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ORIGINAL ARTICLE

Mediators of the improvement in heart failure outcomes with empagliflozin in the EMPA-REG OUTCOME trial

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Abstract

Aims In the EMPA-REG OUTCOME trial, empagliflozin reduced risk of death from heart failure (HF) or hospitalization for heart failure (HHF) versus placebo in patients with type 2 diabetes mellitus (T2DM) and established cardiovascular (CV) disease. We evaluated *post hoc* the degree to which covariates mediated the effects of empagliflozin on HHF or HF death.

Methods and results A mediator had to fulfil the following criteria: (i) affected by active treatment, (ii) associated with the outcome, and finally (iii) adjustment for it results in a reduced treatment effect compared with unadjusted analysis. Potential mediators were calculated as change from baseline or updated mean and evaluated in univariable analyses as time-dependent covariates in Cox regression of time to HHF or HF death; those with the largest mediating effects were then included in a multivariable analysis. Increases in heart rate, log urine albumin-to-creatinine ratio (UACR), waist circumference, and uric acid were associated with increased risk of HHF or HF death; increases in high-density lipoprotein cholesterol, estimated glomerular filtration rate, haematocrit, haemoglobin, and albumin were associated with reduced risk of HHF or HF death. In univariable analyses, change from baseline in haematocrit, haemoglobin, albumin, uric acid, and logUACR mediated 51%, 54%, 23%, 24%, and 27% of the risk reduction with empagliflozin versus placebo, respectively. Multivariable analysis including haemoglobin, logUACR, and uric acid mediated 85% of risk reduction with similar results when updated means were evaluated.

Conclusions Changes in haematocrit and haemoglobin were the most important mediators of the reduction in HHF and death from HF in patients with T2DM and established CV disease treated with empagliflozin. Albumin, uric acid, and logUACR had smaller mediating effects in this population.

Keywords Diabetes; Empagliflozin; SGLT2 inhibitor; Heart failure; Mediation analysis

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Introduction

In the EMPA-REG OUTCOME trial, empagliflozin added to standard of care reduced the risk of 3-point major adverse cardiovascular events (3P-MACE: composite of cardiovascular (CV) death, non-fatal myocardial infarction, or non-fatal stroke) compared with placebo in patients with type 2 diabetes mellitus (T2DM) and established CV disease, driven by a reduction in the risk of CV death.[1] Empagliflozin also reduced the risk of hospitalization for heart failure (HHF) by 35% (hazard ratio [HR] 0.65 [95% CI 0.50, 0.85])[1] and the risk of HHF or death from heart

failure (HF) by 39% (HR 0.61 [95% CI 0.47, 0.79]) compared with placebo.[2]

Several hypotheses have been proposed to explain the effect of empagliflozin on HF outcomes, including effects on plasma volume, blood pressure, hyperglycaemia, ketone levels, body weight, kidney function, and arterial stiffness.[3] As with other large outcomes trials, the EMPA-REG OUTCOME trial was not designed to investigate the actual mechanisms behind the effects of empagliflozin on CV outcomes. Over the course of the study, however, empagliflozin was associated with reductions versus placebo in haemoglobin A1c (HbA1c), weight, waist circumference, uric acid, systolic blood pressure (SBP) and diastolic blood pressure (DBP), a slight reduction in heart rate, and small increases in low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C).[1] There was an initial decrease in estimated glomerular filtration rate (eGFR) with empagliflozin followed by stabilization during prolonged treatment, in contrast to a gradual decline in eGFR in the placebo group.[4] Empagliflozin also led to significant reductions in the urine albumin-to-creatinine ratio (UACR) versus placebo from Week 12, regardless of albuminuria status at baseline.[5] Finally, there was an initial increase in haematocrit and haemoglobin with empagliflozin followed by stabilization.[6] A previous mediation analysis from our group identified changes in haematocrit and haemoglobin to be the strongest mediators of the reduction in CV death with smaller mediation effects observed for glycaemia, UACR, and uric acid.[6] The haematological changes were interpreted as likely reflecting volume contraction, although an effect through augmentation of haematopoiesis via increased erythropoietin production has also been raised as a possibility.[6]

The aim of this *post hoc* mediation analysis was to identify the extent to which treatment differences in covariates during the trial contributed to the reduction in the risk of HHF or death from HF with empagliflozin versus placebo in the EMPA-REG OUTCOME trial.

