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Mechanisms underlying the blood pressure-lowering effects of empagliflozin, losartan and their combination in people with type 2 diabetes: A secondary analysis of a randomized crossover trial

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Abstract

Aim: To study the effects of the sodium-glucose co-transporter-2 (SGLT2) inhibitor empagliflozin, the angiotensin receptor blocker (ARB) losartan, and their combination on blood pressure, while studying the mechanisms potentially involved.

Methods: A total of 24 people with type 2 diabetes (T2D) (age: 66 ± 6 years; body mass index: 31.0 ± 3 kg/m^2; estimated glomerular filtration rate: 90 ml/min/1.73m^2) received a 1-week treatment with empagliflozin 10 mg once daily, losartan 50 mg once daily, their combination, and placebo, in a randomized double-blind crossover design, with 4-week washout periods in between. Blood pressure, arterial stiffness, autonomic nervous system activity and plasma volume, extracellular fluid and serum albumin were assessed.

Results: Versus placebo (139 mmHg), empagliflozin reduced systolic blood pressure (SBP) by 8 mmHg ($P = .001$), losartan by 12 mmHg ($P = .001$) and empagliflozin + losartan by 15 mmHg ($P < .001$). Combination therapy had a larger SBP-lowering effect versus empagliflozin monotherapy (-7 [95% CI -12; -2] mmHg) and numerically larger effects versus losartan monotherapy (-3 [-8; 2] mmHg). Empagliflozin reduced sympathetic nervous system (SNS) activity, arterial stiffness and extracellular fluid, while increasing serum albumin. Losartan reduced SNS activity and arterial stiffness. Combination therapy induced volume contraction variables, together with a reduction in SNS activity and arterial stiffness.

Conclusion: In people with T2D, SGLT2 inhibition in combination with an ARB had a larger blood pressure-lowering effect versus placebo than either of the drugs alone.

Our data further suggest that the mechanisms underlying these blood pressure reductions at least partially differ between these agents.

KEYWORDS
angiotensin receptor blocker, autonomic nervous system activity, blood pressure, empagliflozin, losartan, SGLT2 inhibitor, systemic haemodynamic function, type 2 diabetes

1 | INTRODUCTION

At the time of diagnosis, approximately 60% of people living with type 2 diabetes (T2D) experience hypertension.[1] Hypertension is a major independent risk factor for stroke and death.[1] This may, at least partly, be explained through activation of the renin angiotensin system (RAS) and the sympathetic nervous system (SNS), both of which are associated with macrovascular dysfunction, including increased arterial stiffness and increased vascular tone.[2,3]

Blockade of the RAS pathways with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (ARBs) improves cardiovascular (CV) and kidney outcomes in people with T2D.[4-6] ARBs act by selectively blocking the binding of angiotensin II to the AT1 receptor.[7] In addition, ARBs promote renal sodium and water excretion by decreasing aldosterone secretion.[8] Besides their well-known antihypertensive properties, achieved by blocking the vasoconstrictive actions of angiotensin II, ARBs improve endothelial function[9] and vascular tone.[10,11] Finally, ARBs reduce the collagen content of the arteries and attenuate extracellular matrix remodelling, thereby improving arterial distensibility.[12]

Over the last few years, sodium-glucose co-transporter-2 (SGLT2) inhibitors have been introduced as a therapeutic option for T2D management. These agents induce glycosuria, thereby reducing hyperglycaemia, and reduce the risks of CV disease and end-stage kidney disease in people with or without diabetes and in people with chronic kidney disease (CKD).[13-18] In addition to their glucose-lowering effect, SGLT2 inhibitors lower systolic blood pressure (SBP) with an average of 4 mmHg.[19] The mechanisms underlying their blood pressure-lowering effect remain incompletely understood, but haemodynamic actions including volume contraction could contribute.[20,21] Because the reductions in blood pressure occur without a compensating increase in heart rate (HR), it has been suggested that SGLT2 inhibitors reduce SNS activity.[22-25] Furthermore, improvements in arterial stiffness and endothelial function have been proposed to contribute to the reduction in blood pressure.[26-28]

As SGLT2 inhibitors and RAS blockers are progressively used in combination in people with diabetes, it is of interest to assess their combined effect on blood pressure and systemic haemodynamic function, in particular as their mechanism of action is different, which could result in complementary beneficial actions. Therefore, the aim of this study was to assess the mechanisms underlying the blood pressure reduction with the SGLT2 inhibitor empagliflozin, the ARB losartan, and the empagliflozin + losartan combination compared with placebo in people with T2D.

2 | METHODS

2.1 | Trial design

This was a prespecified secondary analysis of the RECOLAR trial: a phase 4, monocentre, randomized, double-blind, comparator controlled, four-armed crossover mechanistic intervention study conducted from September 2020 to September 2021 at the Amsterdam University Medical Centers (location VUmc, Amsterdam, The Netherlands). The study consisted of 1-week intervention periods for each treatment followed by a 4-week washout period (Figure **S1**). The study protocol, protocol amendments and 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 (ID: NCT04238702).

2.2 | Study population

Participants were recruited from our outpatient clinic database at Diabetes Center Amsterdam UMC. Eligible people were men or postmenopausal women (to prevent confounding effects of the menstrual cycle), aged 45-75 years, with a body mass index of more than 25 kg/m^2 and diagnosed with T2D. For the current treatment of T2D, metformin with or without sulphonylurea derivatives was allowed (stable dose for ≥ 3 months), with HbA1c from 6.5% to 10.5% (48-91 mmol/mol). In the case of previously diagnosed hypertension, only treatment with alpha blockers and/or beta blockers was allowed at a maximum tolerable dose during the study (background medication was actively converted to alpha and/or beta blockers if necessary). Exclusion criteria were a recent (i.e. < 6 months) history of cardiovascular disease (i.e. acute coronary syndrome, stroke or transient ischaemic neurological disorder), diagnosis of heart failure, unstable or rapidly progessing kidney disease, an estimated glomerular filtration rate of less than 60 ml/min per 1.73m^2, 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 within 6 months before inclusion, or the use of non-steroidal 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.

