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ORIGINAL RESEARCH

Association Between Circulating GDF-15 and Cardio-Renal Outcomes and Effect of Canagliflozin: Results From the CANVAS Trial

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BACKGROUND: Studies have suggested that sodium glucose co-transporter 2 inhibitors exert anti-inflammatory effects. We examined the association of baseline growth differentiation factor-15 (GDF-15), a marker of inflammation and cellular injury, with cardiovascular events, hospitalization for heart failure (HF), and kidney outcomes in patients with type 2 diabetes in the CANVAS (Canagliflozin Cardiovascular Assessment Study) and determined the effect of the sodium glucose co-transporter 2 inhibitor canagliflozin on circulating GDF-15.

METHODS AND RESULTS: The CANVAS trial randomized 4330 people with type 2 diabetes at high cardiovascular risk to canagliflozin or placebo. The association between baseline GDF-15 and cardiovascular (non-fatal myocardial infarction, non-fatal stroke, cardiovascular death), HF, and kidney (40% estimated glomerular filtration rate decline, end-stage kidney disease, renal death) outcomes was assessed using multivariable adjusted Cox regression models. During median follow-up of 6.1 years (N=3549 participants with available samples), 555 cardiovascular, 129 HF, and 137 kidney outcomes occurred. Each doubling in baseline GDF-15 was significantly associated with a higher risk of cardiovascular (hazard ratio [HR], 1.2; 95% CI, 1.0–1.3), HF (HR, 1.5; 95% CI, 1.2–2.0) and kidney (HR, 1.5; 95% CI, 1.2–2.0) outcomes. Baseline GDF-15 did not modify canagliflozin's effect on cardiovascular, HF, and kidney outcomes. Canaglifozin treatment modestly lowered GDF-15 compared with placebo; however, GDF-15 did not mediate the protective effect of canagliflozin on cardiovascular, HF, or kidney outcomes.

CONCLUSIONS: In patients with type 2 diabetes at high cardiovascular risk, higher GDF-15 levels were associated with a higher risk of cardiovascular, HF, and kidney outcomes. Canagliflozin modestly lowered GDF-15, but GDF-15 reduction did not mediate the protective effect of canagliflozin.

Key Words: canagliflozin ■ GDF-15 ■ renal and cardiovascular outcomes ■ SGLT2 inhibitor

The CANVAS (Canagliflozin Cardiovascular Assessment Study) trial showed that the sodium glucose co-transporter 2 (SGLT2) inhibitor canagliflozin reduced the risk of hospitalization for heart failure (HF) and slowed progression of kidney function decline in patients with type 2 diabetes at high cardiovascular risk.[1] The underlying mechanisms for these effects are not completely understood. Several mechanisms are thought to be involved, including restoration of tubuloglomerular feedback, reductions in blood pressure, and improvements in vascular function leading to reductions in afterload, as well as reductions in cardiac and kidney inflammation and fibrosis.[2–7]

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CLINICAL PERSPECTIVE

What Is New?

- In a post hoc analysis of patients with type 2 diabetes and high cardiovascular risk from the CANVAS (Canagliflozin Cardiovascular Assessment Study) trial, higher levels of growth differentiation factor-15 (GDF-15), a biomarker released during inflammation and cellular stress, were associated with cardiovascular, heart failure, and kidney outcomes.
- Treatment with canagliflozin attenuated increases in GDF-15 over time.

What Are the Clinical Implications?

- GDF-15 has potential values as a prognostic marker of adverse cardiovascular- and kidney-related outcomes in patients with type 2 diabetes and high cardiovascular risk.
- Although canagliflozin treatment attenuated GDF-15 levels, changes in GDF-15 did not explain the protective effect of canagliflozin on cardiovascular, heart failure, and kidney outcomes.

Nonstandard Abbreviations and Acronyms

CANVAS	Canagliflozin Cardiovascular Assessment Study
GDF-15	growth differentiation factor-15
SGLT2	sodium glucose co-transporter 2
UACR	urine albumin-to-creatinine ratio

Growth differentiation factor-15 (GDF-15) is a stress-induced cytokine released in response to injury or oxidative stress in various organs and is a member of the transforming growth factor-β super family.[8] GDF-15 is released in cardiomyocytes and cells of the collecting duct.[8,9] Higher GDF-15 levels in the systemic circulations are observed in patients with type 2 diabetes, chronic kidney disease, and HF or other cardiovascular diseases.[8,10–14] Observational studies have also shown that elevated GDF-15 is associated with a higher risk of developing HF and kidney failure in patients with type 2 diabetes with or without chronic kidney disease.[8,15,16] A Mendelian randomization study suggested that GDF-15 may be causally involved in cardiovascular disease progression.[17] In addition, in type 2 diabetic GDF-15 knock-out mice, SGLT2 expression in the proximal tubule is reduced, suggesting a possible interaction between SGLT2 and GDF-15.[18]

In this post hoc analysis of the CANVAS trial, we first assessed whether plasma GDF-15 levels are associated with the primary cardiovascular, HF, and kidney outcomes. Second, we investigated the effect of canagliflozin treatment on GDF-15 levels and whether baseline plasma GDF-15 or early changes in GDF-15 mediated the observed beneficial effect of canagliflozin on cardiovascular, HF, and kidney outcomes.