Methods

Trial design

The design of the EMPA-REG OUTCOME trial has been described previously.[1] In brief, adults with T2DM, HbA1c 7–10%, eGFR of ≥30 mL/min/1.73 m^2 and established CV disease were randomized (1:1:1) to receive empagliflozin 10 mg, empagliflozin 25 mg, or placebo, in addition to standard of care. The trial was to continue until ≥691 patients experienced an adjudicated primary outcome event (3P-MACE). The trial was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines and was approved by local authorities. An independent ethics committee or institutional review board approved the clinical protocol at each participating centre. All the patients provided written informed consent before study entry.

For the analyses in this study, data for empagliflozin 10 and 25 mg were pooled. The analysis considered any first by adjudication confirmed HHF or HF death. Thereby, only events that fulfilled the definition as per adjudication charter[1] were considered. Deaths due to causes other than HF led to censoring and intercurrent non-HF cardiovascular events between randomization and first HHF or HF death were not considered in the analysis.

Traditional mediation analysis

A traditional mediation analysis as originally proposed by Baron and Kenny[7] and as described in Inzucchi *et al*.[6] was employed, taking the time-dynamic evolvement of both the potential mediators and the outcome HHF or HF death into account. For a variable to be a mediator of the treatment effect, three conditions have to be fulfilled: 1) the treatment must have an effect on the variable over time; 2) the change in the variable over time must be associated with the outcome; and 3) in an analysis where the variable is included as a time-dependent covariate over time, the effect of treatment on the outcome (represented as the HR) must be reduced compared with the treatment effect in an unadjusted analysis.

Analysis of the effect of treatment on hospitalization for heart failure or heart failure death

The original analysis of the composite of HHF or HF death with empagliflozin versus placebo was based on a Cox proportional hazards model including factors for treatment, age, sex, baseline body mass index (BMI), baseline HbA1c, baseline eGFR, and region, conducted in patients who received ≥1 dose of study drug.[2]

Potential mediators of the effect of empagliflozin on hospitalization for heart failure or heart failure death

Based on evidence from previous studies, we chose potential mediators of the benefit of empagliflozin on HHF or HF death to then undergo *post hoc* evaluation for inclusion in the mediation analysis, grouped by mechanistic category. These potential mediators were glycaemia (HbA1c and fasting plasma glucose [FPG]); CV parameters (SBP, DBP, and heart rate); lipids (LDL-C, HDL-C, triglycerides, and free fatty acids);

adiposity (weight, BMI, and waist circumference); renal function (UACR, eGFR according to Modification of Diet in Renal Disease [MDRD], and CKD-EPI formulae); markers that might represent volume status (haematocrit, haemoglobin, and albumin); and other (uric acid).

Analysis of potential mediation of the reduction in hospitalization for heart failure or heart failure death with empagliflozin

The analysis of mediation condition 1 (treatment effect on the time course of the variable) was performed as described in Inzucchi *et al*.[6] The analyses of mediation conditions 2 and 3 were performed using univariable and multivariable Cox regression models as described in the following.

Univariable analysis

Each variable was analysed as a time-dependent covariate in Cox regression models using two approaches: (i) analysis of the current *change from baseline* to the most recent value available before HHF or HF death and (ii) analysis of the mean value considering all prior values (the 'updated mean' analysis), reflecting the cumulative effect of all prior values of the variable on the risk of HHF or HF death. The models included treatment group, the baseline value of the variable, and the current change or updated mean of the variable as time-dependent covariates. The models provided the estimated HR for HHF or HF death associated with a 1-unit increase in the variable. Mediation condition 2 (change in the variable over time needs to be associated with the outcome HHF or HF death) was regarded as fulfilled for a variable, when the 95% confidence interval of the HR did not include 1 (based on an analysis of pooled treatment groups). The treatment effects estimated from these models were then compared with a model including treatment group alone, and percentage mediation was calculated as described in Inzucchi *et al*.[6] Mediation was indicated if the HR for the composite of HHF or HF death between treatment groups adjusted for the covariate was closer to unity than the HR from the model with treatment group alone (i.e. mediation condition 3 mentioned earlier).

Multivariable analysis

Further analyses investigated how selected individual mediators from the different mechanistic categories jointly contributed to the effect of empagliflozin. Because variables pertaining to the same mechanistic category may be biologically and statistically redundant, only the mediator with the largest mediating effect in the univariable analyses from each of the mechanistic categories was chosen for the multivariable model. As described in Inzucchi *et al*.,[6] a step-up procedure for multivariable model-building was employed to provide a ranking of the different mechanistic categories with regard to their potential as mediators. In each step, the representative variable from the mechanistic category with the largest mediating effect was added. For an investigation of the statistical stability of the results, a bootstrap re-sampling procedure was employed based on 100 bootstrap samples (sampling with replacement) of the same size of the original data set. The unadjusted Cox model and the Cox model adjusted for the finally selected mediators were fitted in each bootstrap sample. The stability of the relationship between the resulting treatment effect estimates (log HR from the unadjusted and adjusted models) was graphically displayed and described by linear regression.

