# How Does Empagliflozin Reduce Cardiovascular Mortality? Insights From a Mediation Analysis of the EMPA-REG OUTCOME Trial

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## OBJECTIVE

**In the EMPA-REG OUTCOME trial involving 7,020 patients with type 2 diabetes and established cardiovascular (CV) disease, empagliflozin given in addition to standard of care reduced the risk of CV death by 38% versus placebo (hazard ratio [HR] 0.62 [95% CI 0.49, 0.77]). This exploratory mediation analysis assesses the extent to which treatment group differences in covariates during the trial contributed to CV death risk reduction with empagliflozin.**

## RESEARCH DESIGN AND METHODS

**Effects of potential mediators, identified post hoc, on the HR for CV death with empagliflozin versus placebo were analyzed by Cox regression models, with treatment group adjusted for the baseline value of the variable and its change from baseline or updated mean (i.e., considering all prior values), each as a time-dependent covariate. HRs were compared with a model without adjustment for covariates. Multivariable analyses also were performed.**

## RESULTS

**Changes in hematocrit and hemoglobin mediated 51.8% and 48.9%, respectively, of the effect of empagliflozin versus placebo on the risk of CV death on the basis of changes from baseline, with similar results in analyses on the basis of updated means. Smaller mediation effects (maximum 29.3%) were observed for uric acid, fasting plasma glucose, and $HbA_{1c}$. In multivariable models, which incorporated effects of empagliflozin on hematocrit, fasting glucose, uric acid, and urine albumin: creatinine ratio, the combined changes from baseline provided 85.2% mediation, whereas updated mean analyses provided 94.6% mediation of the effect of empagliflozin on CV death.**

## CONCLUSIONS

**In this exploratory analysis from the EMPA-REG OUTCOME trial, changes in markers of plasma volume were the most important mediators of the reduction in risk of CV death with empagliflozin versus placebo.**


Empagliflozin, a highly selective sodium–glucose cotransporter 2 (SGLT2) inhibitor, was the first glucose-lowering agent to demonstrate a reduction in cardiovascular (CV) death in patients with type 2 diabetes and high CV risk (1). In the EMPA-REG OUTCOME trial, over a median observation time of 3.1 years, treatment with empagliflozin versus placebo in addition to standard of care led to a 14% reduction

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in the risk of three-point major adverse CV events (MACE [the composite of CV death, nonfatal myocardial infarction, and nonfatal stroke]) (hazard ratio [HR] 0.86 [95% CI 0.74, 0.99]; $P = 0.04$). This was driven by a 38% reduction in the risk of CV death (HR 0.62 [95% CI 0.49, 0.77]; $P < 0.001$) (1). The finding led the U.S. Food and Drug Administration to extend the indication for empagliflozin to include reducing the risk of CV death in patients with type 2 diabetes and established CV disease (2). Empagliflozin also reduced the risk of hospitalization for heart failure (HR 0.65 [95% CI 0.50, 0.85]; $P = 0.002$) and all-cause mortality (HR 0.68 [95% CI 0.57, 0.82]; $P < 0.001$) versus placebo (1).

As with other CV outcome trials, EMPA-REG OUTCOME was not designed to determine the mechanisms underpinning its results. Several explanations for the reduction in CV death with empagliflozin have been proposed, including hemodynamic changes related to plasma volume reduction, a switch in use of fuel, and direct cardiac effects (3–9). The very early reduction in CV death observed in this trial and the heterogeneity of the hazard ratios for the components of three-point MACE suggest that the predominant mechanism, at least in the early part of the trial, was not primarily an attenuation of atherosclerosis, the traditional consideration in CV outcome trials in patients with diabetes. The equally rapid reduction in the risk of hospitalization for heart failure suggests that the cardioprotective benefit of empagliflozin may be related to improved hemodynamic status (10). However, effects on atherogenic processes, the myocardium, ventricular remodeling, or vessel integrity cannot be ruled out because the benefits on CV death were sustained over the course of the trial, and additional mechanisms may have contributed to the reduction in the risk of CV death observed with prolonged treatment. In the current exploratory post hoc mediation analysis of data from the EMPA-REG OUTCOME trial, we identified the extent to which treatment-induced changes in specific variables, either alone or in combination, contributed to the reduction in the risk of CV death observed with empagliflozin versus placebo and, thereby, considered potential mediators of this benefit.

## RESEARCH DESIGN AND METHODS

### Trial Design

The design of the EMPA-REG OUTCOME trial has been described previously (1,11). In brief, adults with type 2 diabetes who were drug-naive with an $HbA_{1c}$ of 7.0–10.0% (53–86 mmol/mol) or were taking any background glucose-lowering medication with an $HbA_{1c}$ of 7.0–9.0% (53–75 mmol/mol) and who had established CV disease were eligible for inclusion. Patients were randomized (1:1:1) to receive empagliflozin 10 mg, empagliflozin 25 mg, or placebo. Glucose-lowering medication was to remain unchanged for the first 12 weeks, although intensification was permitted if the patient had a confirmed fasting glucose level of >240 mg/dL; in cases of medical necessity, dose reduction or discontinuation of background medication could occur. After week 12, investigators were encouraged to adjust glucose-lowering medication to achieve glycemic control according to local guidelines. Throughout the trial, investigators were encouraged to treat other CV risk factors according to local guidelines. The trial was to continue until ≥691 patients experienced an adjudicated primary outcome event (three-point MACE). Patients who prematurely discontinued study medication continued to be followed for ascertainment of CV outcomes and vital status.