METHODS

Patients and Study Design

For this post hoc analysis, we used stored plasma samples obtained during the CANVAS trial. Design, results, and outcomes of this trial have been published previously.[1] In short, the CANVAS trial was a randomized, placebo-controlled, double-blind, multicenter study that assessed the effect of canagliflozin on cardiovascular, renal, safety, and efficacy outcomes in patients with type 2 diabetes who had a history of cardiovascular disease or multiple cardiovascular risk markers. During the trial, blood and urine samples were stored for exploratory biomarker research. Participants eligible for inclusion were randomly assigned in a 1:1:1 ratio to treatment with 100 mg canagliflozin, 300 mg canagliflozin, or placebo. In total, 4330 participants from 24 countries with type 2 diabetes were enrolled. The trial was conducted according to the principles of the Declaration of Helsinki and was approved by the necessary regulatory authorities and ethics committees. All participants provided written informed consent. The trial is registered with ClinicalTrials.gov (identifier NCT01032629).

Eligible participants had type 2 diabetes with a hemoglobin A1c level of ≥58 mmol/mol (7.0%) and ≤91 mmol/mol (10.5%) and were either ≥30 years of age with a history of symptomatic atherosclerotic cardiovascular disease or ≥50 years of age with ≥2 risk factors for cardiovascular disease. Risk factors for cardiovascular disease were defined as a duration of diabetes of ≥10 years, systolic blood pressure >140 mm Hg receiving >1 antihypertensive agent, currently smoking, micro- or macroalbuminuria, or high-density lipoprotein cholesterol level of <1 mmol/L. At inclusion, participants also needed to have an estimated glomerular filtration rate (eGFR) of >30 mL/min per 1.73 m^2 and meet other criteria for inclusion.

Biomarker Assessment

Stored blood plasma samples obtained during the CANVAS trial at baseline and at weeks 52, 156, and 312, were used to measure GDF-15 using the Elecsys GDF-15 electrochemiluminescence immunoassay (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). All GDF-15 measurements occurred between February 27, 2019 and August 8, 2019. A total of 405 samples were measured in duplicate to assess measurement variability. The coefficient of variation of these duplicates was <8.2%. We also assessed

day-to-day laboratory variability in the GDF-15 measurements by analyzing samples with predefined GDF-15 concentrations at multiple time points, together with the CANVAS trial samples. The coefficient of variation of these duplicate control measurements was <8.6%.

Outcomes

The cardiovascular outcome was defined as a composite of non-fatal myocardial infarction, non-fatal stroke, or death attributable to cardiovascular cause. The HF outcome was defined as hospitalization for HF, including subjects with HF at baseline. The composite kidney outcome was defined as a sustained 40% decline of eGFR, end-stage kidney disease (defined as an eGFR <15 mL/min per 1.73 m^2 or need for dialysis or kidney transplantation) or renal death. These end points were adjudicated by an independent adjudication committee using predefined and rigorous end point definitions.

Statistical Analysis

Baseline continuous variables with normal distributions were reported as means with SDs. Baseline variables with skewed distributions were reported as medians with interquartile range. Variables with skewed distributions were natural logarithmic transformed before analysis. Variables in categorical orders were reported as percentages.

Hazard ratios per doubling in baseline GDF-15 were estimated using multivariable Cox proportion hazard regression. In addition, baseline GDF-15 levels were categorized into quartiles, and the hazard ratio (HR) in each quartile was estimated using the first quartile as a common reference. Four consecutive models were built, each adding different covariates to assess the effect of the step-wise addition of covariates on the association between GDF-15 and outcomes. In the first model, age, sex, race, and randomized treatment assignment (canagliflozin or placebo) were included. In the second model, history of cardiovascular disease (yes or no), hemoglobin A1c, systolic and diastolic blood pressure, body mass index, and low-density lipoprotein cholesterol were added. The third model introduced eGFR (calculated with the Modification of Diet in Renal Disease formula) and, in the final model, natural log-transformed urine albumin-to-creatinine ratio (UACR) was added to the above-mentioned covariates. The fully adjusted model was also used to estimate HRs of the association between GDF-15 and outcomes in subgroups defined by randomized treatment assignment, baseline age, sex, eGFR, UACR, and cardiovascular disease history. We assessed C-statistics to assess the discriminative ability of GDF-15.

Few patients (<0.5%) had missing values. These few missing values in continuous normally distributed covariates were imputed as means of the respective covariate, and missing values in continuous not normally distributed covariates were imputed as medians.

The modification of treatment effect of canagliflozin versus placebo on cardiovascular, HF, and kidney outcomes by baseline GFD-15 were explored in Cox proportional hazard regression models. Interactions terms between plasma GDF-15 tertile group and randomized treatment assignment were fitted in relevant Cox models to test for heterogeneity.

The effect of canagliflozin versus placebo on GDF-15 concentrations over time was assessed by calculating the between-group difference in the change from baseline in GDF-15 using linear mixed-effects models. The models included treatment allocation and time as factors, an interaction term between treatment allocation and time, and was adjusted for baseline GDF-15 value and the interaction term between time and baseline GDF-15 value. The variance-covariance matrix was assumed to be unstructured (ie, purely data dependent). Subgroup analyses by baseline (<30 and ≥30 mg/g) and eGFR (<60 and ≥60 mL/min per 1.73 m^2) were performed to explore the consistency of the treatment effect of canagliflozin.

For each outcome, we also provided a descriptive assessment of the percentage of the randomized treatment effect removed with adjustment for change in plasma biomarker levels, as was done previously in the CANVAS trial.[19] For each outcome, the percentage of the treatment effect explained was expressed using the equation: $100\% \times ([HR - HR_{adjusted}]/[HR - 1])$.

All analyses were performed in SAS, version 9.4 (SAS Institute, Cary, NC, USA), and Stata, version 16.1 (StataCorp College Station, TX, USA).

Data Availability

Clinical data from the CANVAS Program are available in the public domain via the Yale University Open Data Access Project (http://yoda.yale.edu/).