2.3 | Randomization and intervention

Participants were randomized to crossover sequences for empagliflozin 10 mg + losartan 50 mg combination therapy, empagliflozin monotherapy, losartan monotherapy and placebo (block size of four, performed by an independent trial pharmacist using computer-generated numbers; Figure **S1**) (NCT04238702). Boehringer Ingelheim (Germany) provided the empagliflozin and matching empagliflozin placebo tablets. The losartan and losartan placebo tablet were bought at Tiofarma (Oud-Beijerland, the Netherlands). The tablets were encapsulated, resulting in identical oral capsules (Trial Pharmacy, Amsterdam UMC, location AMC, Amsterdam, 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 08:00 PM during each treatment period. Adherence was followed up by counting the remaining capsules at all visits.

2.4 | Endpoint measurements

All measurements were taken after 1 week of each treatment. The week before each kidney testing visit, participants adhered to a standardized sodium chloride (9-12 g/d) diet to minimize variation in kidney physiology because of salt intake (compliance checked by 24-h sodium collection) (Figure **S1**B). After an overnight fast, patients arrived at 08:00 AM at the research unit. Prior to each measurement, patients were acclimatized for at least 10 minutes. All measurements were performed in the fasting state, in a semi-supine position, and in a temperature-controlled room (23.0 ± 1.0°C). Measurements were performed at the non-dominant arm comfortably placed at heart level and appropriate cuff sizes were used where applicable.

2.5 | Systemic haemodynamic function

SBP, diastolic blood pressure (DBP) and HR were determined by an automated oscillometric device (Dinamap, GE Healthcare, Little Chalfont, UK). All measurements were performed in triplicate at 1-2 minute intervals; the mean of the three measurements was used for each time point. Pulse pressure was calculated by subtracting DBP from SBP. Stroke volume (SV), cardiac output (CO) and systemic vascular resistance (SVR) were assessed using a non-invasive beat-to-beat finger arterial photoplethysmography BP device (Nexfin, Amsterdam, The Netherlands). A 30-second average was derived using dedicated software (Nexfin@PC version 2, BMEYE, Amsterdam, the Netherlands). The rate-pressure product (RPP), a marker reflecting SNS activity, was calculated as HR × SBP.[29]

2.6 | Pulse wave analysis

To assess arterial stiffness, pulse wave analysis was performed at the radial artery using applanation tonometry with a high fidelity micromanometer (SPT-301; Millar Instruments, Houston, TX) coupled to a SphygmoCor System (Atcor Medical, West Ryde, Australia).[30,31] To obtain adequate pulse wave profiles and high-quality control, the mean of two recordings (of ≥ 12 s) was used, 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.[30,31] 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).

2.7 | HR variability measures

Cardiac autonomic nervous system (ANS) balance was assessed by resting heart rate variability (HRV). Measurements were obtained by using an electrocardiogram (ECG)-equipped Nexfin device for a 5-minute RR-interval period, during which patients were instructed to breath spontaneously (range 10-18 breaths/min) and refrained from sleeping or speaking. ECG measurements were visually inspected and artifacts were manually corrected using linear interpolation. Patients with atrial fibrillation or severe sinus arrhythmias were excluded from analysis. ECG recordings were loaded into Kubios HRV analysis software 2.2 (University of Eastern Finland, Biosignal Analysis and Medical Imaging Group, Kuopio, Finland). After the removal of trend components and additional automated low-level artifact correction, fast Fourier spectral analyses were performed to obtain normalized low frequency (LF; 0.04-0.15 Hz) and high frequency (HF; 0.15-0.5 Hz) bands. The LF/HF ratio, a validated marker for ANS balance,[32] was calculated and used for the current analysis, with LF contributing to the sympathetic activity and HF to the parasympathetic activity.[33,34]

2.8 | Volume variables

Participants collected 24-hour urine samples that ended on the morning of the testing visit. Urinary volume and excretion of sodium and glucose were subsequently measured. Bioimpedance spectroscopy (ImpediMed Limited, Pinkenba, Queensland, Australia) for standardized measurements of extracellular and intracellular volume was performed. Blood was drawn to measure haematocrit (Hct) and haemoglobin (Hb). The percentage change in estimated plasma volume (ePV) was calculated by the Strauss formula[35]:

$$\Delta \text{ePV} = 100 \times [(\text{Hb placebo}/\text{Hb treatment}) \times (1 - \text{Hct treatment}) / (1 - \text{Hct placebo})] - 100.$$

A calculation of ePV was derived from the Strauss formula and compared between treatment arms. The formula used was:

$$ePV = [(1 - Hct)/(Hb \times 0.01)] \times 1000\,(ml).$$

2.9 | Outcome measures and statistical analyses

All measurements were taken after 1 week of each treatment. Primary endpoints included treatment-induced changes in measured glomerular filtration rate (mGFR) and effective renal plasma flow as derived from iohexol and para-aminohippuric acid clearance methodology, respectively, with timed blood sampling.[36] All other secondary endpoints were prespecified and included all derived systemic haemodynamic measures and volume variables. We based our sample size on the expected difference between placebo and the intervention arms in mGFR using Stata version 11 (College Station, TX). Assuming a standard deviation (SD) of 17.0 ml/min and considering $\alpha = .05$ as significant, it was calculated that 23 participants were needed to achieve a power (1 - β) of 90% to detect a mGFR difference of 10 ml/min. Therefore, to have equal arms in the crossover randomization sequence and to account for a dropout, we included 24 participants. Thus, we did not power for the outcomes described here.

