Role of Glycosuria in SGLT2 Inhibitor–Induced Cardiorenal Protection: A Mechanistic Analysis of the CREDENCE Trial

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SGLT2 inhibitors have been shown to provide pronounced reductions in cardiorenal outcomes, including cardiovascular death, heart failure, and renal failure. The mechanisms underlying these benefits remain uncertain. We hypothesized that the effects could be attributed to the elevated glycosuria induced by these drugs. Urine concentrations of glucose, creatinine, and ketones were measured at baseline and after 1 year of treatment with either placebo or canagliflozin 100 mg/day, in approximately 2,600 individuals from the Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) trial (enrolling patients with type 2 diabetes, chronic kidney disease (CKD), and albuminuria). Associations between glycosuria and the primary composite end point from CREDENCE, and secondary outcomes were assessed using Cox proportional hazards models. Canagliflozin treatment increased fractional urinary glucose excretion (± SD) from 3 ± 9% at baseline to 30 ± 26% at year 1 (vs. 5 ± 19% with placebo; $P < 0.001$). Patients in the canagliflozin arm and in the top quartile of urine glucose to creatinine ratio at year 1 were significantly protected for the primary end point (hazard ratio [HR] 0.42; 95% CI 0.30–0.61); similar results were seen for cases of hospitalized heart failure (HR 0.45; 95% CI 0.27–0.73) and all-cause death (HR 0.56; 95% CI 0.39–0.80). These associations persisted when adjustments were made for multiple conventional risk factors. Among patients with type 2 diabetes and CKD treated with canagliflozin, individuals with the highest glycosuria levels had the strongest protection against multiple cardiorenal outcomes.

ARTICLE HIGHLIGHTS

- In cardiovascular outcome trials, SGLT2 inhibitors have been shown to provide marked protection against both cardiovascular morbidity and progression of kidney disease. The mechanisms underlying these effects remain imperfectly understood.
- By using urine samples from patients with type 2 diabetes and kidney disease treated with canagliflozin, we investigated the relationship of glycosuria (i.e., the readout of SGLT2 engagement) with cardiorenal outcomes.
- Individuals with the highest glycosuria levels had the strongest protection against multiple cardiorenal outcomes.
- Glycosuria is mechanistically linked with several effects underlying cardiorenal benefit.

Avoiding glycosuria has been a mainstay in the management of diabetes, with the aim of preventing or reducing symptoms and monitoring metabolic control of the disease. However, over the past decade, the role of the kidney in diabetes has been revisited. Hyperglycemia increases glomerular capillary pressure and single-nephron glomerular filtration rate (GFR), and overactivity of sodium–glucose cotransporters in the proximal renal tubule of patients with type 2 diabetes contributes to their hyperglycemia. Inhibiting sodium–glucose cotransporter 2 (SGLT2) not only improves glycemic control but also yields unexpected benefits for the prevention of

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macrovascular complications (1). In a series of cardiovascular (CV) outcome trials, use of different SGLT2 inhibitors was associated with a large, clinically significant reduction in the risk of CV death and hospitalizations for heart failure (hHF) in a broad range of patients with heart failure (2). Furthermore, a recent meta-analysis conclusively demonstrated that SGLT2 inhibition leads to a consistent reduction in the progression of renal disease (3).

The Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) trial randomized patients with type 2 diabetes and macroalbuminuric kidney disease to receive canagliflozin or placebo and adjudicated a primary composite outcome of renal disease or death from renal or CV disease (4). Several mechanisms have been proposed to explain such a favorable clinical effect; however, to our knowledge, the specific, quantitative contribution of glycosuria itself has not been evaluated in this or any of the other SGLT2 inhibitors cardiorenal outcome trials. To address this question, we used urine samples from a large, representative subsample of the CREDENCE participants.

RESEARCH DESIGN AND METHODS

The CREDENCE trial enrolled 4,401 patients with type 2 diabetes, chronic kidney disease (CKD; estimated glomerular filtration rate [eGFR] $\geq$30 to $<$90 mL/min/1.73 m^2) and albuminuria (urine albumin to creatinine ratio [UACR] $>$300 to $\leq$5,000 mg/g) who were randomized (1:1) to receive canagliflozin 100 mg or placebo (3). The primary composite outcome was end-stage kidney disease (dialysis for at least 30 days, kidney transplantation, or an eGFR of $<$15 mL/min/1.73 m^2 sustained for at least 30 days), doubling of the serum creatinine level from baseline sustained for at least 30 days, or death from renal or CV disease. Secondary outcomes included: *1*) a composite of end-stage kidney disease, doubling of the serum creatinine level, or renal death; *2*) a composite of CV death or hHF; *3*) hHF alone; and *4*) death from any cause.

Details of the trial design and oversight, participants, inclusion and exclusion criteria, randomization, treatment, follow-up, and outcomes have been published (4) (the CREDENCE trial is registered with ClinicalTrials.gov [identifier NCT02065791]). CREDENCE was stopped early after a planned interim analysis, with a final median follow-up of 2.6 years.

Urine samples were obtained from participants who consented to have samples taken for exploratory biomarker research wherever local regulations permitted. All of the samples obtained were analyzed and no retrospective sample selection was done. We had samples from 1,308 participants in the treatment arm and 1,290 in the placebo arm at baseline, and 1,262 and 1,352 participants in those trial arms, respectively, at year 1, totaling 60% of the original cohort. Overall, there were 850 men and 437 women in the canagliflozin arm, and 836 men and 420 women in the placebo group (P = 0.783). This subcohort closely reproduced the main findings of the full cohort for the main outcomes (Supplementary Fig. 1). Samples were stored at −80°C and, in October 2021, were transferred to the Metabolism Unit of the Department of Clinical and Experimental Medicine at University of Pisa (Pisa, Italy) for analysis. Paired samples from the baseline and year 1 collection were assayed in the same run; laboratory operators were blinded to the patients' data.

