# Dapagliflozin Improves the Urinary Proteomic Kidney-Risk Classifier CKD273 in Type 2 Diabetes with Albuminuria: A Randomized Clinical Trial

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## OBJECTIVE

**To evaluate the effect of the sodium–glucose cotransporter 2 inhibitor dapagliflozin on the kidney-risk urinary proteomic classifier (CKD273) in persons with type 2 diabetes (T2D) and albuminuria.**

## RESEARCH DESIGN AND METHODS

**In a double-blind, randomized, controlled, crossover trial, we assigned participants with T2D and urinary albumin to creatinine ratio (UACR) ≥30 mg/g to receive dapagliflozin or matching placebo added to guideline-recommended treatment (ClinicalTrials.gov identifier NCT02914691). Treatment periods lasted 12 weeks, when crossover to the opposing treatment occurred. The primary outcome was change in CKD273 score. Secondary outcomes included regression from high-risk to low-risk CKD273 pattern using the prespecified cutoff score of 0.154. The primary outcome was assessed using paired *t* test between end-to-end CKD273 scores after dapagliflozin and placebo treatment. The McNemar test was used to assess regression in risk category.**

## RESULTS

**A total of 40 participants were randomized and 32 completed the trial with intact proteomic measurements. Twenty-eight (88%) were men, the baseline mean (SD) age was 63.0 (8.3) years, mean (SD) diabetes duration was 15.4 (4.5) years, mean $HbA_{1c}$ was 73 (14) mmol/mol (8.8% [1.3%]), and median (interquartile range) UACR was 154 (94, 329) mg/g. Dapagliflozin significantly lowered CKD273 score compared with placebo (−0.221; 95% CI −0.356, −0.087; *P* = 0.002). Fourteen participants exhibited a high-risk pattern after dapagliflozin treatment compared with 24 after participants placebo (*P* = 0.021).**

## CONCLUSIONS

**Dapagliflozin added to renin-angiotensin system inhibition reduced the urinary proteomic classifier CKD273 in persons with T2D and albuminuria, paving the way for the further investigation of CKD273 as a modifiable kidney risk factor.**


Early detection of diabetic kidney disease (DKD) is essential to initiate treatment to prevent or delay progression of this complication that occurs in 20–40% of all individuals with diabetes (1). To date, elevated albuminuria is the gold standard when assessing early kidney damage in diabetes prior to manifest DKD and is the main

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treatment target for renin-angiotensin system (RAS) inhibitors (1). However, novel urinary proteomic patterns have been shown to predict DKD before the onset of microalbuminuria. Good et al. (2) developed a classifier, called CKD273, based on 273 peptides and peptide fragments that can be used to distinguish people with chronic kidney disease (CKD) from people without CKD. The classifier demonstrates a high specificity and sensitivity, however, without differentiating the underlying type of kidney disease (2). The classifier, associated with kidney fibrosis (3,4), was subsequently identified as an early kidney-risk marker in normoalbuminuric individuals (5), and, in the Proteomic Prediction and Renin Angiotensin Aldosterone System Inhibition Prevention of Early Diabetic Nephropathy in Type 2 Diabetic Patients With Normoalbuminuria (PRIORITY) study, a large, prospective, observational, multicenter study and embedded, randomized, placebo-controlled trial, a high-risk CKD273 score was demonstrated to predict onset of microalbuminuria and decline in estimated glomerular filtration rate (eGFR) in individuals with type 2 diabetes (T2D) and normoalbuminuria (6). Thus, this finding suggests that the CKD273 score may be able to be used to predict progression of kidney disease with fast decline in kidney function at an earlier stage than other known predictors, such as eGFR and albuminuria (6,7).

Although previous studies have shown that treatment with RAS inhibition has been able to improve the CKD273 score (8), no reduction in microalbuminuria incidence was seen after treatment with spironolactone in the randomized controlled trial portion of the PRIORITY study (6). In contrast, sodium–glucose cotransporter 2 (SGLT2) inhibitors have been demonstrated to decrease albuminuria levels (9) and prevent progression of DKD (10,11), but the physiology behind their kidney-protective effect remains elusive.

In the present trial, we investigated whether treatment with the SGLT2 inhibitor dapagliflozin, added to RAS inhibition, improves the urinary proteomic classifier CKD273 in individuals with T2D and albuminuria.

## RESEARCH DESIGN AND METHODS

### Trial Design and Participants

We performed a double-blind, placebo controlled, crossover trial investigating the effect of dapagliflozin, 10 mg/d, versus matching placebo. The primary outcome was change in the urinary proteomic classifier CKD273 score. Secondary outcomes included change in risk category based on CKD273 score using a predefined cutoff of 0.154 (12), change in individual peptide fragments composing the CKD273 classifier, and effect on cardiac function and markers of inflammation, cardiovascular risk factors, and fluid status. Results of dapagliflozin on the effects on cardiac function and markers of inflammation, cardiovascular risk, and fluid status have been published previously (13,14).