All statistical analyses were performed in the per protocol population using SPSS 24.0 (IBM SPSS, Chicago, IL). A linear mixed model analysis was used to compare our outcome measures between treatments and placebo. The model included a random intercept for subject to take into account the dependency of the observations within one subject and included treatment order as a factor to exclude carry over effects. The assumption of a normal distribution was checked by plotting the residuals of the outcome variable. In case the residual was not normally distributed after log transformation, analyses were performed by using the log-transformed variable. Baseline characteristics were summarized using mean and SD or median and interquartile range. As stated, we powered for the treatment effects versus placebo only. Differences, 95% confidence intervals (CIs) and corresponding *P* values were reported for each treatment compared with placebo. However, we provide the comparisons between combination therapy versus empagliflozin monotherapy and losartan monotherapy for SBP, the most important clinical variable, with treatment difference and 95% CIs. A *P* value of less than .05 was considered to indicate statistical significance.

3 | RESULTS

A total of 24 participants were included and randomized to a 1-week treatment with empagliflozin, losartan, empagliflozin + losartan or placebo (Figure **S1**A). One participant withdrew consent after the first visit and was excluded from all analyses. Overall adherence to study medication was 99%. Background medication remained unchanged during the treatment period. In total, three participants were excluded from the current analysis for ANS activity; two participants because of atrial fibrillation and one because of sinus arrhythmia. Full baseline demographic and clinical characteristics are presented in Table 1.

TABLE 1 Baseline characteristics

	N = 24
Age, y	66 ± 6
Male, *n* (%)	21 (87.5)
Weight (kg)	98 ± 15
BMI (kg/m^2)	31.0 ± 3
Ethnicity–White (*n*)	24/24
Diabetes duration, y	11 (IQR 8-16)
Current smoker, *n* (%)	2 (8.3)
Alcohol intake, units/week	4 (IQR 2-11)
ASCVD, *n* (%)	3 (12.5)
Hypertension, *n* (%)	14 (58.3)
eGFR (CKD-EPI), ml/min/1.73m^2	89.5 (IQR 80-90)
Albuminuria, mg/24 h	13 (IQR 6-26)
HbA1c, %	7.4 ± 0.9
Fasting plasma glucose (mmol/L)	8.8 ± 2.3
Systolic blood pressure, mmHg	145 ± 16
Diastolic blood pressure, mmHg	86 ± 7
Medication use	
Platelet aggregation inhibitor, *n* (%)	5 (20.8)
Metformin, *n* (%)	24 (100)
SU, *n* (%)	11 (45.8)
Statin, *n* (%)	17 (70.8)
Beta blocker, *n* (%)	2 (8.3)
Both alpha and beta blocker, *n* (%)	5 (20.8)

Note: Data are represented as mean ± SD, median (IQR) or frequency.
Abbreviations: ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; SU, sulphfonylurea derivative.

3.1 | Systemic haemodynamic function

Compared with placebo, all treatment arms significantly reduced blood pressure (Table 2 and Figure 1). Empagliflozin treatment resulted in a SBP reduction of 8 (95% CI -14; -3) mmHg and a DBP reduction of 3 (95% CI -5; -0.4) mmHg. Losartan reduced SBP by 12 (95% CI -17; -7) mmHg and DBP by 6 (95% CI -8; -3) mmHg, and empagliflozin + losartan reduced SBP by 15 (95% CI -21; -10) mmHg and DBP by 7 (95% CI -9; -4) mmHg. Empagliflozin and losartan combination therapy had a larger SBP-lowering effect versus empagliflozin monotherapy (-7 [95% CI -12; -2] mmHg) and numerically larger effects versus losartan monotherapy (-3 [-8; 2] mmHg).

HR was not affected by any treatment (Table 2). Compared with placebo, none of the treatment arms had an effect on SV, CO or SVR.

