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

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Abstract

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

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

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

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

KEYWORDS
dapagliflozin, heart failure, SGLT2 inhibitor, type 2 diabetes

1 | INTRODUCTION

Sodium-glucose co-transporter-2 (SGLT2) inhibitors induce glycosuria and sodium excretion by inhibiting glucose and sodium reabsorption in the proximal tubule, and several members of this class are approved for the treatment of type 2 diabetes mellitus. The renal inhibition of glucose and sodium reabsorption by SGLT2 inhibitors promotes osmotic/natriuretic diuresis and a reduction in plasma, interstitial and extravascular volume.[1,2] A previous study assessed plasma volume by ^{125}I-human serum albumin, a "gold standard" technique, and demonstrated a reduction of 7% after 12 weeks' treatment with the SGLT2 inhibitor dapagliflozin.[3] The reduction in plasma volume was accompanied by increases in haematocrit and haemoglobin.[3] A decrease in plasma volume reduces ventricular filling pressure and cardiac

workload and may explain some of the beneficial effects regarding hospitalization for heart failure and associated mortality observed in recent cardiovascular outcome trials with SGLT2 inhibitors.[4-6] A post hoc analysis from the EMPAREG cardiovascular outcomes trial for the SGLT2 inhibitor empagliflozin suggested that increases in haematocrit and haemoglobin are important mediators of the reduction in cardiovascular mortality observed in the EMPAREG trial.[7]

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

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

2 | MATERIALS AND METHODS

2.1 | Study design and study population

We used data from a previous study to assess and compare changes in ePV, calculated with the Strauss formula, with mPV.[3] The original study examined the effects of dapagliflozin versus placebo or hydrochlorothiazide in 75 patients with inadequately controlled levels of glycated haemoglobin (HbA1c; ≥ 6.6% and ≤ 9.5%) and blood pressure (systolic blood pressure ≥ 130 and < 165 mmHg, diastolic blood pressure ≥ 80 and < 105 mmHg).[3] Patients were randomly assigned to a 12-week treatment period of dapagliflozin 10 mg/d, hydrochlorothiazide 25 mg/d, or matched placebo. The original mechanistic study enrolled, in a sub-study, 30 patients in whom plasma volume was measured at baseline and at week 12. In the present analysis we included patients in whom plasma volume, haemoglobin and haematocrit were recorded to compare mPV with ePV (N = 10).

Subsequently, we performed a pooled analysis of 13 phase 2b/3 placebo-controlled clinical trials in patients with type 2 diabetes (Figure S1). These studies examined the glucose-lowering effects of dapagliflozin 10 mg/d as monotherapy or in combination with other glucose-lowering drugs in patients with inadequately controlled HbA1c levels. The core study periods were 12 to 24 weeks in duration. The results of these studies were published previously.[9-21]

2.2 | Measurements

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

$$([\{Hb_{baseline}/Hb_{end}\} \times \{(100-Ht_{end})/(100-Ht_{baseline})\}]-1)\times 100,$$

where $Hb_{baseline}$ and $Ht_{baseline}$ are the haemoglobin and haematocrit levels at baseline, and Hb_{end} and Ht_{end} are the haemoglobin and haematocrit levels at end of treatment. The Strauss formula was used on the assumption that there was no or limited change in red blood cell production and red blood cell lifespan during 12 weeks of dapagliflozin therapy.

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

2.3 | Statistical analyses

Baseline characteristics are presented as descriptive statistics. Mean change from baseline in ePV and its 95% confidence interval (CI) were calculated using a longitudinal repeated-measures mixed model with fixed terms for treatment, study, week and week-by-treatment interaction. The Kenward-Roger method was used. If the model did not converge, the Satterthwaite approximation was used. The effect of dapagliflozin on ePV was assessed in various subgroups including subgroups defined by baseline estimated glomerular filtration rate (eGFR), diuretic use, and cardiovascular disease history. Subgroup analyses were performed by adding the subgroup and the interactions subgroup-by-treatment and subgroup-by-week-by-treatment to the model. Pearson or Spearman correlation analyses were performed to calculate correlations between changes in ePV and changes in HbA1c, fasting plasma glucose, systolic blood pressure, eGFR, body weight, and urinary albumin to creatinine ratio (UACR; for the subgroup with baseline UACR >30 mg/g) at week 24 of dapagliflozin therapy.

3 | RESULTS

3.1 | Comparison of mPV and ePV

Plasma volume measurements by ^{125}I -human serum albumin, as well as haemoglobin and haematocrit measurements, were available in ten patients and were included in the present analysis.[3] The median (25th to 75th percentile) baseline mPV was 2539 (2535-2787) mL in the patients who received dapagliflozin and 2547 (2330-2677) mL in the placebo group. Median haemoglobin levels were 14 g/dL in both the

dapagliflozin group and placebo group. Median haematocrit levels were ~40% in both groups, respectively (Table S1).