CV outcome events and deaths were prospectively adjudicated by independent clinical events committees (1,11). Analyses of CV outcomes were prespecified to compare the pooled empagliflozin dose groups with the placebo group.

### Traditional Mediation Analysis

A traditional mediation analysis as originally proposed by Baron and Kenny (12) was used, taking the time-dynamic evolvement of both the potential mediators and the outcome CV death into account. A variable must satisfy several conditions to be a mediator of the treatment effect. Treatment must have an effect on the variable over time, and the change in the variable over time must have an effect on the outcome. As an additional condition, 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 CV Death

The primary analysis of CV death with empagliflozin versus placebo was based on a Cox proportional hazards regression model, with treatment group adjusted for the baseline variables of age, sex, BMI, $HbA_{1c}$, estimated glomerular filtration rate (eGFR), and region, in patients who received one or more doses of study drug (1).

### Analysis of Changes in Variables (Considered Potential Mediators) Over Time

On the basis of evidence from previous studies, potential mediators of the benefit of empagliflozin on CV death were identified post hoc from the variables that were measured and tracked in the trial. Those chosen for analysis involved several mechanistic categories (Table 1): glycemia ($HbA_{1c}$, fasting plasma glucose [FPG]), vascular tone (systolic blood pressure [SBP], diastolic blood pressure [DBP], heart rate), lipids (LDL cholesterol, HDL cholesterol, triglycerides, free fatty acids), adiposity (weight, BMI, waist circumference), renal function (urine albumin-to-creatinine ratio [UACR], eGFR according to MDRD and Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equations), volume status (hematocrit, hemoglobin, albumin), and other (uric acid).

Potential mediators could be physiological parameters conceivably linked to the outcome (e.g., blood pressure) or biochemical markers of such risk (e.g., LDL cholesterol). To be a potential mediator, a variable should satisfy the above-stated conditions. Differences between treatment groups in the longitudinal changes from baseline in each potential mediator were analyzed individually by using a mixed-effects repeated-measures model to establish that treatment had an effect on the variable. The model for each variable included as covariates the baseline value of that variable, baseline $HbA_{1c}$, baseline eGFR, baseline BMI, region, the last week a patient could have had a measurement of that variable, treatment, visit, visit by treatment interaction, baseline $HbA_{1c}$ by visit interaction, and the baseline value of the variable by visit interaction as fixed effects. Because of skewed distributions, triglycerides and UACR were log-transformed before analysis. Each analysis used all data available for patients who received one or more doses of study

**Table 1—Variables analyzed as potential mediators of the effect of empagliflozin versus placebo on risk of CV death in the EMPA-REG OUTCOME trial**

| Mechanistic category and variable | Mean (SE) at baseline | | | Adjusted mean (SE) difference vs. placebo in change from baseline* | | | |
|---|---|---|---|---|---|---|---|
| | | | | Week 12 or 28† | | Week 164 | |
| | Placebo | Empa 10 mg | Empa 25 mg | Empa 10 mg | Empa 25 mg | Empa 10 mg | Empa 25 mg |
| Glycemia | | | | | | | |
| $HbA_{1c}$ (%) | 8.08 (0.02) | 8.08 (0.02) | 8.07 (0.02) | −0.54 (0.02) | −0.60 (0.02) | −0.31 (0.04) | −0.42 (0.04) |
| FPG (mg/dL) | 153.45 (0.91) | 153.23 (0.91) | 151.81 (0.90) | −20.36 (1.12) | −23.80 (1.12) | −8.24 (1.92) | −13.94 (1.91) |
| Vascular tone | | | | | | | |
| SBP (mmHg) | 135.79 (0.36) | 134.91 (0.35) | 135.65 (0.35) | −4.01 (0.40) | −3.72 (0.40) | −3.33 (0.58) | −2.58 (0.58) |
| DBP (mmHg) | 76.83 (0.21) | 76.60 (0.20) | 76.68 (0.20) | −1.48 (0.23) | −1.24 (0.23) | −0.52 (0.34) | −0.19 (0.34) |
| Heart rate (bpm) | 68.41 (0.25) | 68.76 (0.24) | 68.27 (0.25) | −0.63 (0.26) | −1.12 (0.26) | −0.32 (0.39) | −0.60 (0.39) |
| Lipids (mg/dL) | | | | | | | |
| LDL-C | 84.80 (0.74) | 86.29 (0.77) | 85.46 (0.74) | 2.17 (0.82) | 3.27 (0.83) | 0.49 (1.22) | 1.66 (1.22) |
| HDL-C | 44.04 (0.24) | 44.65 (0.25) | 44.49 (0.25) | 1.47 (0.22) | 1.98 (0.22) | 1.18 (0.32) | 2.00 (0.32) |
| TGs | 170.79 (2.53) | 168.49 (2.67) | 172.78 (2.77) | −7.32 (3.03) | −9.99 (3.04) | −2.46 (5.46) | −2.57 (5.43) |
| FFAs | 14.69 (0.15) | 14.50 (0.14) | 14.57 (0.15) | 0.70 (0.20) | 1.01 (0.20) | 1.09 (0.30) | 1.26 (0.30) |
| Renal | | | | | | | |
| UACR (mg/g)‡ | 25.99 (477.32) | 25.51 (452.73) | 25.38 (439.71) | 0.86 (0.82, 0.90) | 0.82 (0.78, 0.87) | 0.78 (0.71, 0.85) | 0.81 (0.74, 0.88) |
| eGFR (MDRD) (mL/min/1.73 $m^2$) | 73.80 (0.44) | 74.34 (0.45) | 74.08 (0.44) | −1.48 (0.31) | −1.96 (0.31) | 2.69 (0.52) | 2.70 (0.52) |
| eGFR (CKD-EPI) (mL/min/1.73 $m^2$) | 75.45 (0.41) | 75.89 (0.41) | 75.68 (0.41) | −1.66 (0.26) | −1.96 (0.26) | 2.49 (0.44) | 2.38 (0.44) |
| Adiposity | | | | | | | |
| Weight (kg) | 86.68 (0.40) | 85.97 (0.39) | 86.53 (0.40) | −1.20 (0.08) | −1.49 (0.08) | −1.60 (0.19) | −1.98 (0.19) |
| BMI (kg/$m^2$) | 30.67 (0.11) | 30.59 (0.11) | 30.62 (0.11) | −0.43 (0.03) | −0.53 (0.03) | −0.56 (0.07) | −0.69 (0.07) |
| WC (cm) | 105.0 (0.3) | 104.8 (0.3) | 104.8 (0.3) | −1.0 (0.1) | −1.1 (0.1) | −1.5 (0.2) | −1.6 (0.2) |
| Volume status | | | | | | | |
| Hematocrit (%) | 41.28 (0.09) | 41.29 (0.09) | 41.44 (0.09) | 2.18 (0.08) | 2.46 (0.08) | 2.57 (0.14) | 2.66 (0.14) |
| Hemoglobin (g/dL) | 13.72 (0.03) | 13.71 (0.03) | 13.76 (0.03) | 0.60 (0.02) | 0.69 (0.02) | 0.78 (0.04) | 0.80 (0.04) |
| Albumin (g/dL) | 4.41 (0.01) | 4.42 (0.01) | 4.43 (0.01) | 0.04 (0.01) | 0.06 (0.01) | 0.04 (0.01) | 0.05 (0.01) |
| Other | | | | | | | |
| Uric acid (mg/dL) | 6.01 (0.03) | 5.91 (0.03) | 5.97 (0.03) | −0.34 (0.03) | −0.35 (0.03) | −0.29 (0.05) | −0.30 (0.05) |

