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Potential contribution of haemoconcentration to changes in lipid variables with empagliflozin in patients with type 2 diabetes: A post hoc analysis of pooled data from four phase 3 randomized clinical trials

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Abstract

Aim: To examine the association between changes in lipids and markers of haemoconcentration (haematocrit and serum albumin) with empagliflozin, a sodium-glucose co-transporter-2 inhibitor, in patients with type 2 diabetes (T2D) using pooled data from four phase 3 randomized trials.

Materials and Methods: Patients with T2D received placebo (n = 825), empagliflozin 10 mg (n = 830) or 25 mg (n = 822) for 24 weeks. In post hoc mediation analyses, we assessed total changes in LDL-cholesterol, HDL-cholesterol, triglycerides, apolipoprotein (Apo) B, and Apo A-I, and changes in these variables associated with, and independent of, changes in haematocrit and serum albumin at week 24 using ANCOVA models.

Results: Empagliflozin versus placebo increased serum LDL-cholesterol, HDL-cholesterol, and Apo A-I, decreased triglycerides (empagliflozin 10 mg only), and (non-significantly) increased Apo B. Empagliflozin modestly increased haematocrit and serum albumin. In mediation analyses, haematocrit changes (increases) with empagliflozin were associated with significant changes (increases) in all lipid variables, including Apo B. Except for triglycerides (non-significant), similar lipid variable associations were observed with serum albumin changes. Haematocrit- and serum albumin-independent changes in lipids with empagliflozin were significant for HDL-cholesterol (increases), mostly significant for triglycerides (decreases), and less so for other lipid fractions.

Conclusion: Haematocrit and serum albumin increases were associated with increases in lipid fractions with empagliflozin. Empagliflozin-associated changes in serum lipids, particularly LDL-cholesterol increases, may be partly attributable to haemoconcentration resulting from increased urinary volume and subsequent volume contraction.

KEYWORDS

antidiabetes drug, clinical trial, empagliflozin, SGLT2 inhibitor, type 2 diabetes

1 | INTRODUCTION

Empagliflozin is a potent and selective sodium-glucose co-transporter-2 (SGLT2) inhibitor[1] that is used in the treatment of type 2 diabetes (T2D) and heart failure with reduced ejection fraction.[2] SGLT2 is located in the nephron proximal tubule and is responsible for approximately 90% of filtered glucose reabsorption, in co-transport with sodium.[3] Previous studies have shown that the inhibition of SGLT2 by empagliflozin leads to increased urinary glucose and, transiently, sodium excretion.[4,5]

Empagliflozin transiently increases urinary volume, probably as a result of the osmotic effects of glucosuria and transient natriuresis.[5] An increase in serum osmolality has been observed with empagliflozin treatment,[5] suggesting that the increase in urine volume and subsequent volume contraction may result in haemoconcentration. A persistent increase in haematocrit has been shown with longer-term empagliflozin treatment,[6] which is consistent with a new steady-state level of volume status (i.e. persistent haemoconcentration probably as a result of volume contraction). Volume contraction might persist beyond the initial transient increase in urinary volume if, for example, the transient increase in fluid loss is incompletely compensated for by an increase in fluid intake or redistribution of fluid between body compartments over time.

Haematocrit and serum or plasma albumin are surrogate markers of changes in plasma volume[7,8] and haematocrit concentrations are commonly used in assessments of changes in estimated plasma volume.[9] A given change in volume (e.g. plasma volume), without change in the amount of solutes, is expected to result in the same relative (percentage) change in the concentration of all solutes irrespective of their initial concentration.

In four phase 3 trials of patients with T2D, empagliflozin 10 or 25 mg once daily reduced HbA1c, weight, and blood pressure versus placebo and was well tolerated.[10-13] The latter is generally in line with findings for the safety profile of other members of the drug class and empagliflozin, which includes an increased risk of adverse events (AEs) such as genital infections and volume depletion.[14-16]

In these trials, small increases in LDL-cholesterol and HDL-cholesterol, and with similar or (non-significantly) lower levels of triglycerides, from baseline were also observed with empagliflozin.[10-13]

To assess whether lipid changes, in particular changes in LDL-cholesterol (as a major pro-atherogenic lipid fraction), with empagliflozin are partly attributable to haemoconcentration as a result of increased urinary volume and volume contraction, we evaluated the contributions of changes in haematocrit and serum albumin to changes in lipid fractions with empagliflozin.

2 | RESEARCH DESIGN AND METHODS

2.1 | Study design and participants

We assessed pooled data from participants with T2D from four phase 3, randomized placebo-controlled trials, receiving empagliflozin 10 mg,

25 mg, or placebo once daily as monotherapy (n = 676), add-on to metformin (n = 637), add-on to metformin + sulphonylurea (n = 666), or add-on to pioglitazone ± metformin (n = 498) for 24 weeks.[10-13] Boehringer Ingelheim was the sponsor of these registrational phase 3 trials for empagliflozin to show efficacy in patients with T2D. The studies were conducted with similar protocols and identical visit structure; as such, individual patient-level data were available and could be combined across the trials to support the presented analyses. The designs and results of these trials have been reported.[10-13]

Key inclusion criteria comprised: T2D diagnosis; HbA1c of 7% or higher and 10% or less (≥53 and ≤86 mmol/mol, respectively); body mass index (BMI) of 45 kg/m^2 or less; and an estimated glomerular filtration rate (eGFR) of 30 mL/min/1.73m^2 or higher (≥50 mL/min/1.73m^2 with monotherapy) at baseline. Blood samples were collected in the fasting state (minimum 10 hours). These studies were approved by the Institutional Review Boards and Independent Ethics Committees, and Competent Authorities of the participating centre. Studies also complied with the Declaration of Helsinki, in accordance with the International Conference on Harmonization Good Clinical Practice guidelines. All study participants provided written informed consent.

2.2 | Endpoints

Absolute changes from baseline were assessed at week 24 for HbA1c and weight; relative changes were assessed for urinary albumin to creatinine ratio (UACR); percentage and absolute changes were assessed for haematocrit, serum albumin, LDL-cholesterol, HDL-cholesterol, triglycerides, apolipoprotein (Apo) B, and Apo A-I. Additionally, the placebo-subtracted percentage and absolute changes in each lipid fraction with empagliflozin 10 and 25 mg, as well as changes associated with and independent of haematocrit and serum albumin changes, were assessed at week 24.