RESULTS

Study Population

In total, 3549 (82.0%) of the 4330 participants in the CANVAS trial had available plasma at baseline. Baseline characteristics of these participants are shown in Table 1. Baseline characteristics were well matched between randomized groups and were representative of the overall trial population. Overall, the mean age of the population was 62.8 years, 33.1% were women, 13.3% had a history of HF, 59.5% had a history of cardiovascular disease, mean body mass index was 32.7, mean hemoglobin A1c was 65.7 mmol/mol (8.2%), mean diabetes duration was 13.5 years,

Table 1. Baseline Characteristics of the CANVAS Cohort With Available GDF-15 Concentrations

Characteristic	Total (N=3549)	Placebo (n=1192)	Canagliflozin (n=2357)
Age, y	62.8 (7.9)	62.5 (7.8)	62.9 (7.9)
Female sex, n (%)	1175 (33.1)	393 (33.0)	782 (33.2)
History of heart failure, n (%)	473 (13.3)	174 (14.6)	299 (12.7)
Duration of diabetes, y	13.5 (7.5)	13.3 (7.5)	13.7 (7.5)
History of cardiovascular disease, n (%)	2113 (59.5)	704 (59.1)	1409 (59.8)
BMI, kg/m^2	32.7 (6.1)	32.6 (6.2)	32.7 (6.1)
Systolic BP, mm Hg	136.7 (15.9)	137.2 (15.7)	136.4 (15.9)
Diastolic BP, mm Hg	77.6 (9.8)	78.2 (9.8)	77.3 (9.8)
Hemoglobin A1c			
mmol/mol	65.7 (9.9)	65.6 (9.9)	65.8 (10.0)
%	8.2 (0.9)	8.2 (0.9)	8.2 (0.9)
eGFR, mL/min per 1.73 m^2	77.0 (18.8)	76.8 (18.9)	77.0 (18.7)
eGFR <60, n (%)	585 (16.5)	210 (17.6)	375 (15.9)
eGFR ≥60, n (%)	2964 (83.5)	982 (82.4)	1982 (84.1)
ACR, mg/g, median (IQR)	11.6 (6.4–34.7)	11.6 (6.2–36.4)	11.6 (6.5–34.3)
Normoalbuminuria, n (%)	2570 (72.4)	854 (71.6)	1716 (72.8)
Microalbuminuria, n (%)	778 (21.9)	257 (21.6)	521 (22.1)
Macroalbuminuria, n (%)	201 (5.7)	81 (6.8)	120 (5.1)
GDF-15, pg/mL	1774 (1242–2514)	1752 (1243–2524)	1791 (1242–2512)

Continuous variables are reported as mean with SD or median with interquartile range. Categorical variables are reported as quantity (n) with percentage. ACR indicates albumin-to-creatinine ratio; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; and IQR, interquartile range.

mean eGFR was 77.0 mL/min per 1.73 m^2, and median GDF-15 level was 1774 pg/mL at baseline.

Pearson correlation coefficients showed generally weak correlations between baseline GDF-15 values and cardiovascular risk markers, except for baseline eGFR, UACR, and age (Figure S1).

Association of Baseline GDF-15 With Cardiovascular, HF, Kidney, and All-Cause Mortality Outcomes

Participants were followed for a median of 6.1 (interquartile range, 5.8 to 6.3) years. During follow-up, 555 (15.6%), 129 (3.6%), and 137 (3.9%) participants experienced the cardiovascular, HF, and kidney outcome, respectively. Cox proportional hazard regression with adjustment for patient demographics and randomized treatment showed that each doubling in GDF-15 was significantly associated with the cardiovascular, HF, and kidney outcomes (Table 2). These associations remained statistically significant after further adjustment for risk markers, including eGFR and UACR, with corresponding HRs per doubling of baseline GDF-15 in the fully adjusted model of 1.2 (95% CI, 1.0–1.3; P=0.01), 1.5 (95% CI, 1.2–2.0; P<0.01), and 1.5 (95% CI, 1.2–2.0; P<0.01) for the cardiovascular, HF, and kidney outcomes, respectively (Table 2). Similar results were obtained in subgroup analyses by treatment assignment, age, sex, UACR, eGFR, and cardiovascular disease history for the cardiovascular, HF, and kidney outcomes (Figure 1). When baseline GDF-15 was analyzed as a categorical variable, the highest quartile of GDF-15 was associated with 2- and 3-fold increased risks of the HF and kidney outcomes, respectively, in the fully adjusted model (Table 2). In an additional analysis we observed that each doubling of baseline GDF-15 was associated with all-cause mortality with a corresponding HR of 1.3 (95% CI, 1.1–1.5) in the fully adjusted model with similar results for subgroups (Table S1 and Figure S2). Assessment of the C-statistics of the models for each outcome showed moderate to good prognostic performance (Table S2).

Effect of Canagliflozin on Cardiovascular, HF, and Kidney Outcomes by Baseline Plasma GDF-15 Levels

In this cohort of CANVAS participants with available GDF-15 concentrations, canagliflozin reduced the risk of the kidney outcome by 44% (HR, 0.56 [95% CI, 0.40–0.79; P<0.01]) compared with placebo. The HRs for the cardiovascular and HF outcomes were 0.91 (95% CI, 0.76–1.08; P=0.28) and 0.82 (95% CI, 0.58–1.17; P=0.28), respectively. There was no evidence that the effect size of canagliflozin for cardiovascular, HF, or kidney outcomes varied by the baseline level of GDF-15 (all P values for heterogeneity >0.07; Figure 2).