Mediation analyses adjusting for changes from baseline in covariates or updated mean covariate values were based on changes before CV death, not at week 12, 28, or 64. bpm, beats per minute; Empa, empagliflozin; FFA, free fatty acid; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TG, triglyceride; WC, waist circumference. *Mixed-model repeated-measures analysis using all data up to individual trial completion in treated patients who had a baseline and postbaseline measurement for the respective outcome. The model included baseline $HbA_{1c}$ and baseline of the outcome in question as linear covariates and baseline eGFR, region, BMI, the last week a patient could have had a measurement of the outcome in question, treatment, visit, visit by treatment interaction, baseline $HbA_{1c}$ by visit interaction, and baseline of the outcome in question by visit interaction as fixed effects. †Week 12 for variables other than heart rate, LDL-C, HDL-C, TGs, and FFAs (which were not measured at week 12). ‡Baseline data are geometric mean (geometric coefficient of variation); differences vs. placebo are adjusted gMean ratio (95% CI) obtained from mixed-model repeated-measures analysis applied on log-transformed data.

drug and had a baseline and postbaseline measurement for the variable in question.

### Analysis of Potential Mediation of the Reduction in CV Death With Empagliflozin

#### *Univariable Analyses*

We considered that empagliflozin could have a direct effect on the risk of CV death or could affect CV death indirectly through its effects on one or more of the aforementioned potential mediators. Each variable was analyzed as a time-dependent covariate in Cox regression models with the outcome time to CV death. The effect of a specific covariate was analyzed by using two approaches.

1. Analysis of the current change from baseline to the most recent value available before CV death, reflecting the acute effect of the variable on the risk of CV death. In the example below, the current change from baseline in a mediator *Y* (*changeY*) is calculated as the difference in the current value to baseline:

$$changeY_{t_i} = Y_{t_i} - Y_0$$

2. Analysis of the mean value considering all prior values (updated mean), reflecting the cumulative effect of all prior values of the variable on the risk of CV death (see example illustration in the Supplementary Material). In the example below, the updated mean of a mediator *Y* (*upmeanY*) is calculated as the updated weighted mean, weighted for the length of time intervals:

$$upmeanY\ (t_i\ until\ t_{i+1}) = \frac{\sum_{0<t_j\le t_i}\left(Y_{t_{j-1}}+Y_{t_j}\right)/2\times(t_j-t_{j-1})}{t_i}$$

The mediating effect of the current change from baseline in a variable, and of the updated mean of the variable, on the treatment effect of empagliflozin versus placebo with respect to CV death were analyzed by using separate Cox regression models, with treatment group adjusted for the baseline value of the variable and either the change from baseline in the variable or the updated mean of the variable as time-dependent covariates. The models provided the estimated HR for CV death associated with a 1-unit increase in the variable (current change from baseline or updated mean). The results were compared with a model adjusting for treatment group alone (i.e., without adjusting for the baseline value of the variable and the longitudinal values of the variable [change or updated mean]).

To be regarded as a mediator, the time-dependent covariate should have an effect on CV death; this was considered to be fulfilled if the 95% CI of the HR did not include 1.0. In addition, the effect of empagliflozin on CV death must be reduced in the analysis adjusted for the time-dependent covariate. Mediation was indicated if the HR for CV death between treatment groups adjusted for the covariate (current change or updated mean) was closer to unity than the HR from the model with treatment group alone. Complete mediation would be indicated by an HR of 1.0 in the model adjusted for the covariate. Percentage mediation was calculated as follows:

$$Mediation\ \% = 100 \times \frac{lnHR - lnHR_C}{lnHR}$$

where *HR* is the HR for the comparison of the treatment groups in the model with treatment group alone, and $HR_C$ is the HR for the comparison of the treatment groups in the model adjusting for the baseline and on-study time-dependent covariate values (change from baseline or updated mean). The closer $HR_C$ is to 1.0, the greater the mediation effect of that covariate on the treatment effect of empagliflozin on the risk of CV death.

