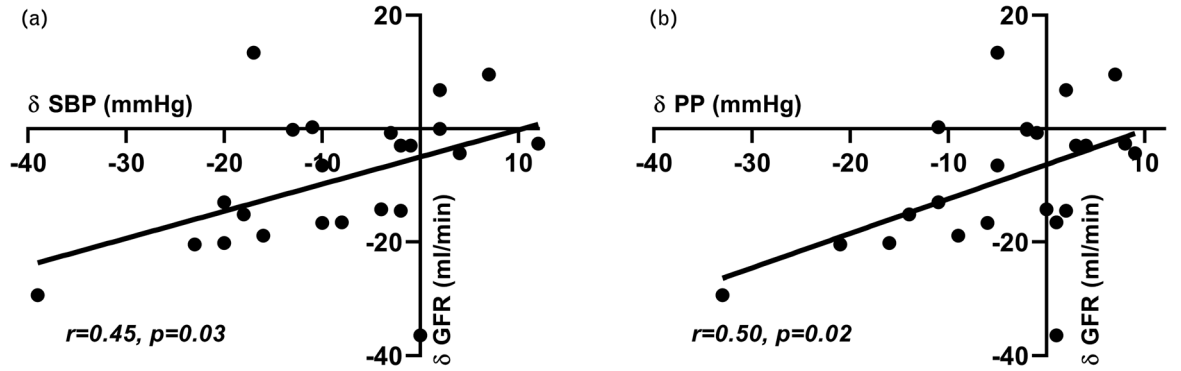

FIGURE 2 Correlations between dapagliflozin-induced changes in (a) SBP and (b) pulse pressure with dapagliflozin-induced changes in glomerular filtration rate. Pearson correlations were used. GFR, measured glomerular filtration rate; PP, pulse pressure.

DISCUSSION

In people with metformin-treated T2D and preserved renal function, dapagliflozin reduced stroke volume, cardiac output, vascular stiffness, and blood pressure from baseline without increasing heart rate or sympathetic nervous system activity, whereas simultaneously lowering GFR, filtration fraction and RVR. That these parameters were unaffected by gliclazide despite similar glucose lowering proves that the effects of dapagliflozin cannot be explained by glucose-lowering alone. Interestingly, the dapagliflozin-induced changes in gold-standard measured renal hemodynamics were almost completely independent of changes in systemic hemodynamics. The ~4 mmHg SBP-lowering effect of SGLT2 inhibitors has been well established in people with T2D [14,15]. In contrast to diuretic agents, like furosemide [16], and other glucose-lowering agents, including GLP1 receptor agonists [17], SGLT2 inhibitors do not cause a compensatory heart rate acceleration [17]. Dapagliflozin lowered SBP by about 8 mmHg in our analysis, which is more than what has been described [14,15], but still, without heart rate acceleration or discernable sympathetic activation. In accordance, empagliflozin did not affect muscle sympathetic nervous system activity while lowering BP in people with T2D [18], while it also did not alter HRV, plasma noradrenalin and adrenalin in people with T1D [19]. Together, this suggests that SGLT2 inhibitor-mediated reductions in effective circulating blood volume/pressure are either too small to activate the sympathetic nervous system, or that potential activation is counteracted by as yet unknown mechanisms. This is clinically relevant as sympathetic activity has been linked to cardiovascular morbidity and mortality [20,21].

SGLT2 inhibitors improve diastolic dysfunction, and maintain or even improve ejection fraction [22–24]. The dapagliflozin-induced reduction in stroke volume from baseline is therefore best explained by reduced preload as a consequence of plasma volume contraction that was previously reported [25].

The consequence of reduced blood pressure at constant HR is a reduction in RPP. This indicates that cardiac workload and myocardial oxygen demand are reduced, and could contribute to the considerable reduction in heart failure hospitalizations with SGLT2 inhibitors, in people both with and without established heart failure at baseline [3,26]. Notably, RPP has strong predictive value for cardiovascular and all-cause mortality [27], and an increase in RPP between heart failure hospitalizations and discharge is associated with increased 30-day mortality and rehospitalization for heart failure [28].