Table 2. Associations of Quartiles and Doubling in GDF-15 With the Cardiovascular, HF, and Kidney Outcomes

	Model 1		Model 2		Model 3		Model 4	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Cardiovascular outcome								
GDF-15								
Quartile 1	(reference)		(reference)		(reference)		(reference)	
Quartile 2	1.0 (0.7–1.2)	0.76	1.0 (0.8–1.3)	0.91	1.0 (0.7–1.3)	0.82	0.9 (0.7–1.2)	0.66
Quartile 3	1.3 (1.0–1.6)	0.06	1.3 (1.0–1.7)	0.05	1.2 (1.0–1.6)	0.10	1.2 (0.9–1.5)	0.27
Quartile 4	1.5 (1.2–1.9)	<0.01	1.5 (1.2–2.0)	<0.01	1.5 (1.1–1.9)	<0.01	1.3 (1.0–1.7)	0.05
Per doubling	1.3 (1.2–1.5)	<0.01	1.3 (1.2–1.5)	<0.01	1.3 (1.1–1.4)	<0.01	1.2 (1.0–1.3)	0.01
HF outcome								
GDF-15								
Quartile 1	(reference)		(reference)		(reference)		(reference)	
Quartile 2	1.6 (0.8–3.2)	0.18	1.5 (0.7–2.9)	0.27	1.4 (0.7–2.9)	0.31	1.3 (0.7–2.7)	0.42
Quartile 3	2.4 (1.3–4.6)	0.01	2.0 (1.1–3.9)	0.03	1.9 (1.0–3.6)	0.06	1.6 (0.8–3.1)	0.16
Quartile 4	3.9 (2.1–7.3)	<0.01	3.1 (1.7–5.9)	<0.01	2.8 (1.5–5.2)	<0.01	2.1 (1.1–4.1)	0.02
Per doubling	2.0 (1.6–2.5)	<0.01	1.8 (1.4–2.4)	<0.01	1.7 (1.3–2.2)	<0.01	1.5 (1.2–2.0)	<0.01
Kidney outcome								
GDF-15								
Quartile 1	(reference)		(reference)		(reference)		(reference)	
Quartile 2	2.2 (1.1–4.4)	0.02	2.3 (1.2–4.6)	0.02	2.3 (1.2–4.6)	0.02	2.0 (1.0–4.0)	0.05
Quartile 3	2.7 (1.4–5.2)	<0.01	2.6 (1.3–5.1)	0.01	2.6 (1.3–5.1)	0.01	1.7 (0.8–3.4)	0.14
Quartile 4	6.1 (3.2–11.4)	<0.01	6.1 (3.2–11.6)	<0.01	6.0 (3.2–11.5)	<0.01	3.4 (1.7–6.6)	<0.01
Per doubling	2.2 (1.7–2.7)	<0.01	2.2 (1.7–2.7)	<0.01	2.2 (1.7–2.7)	<0.01	1.5 (1.2–2.0)	<0.01

Models are adjusted for the following covariates: Model 1: Age, sex, race, and randomized treatment. Model 2: Covariates of model 1+history of cardiovascular disease, hemoglobin A1c, systolic and diastolic blood pressure, body mass index, and low-density lipoprotein cholesterol. Model 3: Covariates of model 2+baseline estimated glomerular filtration rate. Model 4: Covariates of model 3+log transformed baseline urine albumin-to-creatinine ratio. eGFR indicates estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; and HF, heart failure.

Effect of Canagliflozin on Plasma GDF-15

In the placebo group, GDF-15 concentrations increased over time (Figure 3). Canagliflozin attenuated this increase, resulting in a modest least squares mean difference in GDF-15 of –3.4% (95% CI, –6.5% to –0.3%; *P*=0.032) at 3 years and –7.1% (95% CI, –11.6% to –2.4%; *P*=0.004) at 6 years (Figure 3). The least squares mean difference during follow-up between canagliflozin and placebo, considering all measurements, was –3.7% (95% CI, –6.3% to –1.0%; *P*=0.007). The effect of canagliflozin compared with placebo on the difference in GDF-15 over time was consistent in subgroups defined by baseline UACR <30 or ≥30 mg/g or eGFR <60 or ≥60 mL/min per 1.73 m^2 (Table 3).

Proportion of Treatment Effect Explained by Change in GDF-15

Analyses of the proportion of treatment effects on the cardiovascular, HF, and kidney outcomes, explained by the change in the plasma biomarkers, demonstrated that changes in GDF-15 did not explain the effects of canagliflozin on these outcomes (proportion of effect explained 0.1%, 2.3%, and 2.3% for the cardiovascular, HF, and kidney outcomes, respectively).

DISCUSSION

Circulating GDF-15 is a marker of inflammation and cellular injury and is increased in patients with type 2 diabetes, chronic kidney disease, and HF. In this post hoc analysis from the CANVAS trial, we demonstrate that, in patients with type 2 diabetes at high cardiovascular risk, increased levels of circulating GDF-15 are associated with cardiovascular, HF, and kidney outcomes. We also demonstrated that canagliflozin attenuated the increase in GDF-15 over time, although the proportion of canagliflozin's protective effect on the 3 prespecified outcomes could not be explained by the modest reduction in GDF-15 observed.