#### *Multivariable Analyses*

Additional analyses investigated how selected individual mediators from all the mechanistic categories jointly contribute 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 mechanistic category was chosen for the multivariable model. A step-up procedure for multivariable model building was used to provide a ranking of the various 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 resampling procedure was used on the basis of 100 bootstrap samples (sampling with replacement) of the same size as the original data set. The unadjusted Cox model and the Cox model adjusted for the final selected mediators were fitted in each bootstrap sample. The stability of the relationship between the resulting treatment effect estimates (logHR from the unadjusted and adjusted models) was graphically displayed and described by linear regression.

## RESULTS

### Effects of Empagliflozin on the Time Course of Potential Mediators

Table 1 shows the mean of each potential mediator at baseline and the differences with empagliflozin versus placebo in changes from baseline at week 12 or 28 and week 164. Over the course of the study, empagliflozin was associated with small reductions versus placebo in $HbA_{1c}$, weight, waist circumference, uric acid, SBP and DBP, no change in heart rate, and small increases in LDL and HDL cholesterol (1). An initial decrease in eGFR (CKD-EPI) with empagliflozin was observed followed by stabilization during prolonged treatment in contrast to a gradual decline in eGFR in the placebo group (13). Empagliflozin led to significant reductions in UACR versus placebo from week 12, regardless of albuminuria status at baseline (14). An initial increase in hematocrit with empagliflozin was observed followed by stabilization compared with no notable change in the placebo group (Fig. 1).

### Effects of Change in Potential Mediators on the Risk of CV Death

In analyses adjusting for change from baseline, models showed that increases in FPG, heart rate, logUACR, and uric acid were associated with an increased risk of CV death, whereas increases in SBP, HDL cholesterol, eGFR, weight, BMI, hematocrit, hemoglobin, and albumin were associated with a reduced risk of CV death (Supplementary Table 1). In analyses that were based on the updated mean, models showed that increases in $HbA_{1c}$, FPG, heart rate, LDL cholesterol, logUACR, and uric acid were associated with an increased risk of CV death, whereas increases in HDL cholesterol, eGFR, hematocrit, hemoglobin, and albumin were associated with a reduced risk of CV death (Supplementary Table 2).

### Univariable Analysis: Effects of Individual Potential Mediators on the HR for CV Death With Empagliflozin Versus Placebo

Table 2 presents the HRs for CV death with empagliflozin versus placebo after adjusting for the current change from baseline in each covariate and the percentage mediation compared with the HR adjusting for treatment group alone. Treatment group differences in changes from baseline in hematocrit and hemoglobin mediated 51.8% and 48.9%, respectively, of the effect of empagliflozin versus placebo on the reduction in risk of CV death. Changes in albumin and uric acid mediated 25.5% and 24.6%, respectively. The other potential mediators investigated, including $HbA_{1c}$, FPG, weight, BMI, SBP and DBP, LDL and HDL cholesterol, triglycerides, free fatty acids, eGFR, and UACR, had no or negligible effects in these analyses.

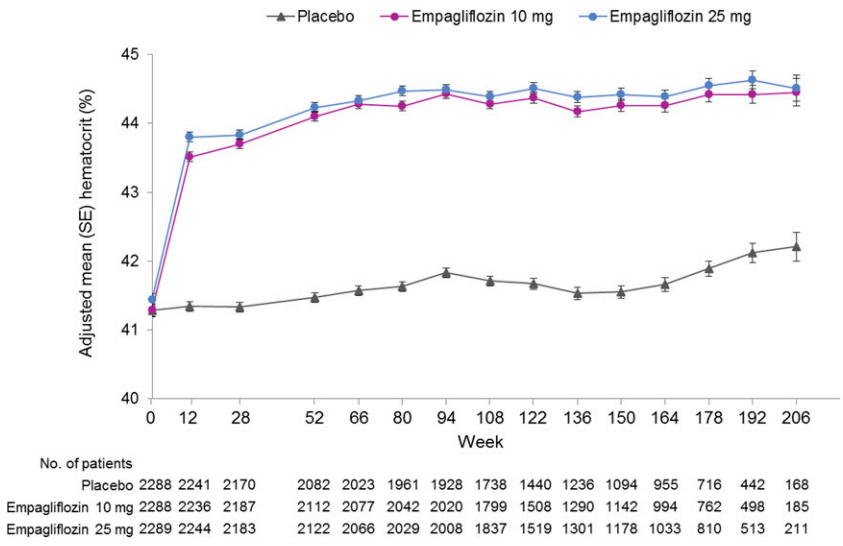


**Figure 1**—Hematocrit over time in patients treated with empagliflozin 10 mg, empagliflozin 25 mg, and placebo. Mixed-model repeated-measures analysis using all data up to individual trial completion in patients treated with one or more doses of study drug who had a baseline and postbaseline measurement.