Median (first to third quartile) changes in mPV and ePV during dapagliflozin treatment were – 9.0 (−11.5 to −5.5)% and – 9.4 (−9.9 to −7.7)% (P = 0.80 vs mPV), respectively. The changes in mPV and ePV during placebo treatment were 5.2 (−2.5 to 7.1)% and 0.3 (−2.0 to 5.0)% (P = 0.96 vs mPV), respectively. Lin's concordance index was 0.6 (P < 0.01; Figure 1). The similar median changes in mPV and ePV during dapagliflozin treatment and the significant Lin's concordance index supported the use of the Strauss formula to assess effects of dapagliflozin on ePV in the pooled clinical trial database.

3.2 | Effects of dapagliflozin on ePV in the pooled clinical trial database

The pooled analysis included 4533 patients, of whom 2295 received dapagliflozin 10 mg/d and 2238 received placebo. Baseline characteristics are shown in Table 1. HbA1c was 8%, haemoglobin was 14 g/dL and haematocrit was 42% in both the dapagliflozin group and the placebo group. Changes in haemoglobin and haematocrit over time are shown in Figure S2.

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

The effects of dapagliflozin versus placebo on ePV observed in the overall population were consistent in various patient subgroups (Figure 3). Specifically, compared to placebo, dapagliflozin reduced ePV by 9.9% (95% CI 7.7 to 12.2) in patients receiving diuretics and by 9.6% (95% CI 8.9 to 10.2) in patients not using diuretics (P value for treatment by subgroup interaction = 0.37). Among patients with a history of cardiovascular disease or heart failure, dapagliflozin compared to placebo reduced ePV by 9.7% (95% CI 8.8 to 10.6). In patients without a history of cardiovascular disease or heart failure ePV was reduced by 9.5% (95% CI 8.7 to 10.3), compared to placebo (P value for treatment by subgroup interaction = 0.66). Dapagliflozin decreased ePV by 9.5% in patients with an eGFR <60 mL/min/1.73m^2, as well as in patients with an eGFR ≥90 mL/min/1.73m^2 (P value for treatment by subgroup interaction = 0.90).

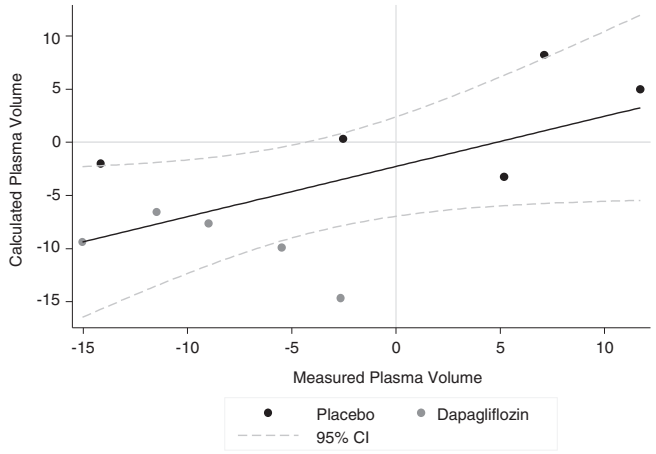


FIGURE 1 Correlation between measured plasma volume, assessed by ^{125}I-human serum albumin, and estimated plasma volume, estimated with the Strauss formula, in a sub-study of patients in whom measured plasma volume as well as haemoglobin and haematocrit values were available. CI, confidence interval