Blood levels of lipids and albumin were measured using serum samples. LDL-cholesterol was calculated by the Friedewald equation (LDL-cholesterol [calculated] = total cholesterol − HDL-cholesterol − [triglycerides/5]) in samples with triglycerides of 400 mg/dL (4.52 mmol/L) or below[17] and measured directly by colorimetric methods (Roche Modular) in samples with triglycerides above 4.52 mmol/L. The use of the Friedewald equation and direct measurement of LDL-cholesterol in samples with triglycerides above 4.52 mmol/L was prespecified in the protocols of each trial. Triglycerides and cholesterol were measured by enzymatic assay (Roche Modular); albumin and HDL-cholesterol by colorimetric methods (Roche Modular); Apo B and Apo A-I by turbidimetric methods (Roche Modular); and haematocrit by electronic cell counter (Beckman Coulter LH series). HbA1c was analysed using the Bio-Rad high-performance liquid chromatography method. Urinary albumin and creatinine concentrations were measured by the turbidimetric method (Roche) and the alkaline picrate-kinetic method (Roche), respectively.

Safety was assessed based on AEs, which were coded according to the Medical Dictionary for Drug Regulatory Activities (MedDRA) version 15.0. AEs of special interest included confirmed hypoglycaemia (plasma glucose ≤ 3.9 mmol/L and/or requiring assistance), AEs consistent with urinary tract infection (based on a prospectively defined search of 67 MedDRA-preferred terms), AEs consistent with genital infection (based on a prospectively defined search of 87 MedDRA-preferred terms), AEs consistent with volume depletion (based on eight MedDRA-preferred terms), and diabetic ketoacidosis.

2.3 | Statistical methods

The analyses we present here were selected post hoc. Changes from baseline in the endpoint in question at week 24 were analysed using an ANCOVA model, including baseline HbA1c and baseline of the endpoint in question as linear covariates and baseline eGFR (Modification of Diet in Renal Disease [MDRD]), study, region, and treatment as fixed effects. Haematocrit, serum albumin, lipids and UACR were safety variables and were analysed using an ANCOVA model in randomized participants who received one or more doses of study medication (treated set). HbA1c and body weight were efficacy endpoints and were analysed using an ANCOVA model in participants in the treated set who had a baseline HbA1c value (i.e. the full analysis set). A last observation carried forward (LOCF) approach was used to impute missing data; only for the efficacy endpoints (i.e. HbA1c and body weight), the values after rescue medication were set to missing.

As a general assessment of potential associations between changes in haematocrit, serum albumin, and lipids, we investigated the correlation between changes in these variables, with LDL-cholesterol as the lipid variable. Regression lines and Spearman coefficients were generated to assess correlations between percentage change from baseline in haematocrit and percentage change in serum albumin, between percentage change in haematocrit and percentage change in LDL-cholesterol, and between percentage change in serum albumin and percentage change in LDL-cholesterol. We did not assess correlations between haematocrit or albumin versus other lipid fractions. As empagliflozin is known to reduce the level of albumin excretion in the urine,[18] we assessed the correlation between changes in serum albumin and urinary albumin concentration as a potential pathway for changes in serum albumin with empagliflozin. All safety analyses were descriptive and performed in the treated set.

Placebo-subtracted total changes in each lipid fraction, as well as placebo-subtracted changes associated with and independent of changes in haematocrit or albumin, were assessed applying separate models for each empagliflozin dose by adding the baseline values, changes from baseline in haematocrit or serum albumin, and their interaction with treatment, as covariates to the ANCOVA. This approach is a mediation analysis, an analysis of associations between variables to quantify the statistical contribution of the change in one variable to the change in another variable.[19,20] Total change in each lipid fraction was estimated separately for each model and was

therefore not identical between models including haematocrit or serum albumin changes, respectively. The mediation effect (%) of the haematocrit change (the mediator) on the lipid fraction change was derived from model estimates as the percentage of the lipid fraction change associated with haematocrit change relative to the total lipid fraction change. The mediation effect of the serum albumin change was derived accordingly. We present the percentage mediation effect only for endpoints that showed a statistically significant overall treatment effect with empagliflozin versus placebo and a statistically significant contribution of the mediator to the overall treatment effect. For other endpoints, we present only the estimated haematocrit- and albumin-associated changes. Additionally, sensitivity analyses using absolute changes were performed.

The use of lipid-lowering drugs was explored; the new use of lipid-lowering drugs was compared between empagliflozin doses and placebo using logistic regression. A sensitivity analysis to the mediation analysis included baseline use of lipid-lowering drugs.

3 | RESULTS

3.1 | Baseline characteristics

In total, 825, 830, and 822 participants were treated with placebo, empagliflozin 10 mg, and empagliflozin 25 mg, respectively, for 24 weeks.

Baseline demographics were similar between treatment groups (Table 1). Mean (standard deviation [SD]) age was 55.6 (10.2) years, mean HbA1c was 7.99% (0.85%) (64 [9.3] mmol/mol), and mean BMI was 28.7 (5.5) kg/m^2. Median (interquartile range) UACR was 9.7 (5.3 to 24.8) mg/g creatinine and, accordingly, most patients presented with normoalbuminuria (78.3%). Mean (SD) baseline levels of total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides were 4.69 (1.08), 2.59 (0.92), 1.27 (0.33), and 1.92 (1.60) mmol/L, respectively, while the mean (SD) baseline levels of Apo B and A-I were 0.91 (0.27) and 1.38 (0.27) g/L, respectively. The mean (SD) baseline haematocrit and serum albumin levels were 41.8% (4.2%) and 45.2 (2.9) g/L, respectively.

Of 2477 participants, approximately one-third received lipid-lowering therapy at baseline, with the majority of these participants receiving statins (Table 1). At baseline, 17.2% received diuretics.