**Table 2—Univariable mediation analysis of risk of CV death with empagliflozin versus placebo: time-dependent covariate analysis adjusting for the change from baseline in each variable**

| | HR for CV death with empagliflozin vs. placebo (95% CI) | Percentage mediation |
|---|---|---|
| Unadjusted | 0.615 (0.491, 0.770) | |
| Adjusted for | | |
| $HbA_{1c}$ | 0.624 (0.496, 0.785) | 3.0 |
| FPG | 0.665 (0.529, 0.837) | 16.1 |
| SBP | 0.593 (0.473, 0.743) | −7.5 |
| DBP | 0.614 (0.490, 0.769) | −0.3 |
| Heart rate | 0.621 (0.495, 0.780) | 2.0 |
| LDL-C | 0.596 (0.475, 0.748) | −6.5 |
| HDL-C | 0.636 (0.506, 0.799) | 6.9 |
| logTG | 0.604 (0.482, 0.758) | −3.7 |
| FFAs | 0.586 (0.463, 0.741) | −9.9 |
| logUACR | 0.649 (0.518, 0.815) | 11.1 |
| eGFR (MDRD) | 0.631 (0.504, 0.790) | 5.3 |
| eGFR (CKD-EPI) | 0.632 (0.505, 0.791) | 5.6 |
| Weight | 0.579 (0.461, 0.727) | −12.4 |
| BMI | 0.578 (0.460, 0.726) | −12.8 |
| WC | 0.598 (0.477, 0.750) | −5.8 |
| Hematocrit | 0.791 (0.626, 1.000) | 51.8 |
| Hemoglobin | 0.780 (0.619, 0.983) | 48.9 |
| Albumin | 0.696 (0.555, 0.873) | 25.5 |
| Uric acid | 0.693 (0.553, 0.869) | 24.6 |

Cox proportional hazards regression analysis in patients treated with one or more doses of study drug. FFA, free fatty acid; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TG, triglyceride; WC, waist circumference.

Table 3 presents the mediating effect after adjusting for the updated mean of each covariate and the baseline value. Adjusting for the updated mean of hematocrit and hemoglobin mediated 51.8% and 45.7%, respectively, of the effect of empagliflozin versus placebo on the reduction in the risk of CV death. Adjusting for the updated mean of albumin and uric acid mediated 31.6% and 18.5%, respectively. Adjusting for the updated mean of $HbA_{1c}$ and FPG mediated 22.8% and 29.3%, respectively, of the treatment group effect, which were notably stronger mediating effects than in the change from baseline analysis (3.0% and 16.1%, respectively). The other potential mediators investigated, including weight, BMI, SBP and DBP, LDL and HDL cholesterol, triglycerides, free fatty acids, eGFR, and UACR, had no or negligible effects in these analyses.

### Multivariable Analysis: Effects of Combinations of Potential Mediators on the HR for CV Death With Empagliflozin Versus Placebo

In the current change from baseline analysis, the strongest mediator was hematocrit, representing the volume category (proportion of the effect of empagliflozin on CV death mediated: 51.8%). The addition of FPG as representative of the glycemia category led to a proportion mediated of 70.9%. The addition of uric acid increased the proportion mediated to 84.4%. Finally, the addition of logUACR as representative of the renal function category led to an estimated HR for CV death with empagliflozin versus placebo of 0.931 (95% CI 0.732, 1.183) (Supplementary Table 3), with the total proportion mediated being 85.2%. In a sensitivity analysis wherein hematocrit was replaced by hemoglobin as representative of the volume category, the estimated HR for CV death with empagliflozin versus placebo was 0.927 (95% CI 0.730, 1.176), thus showing a similar proportion mediated of 84.4%.

In the updated mean analysis, the results were similar. The strongest mediator was again hematocrit (proportion of the effect of empagliflozin on CV death mediated: 51.8%). The addition of FPG in the next step led to a proportion mediated of 84.9%. The addition of uric acid led to a proportion mediated of 92.2%. Finally, the addition of logUACR led to an estimated HR for CV death of 0.974 (95% CI 0.753, 1.261) (Supplementary Table 4), with a total proportion mediated being 94.6%. In the updated mean analysis, the replacement of hematocrit by hemoglobin showed a similar result (proportion mediated: 91.2%). Similarly, replacement of the updated mean FPG by the updated mean $HbA_{1c}$ resulted in 85.1% mediation. Results of stability analyses on the basis of bootstrap resampling are shown in Supplementary Figs. 1 and 2.

## CONCLUSIONS

We conducted this exploratory analysis to identify potential mechanisms underlying the 38% reduction in the risk of CV death observed with empagliflozin versus placebo in patients with type 2 diabetes and established CV disease in the EMPA-REG OUTCOME trial. Our approach used a traditional mediation analysis (15,16), taking the time-dynamic evolvement of the potential mediators and the outcome of CV death into account. This method has been used by others to determine or confirm the underlying mechanisms behind a treatment strategy's effect on disease outcomes (17–19). Therein, change in the point estimate of the HR denoting a treatment effect is measured after sequential controlling for a variety of plausible variables, which are known to be ameliorated with therapy. The variables that bring the HR closest to 1.0 are said to be the major mediators of the treatment effect, suggesting, but not proving, a cause-and-effect relationship. In the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) study, for example, improvement in $HbA_{1c}$ was found to mediate essentially 100% of the benefit of intensive insulin therapy on the development and progression of retinopathy (19). Such an outcome was logical and expected because the goal of that trial was to reduce glycemia, as measured by $HbA_{1c}$, in an effort to reduce long-term microvascular damage. In contrast, multiple mechanisms contribute to the CV risk in patients with type 2 diabetes. The precise contributions of individual hypothesized mechanisms to the reduction in CV death with empagliflozin remain to be elucidated, and a mediation analysis such as this may be used to drive future research.