TABLE 1 Baseline characteristics

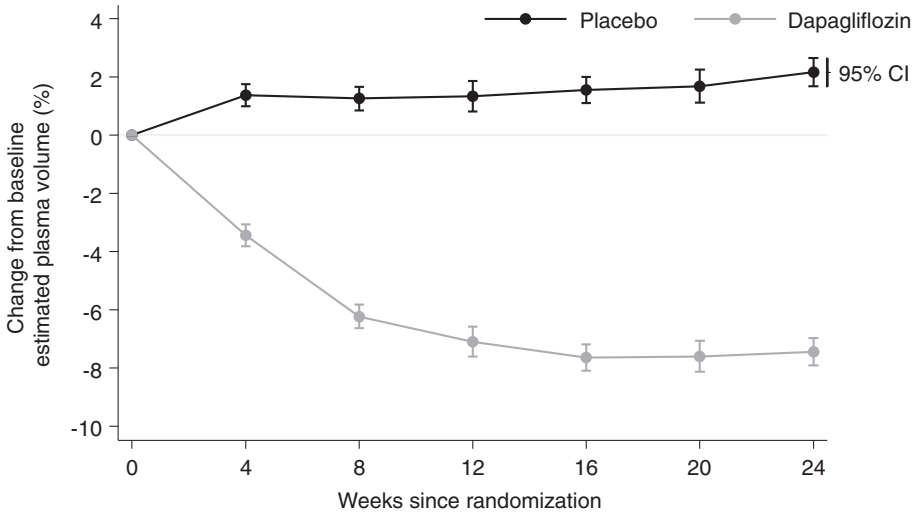
	Placebo (n = 2238)	Dapagliflozin 10 mg (n = 2295)
Age, years	58.9 (9.9)	58.4 (10.0)
Men, n (%)	1312 (58.6)	1320 (57.5)
Women, n (%)	926 (41.4)	975 (42.5)
BMI, kg/m^2	32 (5.8)	32 (5.7)
HbA1c, %	8.2 (0.9)	8.2 (0.9)
Fasting plasma glucose, mg/dL	165.3 (45.3)	165.2 (46.7)
Systolic blood pressure, mmHg	131.6 (14.9)	131.7 (15.4)
Estimated GFR, mL/min/1.73 m^2	82.3 (20.1)	82.8 (20.2)
Median (25th to 75th percentile) UACR, mg/g	10.0 (5.0 to 33.0)	10.0 (5.0 to 33.0)
Haemoglobin, g/dL	14.1 (1.3)	14.1 (1.3)
Haematocrit, %	42.4 (4.0)	42.3 (4.0)
Diuretic use: Yes, n (%)	261 (11.7)	229 (10.0)
Insulin use: Yes, n (%)	779 (34.8)	756 (32.9)
History of CVD/HF at baseline, yes, n (%)	1105 (49.4)	1115 (48.6)
History of PVD/PAD at baseline, yes, n (%)	288 (12.9)	287 (12.5)

Data are mean (SD) unless otherwise stated.
Abbreviations: BMI, body mass index; CVD, cardiovascular disease; GFR, glomerular filtration rate; HbA1c, glycated haemoglobin; HF, heart failure; PAD, peripheral artery disease; PVD, peripheral vascular disease; UACR, urinary albumin/creatinine ratio.

In continuous analyses, there were statistically significant though weak correlations between changes in ePV at week 24 and concurrent changes in HbA1c, fasting plasma glucose, body weight and eGFR during dapagliflozin treatment. ePV did not correlate with systolic blood pressure or UACR (Table 2).

4 | DISCUSSION

The present study showed that the Strauss formula might be a useful equation to estimate changes in plasma volume during dapagliflozin treatment in patients with type 2 diabetes mellitus. Using the formula we observed that dapagliflozin 10 mg/d relative to placebo reduced ePV by 9.6% in a broad population of patients with type 2 diabetes



Number of patients with haemoglobin and haematocrit levels:

Placebo	2238	2185	2108	1156	1852	856	1796
Dapagliflozin	2295	2240	2175	1231	1932	937	1908

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

mellitus. The reduction in ePV was fully present after 8 to 12 weeks of dapagliflozin therapy and was sustained until 24-week follow-up. The effect of dapagliflozin on ePV was consistent in various patient subgroups, highlighting the consistency of this effect among patients with type 2 diabetes mellitus.

To our knowledge, only two small studies have examined the effects of SGLT2 inhibitors on plasma volume in people with diabetes.[3,23] Heerspink et al.[3] found a median (interquartile range) plasma volume change from baseline of −7.3 (−12.4 to −4.8)% after 12 weeks of dapagliflozin treatment. Sha et al.[23] found a mean plasma volume change from baseline of −5.4%, with a difference compared to placebo of −9.7% (95% CI −17.8 to −1.6) after 1 week of treatment with canagliflozin, which was attenuated at week 12. These studies used ^{125}I-labelled human serum albumin or indocyanine green to measure plasma volume. These measurements are cumbersome for patients and time-consuming. Estimation equations have therefore been developed. The Strauss formula was originally developed to estimate changes in plasma volume over time in patients with congestive heart failure and has not yet been used to estimate plasma volume changes in patients with type 2 diabetes mellitus.[8,24] The results of the present study indicate that the Strauss formula may be a useful equation to estimate changes in plasma volume in patients with type 2 diabetes mellitus who receive dapagliflozin or placebo.