TABLE 2 Measures of cardiovascular function

	Placebo	Empagliflozin	Δ EMPA vs. PLC	Sig.	Losartan	Δ LOS vs. PLC	Sig.	Empagliflozin + losartan	Δ EMPA/LOS vs. PLC	Sig.
Blood pressure and heart rate										
Systolic blood pressure, mmHg	139 ± 13	131 ± 14	−8 (−14; −3)	**0.001**	127 ± 14	−12 (−17; −7)	**<0.001**	124 ± 13.4	−15 (−21; −10)	**<0.001**
Diastolic blood pressure, mmHg	84 ± 6	81 ± 7	−3–(−5; −0.4)	**0.02**	78 ± 6	−6 (−8; −3)	**<0.001**	77 ± 7	−7 (−9; −4)	**<0.001**
Heart rate, bpm	67 ± 11	64 ± 10	−3 (−7; 0.8)	0.11	65 ± 9.4	−1.8 (−5.5; 1.9)	0.32	65 ± 11.1	−2.2 (−6; 1.6)	0.25
Haemodynamics										
Stroke volume (ml)	89 ± 12	90 ± 5	1.2 (-3.6; 6.1)	0.62	94 ± 12	2.8 (-2.2; 7.8)	0.26	90 ± 15	1.0 (-3.8; 5.8)	0.68
Cardiac output (L/min)	5.9 ± 1.0	6.1 ± 1.5	0.2 (−0.2; 0.6)	0.35	6.0 ± 1.0	−0.2 (−0.3; 0.6)	0.46	6.0 ± 1.3	0.08 (−0.3; 0.5)	0.72
Systemic vascular resistance (dyn/s/cm)	1461 ± 338	1358 ± 358	−88 (−238; 63)	0.25	1365 ± 321	−82 (−237; 72)	0.29	1364 ± 321	−82 (−233; 69)	0.28
Arterial stiffness										
AIX_HR75	21 ± 7	20 ± 7	−1.5 (−4.2; 1.2)	0.28	20 ± 8	−1.5 (−4.3; 1.3)	0.30	20 ± 7	−1.8 (−4.6; 0.9)	0.19
Pulse pressure (mmHg)	56 ± 10	50 ± 9	−6 (−9.0; −2.3)	**0.001**	49 ± 10	−7 (−10.1; −3.4)	**0.000**	48 ± 9	−8 (−11.8; −4.9)	**0.000**
Autonomic function										
LF/HF ratio	71.1 [52.8–91.4]	57.8 [42.2–75.1]	−7.7 [−17.9; 3.7]	0.17	64.5 [47.7–83.3]	−3.8 [−14.6; 8.5]	0.52	56.2 [40.8–73.3]	−8.6 [−18.7; 2.7]	0.13
Rate pressure product, mmHg*beats min^{-1}	9343 ± 1571	8514 ± 1436	−689 (−1182; −195)	**0.007**	8408 ± 1364	−794 (−1289; −301)	**0.002**	8202 ± 1571	−1120 (−1620; −619)	**0.000**
Volume variables										
Haematocrit (%)	41.6 ± 2.7	41.9 ± 3.0	0.3 (−0.4; 1.1)	0.42	41.3 ± 3.3	−0.3 (−1.0; 0.5)	0.49	42.3 ± 3.3	0.7 (0.0; 1.5)	0.06
Albumin, g/L	37.3 ± 2.0	38.1 ± 2.4	0.9 (0.1; 1.6)	**0.03**	36.7 ± 2.1	−0.6 (−1.4; 0.2)	0.14	38.4 ± 1.8	1.1 (0.4; 1.9)	**0.004**
Extracellular fluid (L)	22.2 ± 4.1	21.7 ± 4.1	−0.5 (−1.0; −0.1)	**0.01**	22.4 ± 4.5	0.2 (−0.2; 0.6)	0.35	21.8 ± 3.9	−0.4 (−0.9; −0.02)	**0.04**
Intracellular fluid (L)	26.2 ± 4.6	26.1 ± 4.9	−0.1 (−0.8; 0.5)	0.69	26.7 ± 5.2	0.5 (−0.2; 1.1)	0.14	26.1 ± 4.6	−0.07 (−0.7; 0.6)	0.82
Estimated plasma volume (ml)	6971 ± 1060	6919 ± 1076	−0.65 [−3.3; 1.9]	0.62	6969 ± 1015	0.18 [−2.4; 2.8]	0.89	6854 ± 1192	−2.14 [−4.8; 0.5]	0.11
Urine volume (ml)	2025 ± 731	2241 ± 778	216 (−46; 516)	0.10	1840 ± 552	−185 (−463; 93)	0.19	2344 ± 803	338 (57; 620)	**0.02**
Glucose excretion (mmol/24 h)	59 ± 147	532 ± 297	473 (385; 558)	**<0.001**	27 ± 46	−32 (−117; 53)	0.46	572 ± 271	513 (425; 598)	**<0.001**
Sodium excretion (mmol/24 h)	177 ± 70	180 ± 89	3 (−28; 33)	0.88	172 ± 72	−5 (−34; 26)	0.79	190 ± 92	13 (−19; 42)	0.46
Body weight, kg	98.5 ± 16.1	97.0 ± 16.1	−1.4 (−2.0; −0.8)	**<0.001**	98.5 ± 16.4	−0.02 (−0.6; 0.7)	0.96	97.4 ± 15.9	−1.1 (−1.7; −0.4)	**0.001**

Note: Measurements of cardiac function at baseline after 7 days of treatment ($N = 20$). Mixed effect models were used to examine outcomes between arms. Data are represented as mean (SD) or geometric mean [95% CI] and absolute changes (95% CI) or percentage change [95% CI]. Significant differences are indicated in bold font.

Abbreviations: AIX@HR75, augmentation index normalized for a HR of 75 bpm; EMPA, empagliflozin; HF, high frequency; LF, low frequency; LOS, losartan; PLC, placebo.