In the current analysis, changes in hematocrit (and hemoglobin) appeared to be the variables with the largest impact on the HR for CV death with empagliflozin versus placebo. Therefore, these variables can be considered important mediators of this benefit, mediating ~50% of the treatment group effect. Results were consistent in analyses that were based on

**Table 3—Univariable mediation analysis of risk of CV death with empagliflozin versus placebo: time-dependent covariate analysis adjusting for the updated mean of each variable**

| | HR for CV death with empagliflozin vs. placebo (95% CI) | Percentage mediation |
|---|---|---|
| Unadjusted | 0.615 (0.491, 0.770) | |
| Adjusted for | | |
| $HbA_{1c}$ | 0.687 (0.543, 0.868) | 22.8 |
| FPG | 0.709 (0.559, 0.898) | 29.3 |
| SBP | 0.610 (0.485, 0.766) | −1.7 |
| DBP | 0.618 (0.493, 0.774) | 1.0 |
| Heart rate | 0.623 (0.497, 0.782) | 2.7 |
| LDL-C | 0.591 (0.471, 0.741) | −8.2 |
| HDL-C | 0.629 (0.500, 0.789) | 4.6 |
| logTG | 0.603 (0.481, 0.757) | −4.1 |
| FFAs | 0.587 (0.463, 0.743) | −9.6 |
| logUACR | 0.672 (0.536, 0.844) | 18.2 |
| eGFR (MDRD) | 0.601 (0.480, 0.752) | −4.7 |
| eGFR (CKD-EPI) | 0.597 (0.477, 0.748) | −6.1 |
| Weight | 0.588 (0.466, 0.741) | −9.2 |
| BMI | 0.588 (0.466, 0.742) | −9.2 |
| WC | 0.602 (0.480, 0.755) | −4.4 |
| Hematocrit | 0.791 (0.620, 1.009) | 51.8 |
| Hemoglobin | 0.768 (0.604, 0.978) | 45.7 |
| Albumin | 0.717 (0.571, 0.900) | 31.6 |
| Uric acid | 0.673 (0.536, 0.845) | 18.5 |

Cox proportional hazards regression analysis in patients treated with one or more doses of study drug. FFA, free fatty acid; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TG, triglyceride; WC, waist circumference.

the current change from baseline or the updated mean. For uric acid and measures of glycemia, modest mediation effects were found, with those of $HbA_{1c}$ and FPG appearing to be stronger in analyses that were based on the updated mean, which assessed the more chronic effects of these measures. In contrast, the mediation effects of other variables, including changes in classical CV risk factors, such as BMI, blood pressure, lipids, and other parameters of renal function, were absent or negligible.

In the multivariable models, a much higher percentage mediation was demonstrated (up to 85.2% in the change from baseline analysis and up to 94.6% in the updated mean analysis). These models incorporated widely disparate effects of empagliflozin, including those on hematocrit, FPG, uric acid, and UACR. These data suggest that although the major influence governing the reduction in CV death may have been plasma volume reduction (as reflected in the increased hematocrit), other variables may have played more modest, yet complementary roles, and multiple mechanisms may be responsible for the reduction in CV death with empagliflozin in patients with type 2 diabetes and established CV disease.

Empagliflozin is a selective inhibitor of SGLT2 in the proximal tubule of the kidney (20). Inhibition of SGLT2 by empagliflozin leads to reduced renal glucose reabsorption and increased urinary glucose excretion (21). Treatment with empagliflozin reduces volume and sodium load through its glucuretic, diuretic, and natriuretic properties (22,23). The initial increase in hematocrit with empagliflozin followed by stabilization during the rest of the trial likely reflect hemodynamic changes related to plasma volume contraction. The same pattern was observed for changes in eGFR with empagliflozin, which also are believed to reflect hemodynamic alterations involving renal blood flow (13). The resulting decrease in circulatory load, especially reduced ventricular filling pressures and cardiac workload, could be an important mechanism behind the mortality benefits seen with empagliflozin (5,9). This finding is supported by the observation that the most frequent modes of CV death are those typically seen in patients with heart failure (sudden death, death as a result of heart failure, and presumed CV death, the latter designated when insufficient data exist for the adjudication committees to attribute a cause of death) (24). Although only 10% of patients in the EMPA-REG OUTCOME trial had heart failure at baseline, the trial population comprised individuals with a mean age of 63 years, of whom 76% had coronary artery disease and 52% were obese. Therefore, many participants in this trial likely had unrecognized left ventricular dysfunction, particularly diastolic dysfunction, which may eventually lead to clinical heart failure with preserved ejection fraction (25). Thus, a tenable conclusion is that a key contributor to the reduction in CV death with empagliflozin is the change in renal sodium and glucose handling with resultant reductions in fluid burden, ventricular stress, and risk of sudden cardiac decompensation.

An increase in erythropoiesis could be a complementary mechanism to the hemodynamic changes reflected by an increase in hematocrit and hemoglobin in patients treated with empagliflozin. Increased erythropoietin and a median 7% increase in red blood cell mass, measured with $^{51}$Cr-labeled erythrocytes, were observed in a small study ($n$ = 30) of the SGLT2 inhibitor dapagliflozin in patients with type 2 diabetes (26). In a larger study, a mean increase in erythropoietin of 30–40% was observed 4 weeks after initiating empagliflozin, which may be related to changes in blood flow between the renal cortex and the medulla (27). The extent to which this mechanism contributes to the CV benefits observed with empagliflozin is unclear, however. Although an improvement in tissue oxygenation in a compromised cellular milieu may be hypothesized to be beneficial, such a robust effect on mortality would be unexpected on the basis of current understanding of oxygen delivery dynamics at hemoglobin concentrations within the normal range (28).