Prior studies have already examined the association between GDF-15 and cardiovascular and kidney outcomes in patients with type 2 diabetes with or without kidney disease.[8,16] We confirm and extend

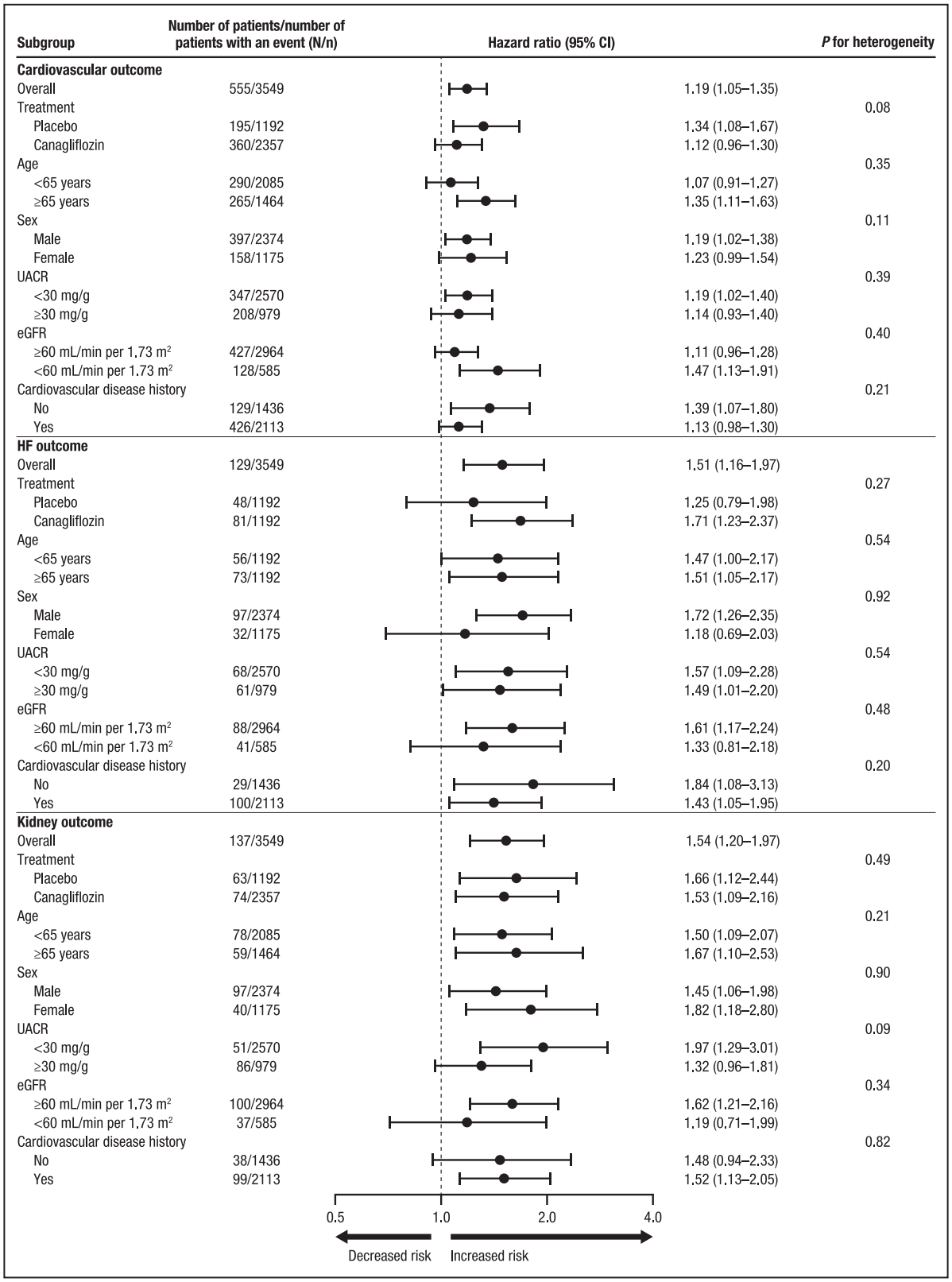

Figure 1. Associations of the doubling in growth differentiation factor-15 with the cardiovascular, heart failure, and kidney outcomes in subgroups defined by treatment assignment, and baseline age, sex, urine albumin-to-creatinine ratio, estimated glomerular filtration rate, and cardiovascular disease history.
eGFR indicates estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; HF, heart failure; and UACR, urine albumin-to-creatinine ratio.

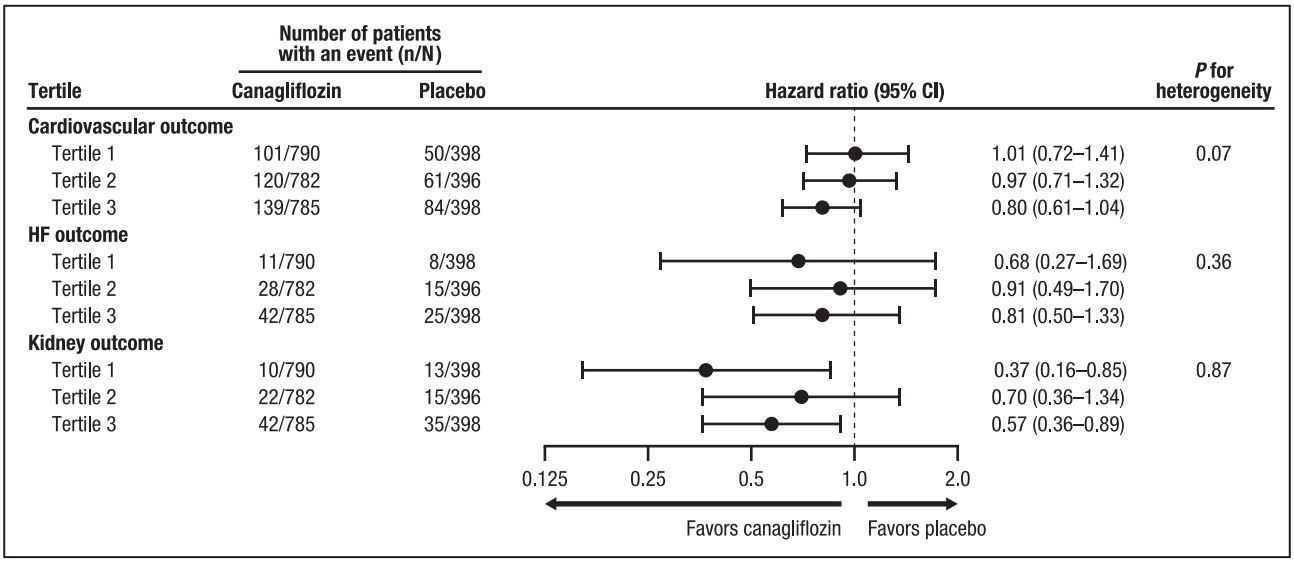

Figure 2. Forest plot of the treatment effect of canagliflozin on cardiovascular, heart failure, and kidney outcomes by tertiles of baseline growth differentiation factor-15 levels.
GDF-15 indicates growth differentiation factor-15; and HF, heart failure.