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

Reduction of plasma volume is one of the hypothesized mechanisms underlying the observed reduction in heart failure events in cardiovascular outcome trials with SGLT2 inhibitors.[4-6] Other underlying mechanisms that are hypothesized to contribute to the beneficial heart failure outcomes are: a reduction of the cardiac afterload by reducing blood pressure and arterial stiffness; reducing inflammatory pathways; improved myocardial energy use as a result of shifts in metabolic substrates from fatty acids to ketone bodies; and activation of energy synthesis and antioxidant pathways in cardiomyocytes by inhibiting the Na^+/H^+ exchanger and consequently decreasing intracellular sodium/calcium while increasing mitochondrial calcium.[27,28] SGLT2 inhibitors also delay the progression of kidney function loss, which may also contribute to heart failure protection.[2,29] According to the prescribing information for SGLT2 inhibitors, they are not recommended for clinical use in patients with impaired kidney function due to reduced glycaemic efficacy; therefore one might expect to observe a smaller effect on ePV in patients with reduced kidney function. However, non-glycaemic effects,[30,31] including

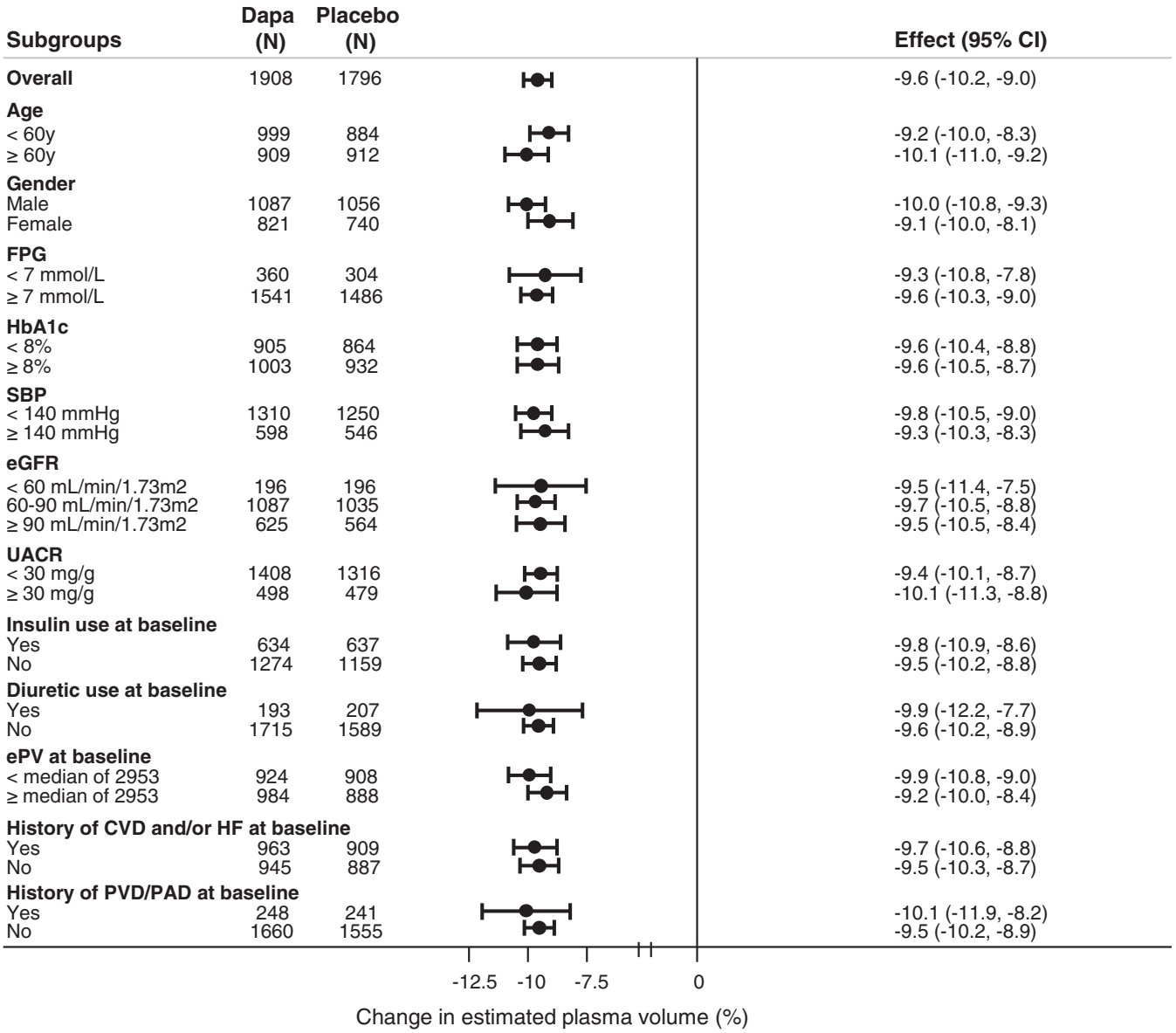


FIGURE 3 Changes from baseline in estimated plasma volume (%) during 24-week treatment with dapagliflozin relative to placebo in various subgroups. CVD, cardiovascular disease; ePV, estimated plasma volume; FPG, fasting plasma glucose; HF, heart failure; PAD, peripheral artery disease; PVD, peripheral vascular disease; UACR, urinary albumin:creatinine ratio. ePV at baseline was calculated with the Kaplan-hakim formula[24]