3.2 | HbA1c and weight changes

Reductions in HbA1c and weight after 24 weeks were significantly greater with empagliflozin than placebo. The placebo-subtracted adjusted mean (95% confidence intervals [CI]) changes from baseline in HbA1c at week 24 were −0.62% (−0.69%, −0.55%) (−6.79 [−7.58, −5.99] mmol/mol) with empagliflozin 10 mg and −0.68% (−0.75%, −0.61%) (−7.44 [−8.23, −6.64] mmol/mol) with empagliflozin 25 mg (P < .001 for both doses vs. placebo). The placebo-subtracted adjusted mean (95% CI) changes from baseline in weight at week 24 were −1.81 (−2.05, −1.57) kg with

TABLE 1 Baseline demographics and clinical characteristics

	Placebo (N = 825)	Empagliflozin 10 mg (N = 830)	Empagliflozin 25 mg (N = 822)	Total (N = 2477)
Male	424 (51.4)	463 (55.8)	464 (56.4)	1351 (54.5)
Age, y	55.7 (10.1)	55.9 (10.3)	55.3 (10.2)	55.6 (10.2)
Race				
White	338 (41.0)	346 (41.7)	339 (41.2)	1023 (41.3)
Asian	467 (56.6)	462 (55.7)	462 (56.2)	1391 (56.2)
Other	20 (2.4)	22 (2.7)	21 (2.6)	63 (2.5)
BMI, kg/m^2	28.6 (5.5)	28.7 (5.5)	28.8 (5.6)	28.7 (5.5)
Weight, kg	78.0 (18.8)	78.7 (18.7)	79.1 (19.0)	78.6 (18.8)
eGFR (MDRD), mL/min/1.73m^2	87.3 (19.9)	87.1 (20.4)	87.8 (21.0)	87.4 (20.4)
HbA1c, % (mmol/mol)	8.03 (0.86) (64 [9.4])	7.98 (0.85) (64 [9.2])	7.96 (0.85) (64 [9.3])	7.99 (0.85) (64 [9.3])
Total cholesterol, mmol/L	4.70 (1.11)	4.67 (1.05)	4.70 (1.09)	4.69 (1.08)
LDL-cholesterol, mmol/L	2.62 (0.93)	2.57 (0.91)	2.57 (0.92)	2.59 (0.92)
HDL-cholesterol, mmol/L	1.26 (0.33)	1.26 (0.32)	1.27 (0.34)	1.27 (0.33)
Triglycerides, mmol/L	1.86 (1.26)	1.95 (1.55)	1.96 (1.91)	1.92 (1.60)
Apo B, g/L	0.92 (0.27)	0.90 (0.27)	0.91 (0.27)	0.91 (0.27)
Apo A-I, g/L	1.37 (0.26)	1.38 (0.27)	1.38 (0.26)	1.38 (0.27)
Haematocrit, %	41.5 (4.2)	41.9 (4.2)	41.9 (4.2)	41.8 (4.2)
Albumin, g/L	45.2 (3.0)	45.3 (2.9)	45.2 (2.9)	45.2 (2.9)
UACR, median (IQR)	9.7 (6.2, 27.4)	9.7 (5.3, 23.9)	9.7 (5.3, 23.0)	9.7 (5.3, 24.8)
UACR				
Normoalbuminuria	635 (77.3)	652 (78.8)	643 (78.6)	1930 (78.3)
Microalbuminuria	157 (19.1)	146 (17.7)	155 (18.9)	458 (18.6)
Macroalbuminuria	29 (3.5)	29 (3.5)	20 (2.4)	78 (3.2)
Time since diagnosis of T2D, y				
≤1	112 (13.6)	139 (16.7)	134 (16.3)	385 (15.5)
>1 to 5	301 (36.5)	289 (34.8)	271 (33.0)	861 (34.8)
>5 to 10	234 (28.4)	216 (26.0)	238 (29.0)	688 (27.8)
>10	178 (21.6)	186 (22.4)	179 (21.8)	543 (21.9)
Systolic blood pressure, mmHg	128.6 (14.6)	129.6 (14.9)	129.0 (15.4)	129.1 (15.0)
Diastolic blood pressure, mmHg	78.0 (8.7)	78.7 (9.0)	78.3 (8.6)	78.3 (8.8)
Lipid-lowering medication[a]	268 (32.5)	294 (35.4)	270 (32.8)	832 (33.6)
Niacin	4 (0.5)	3 (0.4)	5 (0.6)	12 (0.5)
Fibrates	35 (4.2)	28 (3.4)	37 (4.5)	100 (4.0)
Statins	229 (27.8)	260 (31.3)	224 (27.3)	713 (28.8)
Other	22 (2.7)	21 (2.5)	24 (2.9)	67 (2.7)
Diuretics[a]	139 (16.8)	139 (16.7)	147 (17.9)	425 (17.2)

Note: Data are mean (SD) or n (%) in the treated set.

Abbreviations: Apo, apolipoprotein; BMI, body mass index; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; SD, standard deviation; T2D, type 2 diabetes; UACR, urine albumin to creatinine ratio.

[a]Based on selected terms according to the World Health Organization Anatomical Therapeutic Chemical Classification System.

empagliflozin 10 mg and −2.01 (−2.25, −1.76) kg with empagliflozin 25 mg (P < .001 for both doses vs. placebo).

3.3 | Haematocrit and albumin changes

Modest increases in haematocrit and serum albumin were observed with empagliflozin, in contrast to small decreases observed with placebo (Table 2). At week 24, the placebo-subtracted adjusted mean (95% CI) percentage increases from baseline in haematocrit were 5.22% (4.66%, 5.79%) and 5.45% (4.88%, 6.02%) with empagliflozin 10 and 25 mg, respectively (both P < .0001 vs. placebo; Table 2). At week 24, the placebo-subtracted adjusted mean (95% CI) percentage increases from baseline in serum albumin were 1.14% (0.67%, 1.61%) and 0.93% (0.46%, 1.40%) with empagliflozin 10 and 25 mg, respectively (both P < .001 vs. placebo). In the simple correlation analysis,

percentage changes from baseline in haematocrit were weakly correlated with percentage changes from baseline in serum albumin for each treatment group ($P < .0001$; Figure S1).

An 8% decrease in UACR was observed with both empagliflozin doses versus placebo in the overall population (adjusted geometric mean ratio [95% CI] empagliflozin vs. placebo: 0.92 [0.86, 0.99], $P = .034$, for empagliflozin 10 mg, and 0.92 [0.85, 0.99], $P = .023$, for empagliflozin 25 mg). The change in urinary albumin concentration was not significantly correlated with the change in serum albumin in either the placebo or the empagliflozin treatment groups (Spearman correlation coefficients were all positive and <0.06 with P values >.05; otherwise, data not shown).