The difference between SBP and DBP, that is, PP, which was decreased by dapagliflozin, is an indirect marker of arterial stiffness. Empagliflozin also reduced PP in a large group of people with T2D, with greater reductions in older patients (especially >75 years) [15]. In people with T2D, PP is an independent risk factor for cardiovascular disease and lowering PP ameliorates cardiovascular risk [29]. Dapagliflozin also lowered radial artery stiffness when objectified by augmentation indices, confirming previous findings in T1D [19] and T2D [15,30]. In contrast, arterial stiffness, also measured as augmentation index or as carotid–femoral pulse-wave velocity, was unaffected by 4 weeks of dapagliflozin treatment in people with T2D, similar to our population [31]. It should be noted that pulse wave velocity is a more sensitive marker to detect changes in arterial stiffness in older individuals and might have been a better measure in our population [32]. However, as dapagliflozin significantly improved augmentation index, this might not have changed our findings. Others have also found that SGLT2 inhibitors improve pulse wave velocity in people with diabetes [33], confirming improved arterial stiffness with the drug-class.

Together, these systemic hemodynamics effects may explain the cardiovascular protection induced by SGLT2 inhibitors in patients with atherosclerotic disease [2,3]. As we studied the effects of dapagliflozin in parallel with those of gliclazide, and observed no effects of gliclazide, we can conclude that the effects of SGLT2 inhibition occur beyond glucose lowering per se. This is supported by the fact that SGLT2 inhibitors effectively reduce heart failure hospitalizations, and progression of heart failure, irrespective of the presence of diabetes [26]. Other factors that potentially contribute to improved cardiovascular outcomes include a myocardial substrate switch from carbohydrates towards free fatty acid and ketone bodies [34], direct effects on cardiac mitochondria [35–37], increased oxygen delivery secondary to increases in hematocrit [38], direct inhibition of the cardiac sodium-hydrogen exchanger [39,40], weight loss, upregulation of angiotensin 1–7, plasma uric acid reductions, and anti-inflammatory or antioxidative actions [1].

The simultaneous measurement of cardiovascular and renal hemodynamic variables gave us the opportunity to investigate the effects of dapagliflozin on the cardiorenal axis using an integrative approach. We previously demonstrated that dapagliflozin induces an acute reduction in GFR [9], a finding that is also observed with eGFR in large-sized clinical trials [8,41–43]. Although others have also indirectly assessed renal hemodynamic function by ultrasound renal resistive index [31], and directly measured GFR by iohexol clearances [25], we are the first to fully measure renal hemodynamics, including ERPF, in people with T2D. This allowed us to also calculate renal blood flow, renal vascular resistance, and filtration fraction. The dapgliflozin-induced GFR reduction we observed reflects reductions in intraglomerular pressure, and is mediated by tubuloglomerular feedback activation, as we discuss in detail elsewhere [9]. It is believed to be the most important explanation for improved renal outcomes in people with T2D [8,41–43]. We deliberately did not correct for multiple testing to minimize the chance of type 2 error, but still only two associations just reached statistical significance. Added to the fact that significant statistical correlations are not proof of biological pathways, they unlikely represent causality. The absence of (clear) significant correlations suggests that – despite the effects on both sides of the cardiorenal axis – dapagliflozin affects the renal and systemic vasculature independently, thus nuancing the strong cardiorenal axis that is often proclaimed. In this regard, it should be appreciated that although the glucose-lowering efficacy of SGLT2 inhibition directly relates to eGFR, the antihypertensive and renal hemodynamic actions do not [44,45].