Improvements in the updated mean $HbA_{1c}$ and FPG explained 23% and 29%, respectively, of the beneficial effect of empagliflozin on CV death compared with 3% and 16% for the current change from baseline. In the DCCT/EDIC study and the UK Prospective Diabetes Study (UKPDS), CV benefits of intensive glucose control emerged but only after a prolonged follow-up period (29,30). Unlike the UKPDS and the DCCT study, which were designed to achieve glycemic differences, the EMPA-REG OUTCOME trial was designed as a glycemic equipoise trial, and only a modest difference in $HbA_{1c}$

between the empagliflozin and placebo groups was found. Furthermore, unlike the UKPDS with a cumulative follow-up of >10 years (30), trials of shorter duration did not convincingly demonstrate CV benefits from this degree of glucose lowering (31). The smaller mediation effect of glycemia relative to hematological variables, therefore, may reflect these factors. Change in uric acid had a modest mediation effect (24.6% in the analysis adjusting for the change from baseline). Empagliflozin reduces uric acid, possibly as a result of the effect of increased glucose concentration on glucose transporter 9 in the basolateral membrane of the proximal tubule and resulting increased uricosuria (22,32). Serum uric acid has been associated with an increased risk of CV death (33), but evidence is limited regarding the CV benefits of reducing uric acid (34), especially within the normal range and/or to the small degree observed in the EMPA-REG OUTCOME trial.

Limitations of the current analysis include that it was post hoc, and the results can only be considered hypothesis generating, demonstrating possible associations but not necessarily causal relationships. It cannot be inferred that similar changes in these variables achieved with approaches other than empagliflozin treatment will yield the mortality benefits observed. Moreover, only variables measured during the trial could be examined, and in addition to the potential mediators we investigated, other variables such as reductions in glomerular hyperfiltration (35), reductions in arterial stiffness and vascular resistance (36), direct cardiac effects through reductions in myocardial intracellular sodium (37), and a switch in use of cardiac fuel (4,5) have been proposed as important. Finally, although the methods we used in the current analysis have been used previously, they have not been used in the context of CV death. CV death encompasses multiple subcategories with potentially disparate etiologies that may not be influenced by the same mechanisms.

In conclusion, this exploratory investigation into potential mediators of the reduction in risk of CV death with empagliflozin versus placebo in the EMPA-REG OUTCOME trial found that changes in hematocrit and hemoglobin—ostensibly markers of the effects of the drug on volume—appeared to be important mediators of the reduction in mortality risk in univariable and multivariable models. Changes in variables related to glycemia and urate metabolism had smaller mediating effects. These, in addition to changes in UACR, contributed in the multivariable models, suggesting that the underpinnings of empagliflozin's CV mortality benefit are likely multifaceted. In contrast, changes in some traditional CV risk factors, including obesity, blood pressure, lipids, and renal function, made negligible contributions. Ongoing studies, including mechanistic trials, the EMPEROR outcome trials (EMPagliflozin outcomE tRial in Patients With chrOnic heaRt Failure With Reduced Ejection Fraction and EMPagliflozin outcomE tRial in Patients With chrOnic heaRt Failure With Preserved Ejection Fraction) that are evaluating empagliflozin in patients with heart failure with and without diabetes (NCT03057977, NCT03057951), and studies in patients with chronic kidney disease (38), will provide additional physiological insights into the cardioprotective effects of this selective SGLT2 inhibitor.

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## References

1. Zinman B, Wanner C, Lachin JM, et al.; EMPA-REG OUTCOME Investigators. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med 2015;373:2117–2128
2. Boehringer Ingelheim. Jardiance® (empagliflozin) tablets prescribing information. 2016. Available from: http://bidocs.boehringer-ingelheim.com/BIWebAccess/ViewServlet.ser?docBase=renetnt&folderPath=/Prescribing+Information/PIs/Jardiance/jardiance.pdf. Accessed 18 January 2017
3. Heerspink HJ, Perkins BA, Fitchett DH, Husain M, Cherney DZ. Sodium glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: cardiovascular and kidney effects, potential mechanisms, and clinical applications. Circulation 2016;134:752–772
4. Mudaliar S, Alloju S, Henry RR. Can a shift in fuel energetics explain the beneficial cardiorenal outcomes in the EMPA-REG OUTCOME study? A unifying hypothesis. Diabetes Care 2016;39:1115–1122
5. Sattar N, McLaren J, Kristensen SL, Preiss D, McMurray JJ. SGLT2 inhibition and cardiovascular events: why did EMPA-REG Outcomes surprise and what were the likely mechanisms? Diabetologia 2016;59:1333–1339
6. Ferrannini E, Baldi S, Frascerra S, et al. Shift to fatty substrate utilization in response to sodium-glucose cotransporter 2 inhibition in subjects without diabetes and patients with type 2 diabetes. Diabetes 2016;65:1190–1195
7. Marx N, McGuire DK. Sodium-glucose cotransporter-2 inhibition for the reduction of cardiovascular events in high-risk patients with diabetes mellitus. Eur Heart J 2016;37:3192–3200
8. Jørgensen NB, Pedersen J, Vaag AA. EMPA-REG: glucose excretion and lipid mobilization - not storage - saves lives. J Diabetes Complications 2016;30:753
9. Rajasekeran H, Lytvyn Y, Cherney DZ. Sodium-glucose cotransporter 2 inhibition and cardiovascular risk reduction in patients with type 2 diabetes: the emerging role of natriuresis. Kidney Int 2016;89:524–526
10. Fitchett D, Zinman B, Wanner C, et al.; EMPA-REG OUTCOME® Trial Investigators. Heart failure outcomes with empagliflozin in patients with type 2 diabetes at high cardiovascular risk: results of the EMPA-REG OUTCOME® trial. Eur Heart J 2016;37:1526–1534
11. Zinman B, Inzucchi SE, Lachin JM, et al. Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empagliflozin (EMPA-REG OUTCOME™). Cardiovasc Diabetol 2014;13:102
12. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J Pers Soc Psychol 1986;51:1173–1182
13. Wanner C, Inzucchi SE, Lachin JM, et al.; EMPA-REG OUTCOME Investigators. Empagliflozin and progression of kidney disease in type 2 diabetes. N Engl J Med 2016;375:323–334
14. Wanner C, Zinman B, Inzucchi SE, et al. Effect of empagliflozin on albuminuria in patients with