Analytical Methods

Creatinine, glucose, β-hydroxybutyrate (β-OH), and acetoacetate (AcAc) were measured by in-house automated spectrophotometric enzymatic methods on a Beckman UniCel DXC600 Synchron Analyzer (Fullerton, CA) (5,6). Within-assay and between-assay coefficients of variation ranged between $<$1% and $<$7% for all.

Calculations

Total GFR was calculated as eGFR divided by 1.73 and multiplied by the body surface area (calculated by Watson formula [7]). Urinary glucose excretion (UGE) is given as the ratio of urine glucose to creatinine (G/Cr). For easy reference to common units, UGE is also expressed in grams per 24 h; this was done by multiplying the urine glucose concentration value (in milligrams per deciliter) by 25, which is equivalent to assuming a 24-h urine output of 2.5 L (8). Previous studies using timed urine collections over 24 h with canagliflozin administration in patients with type 2 diabetes (9,10) measured 24-h urine output and reported values of $>$3 L/day (one small study with Japanese participants (11) had values in the range of 2.3–3.1 L/day). Fractional UGE was calculated as the ratio of UGE to filtered glucose load (the latter was estimated as the product of total GFR, in milliliters per minute, and average plasma glucose concentration [estimated from HbA_{1c} (12)]). The proximal tubule ATP cost of glucose reabsorption by SGLT2 was estimated by multiplying the fasting plasma glucose concentration by total eGFR and dividing the result by 3 (assuming a 1:1 glucose to sodium stoichiometry at SGLT2 and that extrusion of sodium from the proximal tubular cell to the interstitium through the basolateral membrane costs 1 mol of ATP for each 3 mol of sodium (13–15)). This calculation is a systematic underestimate of the 24-h value in that it uses fasting glucose; in addition, it assumes minimal SGLT1-mediated glucose reuptake in the proximal tubule. Urine albumin to creatinine ratio (UACR) was expressed in milligrams per gram.

Statistical Analysis

Data are presented as mean ± SD or median [interquartile range] for continuous variables showing a nonnormal distribution (by Shapiro–Wilk test). Group comparisons were analyzed by χ^2 statistics (for nominal variables) and by Student t test or Wilcoxon signed rank test for normally and nonnormally distributed variables, respectively. Cumulative event curves for the time to first end point were computed by the Kaplan-Meier estimator and compared by the log-

rank test. Univariate and multivariate Cox proportional hazards models were used to test the association of urine parameters with end points. Associations were expressed as hazard ratios (HRs) and 95% CIs. All analyses were performed using JMP 16.2.0 (JMP Statistical Discovery).

Ethics

CREDENCE was approved by the ethics committees at each study site. All participants provided written informed consent.

Data and Resource Availability

The data-sharing policy of Janssen Pharmaceutical Companies of Johnson & Johnson is available at https://www.janssen.com/clinical-trials/transparency. As noted at that webpage, requests for access to the study data can be submitted through Yale Open Data Access Project site at https://yoda.yale.edu. No applicable resources were generated or analyzed during this study.

RESULTS

Urine substrate concentrations were measured at baseline and 1 year after randomization (Table 1). At baseline, values of urine glucose, β-OH, and AcAc concentrations were similar between the placebo and canagliflozin arms. At year 1, urine glucose, β-OH, and AcAc concentrations increased significantly with canagliflozin, whereas none of these substrates changed in the placebo arm. Both urine creatinine and UACR decreased significantly after treatment with canagliflozin versus placebo (possibly also because of increased urine volume). Fasting plasma free fatty acid (FFA) levels increased at year 1 in the canagliflozin arm but not in the placebo arm (by 29 [348] vs. −10 [316] μmol/L, respectively; $P = 0.0044$). Likewise, at year 1, fasting plasma β-OH levels were only increased with canagliflozin (by +22 [186] vs. −6 [168] μmol/L placebo; $P = 0.0001$).

Characteristics of Urinary Glucose Excretion

In the cohort as a whole, median urine G/Cr was 0.24 mg/mg at baseline ($n = 2,598$) and increased to a median 15.8 mg/mg with canagliflozin treatment ($n = 1,362$)—with a very large spread of values in both cases. The corresponding estimated 24-h UGE averaged 6.0 g at baseline and increased to 34.4 g with canagliflozin treatment. Baseline G/Cr decreased across categories of decreasing eGFR (Fig. 1*A*); likewise, year 1 G/Cr values were significantly lower at lower eGFR categories (Fig. 1*B*). By log-linear regression, G/Cr was positively associated with eGFR ($r = 0.11$; $n = 1,142$; $P = 0.0003$). Values above the mean value of the highest eGFR category (i.e., 20 mg/mg; red dotted line in Fig. 1*B*) were as follows: 32%, 30%, 22%, 19%, and 14% for the groups with eGFR ≥90, 60 to <90, 45 to <60, 30 to <45, and 15 to <30 mL/min/1.73 m^2, respectively. Overall, after randomization, 42% of participants in the canagliflozin arm compared with 5% of participants in the placebo arm had urine G/Cr above this level. On the other hand, at year 1, G/Cr was below the detection limit in 4% of the canagliflozin arm compared with 18% of the placebo arm. Both at baseline and year 1, UACR increased steeply with progressively lower eGFR ($P < 0.0001$ for both) (Fig. 2*A* and *B*).

Fractional UGE averaged 3% with placebo and increased 10-fold (to an average of 30%) with canagliflozin (Table 1); hematocrit decreased across diminishing eGFR (Fig. 2*C* and *D*). Urine concentrations of β-OH and AcAc were directly correlated with urine glucose concentrations at baseline as well as at year 1 (data not shown).