Results

Incidence of hospitalization for heart failure or heart failure death

In the overall population, the number of patients with HHF in the placebo (N = 2333) and pooled empagliflozin (N = 4687) treatment groups was 95 (4.1%) and 126 (2.7%), respectively.[1] In addition, the incidence of HF death was 19 (0.8%) and 11 (0.2%) in the placebo and pooled empagliflozin groups, respectively.[1]

Effects of empagliflozin on the time course of potential mediators

Effects of empagliflozin on the time course of potential mediators have been reported by Inzucchi *et al*.[6] For all considered variables, mediation condition 1 was therefore regarded as fulfilled.

Effects of change in potential mediators on the risk of hospitalization for heart failure or heart failure death

When the effect of the *change from baseline* of potential mediators adjusted for treatment group and the respective baseline value on the risk of HHF or HF death were analysed, increases in heart rate, logUACR, waist circumference, and uric acid and reductions in eGFR were associated with an increased risk of HHF or HF death, while increases in HDL-C, haematocrit, haemoglobin, and albumin were associated with a reduced risk of HHF or HF death (*Table 1*). These covariates fulfilled mediation condition 2 and therefore entered the analysis to determine mediation.

In analyses based on the *updated mean*, increases in heart rate, logUACR, waist circumference, and uric acid, but reductions in eGFR, were associated with an increased risk of HHF

Table 1 Association of variables with risk of HHF or HF death: Time-dependent covariate analysis for each variable, adjusted for the baseline value of each variable

	Change from baseline		Updated mean	
	HR for HHF or HF death	95% CI	HR for HHF or HF death	95% CI
Association with a 1-unit increase in				
Heart rate (b.p.m.)	1.043	1.030, 1.056	1.059	1.036, 1.082
HDL-C (mg/dL)	0.973	0.954, 0.992	0.965	0.941, 0.991
logUACR (1.0 measured on log-scale [log (mg/g)])	1.585[a]	1.406, 1.787	1.723[b]	1.455, 2.039
eGFR (MDRD) (mL/min/1.73 m^2)	0.961	0.950, 0.972	0.958	0.943, 0.974
eGFR (CKD-EPI) (mL/min/1.73 m^2)	0.960	0.949, 0.971	0.957	0.941, 0.973
Waist circumference (cm)	1.030	1.008, 1.053	1.040	1.008, 1.073
Haematocrit (%)	0.894	0.862, 0.928	0.912	0.859, 0.968
Haemoglobin (g/dL)	0.668	0.598, 0.746	0.687	0.569, 0.830
Albumin (g/dL)	0.173	0.115, 0.258	0.100	0.048, 0.209
Uric acid (mg/dL)	1.327	1.202, 1.465	1.344	1.162, 1.553

BMI, body mass index; CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration formula; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; MDRD, Modification of Diet in Renal Disease formula; SBP, systolic blood pressure; UACR, urine albumin-to-creatinine ratio.
Analyses were adjusted for treatment. Cox regression analysis in patients treated with ≥1 dose of study drug.
[a]The HR per unit change in the logUACR corresponds to a 100(1.1 ^ log(1.585) − 1) = 4.5% increase in risk per 10% higher UACR (mg/g) (95% CI [3.3%, 5.7%]), where ' ^ ' represents 'to the power of' and log is the natural logarithm.
[b]The HR per unit change in the logUACR corresponds to a 100(1.1 ^ log(1.723) − 1) = 5.3% increase in risk per 10% higher UACR (mg/g) (95% CI [3.6%, 7.0%]), where ' ^ ' represents 'to the power of' and log is the natural logarithm.

or HF death, while increases in HDL-C, haematocrit, haemoglobin, and albumin were associated with a reduced risk of HHF or HF death (*Table 1*). These covariates similarly fulfilled mediation condition 2 and proceeded into the analysis to determine mediation.

Univariable analysis: Effects of individual potential mediators on the risk of hospitalization for heart failure or heart failure death with empagliflozin versus placebo

Treatment group differences in the changes from baseline in haematocrit and haemoglobin mediated 51% and 54%, respectively, of the effect of empagliflozin versus placebo on the reduction in risk of HHF or HF death (*Figure 1*). Equivalent percentages for changes in albumin, uric acid, and logUACR were 23%, 24%, and 27%, respectively.