3.4 | Lipid fraction changes

Compared with placebo, increases from baseline in LDL-cholesterol, HDL-cholesterol, Apo B (non-significantly), and Apo A-I, and decreases from baseline in triglycerides, were observed with empagliflozin at week 24 (Tables 2 and S1). The placebo-subtracted adjusted mean (95% CI) percentage increases from baseline in LDL-cholesterol were 3.40% (0.42%, 6.38%) and 4.44% (1.47%, 7.42%) with empagliflozin 10 and 25 mg, respectively (both $P < .05$). Corresponding percentage increases from baseline in HDL-cholesterol were 5.47% (3.97%, 6.96%) and 5.11% (3.61%, 6.60%) with empagliflozin 10 and 25 mg, respectively (both $P < .001$) (Table 2).

Week 24 placebo-subtracted adjusted mean (95% CI) percentage changes in triglycerides from baseline reached statistical significance with empagliflozin 10 mg only: -6.90% (-11.32%, -2.48%) for empagliflozin 10 mg ($P < .01$) and -3.58% (-7.99%, 0.84%) for empagliflozin 25 mg (Table 2).

The placebo-subtracted adjusted mean (95% CI) percentage increases from baseline in Apo A-I at week 24 were 3.60% (1.86%, 5.33%) and 3.59% (1.85%, 5.33%) with empagliflozin 10 and 25 mg, respectively (both $P < .0001$; Table 2). For Apo B, placebo-subtracted percentage changes from baseline at week 24 were 1.48% (-0.64%, 3.60%) for empagliflozin 10 mg and 2.04% (-0.09%, 4.16%) for empagliflozin 25 mg, neither reaching statistical significance.

For all lipid fractions, the absolute placebo-subtracted changes from baseline were in the same direction as the percentage change from baseline (Tables 2 and S1).

3.5 | Associations of changes in haematocrit and albumin to changes in lipid fractions

In the following, descriptions related to changes in variables pertain to percentage changes unless otherwise stated; the direction of change in variables, typically specified in brackets (increase or decrease) refers to the direction of the mean change of all participants in that variable and not exclusively to those with an increase or decrease in that variable.

In simple correlation analyses, LDL-cholesterol change (increase) from baseline was significantly correlated with haematocrit change

(increase) from baseline (Figure S2), as well as with serum albumin change (increase) from baseline (Figure S3) in each treatment group. Accordingly, in mediation analyses (accounting for the differential treatment effect of the change in the mediator on the placebo-subtracted change of the lipid endpoint), the changes (increases) in LDL-cholesterol that were associated with changes (increases) in haematocrit and serum albumin, were statistically significant with both doses of empagliflozin at week 24 (Figure 1A, Table S1). The proportion of the total change (increase) in LDL-cholesterol that was associated with the change (increase) in haematocrit (the percentage mediated) was 60.2% with empagliflozin 10 mg, and 48.6% with empagliflozin 25 mg (Table S1). The proportion of the total change (increase) in LDL-cholesterol that was associated with the change (increase) in serum albumin was 42.9% with empagliflozin 10 mg, and 22.3% with empagliflozin 25 mg (Table S1). The change (increase) in LDL-cholesterol that was independent of the change (increase) in haematocrit did not reach significance for either empagliflozin dose. The change in LDL-cholesterol (increase) that was independent of the change (increase) in serum albumin reached statistical significance with empagliflozin 25 mg only (Figure 1A).

For both empagliflozin doses, the changes (increases) in HDL-cholesterol associated with haematocrit and serum albumin changes (increases) were significant at week 24 (Figure 1B; Table S1). Likewise, the changes (increases) in HDL-cholesterol that were independent of haematocrit or serum albumin changes (increases) were significant in both empagliflozin groups (Figure 1B).

Despite an overall decrease in triglycerides with both empagliflozin doses (reaching significance vs. placebo with empagliflozin 10 mg only), the changes (increases) in haematocrit were also associated with significant changes (increases, rather than decreases) in triglycerides at week 24 with both doses. A similar pattern was observed between serum albumin and triglycerides, but the association between the changes (increases) in triglycerides associated with changes (increases) in serum albumin did not reach statistical significance with either empagliflozin dose (Figure 1C; Table S1). By contrast, the changes (decreases) in triglycerides that were independent of changes (increases) in haematocrit were significant with both doses, and the change (decrease) in triglycerides independent of the change (increase) in serum albumin was significant with empagliflozin 10 mg (Figure 1C).

With both empagliflozin doses, the changes (increases) in Apo B and Apo A-I that were associated with changes (increases) in haematocrit and serum albumin were significant at week 24 (Figure 2A, B; Table S1). While there were no significant changes in Apo A-I or Apo B independent of haematocrit, changes (increases) in Apo A-I (but not Apo B) that were independent of changes (increases) in serum albumin were significant with both empagliflozin doses (Figure 2A,B).

As observed with LDL-cholesterol, for HDL-cholesterol and Apo A-I, the percentage mediated effect was larger for haematocrit-associated changes compared with albumin-associated changes (Table S1).

Analyses of the magnitude of changes in absolute levels of each lipid fraction that were associated with or independent of absolute changes in haematocrit and serum albumin were generally aligned

TABLE 2 Changes from baseline in haematocrit, albumin, and lipid fractions at week 24