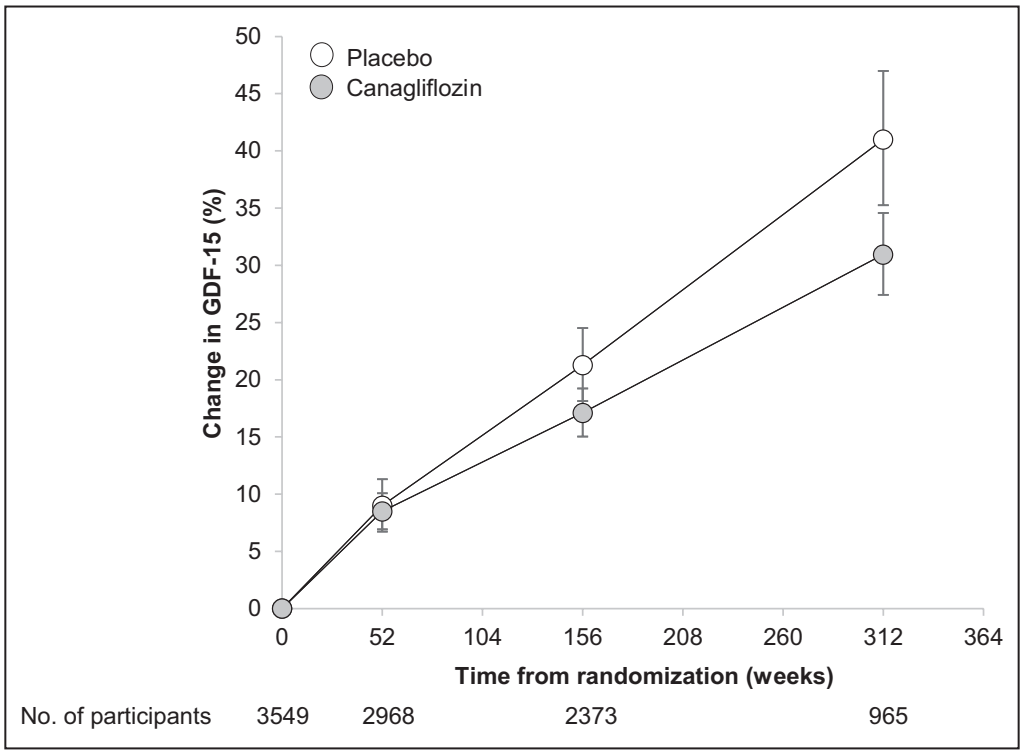

Figure 3. Change in plasma growth differentiation factor-15 over time in the canagliflozin and placebo groups.
GDF-15 indicates growth differentiation factor-15.

these findings to a large, heterogeneous population of patients of various ethnicities with type 2 diabetes at high cardiovascular risk, who were treated according to contemporary guidelines. We also showed that these associations were consistent across various patient subgroups defined by baseline demographic and clinical laboratory parameters. The comparability of our results with previous findings in different populations

Table 3. Changes in Plasma GDF-15 in the Placebo- and Canagliflozin-Treated Groups Over Time in Subgroups Defined by Baseline UACR and eGFR

	Baseline GDF-15 in canagliflozin (pg/mL)	Baseline GDF-15 in placebo (pg/mL)	Canagliflozin change (%) (95% CI)	Placebo change (%) (95% CI)	Placebo corrected effect canagliflozin (%) (95% CI)	*P* interaction
GDF-15						
Treatment						
UACR						
<30 mg/g	1686	1606.5	16.1 (14.0 to 18.2)	20.4 (17.2 to 23.6)	−3.6 (−6.6 to −0.5)	0.95
≥30 mg/g	2012	2007.5	26.1 (22.4 to 30.0)	31.1 (25.4 to 37.0)	−3.8 (−8.8 to 1.5)	
eGFR						
<60 mL/min per 1.73 m^2	2246	2075	32.1 (28.6 to 35.7)	35.2 (30.0 to 40.5)	−3.2 (−6.1 to −0.3)	0.46
≥60 mL/min per 1.73 m^2	1706	1622	14.4 (12.5 to 16.3)	19.3 (16.4 to 22.3)	−4.2 (−7.0 to −1.2)	

eGFR indicates estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; and UACR, urine albumin-to-creatinine ratio.

highlights the prognostic value of baseline GDF-15 for adverse cardio-kidney outcomes.

A previous experimental study in a GDF-15 knockout diabetic mice model reported increased glycosuria because of decreased tubular SGLT2 expression, suggesting that, at low GDF-15 levels, SGLT2 activity may be decreased.[18] To assess whether these experimental findings have therapeutic implications, we assessed the effect of canagliflozin according to baseline GDF-15 levels and observed consistent effects of canagliflozin on cardiovascular, HF, and kidney outcomes, regardless of baseline GDF-15 levels. Whereas the relative risk reductions were consistent, the absolute benefits of canagliflozin in preventing cardiovascular, HF, and kidney outcomes were greater in the highest tertile of baseline GDF-15 levels because of the higher absolute risk among these participants.