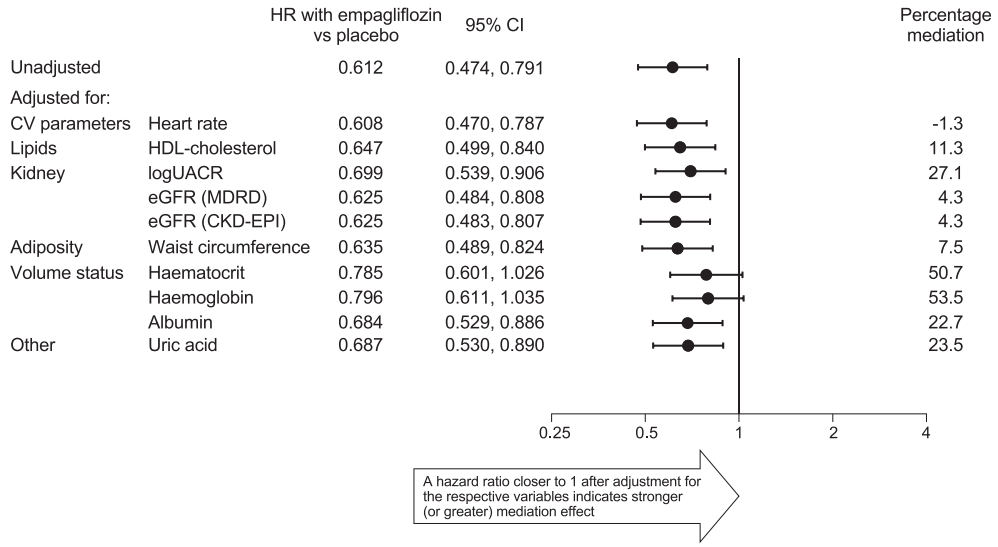
Changes in the updated mean of haematocrit and haemoglobin mediated 37% and 44%, respectively, of the effect of empagliflozin versus placebo on the reduction in the risk of HHF or HF death (*Figure 2*); adjusting for the updated mean of albumin, uric acid, and logUACR resulted in a mediation of 25%, 22%, and 26%, respectively.

Multivariable analysis: Effects of combinations of potential mediators on the hazard ratio for hospitalization for heart failure or heart failure death with empagliflozin versus placebo

A multivariable analysis of the current change from baseline, including haemoglobin as the strongest representative of the volume category, logUACR as representative of the renal function category, and uric acid, led to an estimated HR for HHF or HF death with empagliflozin versus placebo of 0.927 (95% CI 0.710, 1.212; *Table 2*). Therefore, the total proportion mediated by this group of variables was 85% (*Figure 3*). The results on mediation of the treatment effect were stable over the bootstrap samples, as there was high correlation between the unadjusted and the adjusted logHR over the bootstrap samples, the estimated slope of the regression line was near 1, and the estimated intercept of 0.37 was of similar magnitude as the difference of 0.42 between the unadjusted and adjusted logHR from the original data. The median of the proportion mediated estimated in the 100 bootstrap samples was 82% (*Figure 4*). In a further analysis, where haemoglobin was replaced by haematocrit as representative of the volume category from the univariable analysis, the estimated HR for HHF or HF death with empagliflozin versus placebo was 0.900 (95% CI 0.687, 1.180), showing a slightly reduced proportion mediated of 79%.

A multivariable analysis of the updated mean, including haemoglobin, logUACR, and uric acid, led to an estimated HR for HHF or HF death with empagliflozin versus placebo of 0.880 (95% CI 0.663, 1.168; *Table 2*). Therefore, the total proportion mediated by this group of variables was 74% (*Figure 3*). The results on mediation of the treatment effect were again stable over the bootstrap samples, represented by the intercept of 0.35 estimated from linear regression, corresponding to the estimated difference between unadjusted and adjusted logHR, which was equal to 0.36 in the original data. The median of the proportion mediated estimated in the 100 bootstrap samples was 73% (*Figure 4*). In a further analysis where haemoglobin was replaced by haematocrit as the representative of the volume category

Figure 1 Univariable mediation analysis of risk of HHF or HF death with empagliflozin versus placebo: Time-dependent covariate analysis adjusting for the change from baseline in each variable.



Cox regression analysis in patients treated with ≥1 dose of study drug.
BMI, body mass index; CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration formula; CV, cardiovascular; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HF, heart failure; HHF, hospitalization for heart failure; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; MDRD, Modification of Diet in Renal Disease formula; SBP, systolic blood pressure; UACR, urine albumin-to-creatinine ratio.

from the univariable analysis, the estimated HR for HHF or HF death with empagliflozin versus placebo was 0.839 (95% CI 0.628, 1.120), showing a slightly reduced proportion mediated of 64%.