Relation of Glycosuria to Trial End Points

For the primary trial end point—CV death plus composite renal end point—as well as for the other major end points (composite renal end point, CV death plus hHF, and hHF)—

Table 1—Urine measurements

	Placebo			Canagliflozin			
	Baseline	Year 1	∂	Baseline	Year 1	∂	*P**
Participants, *n*	1,290	1,352	–	1,308	1,362	–	–
Glucose (mg/dL)	226 ± 663	267 ± 739	27 ± 768	230 ± 632	1,374 ± 1,340	1,129 ± 1,364	<0.0001
Creatinine (mg/dL)	90 ± 55	91 ± 57	1 ± 61	87 ± 53	79 ± 43	−8 ± 55	<0.0001
G/Cr (mg/mg)	0.24 [1.09]	0.24 [1.29]	0 [0.42]	0.25 [1.23]	15.8 [29.2]	12.5 [28.1]	<0.0001
β-OH (μmol/L)	27 ± 91	29 ± 95	2 ± 118	29 ± 124	48 ± 313	21 ± 338	0.0012
β-OH to creatinine ratio (nmol/g)	0.0 [26.9]	0.0 [28.7]	0 [10.0]	0.0 [28.1]	2.4 [49.3]	0.0 [24.0]	<0.0001
Acetoacetate (μmol/L)	32 ± 40	34 ± 90	2 ± 96	33 ± 58	42 ± 99	10 ± 111	<0.0001
Acetoacetate to creatinine ratio (nmol/g)	28.0 [22.2]	28.2 [22.1]	0 [20.2]	28.7 [23.6]	35.3 [31.3]	3.9 [26.2]	<0.0001
UACR (mg/g)	886 [1,266]	1,016 [1,819]	83 [1,034]	920 [1,283]	593 [1,311]	−192 [810]	<0.0001
Fractional glucose excretion (%)	3 ± 8	5 ± 19	2 ± 20	3 ± 9	30 ± 26	27 ± 27	<0.0001

Data are reported as mean ± SD or median [interquartile range]. **P* for the difference between placebo and canagliflozin ∂ by Wilcoxon signed rank test.

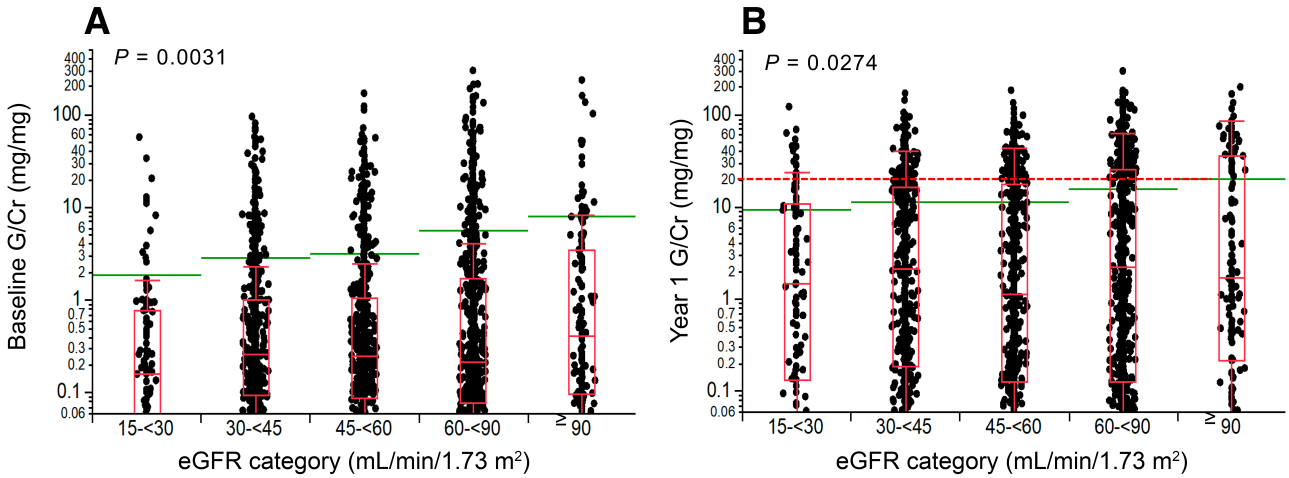

Figure 1—Box plots of baseline urinary glucose excretion ratio (G/Cr; *n* = 2,598) by eGFR category (*A*) and year 1 G/Cr (*n* = 2,714) by eGFR category (*B*). Green segments are mean category values; *P* values were determined by Wilcoxon signed rank test. The red dotted line is the mean value of the highest eGFR category. For both distributions, note the wide dispersion of G/Cr values despite the log scale.

cumulative risk increased in step with progressively lower eGFR category (Supplementary Fig. 2).

The association of glycosuria with outcomes was explored by using the top quartile (35 [25] mg/mg) of the whole year 1 G/Cr distribution, representing 43% of the participants in the canagliflozin arm and 7% of those in the placebo arm. Participants in this quartile who were treated with canagliflozin were significantly protected against the primary composite end point (Fig. 3) (using the top quartile of UGE yielded a superimposable result,