The mechanisms explaining how SGLT2 inhibitors decrease cardiovascular and kidney events is an area of great research interest. As a general inflammatory and tissue injury stress marker, it is interesting that GDF-15 levels were modestly reduced by canagliflozin. These effects became apparent after 3 years of treatment and were consistent in patients with preserved and impaired kidney function. These results contrast to a prior study of empagliflozin, which reported that empagliflozin increased GDF-15 levels.[20] However, there are important differences between our trial and the empagliflozin study. First, our study was much larger, involving 3549 patients compared with only 72 in the prior study. In addition, we compared the effect of canagliflozin with placebo, whereas the prior study did not include a control group. Although canagliflozin modestly reduced GDF-15, adjusting the treatment effect of canagliflozin for changes in GDF-15 demonstrated that GDF-15 did not explain the protective effect of canagliflozin on cardiovascular, HF, and kidney outcomes. Thus, although GDF-15 is a prognostic marker, the beneficial effects of canagliflozin are unlikely to be mediated through molecular pathways represented by GDF-15.

The downstream signaling pathways for how GDF-15 is associated with adverse cardiovascular and kidney outcomes is incompletely understood, but it is thought that the effect might be mediated by different pathways, such as glial cell–derived neurotrophic factor receptor α-like, endothelial nitric oxide synthase, SMAD 2 and 7, and nuclear factor kappa B.[21–23] Some studies indicate that GDF-15 is released in the setting of damage and may exert a preventative role through attenuation of interstitial fibrosis in the kidneys and prevents hypertrophy and reduces the formation of cardiac lesions.[23–27] It is unclear whether the increase in circulating GDF-15 in various diseases is a response to injury to prevent further damage or marks a failure to protect the heart and kidney. GDF-15 is elevated in various chronic diseases including diabetes, cancer, cardiovascular disease, and autoimmune diseases suggesting it is involved in the pathophysiology of multiple diseases.[10,28–31] Because GDF-15 is elevated in different diseases, the clinical utility of GDF-15 as a diagnostic marker is limited. However, GDF-15 has been shown to predict clinical end points in these different diseases illustrating its utility as a prognostic risk marker.

This study has some limitations. First, because the design of the study was post hoc, no causality can be inferred between GDF-15 and the outcomes. It is likely, as shown with the mediation analyses, that GDF-15 reflects other molecular pathways that are mediating effects to prevent cardiovascular, HF, and kidney events. Second, although we measured samples obtained during a large multicenter clinical trial, the results can only be applied to patients with similar characteristics to the CANVAS trial cohort. However, the consistency in subgroup analyses and consistent findings in the literature support the generalizability of GDF-15 as a risk marker for cardiovascular, HF, and kidney outcomes.

Lastly, the attenuation in GDF-15 in the canagliflozin group compared with the placebo group after 3 years of follow-up could be the result of improved disease status rather than a treatment effect of canagliflozin per sé.

In conclusion, we confirm the prognostic association of GDF-15 with cardiovascular, HF, and kidney outcomes in patients with type 2 diabetes and established cardiovascular disease or who were at high cardiovascular risk. In addition, treatment with canagliflozin attenuated elevations of GDF-15 over time. This effect was consistent in patient subgroups but did not explain the protective effect of canagliflozin on cardiovascular, HF, or kidney outcomes.

ARTICLE INFORMATION

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Sen measured all samples. Sen, Li, and Heerspink conducted statistical analysis. Sen and Heerspink wrote the first draft of the manuscript. Neuen, Perkovic, and Mahaffey were involved in data collection. All authors contributed to data interpretation, provided input into subsequent drafts, and approved the final version for submission. All authors reviewed and approved the manuscript.

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Supplementary Material

Tables S1–S2
Figures S1–S2

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SUPPLEMENTAL MATERIAL

Table S1. Associations of quartiles and doubling in GDF-15 with the all-cause mortality outcome by four different models.

		Model 1		Model 2		Model 3		Model 4	
		Hazard ratio (95% CI)	***P* value**	**Hazard ratio (95% CI)**	***P* value**	**Hazard ratio (95% CI)**	***P* value**	**Hazard ratio (95% CI)**	***P* value**
		All-cause mortality outcome							
GDF-15									
	Quartile 1	(reference)		(reference)		(reference)		(reference)	
	Quartile 2	1.05 (0.74, 1.49)	0.77	1.06 (0.75, 1.50)	0.74	1.04 (0.74, 1.48)	0.81	1.00 (0.70, 1.41)	0.99
	Quartile 3	1.64 (1.19, 2.25)	<0.01	1.63 (1.18, 2.24)	<0.01	1.56 (1.13, 2.16)	0.01	1.40 (1.01, 1.95)	0.04
	Quartile 4	2.03 (1.49, 2.77)	<0.01	2.03 (1.48, 2.78)	<0.01	1.91 (1.38, 2.63)	<0.01	1.60 (1.15, 2.23)	0.01
	Per doubling	1.50 (1.31, 1.72)	<0.01	1.51 (1.31, 1.74)	<0.01	1.46 (1.26, 1.69)	<0.01	1.32 (1.14, 1.54)	<0.01

Table S2. C-statistics of the Cox-proportional hazard regression models used to assess the association of the doubling in GDF-15 with each outcome.