| Lipid fraction	Placebo	Empagliflozin 10 mg	Empagliflozin 25 mg
Haematocrit (%)			
Baseline	41.48 (0.15)	41.93 (0.14)	41.88 (0.14)
Percentage change from baseline	−1.14 (0.20)	4.08 (0.20)	4.31 (0.20)
Difference versus placebo		5.22 (4.66, 5.79)***	5.45 (4.88, 6.02)***
Absolute change from baseline	−0.51 (0.08)	1.61 (0.08)	1.73 (0.08)
Difference versus placebo		2.11 (1.89, 2.33)***	2.24 (2.02, 2.46)***
Albumin (g/L)			
Baseline	45.20 (0.10)	45.25 (0.10)	45.17 (0.10)
Percentage change from baseline	−0.40 (0.17)	0.74 (0.17)	0.53 (0.17)
Difference versus placebo		1.14 (0.67, 1.61)***	0.93 (0.46, 1.40)***
Absolute change from baseline	−0.24 (0.08)	0.27 (0.08)	0.17 (0.08)
Difference versus placebo		0.51 (0.30, 0.72)***	0.41 (0.20, 0.62)***
LDL-cholesterol (mmol/L)			
Baseline	2.62 (0.03)	2.57 (0.03)	2.57 (0.03)
Percentage change from baseline	4.02 (1.07)	7.42 (1.08)	8.46 (1.07)
Difference versus placebo		3.40 (0.42, 6.38)*	4.44 (1.47, 7.42)**
Absolute change from baseline	0.02 (0.02)	0.08 (0.02)	0.10 (0.02)
Difference versus placebo		0.06 (−0.00, 0.11)	0.08 (0.02, 0.14)**
HDL-cholesterol (mmol/L)			
Baseline	1.26 (0.01)	1.26 (0.01)	1.27 (0.01)
Percentage change from baseline	1.11 (0.54)	6.57 (0.54)	6.21 (0.54)
Difference versus placebo		5.47 (3.97, 6.96)***	5.11 (3.61, 6.60)***
Absolute change from baseline	0.00 (0.01)	0.07 (0.01)	0.07 (0.01)
Difference versus placebo		0.07 (0.05. 0.09)***	0.06 (0.05, 0.08)***
Triglycerides (mmol/L)			
Baseline	1.86 (0.04)	1.95 (0.05)	1.96 (0.07)
Percentage change from baseline	9.23 (1.59)	2.33 (1.59)	5.65 (1.59)
Difference versus placebo		−6.90 (−11.32, −2.48)**	−3.58 (−7.99, 0.84)
Absolute change from baseline	0.03 (0.04)	−0.11 (0.04)	−0.02 (0.04)
Difference versus placebo		−0.14 (−0.25, −0.03)*	−0.06 (−0.17, 0.05)
Apo B (g/L)			
Baseline	0.92 (0.01)	0.90 (0.01)	0.91 (0.01)
Percentage change from baseline	4.44 (0.77)	5.92 (0.76)	6.47 (0.77)
Difference versus placebo		1.48 (−0.64, 3.60)	2.04 (−0.09, 4.16)
Absolute change from baseline	0.02 (0.01)	0.03 (0.01)	0.03 (0.01)
Difference versus placebo		0.01 (−0.01, 0.02)	0.01 (−0.01, 0.03)
Apo A-I (g/L)			
Baseline	1.37 (0.01)	1.38 (0.01)	1.38 (0.01)
Percentage change from baseline	2.06 (0.63)	5.65 (0.62)	5.65 (0.63)
Difference versus placebo		3.60 (1.86, 5.33)***	3.59 (1.85, 5.33)***
Absolute change from baseline	0.01 (0.01)	0.06 (0.01)	0.06 (0.01)
Difference versus placebo		0.05 (0.03, 0.07)***	0.05 (0.03, 0.07)***

Note: Baseline data are mean (SE), changes from baseline are adjusted mean (SE) and differences versus placebo are adjusted mean (95% CI). Treated set.
Abbreviations: Apo, apolipoprotein; SE, standard error.
***$P < .001$;
**$P < .01$;
*$P < .05$ for difference versus placebo in change from baseline.

FIGURE 1 Contribution of percentage changes in haematocrit and albumin to percentage changes in A, LDL-cholesterol, B, HDL-cholesterol, and C, triglycerides at week 24. Treated set (LOCF-IR). *Difference versus placebo $P < .05$ (ANCOVA). LOCF-IR, last observation carried forward including values after rescue; SE, standard error
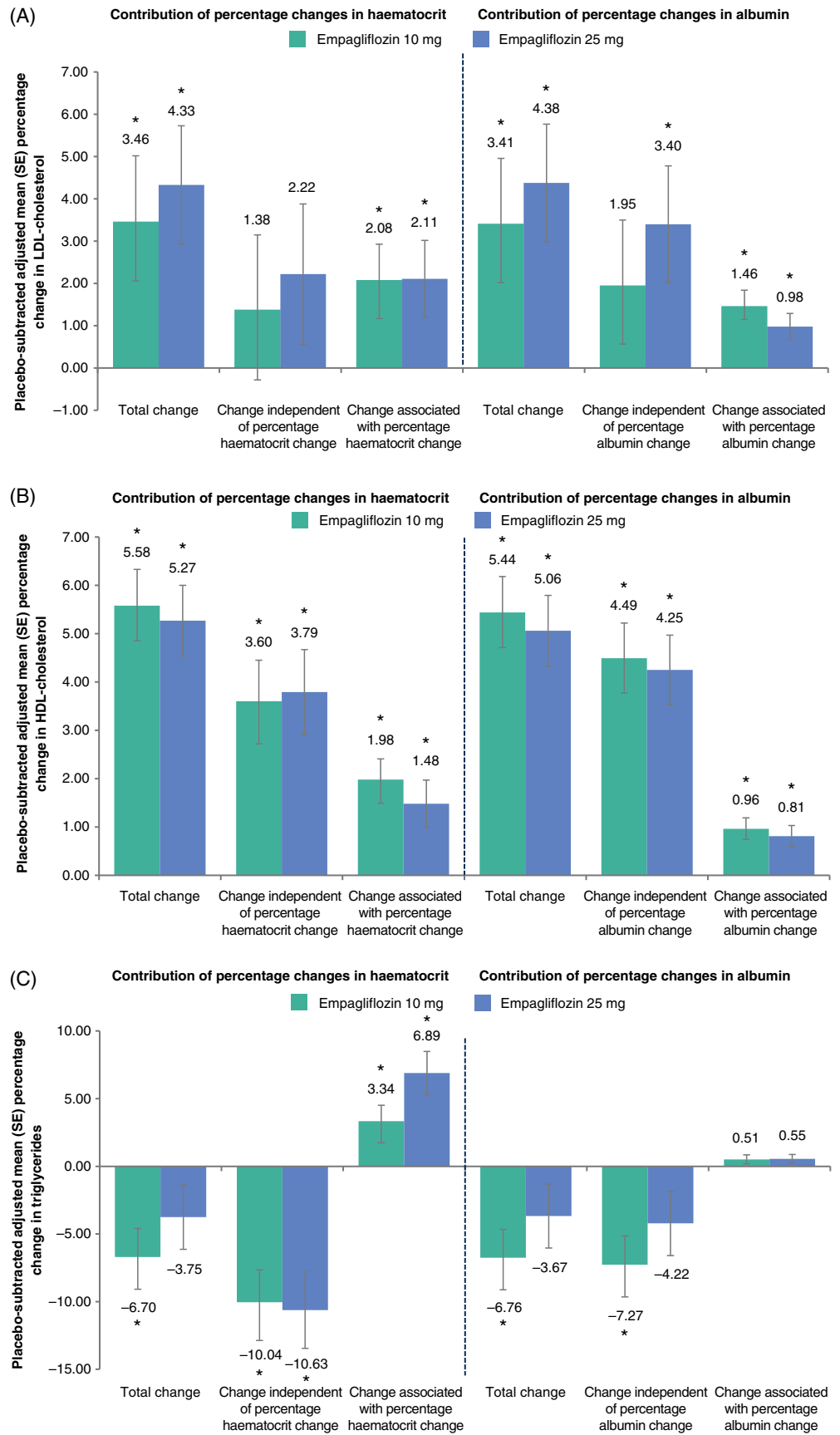
with percentage change analyses (Figures S4–S8; Table S1). The only notable difference in the analyses was that the absolute change in Apo B independent of the absolute change (increase) in haematocrit showed a significant decrease for Apo B with both empagliflozin doses, whereas these changes did not reach statistical significance in analyses of percentage changes (as described above) but were directionally identical (Figures 2A and S7).