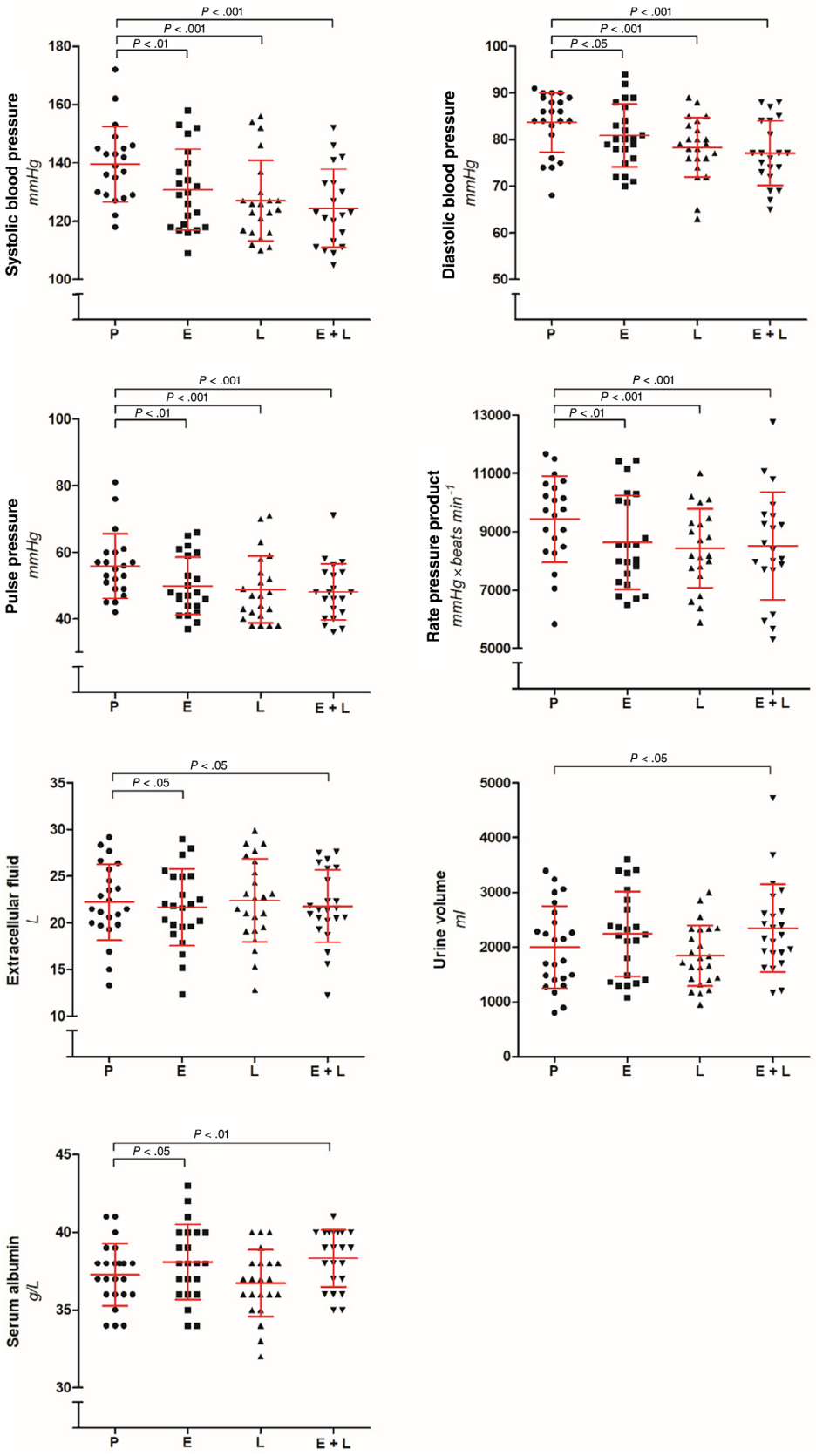

FIGURE 1 Changes in blood pressure and potentially involved mechanisms. Changes in blood pressure, autonomic function and indirect measurements of plasma volume. Datapoints represent mean with SEM. P = Placebo, E = Empagliflozin, L = Losartan, E + L = Empagliflozin + Losartan. Statistically significant mean differences of treatments compared with placebo are indicated with brackets

3.2 | Arterial stiffness

None of the treatments had a significant effect on AIX@HR75, while pulse pressure was significantly reduced in all treatment arms (Table 2). Compared with placebo, empagliflozin reduced pulse pressure by 6 (95% CI -9.0; -2.3) mmHg, losartan by 7 (95% CI -10.1; -3.4) mmHg and empagliflozin + losartan by 8 (95% CI -11.8; -4.9) mmHg.

3.3 | ANS activity

No effect on LF/HF ratio was observed in any of the treatment arms. RPP was significantly reduced in all arms compared with placebo. Empagliflozin reduced RPP by 689 (95% CI -1182; -195) mmHg*beats min^{-1}, losartan by 794 (95% CI -1289; -301) mmHg*beats min^{-1} and empagliflozin + losartan by 1120 (95% CI -1620; -619) mmHg*beats min^{-1}.

3.4 | Body composition and indicators of plasma volume

Compared with placebo, empagliflozin and empagliflozin + losartan significantly decreased body weight by 1.4 kg (95% CI -2.0; -0.8) and 1.1 kg (95% CI -1.7; -0.4), respectively, while losartan did not change body weight (Table 2). Hct numerically increased by 0.3 (95% CI -0.4; 1.1)% and 0.7 (95% CI 0.0; 1.5)% during empagliflozin and empagliflozin + losartan treatment, respectively, but this did not reach statistical significance. Serum albumin was significantly increased by empagliflozin and empagliflozin + losartan treatment, but did not change during losartan monotherapy (Table 2).

Compared with placebo, empagliflozin and empagliflozin + losartan significantly decreased extracellular fluid by 0.5 L (95% CI -1.0; -0.1) and 0.4 L (95% CI -0.9; -0.02), respectively, while losartan did not change extracellular fluid (Table 2). No changes in intracellular fluid were observed.

No significant alterations in estimated plasma volume were observed in the empagliflozin and losartan arms, while empagliflozin + losartan tended to reduce estimated plasma volume by 2.1% (95% CI -4.8; 0.5), not reaching statistical significance (Table 2).

Urinary volumes were significantly increased by empagliflozin + losartan (+338 ml; 95% CI 57; 620) and tended to increase by empagliflozin monotherapy (+235 ml; 95% CI -46; 516), while no changes were observed in the losartan monotherapy arm (Table 2). There were no significant associations between changes in blood pressure and changes in urine volume, extracellular fluid or estimated plasma volume (data not shown).

4 | DISCUSSION

The first goal of this secondary prespecified analysis of a randomized, double-blind, crossover trial was to determine whether combined RAS inhibition and SGLT2 inhibition had larger effects on blood pressure than either of the drugs alone, as compared with placebo. Second, we investigated the involved mechanisms underlying these blood pressure-lowering effects. Compared with placebo, we observed a greater reduction in blood pressure in the empagliflozin + losartan combination arm than the effects of the empagliflozin and losartan monotherapy arms versus placebo. In addition, combination therapy had a significant larger effect on SBP than empagliflozin monotherapy and a numerically larger effect versus monotherapy. This may indicate that combination therapy may induce synergistic effects on blood pressure lowering, which is in line with a recently published meta-analysis,[37] although we were underpowered to formally test this.