effects on ePV as observed in the present study, persist among patients with eGFR levels <60 mL/min/1.73m^2. Presumably the natriuretic and osmotic diuretic effects persist in patients with a moderate decreased kidney function. Yet the proportion of patients in the present analysis with moderate to severe chronic kidney disease was relatively small; only one patient had an eGFR <30 mL/min/1.73m^2. Hence, we cannot extrapolate our findings to this population with severe loss of renal function. In addition, our analysis demonstrated that effects on ePV were consistent regardless of diuretic use or prevalent heart failure. These findings were also observed in recent cardiovascular outcome trials that showed that effects of SGLT2 inhibitors were not modified by baseline diuretics use or presence of congestive heart failure.[4-6]

The present study has some limitations. First, the number of patients in whom both ePV and mPV was determined was small. Although changes in ePV corresponded with the changes in mPV, we acknowledge that the Strauss formula should be validated in larger cohorts of patients with diabetes mellitus without heart failure. Second, ePV remains a proxy for actual plasma volume. The Strauss formula uses changes in haematocrit and haemoglobin, which could have been influenced by dapagliflozin-induced changes in erythropoietin.[3] We cannot exclude the possibility that dapagliflozin has an effect on red blood cell production or turnover. Accordingly, changes in ePV may not only reflect changes in volume status and may be an overestimation of the true change; however, it is interesting that the change in ePV comparing dapagliflozin with placebo in the pooled

TABLE 2 Pearson correlations between percentage change from baseline at 24 weeks in estimated plasma volume and change from baseline in various cardiovascular risk markers during dapagliflozin treatment

	ePV	*P*
HbA1c	−0.08	<.01
Fasting plasma glucose	−0.05	.04
Systolic blood pressure	−0.01	.62
Estimated GFR	0.15	<.01
Body weight	0.09	<.01
UACR[a]	−0.07	.10

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

[a]Only patients with baseline UACR >30 mg/g were included.

analysis was similar to the change in mPV in the mechanistic study. The notion that ePV may also be affected by direct effects on haematopoiesis might explain the relatively slow onset of the reduction of ePV in the pooled analysis, which was expected to occur faster. Increased haematocrit can improve the myocardial oxygen delivery, which may also play a beneficial role. Imaging studies such as DAPACARD (NCT03387683) and SIMPLE (NCT03151343) will specifically investigate intra-cardiac oxygen consumption. These studies may provide additional insight into the mechanism behind the cardiovascular and heart failure benefits of SGLT2 inhibitors. In addition, further studies in broader populations with type 2 diabetes are needed, such as those with and without congestive heart failure and with different stages of chronic kidney disease, to confirm and generalize our results.

To conclude, dapagliflozin significantly reduced estimated plasma volume in a broad range of patients with type 2 diabetes. Ongoing studies such as DAPA-HF and DELIVER in patients with heart failure with reduced or preserved ejection fraction (NCT03036124 and NCT03619213) as well as mechanistic studies will provide additional insight into the cardioprotective effects of this SGLT2 inhibitor.

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CONFLICT OF INTEREST

C.C.J.D. reports no conflicts of interest. H.J.L.H. is a consultant for and received honoraria from AbbVie, Astellas, Astra Zeneca, Boehringer Ingelheim, Fresenius, Janssen and Merck. He has a policy that all honoraria are paid to his employer. C.D.S., P.J.G. and D.W.B. are employees and stockholders of AstraZeneca. V.C. is a former employee of AstraZeneca and owns AstraZeneca stock.

AstraZeneca was involved in the design, execution and analysis of each original study.

AUTHOR CONTRIBUTIONS

C.C.J.D. and H.J.L.H. designed the study, performed the data analysis and interpretation, and wrote the first draft of the manuscript. C.D.S., P.J.G. and D.W.B. contributed to data collection, analysis and interpretation, and contributed to critical revisions of the manuscript. V.C. was involved in the analysis and interpretation of the pooled data.

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SUPPORTING INFORMATION

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

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