Outcomes for both percentage and absolute changes for each lipid fraction were similar when the analyses were repeated with additional adjustment for baseline use of lipid-lowering drugs (data not shown). The proportion of participants with a change in use of lipid-lowering drugs was comparable between treatment groups (placebo:

n = 34 [4.2%]; empagliflozin 10 mg: n = 41 [5.2%]; empagliflozin 25 mg: n = 36 [4.5%] – odds ratio [95% CI] vs. placebo: empagliflozin 10 mg, 1.3 [0.8, 2.1], $P = .26$; empagliflozin 25 mg, 1.1 [0.7, 1.9], $P = .58$).

3.6 | Safety

The proportions of participants with one or more AE were similar across treatment groups (63.3% with placebo, 61.4% with empagliflozin 10 mg, and 60.8% with empagliflozin 25 mg). Confirmed hypoglycaemic AEs were reported in 2.9% of placebo-treated
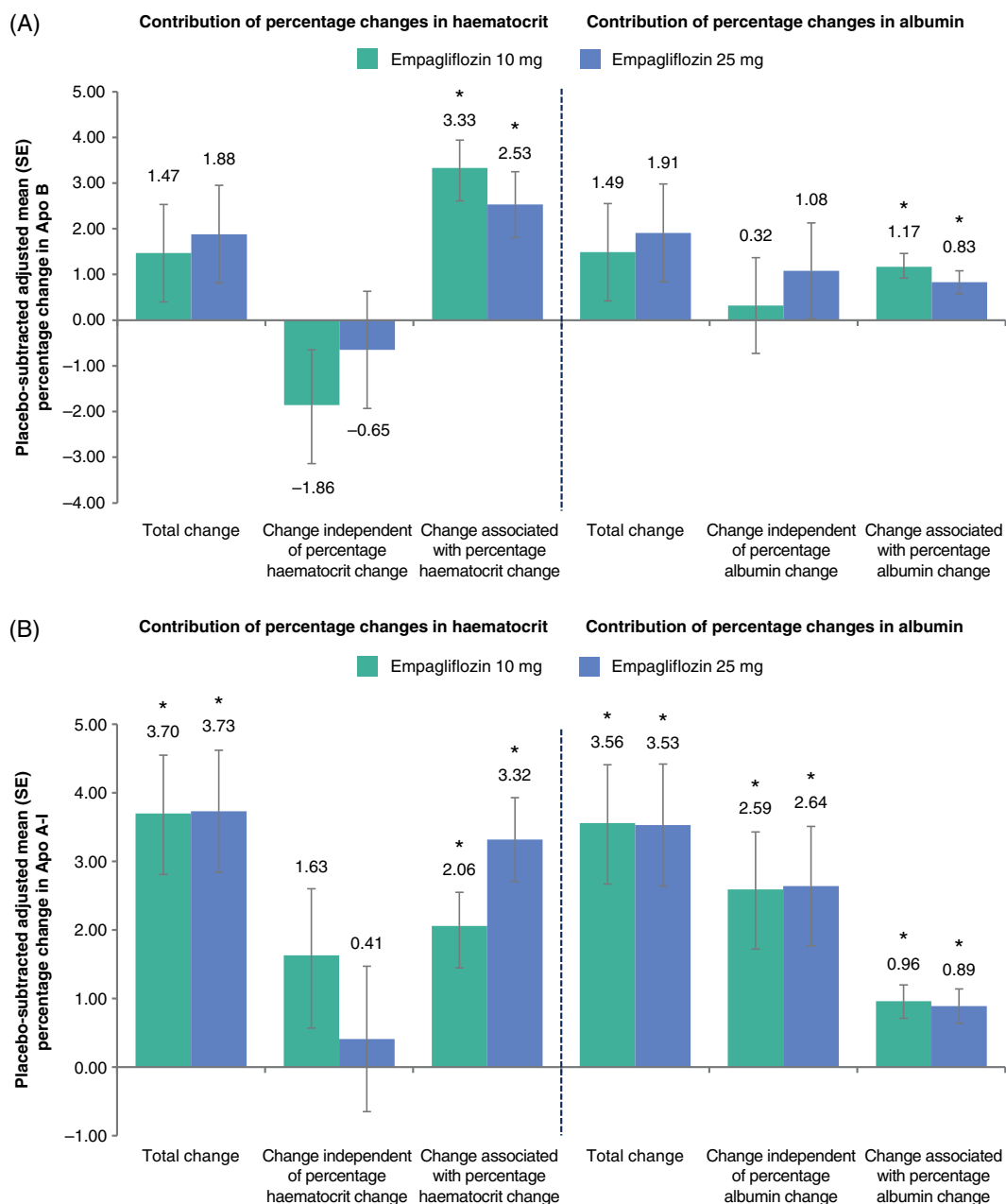

FIGURE 2 Contribution of percentage changes in haematocrit and albumin to percentage changes in A, Apo B, and B, Apo A-I at week 24. Treated set (LOCF-IR). *Difference versus placebo $P < .05$ (ANCOVA). Apo, apolipoprotein; LOCF-IR, last observation carried forward including values after rescue; SE, standard error

participants, 5.2% of empagliflozin 10 mg, and 4.0% of empagliflozin 25 mg participants, none of whom required assistance.

AEs consistent with urinary tract infections were reported in 8.2%, 9.3%, and 7.5% of participants in the placebo, empagliflozin 10 mg, and empagliflozin 25 mg groups, respectively. AEs consistent with genital infection were reported in 0.7%, 4.2%, and 3.6%, and AEs consistent with volume depletion were reported in 0.2%, 0.5%, and 0.1% of participants in the placebo, empagliflozin 10 mg, and empagliflozin 25 mg groups, respectively. No diabetic ketoacidosis AEs were reported.