ARBs have antihypertensive properties, achieved by blocking the vasoconstrictive actions of angiotensin II.[10] In addition, ARBs reduce systemic vascular resistance[9] and improve endothelial function,[11] potentially being factors contributing to the blood pressure-lowering effect observed in the losartan monotherapy arm. Furthermore, ARBs reduce the collagen content of the arteries and attenuate extracellular matrix remodelling, thereby improving arterial distensibility.[38] A reduction in pulse pressure leads to improvement of arterial distensibility and thereby reduction of arterial stiffness.[39] We observed a significant reduction in pulse pressure in the losartan arm compared with placebo. This may indicate that a reduction in arterial stiffness may play a role in the blood pressure-lowering effect of losartan.

Angiotensin II exerts different actions on the SNS, including a central action to increase sympathetic stimulation, stimulatory effects on sympathetic ganglia and adrenal medulla, and actions at sympathetic nerve endings that serve to facilitate sympathetic neurotransmission. There is considerable evidence that the actions of endogenous angiotensin II on the SNS enhance the cardiovascular responses elicited by the activation of the SNS.[40] By inhibiting angiotensin II using ARBs, these actions on SNS activity are consequently diminished. We indeed observed a reduction in SNS activity, mainly by a reduction in RPP, which may indicate a dampening effect of ARBs on SNS activity. In this trial, we did not find any changes in volume variables in the losartan monotherapy arm compared with placebo, suggesting that a reduction in arterial stiffness and SNS activity contributes to a larger extent than volume contraction to the blood pressure-lowering effect of RAS inhibition.

We also investigated the contributing factors that could explain the reduction in blood pressure with the SGLT2 inhibitor empagliflozin. One of the underlying factors may be volume contraction, secondary to (osmotic) diuresis, which leads to a reduction in preload and afterload.[20,41] In the current study, compared with placebo, empagliflozin induced a significant reduction in extracellular fluid together with an increase in serum albumin, suggesting volume contraction. However, despite a significant increase in glucose excretion, empagliflozin did not change ePV. As extracellular volume is the sum of plasma volume and interstitial volume, this may suggest that, although not directly measured, a proportionally larger decrease in interstitial volume occurred, which is consistent with the results suggested by Hallow et al. in a modelling study of SGLT2 inhibitor effects,[42] and reflected by the lack of change in Hct. In addition, we did not find any changes in sodium excretion, which is in concordance with previous studies showing that the natriuretic effect with SGLT2 inhibitors is modest and transient,[43-45] possibly because of compensatory kidney mechanisms.

Normally, a reduction in blood pressure promotes a baroreflex-mediated increase in SNS activity, leading to an increase in HR. SGLT2 inhibitors, however, reduce blood pressure without an increase in HR, which may suggest a diminishing of SNS activity.[23] In line with this knowledge, we did not observe an increase in HR in the

empagliflozin arm. This premise was underlined by a concomitant decrease in RPP[29] and LF:HF ratio, although the latter did not reach significance, supporting the potential role of reduced SNS activity. However, whether the effect of empagliflozin on SNS activity contributes to the long-term blood pressure reduction cannot be ascertained.

In people with T2D and type 1 diabetes, empagliflozin led to a reduction in arterial stiffness, which has been associated with improved blood pressure control.[46,47] Although we did not find a significant decrease in the variable AIX@HR75, possibly because of the short-term treatment period, we observed a significant reduction in pulse pressure following empagliflozin treatment, which is strongly correlated with arterial stiffness.[48]

Finally, empagliflozin exerts a consistent significant weight-lowering effect in patients with T2D.[49,50] Weight loss reduces blood pressure through a variety of mechanisms including anti-inflammatory effects.[51,52] In this study, compared with placebo, empagliflozin significantly reduced body weight and this reduction was associated with reductions in SBP ($r = 0.4$; $P = .07$). Together, these findings suggest that volume contraction, a reduction in SNS activity, improvement in arterial stiffness and body weight reduction are contributing factors to the blood pressure reduction observed with SGLT2 inhibition therapy.

Given that the largest reduction in SBP was observed in the combination therapy arm, we believe that these agents have beneficial combined effects. This is also based on the finding that both drugs reduced arterial stiffness and SNS activity; empagliflozin, moreover, induced volume contraction and body weight reductions.

While the double-blind, randomized, four-armed crossover design is a major strength, we also acknowledge some limitations. First, we powered on the different treatment arms compared with placebo and had insufficient power to compare combination therapy with either of the monotherapies alone for all endpoints. Instead we performed this analysis for SBP, the most clinically relevant variable that was obtained. Second, we were unable to reproduce the RAS inhibition-induced reduction in SVR, which may be explained by the non-invasive measurement of SVR, which is not the gold-standard invasive Fick or thermodilution method to assess systemic haemodynamics,[53] although the non-invasive measurement devices are well validated against intra-arterial measurements.[54] We also did not measure endothelial function. Moreover, 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.[55] Finally, we measured the acute effects of the interventions on systemic haemodynamic function, while longer treatment duration could elicit different effects, and we admit limited generalizability as our participants included White men and postmenopausal women.

In conclusion, we show that SGTL-2 inhibition combined with the RAS blocker losartan has a larger blood pressure-lowering effect than either of the agents alone when compared with placebo. We also show that the drugs have both overlapping and different regulatory effects on systemic haemodynamic function. Future dedicated and sufficiently powered studies should address the role for RAS blockade-SGLT2 inhibitor combination therapy in people with diabetes and hypertension.

AUTHOR CONTRIBUTIONS

RAS and DHvR designed and set up the trial. ACH and CMM were involved in sample collection and/or analysis. RAS performed statistical analysis. RAS and DHvR wrote the first draft of the paper, and the submitted version was approved by all authors.