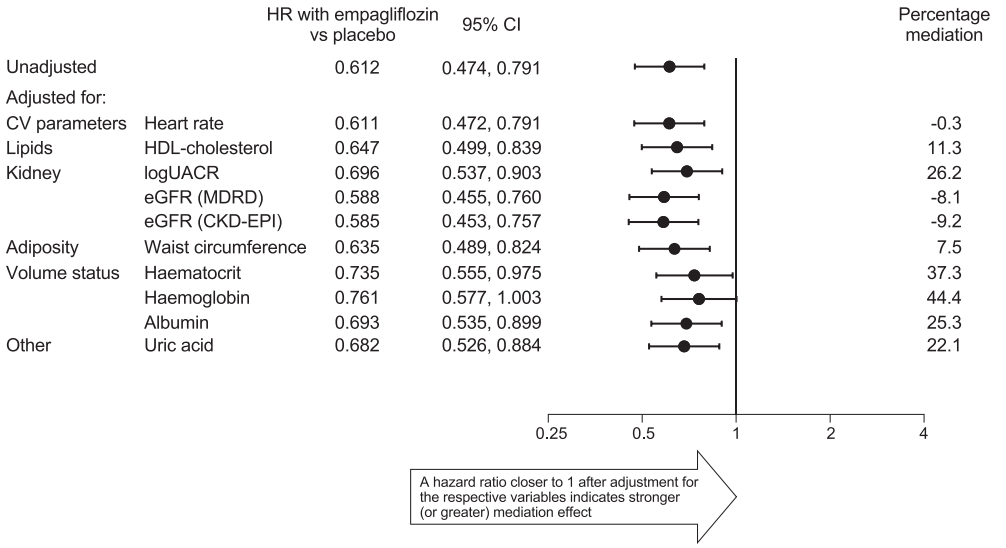
Discussion

In this exploratory mediation analysis, changes in haematocrit and haemoglobin were important mediators of the reduction in risk of HHF or HF death with empagliflozin versus placebo in the EMPA-REG OUTCOME trial, with mediations of up to 51% and 54%, respectively. Results were similar regardless of whether the analyses were based on current change from baseline or updated mean, confirming the robustness of the findings. Changes in albumin, uric acid, and logUACR each mediated 22–27% of the effect on the reduction in risk of HHF or HF death with empagliflozin in the univariable analyses, whereas measures related to glycaemia, blood pressure, heart rate, lipids, eGFR, and adiposity appeared to have small or negligible mediation effects or did not qualify as mediators.

Although empagliflozin has a beneficial effect on established risk factors for HF such as BP and weight, the current and other analyses suggest that the beneficial CV effects observed in EMPA REG OUTCOME may largely be mediated independently of control of conventional risk factors. This is consistent with analyses showing that the effect of empagliflozin on HHF in EMPA-REG OUTCOME was independent of glycaemic control,[8] and as well as after adjustment for control of BP, or lipid levels during the trial.[9,10] In our analyses, UACR, which is not a true renal functional parameter but may merely reflect a change in glomerular pressure or less vascular stress leading to reduced transendothelial permeability (as opposed to a change in eGFR), seemed to be a partial mediator. This may however be explained by the established dynamics of eGFR during the trial (an initial dip followed by stabilization) making it questionable for the statistical models applied. Moreover, a previous similar exploratory mediation analysis of EMPA-REG OUTCOME[6] reported that changes in haematocrit and haemoglobin appeared to be the most important mediators of the reduction in risk of CV death with empagliflozin, indicating an overlap in the potential mechanisms behind the reduced risk of these endpoints.

Haematocrit and haemoglobin are markers of both plasma volume and blood oxygen-carrying capacity. The osmotic diuresis induced by SGLT2 inhibition is accompanied by natriuresis as well, resulting in increases in urine volume[11] and reductions in plasma volume. This has been confirmed using direct measures in a small mechanistic trial involving patients with HF.[12] In EMPA-REG OUTCOME, the initial increase in haematocrit with empagliflozin followed by stabilization with

Figure 2 Univariable mediation analysis of risk of HHF or HF death with empagliflozin versus placebo: Cox regression analysis adjusting for the updated mean of each variable as a time-dependent covariate.



Cox regression analysis in patients treated with ≥1 dose of study drug.
BMI, body mass index; CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration formula; CV, cardiovascular; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HF, heart failure; HHF, hospitalization for heart failure; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; MDRD, Modification of Diet in Renal Disease formula; SBP, systolic blood pressure; UACR, urine albumin-to-creatinine ratio.