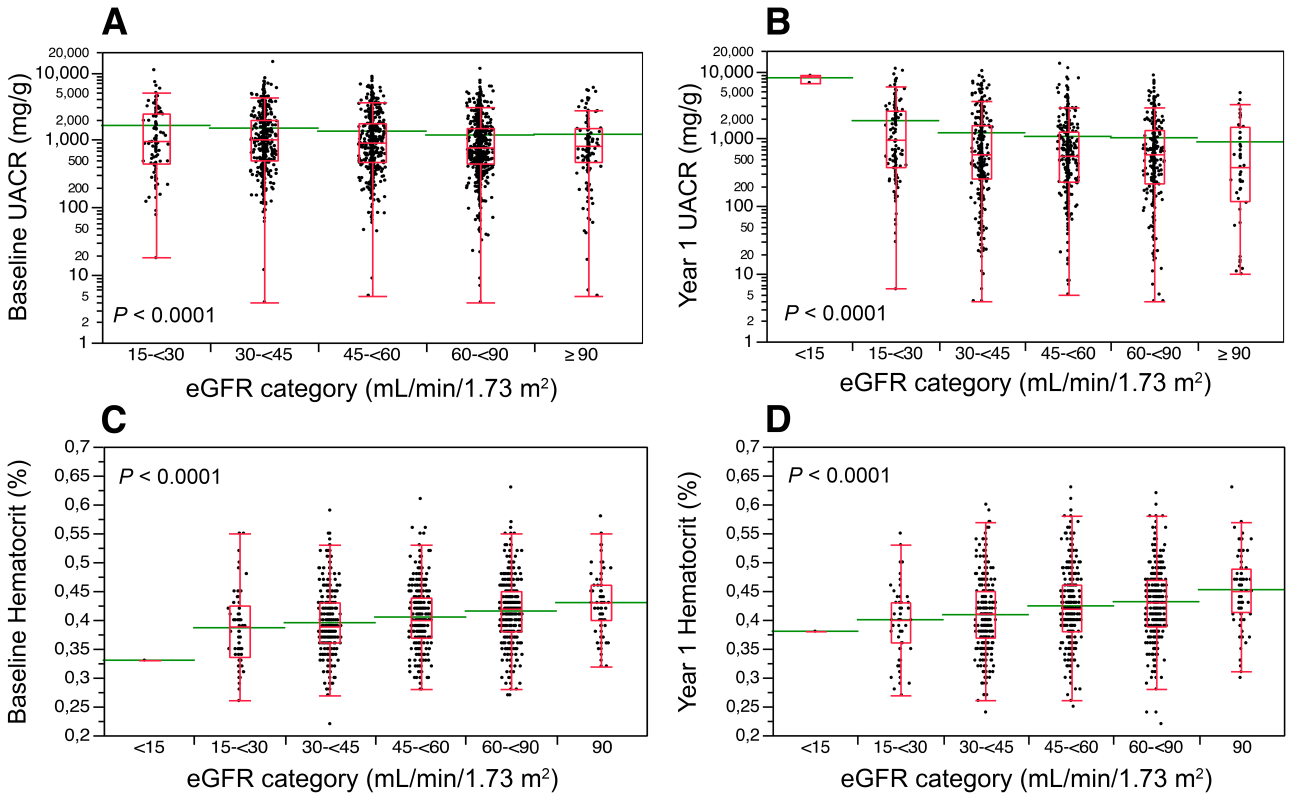

Figure 2—Box plots of baseline UACR (*n* = 2,543) by eGFR category (*A*) and year 1 UACR (*n* = 2,328) by eGFR category (*B*). For both UACR distributions, note the wide dispersion of values despite the log scale, baseline hematocrit (*n* = 2,335) by eGFR category (*C*), and year 1 hematocrit (*n* = 2,248) (*D*) by eGFR category. Green segments are mean category values; *P* values were determined by Wilcoxon signed rank test.

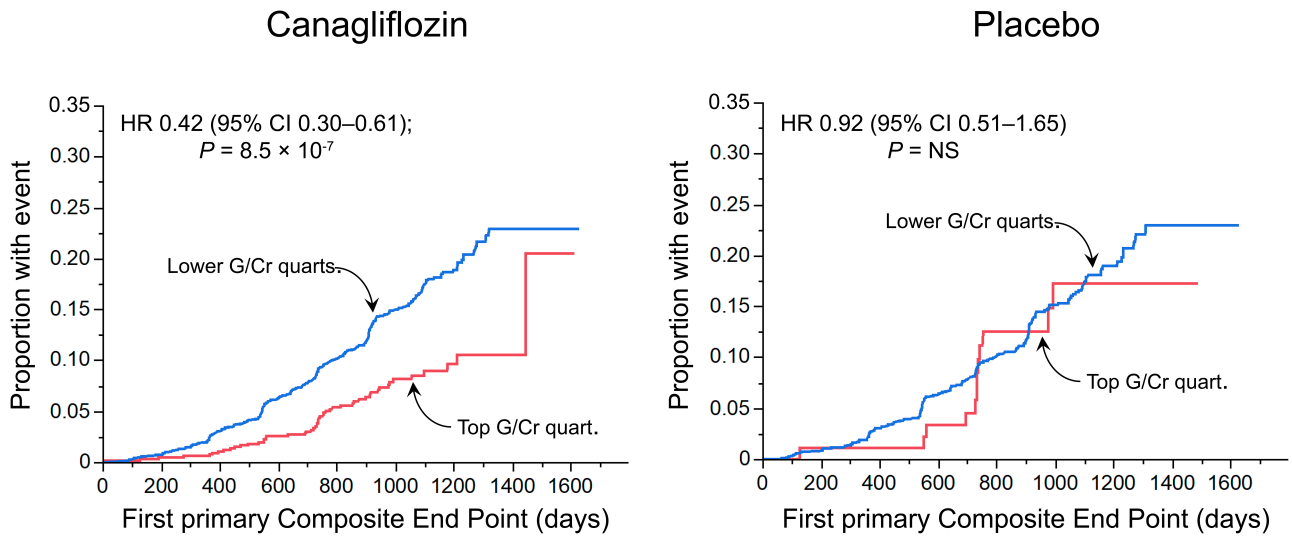

Figure 3—Kaplan-Meier plots of the primary composite end point by G/Cr quartile (quart.; top quartile vs. lower quartiles) in the canagliflozin and placebo arm.