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CONFLICT OF INTEREST

RAS, ACH, CMM and MMS have no conflicts of interest. DHvR 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). HJLH has received research support from AbbVie, AstraZeneca, Boehringer Ingelheim and Janssen; fees to his institution for his participation in advisory boards for Merck, Mitsubishi Tanabe, Janssen and Mundipharma; as a consultant for AbbVie, Retrophin, Boehringer Ingelheim and Novo Nordisk; and for participation in steering committees for Janssen, Gilead, Bayer, Chinook and CSL Pharma.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Chen G, McAlister FA, Walker RL, Hemmelgarn BR, Campbell NR. Cardiovascular outcomes in Framingham participants with diabetes: the importance of blood pressure. *Hypertension*. 2011;57(5):891-897.

2. Naha S, Gardner MJ, Khangura D, Kurukulasuriya LR, Sowers JR. In: Feingold KR, Anawalt B, Boyce A, et al., eds. *Diabetes and hypertension*. South Dartmouth, MA: Endotext; 2000.
3. Perin PC, Maule S, Quadri R. Sympathetic nervous system, diabetes, and hypertension. *Clin Exp Hypertens*. 2001;23(1–2):45-55.
4. Tatti P, Pahor M, Byington RP, et al. Outcome results of the Fosinopril versus amlodipine cardiovascular events randomized trial (FACET) in patients with hypertension and NIDDM. *Diabetes Care*. 1998;21(4): 597-603.
5. Lewis EJ, Hunsicker LG, Clarke WR, et al. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med*. 2001;345(12): 851-860.
6. Brenner BM, Cooper ME, de Zeeuw D, et al. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med*. 2001;345(12):861-869.
7. Schmieder RE. Mechanisms for the clinical benefits of angiotensin II receptor blockers. *Am J Hypertens*. 2005;18(5 Pt 1):720-730.
8. Scott JH, Menouar MA, Dunn RJ. *Physiology, Aldosterone*. Treasure Island, FL: Statpearls Publishing; 2022.
9. Podzolkov VI, Bulatov VA, Son EA, Os I. Central and peripheral hemodynamic effects of losartan and in combination with hydrochlorothiazide in mild to moderate essential hypertension. *Blood Press*. 2003; 12(4):239-245.
10. Baan J Jr, Chang PC, Vermeij P, Pfaffendorf M, van Zwieten PA. Effects of losartan on vasoconstrictor responses to angiotensin II in the forearm vascular bed of healthy volunteers. *Cardiovasc Res*. 1996; 32(5):973-979.
11. Kim JH, Oh SJ, Lee JM, et al. The effect of an angiotensin receptor blocker on arterial stiffness in type 2 diabetes mellitus patients with hypertension. *Diabetes Metab J*. 2011;35(3):236-242.
12. Omboni S. Do arterial stiffness and wave reflections improve more with angiotensin receptor blockers than with other antihypertensive drug classes? *J Thorac Dis*. 2016;8(7):1417-1420.
13. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med*. 2015; 373(22):2117-2128.
14. Heerspink HJL, Stefansson BV, Correa-Rotter R, et al. Dapagliflozin in patients with chronic kidney disease. *N Engl J Med*. 2020;383(15): 1436-1446.
15. Perkovic V, Jardine MJ, Neal B, et al. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N Engl J Med*. 2019; 380(24):2295-2306.
16. Cannon CP, Pratley R, Dagogo-Jack S, et al. Cardiovascular outcomes with ertugliflozin in type 2 diabetes. *N Engl J Med*. 2020;383(15): 1425-1435.
17. Neal B, Perkovic V, Matthews DR. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med*. 2017;377(21): 2099.
18. Wiviott SD, Raz I, Bonaca MP, et al. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. 2019;380(4):347-357.
19. Kario K, Ferdinand KC, O'Keefe JH. Control of 24-hour blood pressure with SGLT2 inhibitors to prevent cardiovascular disease. *Prog Cardiovasc Dis*. 2020;63(3):249-262.
20. Inzucchi SE, Zinman B, Fitchett D, et al. How does Empagliflozin reduce cardiovascular mortality? Insights from a mediation analysis of the EMPA-REG OUTCOME trial. *Diabetes Care*. 2018;41(2): 356-363.
21. Wanner C, Inzucchi SE, Lachin JM, et al. Empagliflozin and progression of kidney disease in type 2 diabetes. *N Engl J Med*. 2016;375(4): 323-334.
22. Jordan J, Tank J, Heusser K, et al. The effect of empagliflozin on muscle sympathetic nerve activity in patients with type II diabetes mellitus. *J Am Soc Hypertens*. 2017;11(9):604-612.
23. Scheen AJ. Effect of SGLT2 inhibitors on the sympathetic nervous system and blood pressure. *Curr Cardiol Rep*. 2019;21(8):70.
24. Zelniker TA, Braunwald E. Mechanisms of cardiorenal effects of sodium-glucose cotransporter 2 inhibitors: JACC state-of-the-art review. *J Am Coll Cardiol*. 2020;75(4):422-434.
25. van Bommel EJM, Smits MM, Ruiter D, et al. Effects of dapagliflozin and gliclazide on the cardiorenal axis in people with type 2 diabetes. *J Hypertens*. 2020;38:1811-1819.
26. Filippatos TD, Tsimihodimos V, Elisaf MS. Mechanisms of blood pressure reduction with sodium-glucose co-transporter 2 (SGLT2) inhibitors. *Expert Opin Pharmacother*. 2016;17(12):1581-1583.
27. Lopaschuk GD, Verma S. Mechanisms of cardiovascular benefits of sodium glucose co-transporter 2 (SGLT2) inhibitors: a state-of-the-art review. *JACC Basic Transl Sci*. 2020;5(6):632-644.
28. Solini A, Giannini L, Seghieri M, et al. Dapagliflozin acutely improves endothelial dysfunction, reduces aortic stiffness and renal resistive index in type 2 diabetic patients: a pilot study. *Cardiovasc Diabetol*. 2017;16(1):138.
29. Valensi P, Chiheb S, Fysekidis M. Insulin- and glucagon-like peptide-1-induced changes in heart rate and vagosympathetic activity: why they matter. *Diabetologia*. 2013;56(6):1196-1200.
30. Chen CH, Nevo E, Fetics B, et al. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. *Validation of generalized transfer function. Circulation*. 1997;95(7): 1827-1836.
31. Pauca AL, O'Rourke MF, Kon ND. Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. *Hypertension*. 2001;38(4):932-937.
32. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation*. 1996;93(5):1043-1065.
33. Shaffer F, Ginsberg JP. An overview of heart rate variability metrics and norms. *Front Public Health*. 2017;5:258.
34. Shaffer F, Meehan ZM. A practical guide to resonance frequency assessment for heart rate variability biofeedback. *Front Neurosci*. 2020;14:570400.
35. Fudim M, Miller WL. Calculated estimates of plasma volume in patients with chronic heart failure-comparison with measured volumes. *J Card Fail*. 2018;24(9):553-560.
36. van Bommel EJM, Muskiet MHA, van Baar MJB, et al. 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. *Kidney Int*. 2020;97(1):202-212.
37. Tian B, Deng Y, Cai Y, Han M, Xu G. Efficacy and safety of combination therapy with sodium-glucose cotransporter 2 inhibitors and renin-angiotensin system blockers in patients with type 2 diabetes: a systematic review and meta-analysis. *Nephrol Dial Transplant*. 2022; 37(4):720-729.
38. Struthers AD, MacDonald TM. Review of aldosterone- and angiotensin II-induced target organ damage and prevention. *Cardiovasc Res*. 2004;61(4):663-670.
39. Lacombe F, Dart A, Dewar E, Jennings G, Cameron J, Laufer E. Arterial elastic properties in man: a comparison of echo-Doppler indices of aortic stiffness. *Eur Heart J*. 1992;13(8):1040-1045.
40. Reid I. Interactions between ANG II, sympathetic nervous system, and baroreceptor reflexes in regulation of blood pressure. *Endocrinol Metab*. 1992;262(6):E763-E778.
41. Verma S, McMurray JJV. SGLT2 inhibitors and mechanisms of cardiovascular benefit: a state-of-the-art review. *Diabetologia*. 2018;61(10): 2108-2117.
42. Hallow KM, Helmlinger G, Greasley PJ, McMurray JJV, Boulton DW. Why do SGLT2 inhibitors reduce heart failure hospitalization? A