4 | DISCUSSION

This analysis explored whether changes in lipid variables following empagliflozin treatment could be partly explained by haemoconcentration (as estimated by changes in haematocrit and serum albumin), probably as a result of increased urinary volume and subsequent volume contraction. The observed increases in haematocrit and serum albumin following 24 weeks of treatment with empagliflozin may arise from reduced plasma volume, following an early increase in urinary volume.[5,21-23] In support of this, our analyses show that changes (increases) in haematocrit and serum albumin, as surrogate markers of volume status (e.g. haemoconcentration), were weakly correlated. We observed significant increases in the serum levels of several lipids and lipoproteins with empagliflozin (i.e. LDL-cholesterol, HDL-cholesterol, and Apo A) and a lowering of serum triglycerides. Serum Apo B levels were increased with empagliflozin; however, this did not reach statistical significance. Mediation analyses showed changes (increases) in haematocrit to be significantly associated with changes (increases) in all assessed lipid fractions. Likewise, changes (increases) in serum albumin were significantly associated with changes (increases) in all lipid fractions, except for triglycerides.

For Apo A-I, present on HDL particles, and Apo B, present on both cholesterol-rich LDL and triglyceride-rich very low-density lipoprotein (VLDL) particles,[24] we found several similarities in the haematocrit and serum albumin mediation analyses, as in the corresponding analyses for HDL-cholesterol, LDL-cholesterol, and triglycerides. Such similarities might support our conclusions of associations between changes in haematocrit or albumin and the various lipid fractions, as the measurements of Apo A-I and Apo B are performed with different methods (assays) than those for the corresponding lipid levels.

For LDL-cholesterol, concomitant changes (increases) in haematocrit and serum albumin explained a sizeable proportion (∼20%-60%) of the significant LDL-cholesterol total percentage increase. For Apo B, while the overall increase was not significant, the portion of Apo B levels that were associated with the change (increase) in haematocrit or serum albumin showed, as for LDL-cholesterol, a statistically significant increase. By contrast, haematocrit- and/or serum albumin-independent changes in LDL-cholesterol and Apo B mostly did not reach statistical significance. For example, in the majority of mediation analyses for LDL-cholesterol, the CI for the

haematocrit- and/or serum albumin-independent changes contained zero (i.e. were non-significant). Consequently, the haematocrit- and/or serum albumin-independent effect of empagliflozin on LDL-cholesterol could be zero. This might mean that the remaining part, the haematocrit- and/or serum albumin-associated changes (i.e. haemoconcentration), cannot be ruled out as having contributed to 100% of the placebo-subtracted increase from baseline in LDL-cholesterol with empagliflozin, although we suspect the truth is probably closer to the percentage mediated effects stated in Table S1. For instance, the statistically significant serum albumin-independent change (increase) in LDL-cholesterol with empagliflozin 25 mg suggests only a partial contribution of haemoconcentration to the increase in LDL-cholesterol.

For HDL-cholesterol, and to a lesser extent for triglycerides and Apo A-I, changes both dependent and independent of changes in haematocrit and/or serum albumin typically reached statistical significance.

We observed only modest or negligible dose-dependent differences in the investigated variables across the analyses. This is in line with the dose-response relationship of empagliflozin observed with similar/other variables in previous studies, such as HbA1c, glucosuria, and haematocrit.[4,25-27]

These findings suggest that changes (increases) in LDL-cholesterol during empagliflozin treatment may be driven by haemoconcentration, whereas changes in HDL-cholesterol, triglycerides, Apo A-I, and, to some extent, Apo B, may also be significantly regulated by other mechanisms, independent of haemoconcentration.

In addition to possible haemoconcentration, non-volume mechanisms might also contribute to the increase in LDL-cholesterol during empagliflozin treatment, as previously suggested in animal studies for SGLT2 inhibitors (e.g. reduced LDL-receptor expression).[28,29] Non-volume mechanisms might also contribute to changes in other lipid fractions. For example, levels of HDL-cholesterol and triglycerides are known to be sensitive to changes in glucose-related metabolic variables.[24] Accordingly, we observed a significant association of these lipid fractions independent of changes (increases) in haematocrit and serum albumin (i.e. independent of possible haemoconcentration). Notably, changes in triglycerides both independent of and associated with changes in haematocrit and/or serum albumin were mostly statistically significant, but in opposite directions. This suggests that the observed net changes in triglycerides with empagliflozin treatment are a function of the opposing effects of possible haemoconcentration (tending to increase triglycerides levels) and changes in other variables, such as improvements in blood glucose, body weight, and possibly insulin sensitivity (tending to decrease triglycerides levels).[30]

Changes (increases) in HDL-cholesterol both independent of and associated with changes (increases) in haematocrit and serum albumin were statistically significant. The opposite relationship between metabolic-induced changes in HDL-cholesterol and triglycerides (typically HDL-cholesterol increases if triglycerides decrease, and vice versa) is well known.[24] Accordingly, the change (increase) in HDL-cholesterol in this study was in the opposite direction to that of triglycerides (decrease) for the part possibly driven by metabolic

changes (i.e. independent of changes [increases] in haematocrit and/or serum albumin), and mostly in the same direction as that of triglycerides (increase) for the part possibly related to haemoconcentration (i.e. associated with changes [increases] in haematocrit and/or serum albumin). As expected, directionally similar associations were observed for Apo A-I as for HDL-cholesterol, in support of these considerations.

In the present analyses, the overall lack of significant increase in Apo B, despite the possible influence of haemoconcentration, could be a result of the concomitant lowering of triglycerides (as triglyceride-rich particles such as VLDL carry one molecule of Apo B).[24] The significant absolute decrease in Apo B that was independent of the absolute increase in haematocrit appears to support this hypothesis, as does the opposing concomitant significant absolute increase in Apo B that was associated with the absolute increase in haematocrit (possibly reflecting haemoconcentration), that is, similar to the associations for triglycerides.