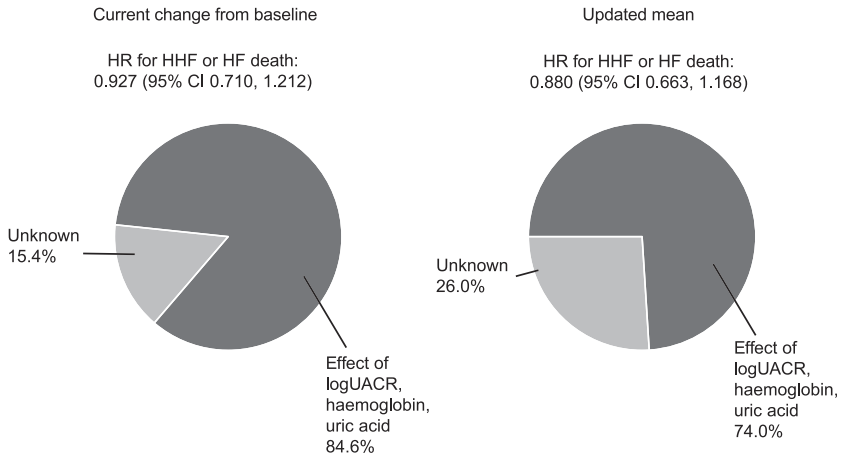
Table 2 Final multivariable analysis of the current change from baseline and the updated mean built from a step-up procedure including variables from different mechanistic categories leading to maximal mediation of treatment effect

	Change from baseline			Updated mean		
	HR for HHF or HF death	95% CI	Percentage mediation	HR for HHF or HF death	95% CI	Percentage mediation
Effect of empagliflozin versus placebo adjusted for logUACR, haemoglobin, uric acid	0.927	0.710, 1.212	84.6	0.880	0.663, 1.168	74.0
Association with a 1-unit increase in						
logUACR (1.0 measured on log-scale log [mg/g])	1.546[a]	1.377, 1.735	–	1.671[b]	1.414, 1.976	–
Haemoglobin (g/dL)	0.738	0.664, 0.821	–	0.763	0.639, 0.911	–
Uric acid (mg/dL)	1.251	1.135, 1.379	–	1.233	1.062, 1.431	–

Effects of treatment and variables on risk of HHF or HF death (including the change from baseline in each variable as a time-dependent covariate, adjusted for the baseline value of each variable). Cox regression analysis in patients treated with ≥1 dose of study drug.
CI, confidence interval; HR, hazard ratio; UACR, urine albumin-to-creatinine ratio.
[a]The HR per unit change in the logUACR corresponds to a 100(1.1 ^ log(1.546) − 1) = 4.2% increase in risk per 10% higher UACR (mg/g) (95% CI [3.1%, 5.4%]), where ' ^ ' represents 'to the power of' and log is the natural logarithm.
[b]The HR per unit change in the logUACR corresponds to a 100(1.1 ^ log(1.671) − 1) = 5.0% increase in risk per 10% higher UACR (mg/g) (95% CI [3.4%, 6.7%]), where ' ^ ' represents 'to the power of' and log is the natural logarithm.

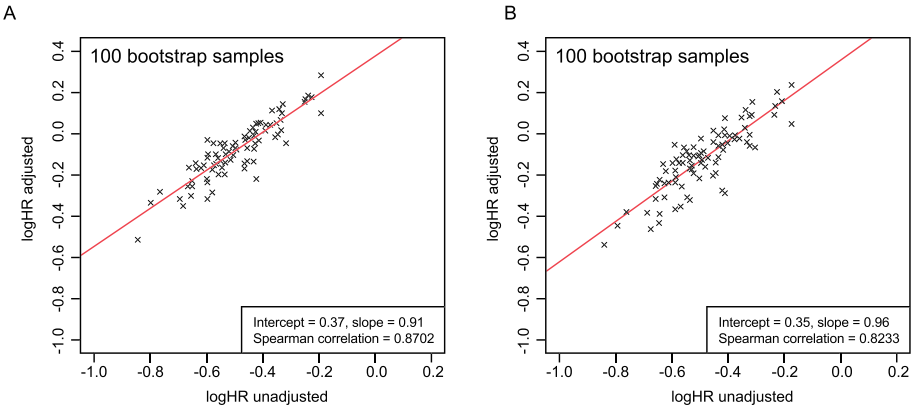
long-term treatment[6] likely reflected this haemodynamic change, presumably resulting in improved cardiac working conditions with decreased cardiac preload and afterload.[13] Importantly, this reduction in plasma volume occurred without increased heart rate, increased risk of hyperkalaemia, or increased frequency of volume-related adverse events, including acute kidney injury, as compared with placebo. Some studies have suggested separate or contributory effects from increased erythropoiesis on the increases in haematocrit and haemoglobin, with resulting improvement in oxygen supply.[14] In a small study of patients with T2DM, dapagliflozin induced a median 7% increase in red blood cell mass using measurements with ^{51}Cr-labelled erythrocytes.[15] In another study, 4 weeks of treatment with empagliflozin led to a 31% increase in circulating erythropoietin concentrations,[16] an effect potentially related to changes in kidney oxygen consumption.[17] Links between erythropoietin concentrations and changes in haematocrit and haemoglobin have not been

Figure 3 Final multivariable analysis of kidney, volume, and other variables categories and the proportion of maximal mediation of treatment effect.