differential volume regulation hypothesis. *Diabetes Obes Metab*. 2018;20(3):479-487.
43. Scholtes RA, Muskiet MHA, van Baar MJB, et al. Natriuretic effect of two weeks of Dapagliflozin treatment in patients with type 2 diabetes and preserved kidney function during standardized sodium intake: results of the DAPASALT trial. *Diabetes Care*. 2021; 44(2):440-447.
44. Tanaka H, Takano K, Iijima H, et al. Factors affecting Canagliflozin-induced transient urine volume increase in patients with type 2 diabetes mellitus. *Adv Ther*. 2017;34(2):436-451.
45. Iijima H, Kifuji T, Maruyama N, Inagaki N. Pharmacokinetics, pharmacodynamics, and safety of Canagliflozin in Japanese patients with type 2 diabetes mellitus. *Adv Ther*. 2015;32(8): 768-782.
46. Chilton R, Tikkanen I, Cannon CP, et al. Effects of empagliflozin on blood pressure and markers of arterial stiffness and vascular resistance in patients with type 2 diabetes. *Diabetes Obes Metab*. 2015; 17(12):1180-1193.
47. Cherney DZ, Perkins BA, Soleymanlou N, et al. The effect of empagliflozin on arterial stiffness and heart rate variability in subjects with uncomplicated type 1 diabetes mellitus. *Cardiovasc Diabetol*. 2014; 13:28.
48. Mitchell GF. Arterial stiffness and wave reflection in hypertension: pathophysiologic and therapeutic implications. *Curr Hypertens Rep*. 2004;6(6):436-441.
49. Kovacs CS, Seshiah V, Swallow R, et al. Empagliflozin improves glycaemic and weight control as add-on therapy to pioglitazone or pioglitazone plus metformin in patients with type 2 diabetes: a 24-week, randomized, placebo-controlled trial. *Diabetes Obes Metab*. 2014; 16(2):147-158.
50. Haring HU, Merker L, Seewaldt-Becker E, et al. Empagliflozin as add-on to metformin plus sulfonylurea in patients with type 2 diabetes: a 24-week, randomized, double-blind, placebo-controlled trial. *Diabetes Care*. 2013;36(11):3396-3404.
51. Samaras K, Viardot A, Lee PN, et al. Reduced arterial stiffness after weight loss in obese type 2 diabetes and impaired glucose tolerance: the role of immune cell activation and insulin resistance. *Diab Vasc Dis Res*. 2013;10(1):40-48.
52. Cooper JN, Buchanich JM, Youk A, et al. Reductions in arterial stiffness with weight loss in overweight and obese young adults: potential mechanisms. *Atherosclerosis*. 2012;223(2):485-490.
53. Zhang Z, Ansari S, Wang L, Aaronson KD, Golbus JR, Oldham KR. Non-invasive systemic vascular resistance estimation using a photoplethysmogram and a piezoelectric sensor. *IFAC*. 2021;54(20):298-303.
54. Bogert LW, Wesseling KH, Schraa O, et al. Pulse contour cardiac output derived from non-invasive arterial pressure in cardiovascular disease. *Anaesthesia*. 2010;65(11):1119-1125.
55. McEniery CM, Yasmin HIR, Qasem A, et al. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff collaborative trial (ACCT). *J Am Coll Cardiol*. 2005; 46(9):1753-1760.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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