type 2 diabetes and high cardiovascular risk. J Am Soc Nephrol 2016;27:552A
15. VanderWeele TJ. Mediation: introduction and regression-based approaches. In *Explanation in Causal Inference Methods for Mediation and Interaction*. New York, Oxford University Press, 2015, p. 20–65
16. MacKinnon DP. *Introduction to Statistical Mediation Analysis*. New York, Taylor & Francis, 2008
17. Mittelman MS, Haley WE, Clay OJ, Roth DL. Improving caregiver well-being delays nursing home placement of patients with Alzheimer disease. Neurology 2006;67:1592–1599
18. Colón-Emeric CS, Mesenbrink P, Lyles KW, et al. Potential mediators of the mortality reduction with zoledronic acid after hip fracture. J Bone Miner Res 2010;25:91–97
19. Lachin JM, White NH, Hainsworth DP, Sun W, Cleary PA, Nathan DM; Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Research Group. Effect of intensive diabetes therapy on the progression of diabetic retinopathy in patients with type 1 diabetes: 18 years of follow-up in the DCCT/EDIC. Diabetes 2015;64:631–642
20. Grempler R, Thomas L, Eckhardt M, et al. Empagliflozin, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterisation and comparison with other SGLT-2 inhibitors. Diabetes Obes Metab 2012;14:83–90
21. Heise T, Seewaldt-Becker E, Macha S, et al. Safety, tolerability, pharmacokinetics and pharmacodynamics following 4 weeks' treatment with empagliflozin once daily in patients with type 2 diabetes. Diabetes Obes Metab 2013;15:613–621
22. Heise T, Jordan J, Wanner C, et al. Pharmacodynamic effects of single and multiple doses of empagliflozin in patients with type 2 diabetes. Clin Ther 2016;38:2265–2276
23. Heise T, Jordan J, Wanner C, et al. Acute pharmacodynamic effects of empagliflozin with and without diuretics in patients with type 2 diabetes. Clin Ther 2016;38:2248–2264.e5
24. Fitchett D, Inzucchi SE, Lachin JM, et al. Further exploration of the cardiovascular mortality reduction with empagliflozin in patients with type 2 diabetes and cardiovascular disease in EMPA-REG OUTCOME. J Am Coll Cardiol. In press
25. Low Wang CC, Hess CN, Hiatt WR, Goldfine AB. Clinical update: cardiovascular disease in diabetes mellitus: atherosclerotic cardiovascular disease and heart failure in type 2 diabetes mellitus - mechanisms, management, and clinical considerations. Circulation 2016;133:2459–2502
26. Lambers Heerspink HJ, de Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. Diabetes Obes Metab 2013;15:853–862
27. Ferrannini E, Baldi S, Frascerra S, et al. Renal handling of ketones in response to sodium-glucose co-transporter-2 inhibition in patients with type 2 diabetes. Diabetes Care 2017;40:771–776
28. Messmer K. Dependance of oxygen delivery on hematocrit. In *Oxygen Transport to Tissue XVI*. Hogan MC, Mathieu-Costello O, Poole DC, Wagner PD, Eds. New York, Springer Science+Business Media, 1994, p. 379–380
29. Nathan DM, Genuth S, Lachin J, et al.; Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977–986
30. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2008;359:1577–1589
31. Giorgino F, Home PD, Tuomilehto J. Glucose control and vascular outcomes in type 2 diabetes: Is the picture clear? Diabetes Care 2016;39(Suppl. 2):S187–S195
32. Chino Y, Samukawa Y, Sakai S, et al. SGLT2 inhibitor lowers serum uric acid through alteration of uric acid transport activity in renal tubule by increased glycosuria. Biopharm Drug Dispos 2014;35:391–404
33. Odden MC, Amadu AR, Smit E, Lo L, Peralta CA. Uric acid levels, kidney function, and cardiovascular mortality in US adults: National Health and Nutrition Examination Survey (NHANES) 1988-1994 and 1999-2002. Am J Kidney Dis 2014;64:550–557
34. Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. N Engl J Med 2008;359:1811–1821
35. Cherney DZ, Perkins BA, Soleymanlou N, et al. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. Circulation 2014;129:587–597
36. Chilton R, Tikkanen I, Cannon CP, et al. Effects of empagliflozin on blood pressure and markers of arterial stiffness and vascular resistance in patients with type 2 diabetes. Diabetes Obes Metab 2015;17:1180–1193
37. Baartscheer A, Schumacher CA, Wüst RC, et al. Empagliflozin decreases myocardial cytoplasmic Na(+) through inhibition of the cardiac Na(+)/H(+) exchanger in rats and rabbits. Diabetologia 2017;60:568–573
38. Boehringer Ingelheim. Empagliflozin (Jardiance®) to be studied in chronic kidney disease. 2017. Available from: https://www.boehringer-ingelheim.com/press-release/empagliflozin-be-studied-chronic-kidney-disease. Accessed 6 September 2017