HR 0.45 [95% CI 0.32–0.64] for canagliflozin; HR 0.94 [95% CI 0.51–1.73] for placebo). These individuals were also protected against the primary renal end point, CV plus hHF, hHF, and all-cause death (Fig. 4). For each these end points, being in the top year 1 G/Cr quartile replaced canagliflozin treatment in a bivariate Cox

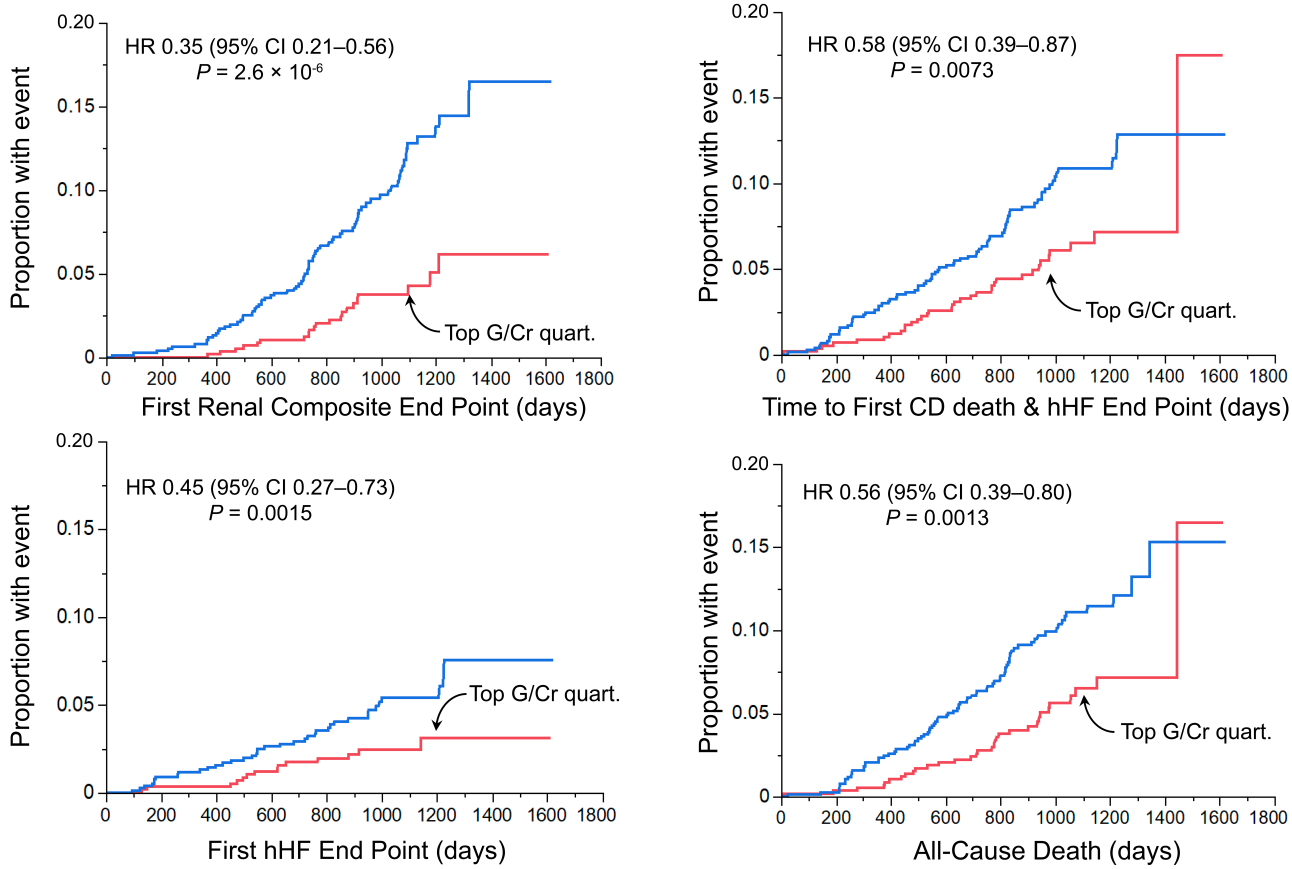

Figure 4—Kaplan-Meier plots of renal composite end point, hHF, or CV death, hHF, and all-cause death by G/Cr quartile (top quartile vs. lower quartiles).

proportional hazard model. For example, for the primary composite end point, canagliflozin treatment alone had an HR of 0.70 (95% CI 0.59–0.82; $P = 1.6 \times 10^{-5}$); upon adding the top quartile of year 1 G/Cr (HR 0.50 [95% CI 0.37–0.69]; $P < 7.8 \times 10^{-6}$) in the Cox model, the HR for canagliflozin was no longer significantly different from unity (HR 0.92 [95% CI 0.74–1.16]; $P = 0.4906$). Of note, participants in the top G/Cr quartile were also protected against major adverse cardiovascular events (HR 0.63 [95% CI 0.47–0.85]; $P = 0.0013$) and fatal and nonfatal myocardial infarction (HR 0.47 [95% CI 0.28–0.82]; $P = 0.0032$). Testing all quartiles together yielded similar results (Supplementary Fig. 3).

The clinical phenotype of participants in the top year 1 G/Cr distribution is strikingly similar to that of the other quartiles (including use of cardioprotective and antihyperglycemic drugs) except for significantly higher eGFR, plasma glucose, and HbA_{1c}, and lower UACR (Table 2). Although the current CREDENCE database may not be large enough to test the association of G/Cr with outcomes in multiple subgroups of eGFR, G/Cr (top quartile) was significantly associated with time to first CV plus hHF (HR 0.66; 95% CI 0.45–0.97) while accounting for year 1 eGFR (as a continuous variable; HR 0.89 [95% CI 0.85–0.93] for each 5 mL/min/1.73 m^2 eGFR). Likewise, the G/Cr (top quartile) was significantly associated with time to first primary composite outcome (HR 0.70; 95% CI 0.51–0.97) while accounting for year 1 eGFR (HR 0.72 [95% CI 0.68–0.75] for each 5 mL/min/1.73 m^2 eGFR) (Supplementary Fig. 4).