HF, heart failure; HHF, hospitalization for heart failure; HR, hazard ratio; UACR, urine albumin-to-creatinine ratio.

Figure 4 Statistical stability of mediation of treatment effect in the multivariable model including (A) the change from baseline and (B) the updated mean of UACR, haemoglobin, and uric acid as time-dependent covariates.



HR, hazard ratio; UACR, urine albumin-to-creatinine ratio.

adequately assessed, however. In EMPA-REG OUTCOME, erythropoietin was not measured. In our analyses, serum albumin levels seemed to be a weaker mediator than haematocrit and haemoglobin, although albumin may not be as suitable a marker for plasma volume, given that it is also influenced by other factors, including hepatic function and nutritional status. Moreover, the impact of increases in haematocrit levels *per se* on clinical outcomes is less clear. Despite an inverse association between change in in-hospital haematocrit levels and post-discharge HF outcomes,[18] tolvaptan, which results in haemoconcentration, did not impact long-term mortality or HF morbidity in patients hospitalized with HF with reduced ejection fraction (EF) (HFrEF),[19] as later confirmed in a meta-analysis.[20] This differential impact on long-term clinical outcomes observed with tolvaptan versus SGLT2 inhibitors suggests that the mechanism by which haematocrit is increased is of importance rather than the increased level itself. It should be kept in mind that a mediation analysis does not intend to establish treatment independent causality between the changes in the identified mediator and outcome. Therefore, if another drug causes similar changes in an identified mediator, this does not necessarily imply that this drug will have a comparable effect on outcome as observed with empagliflozin.

Reduction in plasma volume and accompanying decongestion could, from a pathophysiological point of view, potentially contribute to improved outcomes in patients with either HFrEF or HF with preserved EF (HFpEF).[3] The effects of dapagliflozin in patients with HFrEF were confirmed in the DAPA-HF trial.[21] In the EMPEROR-Reduced trial, patients

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with HFrEF treated with empagliflozin had a significantly lower risk of CV death or HHF.[22] Of particular interest is the possible benefit for patients with HFpEF, because there is currently no drug with a proven benefit on mortality or HHF for these patient populations.[23,24] Previous mechanistic studies have shown that empagliflozin has the ability to influence diastolic function and left ventricular (LV) remodelling by reducing LV mass.[25] Recently published *post hoc* data from CANVAS support this hypothesis, demonstrating a similar reduction in HF events by canagliflozin in patients with HFrEF and HFpEF based on a retrospective review of EF measurements performed during HF admission.[26] In addition, in the SOLOIST-WHF trial, sotagliflozin versus placebo reduced the risk of the primary endpoint (CV death and HHF) in patients with T2DM who were recently hospitalized for worsening HF. This benefit appeared consistent across subgroups stratified by EF, although the small sample size for HFpEF and early trial termination made it difficult to make firm conclusions.[27] There are a number of ongoing trials evaluating SGLT2 inhibitors in patients with HFpEF including EMPEROR-Preserved with empagliflozin and the DELIVER trial with dapagliflozin.

Increased heart rate carries worsened prognosis in patients with diabetes and cardiovascular disease,[28] or with HF.[29] Some medications, such as conventional diuretics and glucagon-like peptide 1 receptor agonists, are known to increase heart rate. Indeed, two studies that explored the effect of liraglutide versus placebo in acute and chronic HFrEF[30,31] demonstrated no clinical benefit but rather a numerically increased risk of clinical events was observed. In both trials, heart rate was increased and discussed as a potential contributing factor to the lack of benefit. With this in mind, although the reduction in heart rate observed with empagliflozin was not identified in this analysis as a mediator of empagliflozin's treatment effect, the lack of any increase in heart rate, which typically accompanies reductions in plasma volume, may be important.