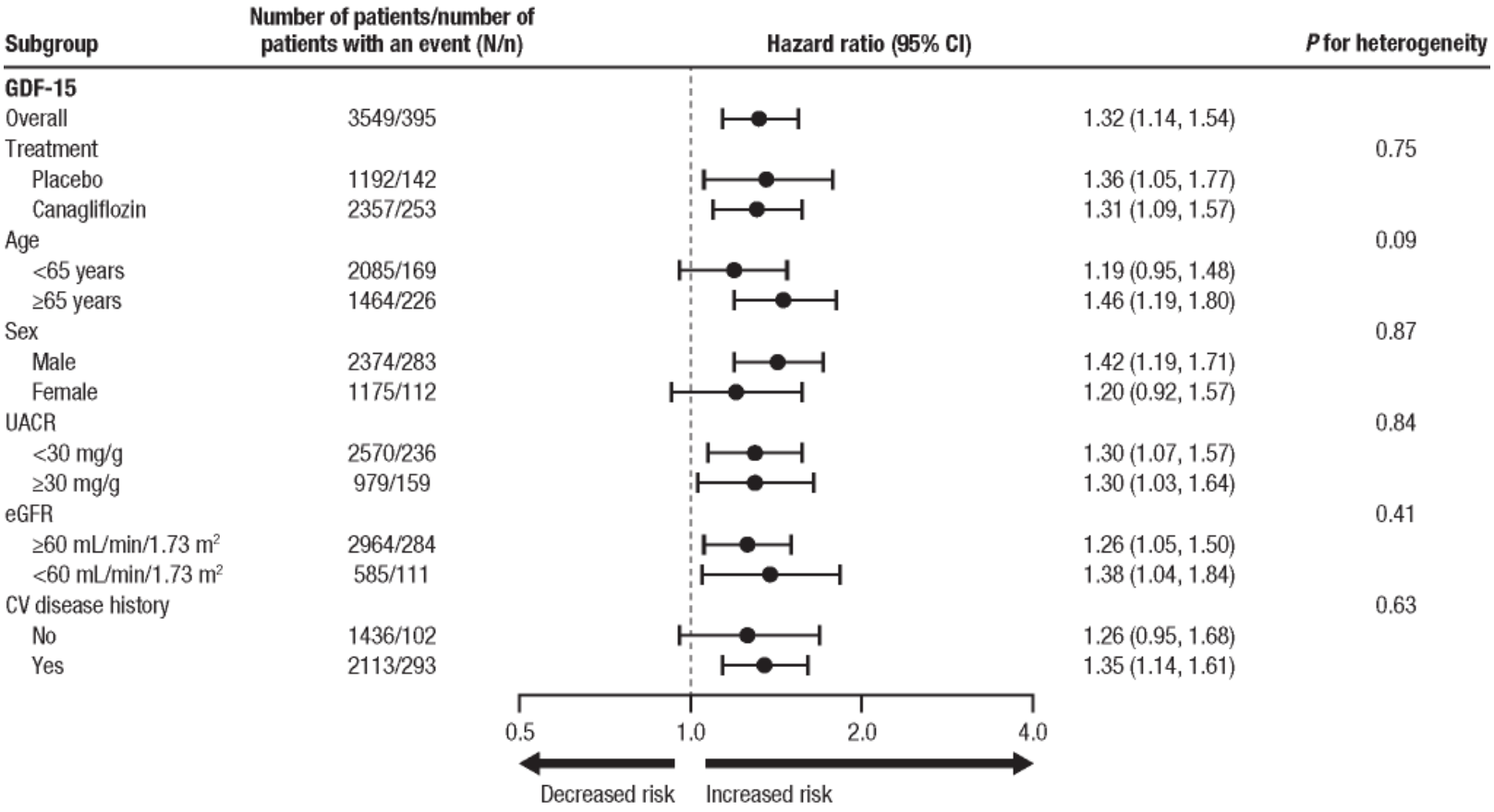
Outcome	**Model 1** C-statistic (95% CI)	**Model 2** C-statistic (95% CI)	**Model 3** C-statistic (95% CI)	**Model 4** C-statistic (95% CI)
Cardiovascular	0.5922 (0.5667, 0.6176)	0.6713 (0.6476, 0.6949)	0.6726 (0.6489, 0.6963)	0.6805 (0.6569, 0.7040)
Heart failure	0.6971 (0.6544, 0.7399)	0.7873 (0.7516, 0.8223)	0.7904 (0.7550, 0.8259)	0.8014 (0.7664, 0.8365)
Kidney	0.6838 (0.6395, 0.7281)	0.7258 (0.6839, 0.7578)	0.7258 (0.6839, 0.7678)	0.7992 (0.7597, 0.8388)
All-cause mortality	0.6566 (0.6277, 0.6855)	0.6969 (0.6695, 0.7244)	0.6985 (0.6709, 0.7261)	0.7132 (0.6859, 0.7406)

Figure S1. Pearson correlation test of baseline GDF-15 with covariates used in the assessment of the association of baseline GDF-15 with CV, HF, and kidney outcomes.

	GDF-15
Age (years)	0.28
Sex (male/female)	0.04
Race	-0.03
Treatment with canagliflozin (yes/no)	0.00
History of CV disease (yes/no)	0.03
HbA1c	0.02
Systolic blood pressure	0.00
Diastolic blood pressure	-0.17
Body mass index	-0.02
Low-density lipoprotein	-0.17
eGFR	-0.31
UACR	0.24

GDF-15: growth differentiation factor-15; CV: cardiovascular; HF: heart failure; eGFR: estimated glomerular filtration rate; UACR: urine albumin-to-creatinine ratio.

Figure S2. Associations of the doubling in GDF-15 with the all-cause mortality outcome by subpopulations defined by treatment assignment, age, sex, UACR, eGFR, and CV disease history.



GDF-15: growth differentiation factor-15; CV: cardiovascular; UACR: urine albumin-to-creatinine ratio; eGFR: estimated glomerular filtration rate; CI: confidence interval.