In a multivariate Cox model of the primary composite end point, older age, BMI, and eGFR were negative predictors, whereas higher HbA_{1c} and systolic blood pressure were

Table 2—Baseline clinical characteristics of participants in the top quartile of year 1 G/Cr compared with lower quartiles

	Top quartile	Lower quartiles	*P**
Participants, *n*	679	2,036	—
Year 1 G/Cr (mg/mg)	35 [25]	0.4 [4]	<0.0001
Year 1 24-h UGE (g/day)	57 [40]	1 [8]	<0.0001
Ratio of male to female participants	426/252	1,373/663	0.0289
Age (years)	62.6 ± 8.9	63.5 ± 9.2	0.0216
BMI (kg/m^2)	31.4 ± 6.3	32.0 ± 6.2	0.0358
Diabetes duration (years)	15 [10]	15 [11]	ns
HbA_{1c} (%)	8.59 ± 1.43	8.13 ± 1.24	<0.0001
Fasting glucose (mg/dL)	176 ± 69	156 ± 57	<0.0001
eGFR (mL/min/1.73 m^2)	60.5 ± 18.5	55.4 ± 18.1	<0.0001
UACR (mg/g)	853 [1,101]	941 [1,340]	<0.0001
Systolic blood pressure (mmHg)	139 ± 16	141 ± 16	NS
Diastolic blood pressure (mmHg)	78 ± 9	78 ± 10	NS
LDL cholesterol (mg/dL)	97 ± 44	94 ± 40	NS
HDL cholesterol (mg/dL)	44 ± 13	45 ± 14	NS
Triglycerides (mg/dL)	164 [124]	159 [114]	NS
Smoking (%)	16	14	NS
Prior CV disease (%)	49	52	NS
Prior HF (%)	100	100	NS
Use of metformin (%)	63	59	NS
Use of sulfonylureas (%)	26	27	NS
Use of insulin (%)	67	67	NS
Use of GLP-1 RA (%)	4	5	NS
Use of statins (%)	70	72	NS
Use of RAAS inhibitors (%)	100	100	NS
Use of diuretics (%)	46	51	NS
Use of antithrombotics (%)	63	62	NS

Data reported as mean ± SD or mean [interquartile range]. HF, heart failure; RA, receptor agonist; RAAS, renin-angiotensin-aldosterone system. **P* value is by χ^2 for categorical variables, Student *t* test for normally distributed variables, and Wilcoxon signed rank test for variables with nonnormal distribution.

positive predictors; UACR was a strong positive predictor and G/Cr a negative predictor. A similar pattern of predictors was seen for all-cause death (Fig. 5). Nominal logistic fit of these variables to the primary composite end point yielded a receiver operating characteristic of 0.76, with 73% sensitivity and 66% specificity.

Finally, we sought to determine which participants in the canagliflozin arm did not respond to the treatment. We calculated individual eGFR slopes between baseline and week 156 and defined as nonresponders those participants falling in the top quintile of the slope distribution (i.e., an eGFR decline >5.72 mL/min/1.73 m^2 per year). As expected, the HR for the primary composite end point was ninefold higher in nonresponders than responders (HR 9.01 [95% CI 6.46–12.67]; $P < 0.0001$). The clinical phenotype shows that nonresponders were younger than responders, had a lower fasting glucose level but higher eGFR, lower year 1 G/Cr and UGE, and twice the level of proteinuria (Table 3).

DISCUSSION

The present analysis established the following characteristics of glycosuria: *1*) over at least 1 year, its amount is consistently large in response to 100 mg/day canagliflozin, estimated 24-h UGE exceeding 80 g in 10% of the cases (by comparison, the corresponding values from timed urine collection were 88–119 g in patients with well-controlled type 2 diabetes and preserved renal function [16] and 44 g in normoglycemic participants [17]); *2*) glycosuria is also higher in people with persistent hyperglycemia, especially in individuals with a relatively preserved eGFR, but mostly below the levels induced by SGLT2 inhibition (in the placebo arm, only 84 participants in the top quartile of HbA_{1c} [9.0–14.2%] had an estimated UGE >14 g/24 h); and *3*) the interindividual spread of glycosuria values is wide, enough to cover eGFRs ranging from 30 to 90 mL/min/1.73 m^2 (Fig. 1). Thus, so long as there are sufficient numbers of participants in lower eGFR classes with a high glycosuria, favorable outcomes will emerge nonetheless; likewise, cardiorenal protection by SGLT2 inhibition is expected to occur in individuals without diabetes, as recently confirmed by the Dapagliflozin and Prevention of Adverse Outcomes in Chronic Kidney Disease (DAPA-CKD) and Study of Heart and Kidney Protection with Empagliflozin (EMPA-KIDNEY) trials (18,19); *4*) by survival analysis, glycosuria per se is associated with a lower incidence of the primary composite end point of CREDENCE across eGFR categories, statistically replacing canagliflozin treatment as a predictor; and *5*) glycosuria is also associated with protection against other relevant end points, including all-cause death.