Considering that the correlations are hypothesis generating at best, it is interesting to see that the dapagliflozin-induced decrease in SBP correlated positively with the decrease in GFR. A relationship that is physiologically explainable: decreased blood pressure could reduce glomerular pressure and hence GFR. Using Gomez formulas, we have previously calculated that dapagliflozin reduces intraglomerular pressure [9], suggesting that this could be the potential mechanism. However, only the change in SBP, but not the changes in DBP and MAP, was associated with changes in GFR, making it improbable that reductions in BP are directly responsible for the drop in GFR. Alternatively, there could be a role for arterial stiffness in regulation of GFR. Increases in PP and pulse wave velocity, both measures of arterial stiffness, have indeed been associated with declining eGFR in older people [46], and PP also significantly correlated with the changes in GFR in our study. However, the absence of an association between changes in augmentation index and changes in GFR make arterial stiffness a less likely mediator of the observed GFR changes.

It should be noted that our trial was primarily designed to compare the effects of dapagliflozin versus gliclazide on measured renal hemodynamics during glucose clamps, and therefore, lacked power to perform between-group analyses for the fasting systemic variables presented here. We, therefore, primarily analyzed the data within both treatment arms. Still, we are the first to simultaneously study the cardiovascular and renal effects of SGLT2 inhibition with an active comparator group, and importantly observed no changes in cardiorenal hemodynamics in our control group despite similar glucose lowering. As the study population was relatively homogenous, our findings should not be translated to people with kidney disease or heart failure. The fact that our participants did not suffer from cardiorenal failure, however, also means that we were able to study the effects in people with relatively normal physiology, and thus, more hemodynamic flexibility. Lastly, it is possible that the strong relationship between cardiovascular and renal disease depends on global structural (fibrotic) changes, and that 12 weeks of treatment is too short to influence such changes.

In conclusion, our findings indicate that 12 weeks of dapagliflozin treatment influences systemic and renal hemodynamics independently and beyond glucose lowering in people with T2D and preserved renal function.

ACKNOWLEDGEMENTS

We are very grateful for all the time and commitment of our study participants without whom we could have never finished the demanding protocol. We would also like to acknowledge the help of the assistants and technicians that were indispensable in the process of data collection: Jeanette Boerop, Ingrid Knufman, Renée de Meijer and Sandra Gassman. Finally, we thank Adele Dijk, Nel Willekes-Koolschijn, and Hiltjo Kuiper for their much appreciated technical laboratory assistance.

Author contributions: E.J.M.vB., M.M.S., M.H.A.M., M.N., M.H.H.K., J.A.J., and D.H.vR. designed and set up the trial. E.J.M.vB., M.M.S., D.R., M.H.A.M., D.T., and H.J.L.H. were involved in sample collection and/or analysis. E.J.M.vB. and M.M.S. performed statistical analysis. E.J.M.vB., M.M.S. and D.H.vR. wrote the first draft of the article. The submitted version was approved by all authors. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Source(s) of funding: This trial was funded by AstraZeneca as an investigator-initiated study. The funder had no role in the study design, the analyses or interpretation of the data, or drafting the manuscript. The funder had no role in the decision to submit this manuscript for publication.

Conflicts of interest

E.J.M.vB., M.M.S., D.R., M.H.H.K., and J.A.J. have no conflicts of interest. M.H.A.M. is a consultant and speaker for Eli Lilly & Co, Sanofi and Novo Nordisk; all honoraria are paid to his employer (AUMC, location VUMC). M.N. received an unrestricted investigator-initiated grant from Astra Zeneca on SGLT2i and lipid fluxes. D.T. reports grants from ZONMW and from Chiesi Pharmaceuticals. H.J.L.H. is a consultant for and receives honoraria (to his employer) from AbbVie, Astellas, Astra-Zeneca, Janssen, Reata Pharmaceuticals, and ZS-Pharma. D.H.vR. has acted 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-Lilly Diabetes Alliance, AstraZeneca and Novo Nordisk all honoraria are paid to his employer (AUMC, location VUMC).

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