The magnitude of changes in lipid levels that were either associated with or independent of changes in haematocrit and serum albumin varied among lipid variables. Typically, haematocrit-associated changes were more pronounced than albumin-associated changes. Besides changes in fluid status as a common mechanism, levels of haematocrit (i.e. red blood cells produced in the bone marrow) and serum albumin (i.e. protein produced in the liver) are regulated by different mechanisms. The observed differences between the percentage changes in these variables suggest mechanisms for the regulation of haematocrit and/or serum albumin beyond changes in fluid status during empagliflozin treatment. For example, for haematocrit, changes in tissue-oxygenation may be relevant during SGLT2-inhibitor treatment (detailed discussions have been reported previously elsewhere).[31-34] This underscores the relevance of investigating both changes in haematocrit and serum albumin in our present analysis, that is, to mitigate any effect of non-volume status mechanisms that may explain the observed associations between changes in haematocrit or serum albumin with changes in lipid levels. Studies of measured plasma volume support a volume contraction with SGLT2 inhibitors, including empagliflozin,[31,35-37] which is in line with the observed increases in both haematocrit and serum albumin levels, as markers of haemoconcentration, despite differences in magnitude between them in our study.

Dyslipidaemia, including elevated LDL-cholesterol, is a risk factor for cardiovascular disease in patients with T2D.[38-40] In the EMPA-REG OUTCOME trial, empagliflozin versus placebo reduced the risk of cardiovascular events in patients with T2D and cardiovascular disease, driven by a relative risk reduction in cardiovascular death of 38%.[6] This included a reduction in atherosclerosis-related outcomes, for example, myocardial infarctions with empagliflozin,[41] a finding that is supported by animal models of atherosclerosis.[42,43] Multiple mechanisms have been proposed for the observed benefits of empagliflozin and other SGLT2 inhibitors on cardiovascular events, including effects on volume status as well as on cardiac energetics and electrolyte transporters.[44,45] In EMPA-REG OUTCOME, haematocrit values on treatment were approximately 5% higher in the empagliflozin groups

versus placebo and empagliflozin was also associated with small increases from baseline in LDL-cholesterol and HDL-cholesterol[6]; thus, our results are broadly similar to those in EMPA-REG OUTCOME. In a mediation analysis from EMPA-REG OUTCOME, the change in haematocrit was identified as one of the key potential mediators of (i.e. a possible contributor to) the reduction in risk of cardiovascular death, whereas changes in lipid levels, including LDL-cholesterol, were not.[46] Consequently, even if caused by volume contraction, any increase in atherogenic lipid fractions, such as LDL-cholesterol, did not offset a more general beneficial effect in reducing cardiovascular events, including myocardial infarction, with empagliflozin in EMPA-REG OUTCOME.

Despite the potential volume contraction with empagliflozin, events consistent with volume depletion were infrequent in the current analysis. In EMPA-REG OUTCOME, the proportions of participants with events consistent with volume depletion were similar with empagliflozin versus placebo (5.1% vs. 4.9%, respectively),[6] which is consistent with pooled analyses of phase 1–3 trials, except for participants aged older than 75 years, among whom rates were higher with empagliflozin 10 and 25 mg versus placebo (3.2 and 3.0 vs. 2.3/100 patient-years, respectively).[16]

The limitations of our analyses include that they are unable to prove causality in either direction (and so should be considered hypothesis-generating), that they were performed post hoc, and were restricted to patients with T2D. Additionally, urinary volume was not measured in the studies, and we assessed volume contraction/haemoconcentration indirectly by use of haematocrit and serum albumin as markers of volume/concentration status. Because of the small changes from baseline for some lipids, analysing percentage or proportional changes may provide more conservative estimates. However, our findings were generally consistent between the percentage and absolute changes in both haematocrit and serum albumin and their associations with changes in each lipid fraction.

We assessed the potential impact of empagliflozin-induced changes (reductions) in urinary albumin excretion to potentially confound the corresponding changes (increases) in serum albumin. Reassuringly, we did not find evidence of such potential bias. We measured lipid concentrations rather than function or particle number, which are other relevant aspects of lipoprotein-related cardiovascular risk.[47,48] We used the Friedewald formula to calculate LDL-cholesterol (plus direct LDL-cholesterol measurement in samples with triglycerides above 400 mg/dL [>4.52 mmol/L]), as prespecified for each trial, rather than a more recent proposed formula to calculate LDL-cholesterol allowing for higher triglycerides levels.[49]

In addition, diet/nutritional changes can affect lipid metabolism[50] and SGLT2 inhibitor-related changes in nutrient intake and/or a caloric deficit from glucosuria have previously been shown.[51,52] Data on diet and urinary glucose excretion (i.e. caloric loss) were not obtained in the studies used in our present analyses. We would not expect, however, that the modest weight loss of about 2.5% (placebo-corrected) seen in the empagliflozin group would raise LDL-cholesterol levels. We cannot rule out an effect of weight loss on the increase in HDL-cholesterol or the minimal reductions seen in serum triglyceride levels.

Use and changes in use of lipid-lowering medications during the trial could have affected our results; however, approximately one-third of participants used lipid-lowering medications at baseline, and adjustment for baseline use of lipid-lowering medications did not change the conclusions of the mediation analyses. Also, the proportion of participants with changes in lipid-lowering medications was small and balanced between treatment groups (~5% in each group), and therefore not probable to majorly impact our conclusions.

In summary, findings from our analysis suggest that in patients with T2D, empagliflozin-associated changes in lipids, especially increases in LDL-cholesterol, might be partly attributable to haemoconcentration, probably as a result of increased urinary volume and subsequent volume contraction.

CONFLICT OF INTEREST
H.N.G. has received grants from AstraZeneca, Pfizer, and Amgen; has served on steering committees at Reverlogix and Kowa; and on advisory boards for Silence Therapeutics, Amgen, Merck, Regeneron, AstraZeneca, and Kowa. N.S. has consulted for Amgen, Boehringer Ingelheim, Eli Lilly and Company, Napp, Novo Nordisk, Pfizer, and Sanofi; and has received grant support from Boehringer Ingelheim. S.S.L., D.N., and A.S. are employees of Boehringer Ingelheim, and S.S.L. owns shares in Novo Nordisk A/S as well as shares in dynamically traded investment funds, which may own stocks from pharmaceutical companies.

AUTHOR CONTRIBUTIONS
All authors contributed to the interpretation of data, drafting of the article, and reviewing the article for important intellectual content.

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DATA AVAILABILITY STATEMENT
The sponsor of the study (Boehringer Ingelheim) is committed to responsible sharing of clinical study reports, related clinical documents, and patient-level clinical study data. Researchers are invited to submit inquiries via the following website: https://trials.boehringer-ingelheim.com

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