The interpretation of these findings is complex, but some mechanisms emerge clearly. Increased glycosuria triggers a chain of metabolic reactions that includes acute glucose deficit → lower glycemia → lower insulinemia → disinhibited lipolysis → higher circulating FFAs and ketones → switch in substrate provision to end organs. In the present study's data, the increased ketonemia and the presence of ketones in the urine in the canagliflozin arm document the end step in the sequence (i.e., the enhanced use of fatty substrates). Of note, this sequence has been outlined in a previous study treating patients with type 2 diabetes with empagliflozin (16) and has emerged from the metabolic analysis of the Canagliflozin Cardiovascular Assessment Study (CANVAS) trial of canagliflozin, in which higher circulating FFA

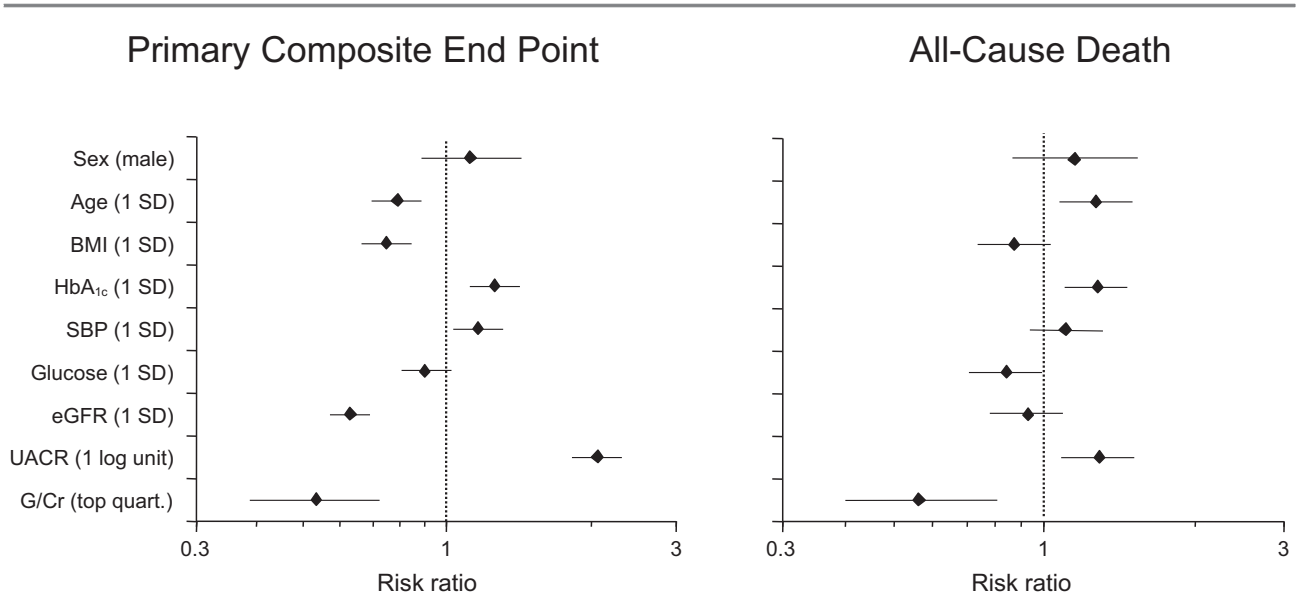

Figure 5—Multivariate Cox regression of the primary composite end point and all-cause death (canagliflozin arm). Dots are odds ratios, lines are 95% CIs calculated for 1 SD of continuous variables, and 1 log unit for UACR. Quart., quartile; SBP, systolic blood pressure.

Table 3—Baseline clinical characteristics of nonresponders to canagliflozin

	Nonresponders	Responders	*P**
Participants, *n*	252	1,078	—
Year 1 G/Cr (mg/mg)	8.5 [25.5]	17.3 [29.5]	<0.0001
Year 1 UGE (g/day)	13 [39]	31 [50]	<0.0001
Ratio of male to female participants	163/89	713/365	ns
Age (years)	61.3 ± 9.5	63.6 ± 8.9	0.0007
BMI (kg/m^2)	31.6 ± 7.1	31.9 ± 6.0	ns
Diabetes duration (years)	15 [10]	15 [12]	ns
HbA_{1c} (%)	8.63 ± 1.28	8.27 ± 1.31	ns
Fasting glucose (mg/dL)	156 ± 59	164 ± 63	0.0240
eGFR (mL/min/1.73 m^2)	61.0 ± 18.9	55.6 ± 18.0	<0.0001
UACR (mg/g)	1,660 [2,199]	796 [1,044]	<0.0001
Systolic blood pressure (mmHg)	143 ± 17	140 ± 16	0.0090
Diastolic blood pressure (mmHg)	78 ± 10	77 ± 9	ns
LDL cholesterol (mg/dL)	106 ± 50	93 ± 40	0.0006
HDL cholesterol (mg/dL)	46 ± 17	45 ± 13	ns
Triglycerides (mg/dL)	158 [125]	164 [111]	ns
Smoking (%)	14	15	ns
Prior CV disease (%)	54	51	ns
Use of metformin (%)	62	59	ns
Use of sulfonylureas (%)	21	25	ns
Use of insulin (%)	75	67	0.0095
Use of GLP-1 RA (%)	6	4	ns
Use of statins (%)	67	76	0.0048
Use of RAAS inhibitors (%)	100	100	ns
Use of diuretics (%)	55	49	ns
Use of antithrombotics (%)	62	65	ns

Data reported as mean ± SD or mean [interquartile range]. RA, receptor agonist; RAAS, renin-angiotensin-aldosterone system. *P value is by χ^2 for categorical variables, Student t test for normally distributed variables, and Wilcoxon signed rank test for variables with nonnormal distribution.

concentrations were associated with a significant reduction in hHF (20).