Studies suggest that uric acid is linked to HF via endothelial dysfunction and oxidative stress. Uric acid levels have been shown to be associated with an increased risk of incident HF,[32] and in EMPA-REG OUTCOME there was a trend towards an increased risk of HHF with higher baseline uric acid levels.[33] Our mediation analysis of CV death from EMPA-REG OUTCOME found similar results with uric acid mediating around 19%–25% of the effect from empagliflozin on CV death.[6] This is in line with a Mendelian randomization study that demonstrated a likely causal relationship between uric acid, CV death, and sudden cardiac death.[34] While some data also support a causal relationship between uric acid levels and HF outcomes, notably, two smaller studies evaluating uric acid level lowering in patients with HFrEF did not observe an improvement in clinical outcomes despite significant decreases in uric acid levels.[35,36] These discrepancies underscore the challenges of drawing conclusions regarding causality based on *post hoc* analyses such as mediation analyses.

The multivariable analyses suggested that 74–85% of the effects on HHF or HF death were mediated by the combined changes in haematocrit/haemoglobin, urine albumin excretion, and uric acid concentrations, indicating that the underlying mechanisms of empagliflozin in the reduction in HF outcomes may be not only multifaceted, but also not fully explained by those variables measured in the trial. There are a number of other factors that could be explored in future studies including potential effects on central pressures, myocardial wall stress, mitochondrial dysfunction, energy balance, ketone levels, and oxidative stress. Interestingly, our results are consistent with a recent report from the CANVAS Program demonstrating that erythrocyte concentration, uric acid, and UACR were the strongest mediators of the reduction in HHF observed with canagliflozin.[37]

Limitations of this analysis include that it was *post hoc* and the results can only be considered hypothesis generating. In addition, as mentioned, the finding of a mediation effect does not prove that the described empagliflozin-induced changes in that covariate resulted directly in the improved outcome. It also cannot be inferred that similar changes in these variables achieved with approaches other than empagliflozin treatment will have similar effects on HF outcomes. The sensitivity of a mediation analysis is furthermore dependent on which variables were measured in the study. Thus, other effects of empagliflozin, such as the associated reductions in LV mass observed in mechanistic trials with empagliflozin,[38] may have an effect on HHF outcomes[3] but could not be assessed in this analysis because they were not measured. Finally, the possibility of collider bias exists but is regarded as low. In a typical confounder model, the confounder affects both the exposure and the outcome. In a collider model, the exposure and the outcome both affect the 'collider' that then distorts the association of the exposure with the outcome. From our clinical knowledge, the likelihood that any of the identified major mediators in our analysis (haematocrit, UACR, or uric acid) were influenced by the outcome (HHF or HF death), thus distorting the association between the outcome and empagliflozin, is very low.

In conclusion, an exploratory investigation into potential mediators of the reduction in risk of HHF or HF death with empagliflozin versus placebo in patients with T2DM and established CV disease in the EMPA-REG OUTCOME trial found that, as with CV mortality in our prior analysis, changes in haematocrit and haemoglobin appeared to be the most important mediators of the reduction in risk. However, these may be serving merely as surrogates for the true underlying mediators. For example, haematocrit, haemoglobin, and albumin may largely be reflecting at least to some degree haemoconcentration due to plasma volume reduction, and, as a result, reductions in central pressures and ventricular off-loading. Changes in uric acid and logUACR had smaller mediating effects and may underscore the likely multifactorial underlying mechanisms of empagliflozin on HF outcomes.

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Further study is required to examine whether these factors are actually responsible for the reduction in observed HF outcomes.

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Conflict of interest

D.F. has received honoraria from Sanofi, Merck & Co., Amgen, AstraZeneca, Eli Lilly and Company, and Boehringer Ingelheim and has served on the data and safety monitoring board for Novo Nordisk. S.E.I. has received honoraria for lectures, advisory work and/or clinical trial leadership from AstraZeneca, Boehringer Ingelheim, Novo Nordisk, Sanofi/Lexicon, Merck, vTv Therapeutics, and Abbott/Alere. B.Z. has received research grants awarded to his institution from Boehringer Ingelheim, AstraZeneca, and Novo Nordisk and honoraria from Janssen, Sanofi, Eli Lilly and Company, Boehringer Ingelheim, Novo Nordisk, and Merck. C.W. reports honoraria from AstraZeneca, Boehringer Ingelheim, Eli Lilly and Company, MSD, and Sanofi. J.M.L. reports no dualities within the past 3 years relevant to the content of this article. F.Z. has served on the board of Boston Scientific; received consulting fees from Novartis, Takeda, AstraZeneca, Boehringer Ingelheim, GE Healthcare, Relypsa, Servier, Boston Scientific, Bayer, Johnson & Johnson, and Resmed; speakers' fees from Pfizer and AstraZeneca; and is CardioRenal cofounder. M.S., C.S., and K.O. received an institutional research grant from Boehringer Ingelheim to conduct these analyses. A.P.O, A.S., S.H., and E.B. are employed by Boehringer Ingelheim. J.T.G. was employed by Boehringer Ingelheim at the time of the study.

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