Simultaneously, SGLT2-induced glycosuria causes osmotic diuresis, which, in turn, leads to systemic fluid offloading and a degree of blood volume contraction, resulting in a rapid reduction in eGFR (in this data set, −5 [10] vs. −1 [9] mL/min/1.73 m^2; $P < 0.0001$ canagliflozin vs. placebo at week 3) and a persistent increase in hematocrit (+0.02 [0.04]; $P < 0.0001$ vs. −0.01 [0.04] of placebo at 1 year). The close numeric parallelism between glycosuria and hematocrit in the present data set is a clear signal of this mechanism. Studies have shown suppression of hepcidin in polymorphonuclear cells (21), with increased erythropoietin release and erythropoiesis (extensively reviewed by Packer (22,23)), which has led some to argue for pleiotropism of SGLT2 inhibitors. Pleiotropism presupposes that SGLT2 is expressed in multiple tissues. To our knowledge, SGLT2 is only functionally expressed at a high density in the apical membrane of the early proximal tubules in the kidney cortex (13–15), and no other target for highly specific SGLT2 inhibitors has been convincingly identified thus far, with the notable exception of sodium–hydrogen exchanger-3, as recently shown in vivo in healthy volunteers (24). Therefore, increased erythropoietin release, itself, may be viewed as a secondary effect of glycosuria, possibly via changes in intrarenal oxygenation (22,23).

Despite its primacy, higher glycosuria did not identify a clear clinical phenotype, because most baseline clinical variables were similar between participants in the top quartile of G/Cr and the rest of the cohort (except for the determinants of glycosuria themselves, namely, HbA_{1c}, plasma glucose, and eGFR). The one difference that stood out was the higher albuminuria in the participants in the lower quartiles (Table 2), which, in the whole data set, tracked progressive renal dysfunction (Fig. 1) and a higher incidence of outcomes (Fig. 5). Similarly, when we sought to identify canagliflozin nonresponders based on a faster rate of eGFR

decline (Table 3), the clinical phenotype of these individuals was characterized not only by lesser glycosuria but also by twice the levels of albuminuria, in support of a major risk role for albuminuria in the rapid progression of renal failure (Table 3). In a Cox model with year 1 G/Cr and UACR as factors for the primary composite end point, both G/Cr and UACR were predictors (HR 0.49 [95% CI 0.33–0.73] for G/Cr, $P = 0.004$; and HR 3.07 [95% CI 2.18–4.34] for UACR, $P < 0.0001$), with no significant interaction. Results were similar when testing G/Cr separately in the top and lower quartiles of UACR (Supplementary Fig. 4). This result shows that the benefit of glycosuria is independent of, and opposed to, the toxic effect of albuminuria on the progression of CKD.

Finally, when G/Cr was included in a fully adjusted Cox model, it retained a significant protective role against the primary composite end point independently of the high risk carried by albuminuria (Fig. 5). Thus, the primary effect of canagliflozin on inhibiting glucose reabsorption can provide benefit even in patients with severely compromised renal function. Strikingly, in the same fully adjusted model for all-cause death, G/Cr still had a significant protective role.

Higher G/Cr was also strongly protective against the renal-specific outcome (Fig. 4), thereby incorporating the effect of canagliflozin treatment. The mechanisms by which glycosuria is linked to renal protection in humans with type 2 diabetes are incompletely understood (25,26). Diabetic kidney disease is a multifactorial condition, and SGLT2 inhibition affects several functions (27). The data from the present study offer some clues. First, in our participants, the baseline proximal tubule ATP cost of reabsorbing the entire 24-h filtered load was estimated to amount to 0.25 [0.20] mol/day. By assuming that canagliflozin only inhibits SGLT2 cotransporters, it can be calculated that the "saved" ATP cost amounted to 0.05 [0.09] mol/day (i.e., a significant ∼20% energy relief for the proximal tubule) (28). Because distal tubular effects may cancel or modulate the proximal phenomena, it is difficult to gauge the net quantitative benefit of proximal ATP saving for renal function. For example, a recent study of patients with type 2 diabetes in which the authors used blood oxygen level–dependent MRI to measure tissue oxygenation, demonstrated that 32 weeks of treatment with empagliflozin resulted in a reduction of medullary oxygenation, a driver of erythropoietin release and erythrocytosis (22,23), but no change in whole-kidney perfusion (29). Clearly, more studies are needed to assess the intrarenal energy partition under different circumstances (e.g., CKD or normal renal function, diabetes or normoglycemia). Second, the appearance of ketones in the urine of participants receiving canagliflozin indicates an increased availability of these thrifty substrates for renal tissues as well as substrate-independent beneficial effects on nutrient-deprivation signaling and cellular stress (30). Finally, it is possible that natriuresis contributes to renal protection. For example, it has been argued that increased delivery of the sodium escaping proximal reabsorption because of SGLT2 inhibition would induce adenosine release at the macula densa, with subsequent vasoconstriction of the afferent arteriole and relief of intraglomerular pressure. Such tubulo-glomerular feedback has been convincingly shown in patients with type 1 diabetes (31), but that finding was not reproduced in patients with type 2 diabetes (32). In general, other than in acute or subacute sodium balance studies (16), it has proven difficult to directly quantitate long-term SGLT2-induced natriuresis given the multiple mechanisms of sodium exchange along the nephron, and more studies are needed.

In summary, quantitative analysis supports the concept that glycosuria is the sine qua non and the trigger of most, if not all, changes that follow SGLT2 inhibition in patients with type 2 diabetes and renal impairment. Some of these changes are rapid and direct (e.g., hemodynamics, substrate utilization shift), others are delayed and mediated (e.g., erythropoiesis, gene programs (21,22)), but they involve enhanced glycosuria as the requisite.

Strengths of the analysis are the large population sample size, the placebo-controlled design, the centralized assays, and the adjudicated outcomes. Limitations are the lack of similar data in individuals without type 2 diabetes, such as those recruited into the EMPA-KIDNEYstudy, across degrees of kidney dysfunction.

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