Participants were eligible for inclusion if they had T2D, $HbA_{1c}$ >58 mmol/mol (7.5%), urinary albumin to creatinine ratio (UACR) ≥30 mg/g in two of three consecutive first morning-void urine samples, eGFR ≥45 mL/min/1.73 $m^2$, and stable treatment with an RAS inhibitor. Candidates were excluded if they had a recent cardiovascular event, congestive heart failure defined as New York Heart association class IV, or unstable or acute congestive heart failure. A complete list of inclusion and exclusion criteria is presented in Supplementary Table 1.

After screening, participants were randomized to receive dapagliflozin 10 mg once daily or matching placebo in a 1:1 ratio added to standard treatment. After 12 weeks, crossover occurred and participants continued with the opposite treatment for another 12 weeks (Supplementary Fig. 1). All participants gave informed written consent. The trial was conducted at Steno Diabetes Center Copenhagen, Denmark; was approved by the Regional Ethics Committee of the Capital Region in Denmark and the Danish Medicines Agency; and was carried out in accordance with the Declaration of Helsinki. The trial was registered with ClinicalTrials.gov (NCT02914691) and EudraCT (2015–000335–32).

### Study Medication and Randomization Procedure

The Capital Region Pharmacy, Copenhagen, Denmark, performed the randomization and masking of study medication. Participants, study personnel, and investigators were all masked to study medication. Compliance of treatment administrations was assessed by pill count at each visit, and participants were excluded from analysis if their adherence was below 80%.

### Urinary Proteomics

Urinary proteomic analysis was performed using capillary electrophoresis coupled to mass spectrometry, as previously described (15,16). Urinary samples were processed and analyzed using a support vector machine to generate proteomic-classifier scores. Sample peaks were normalized against 29 urinary peptides as an internal standard (17). These procedures have previously been described and validated (2,18,19). Performance characteristics, including variability and reproducibility, have been described in detail (20,21). The CKD273 classifier score was calculated as a continuous variable, as well as categorized in a high-/low-risk pattern based on the cutoff score ≤0.154. This categorization has been previously explored (12) and validated (6) as an early marker of increased risk of kidney disease in T2D.

### Procedures

A validated oscillometric cuff device (Takeda, A&D Medical, Tokyo, Japan) was used for the evaluation of 24-h blood pressure (BP) at baseline and after each treatment period. Measurements were carried out every 15 min during daytime and every 30 min during nighttime. The mean of all recordings of systolic and diastolic BPs across the 24 h were calculated. In addition, at each visit, the mean of three consecutive office BP measurements were collected. The 2021 Chronic Kidney Disease Epidemiology Collaboration formula (22) was applied for the calculation of serum creatinine-based eGFR, whereas glomerular filtration rate (GFR) was measured by quantification of $^{51}$Cr-EDTA clearance at the end of each treatment period.

### Statistical Analysis

Because this was an explorative trial studying the effect of dapagliflozin on urinary proteomic patterns, no formal power calculation was performed. Normally distributed variables at baseline (visit 1) are presented as mean (SD), and nonnormally distributed variables are presented as median (interquartile range). Paired *t* tests were used to assess the difference between end-to-end values of CKD273 score and clinical factors, and the Mann-Whitney *U* test was used to assess differences in UACR. Furthermore, the Mann-Whitney *U* test was used to test for differences between baseline and end-of-treatment changes

after the dapagliflozin and placebo treatment periods. The McNemar test was performed to investigate the difference between presence of a high-risk CKD273 classifier pattern. Pearson correlation analyses were performed to assess the relationship between UACR and CKD273 at baseline and after treatment with dapagliflozin. Likewise, Pearson correlation analyses were performed to assess the relationship of treatment differences between dapagliflozin and placebo in clinical covariates and CKD273. Mann-Whitney *U* tests were performed to investigate the effect of dapagliflozin on the single peptide fragments composing the CKD273 classifier. These *P* values were corrected for false discovery rate using the Benjamini–Hochberg method (23).

Given the explorative nature of the trial, a sensitivity analysis was performed on the primary end point using a linear mixed-effects model to calculate the end-to-end differences between dapagliflozin and placebo on CKD273 score. The model was fitted using treatment, sequence, and period as fixed effects and random intercepts for all participants. The *P* value was calculated using Kenward-Roger approximation for the degrees of freedom, and 95% CIs for the fixed effects were calculated using bootstrapping with 1,000 iterations. Adjustment was performed including sex, baseline age, $HbA_{1c}$, 24-h systolic BP, UACR, and GFR. All statistical analyses were performed using R, version 4.1.2 (R Core Team, Vienna Austria) and RStudio, version 2021.09.1 (RStudio, PBC, Boston, MA, USA).

## RESULTS

### Participants and Baseline Characteristics

Of 77 screened individuals, 40 were randomized to either dapagliflozin and then placebo or placebo and then dapagliflozin. One participant died due to cardiac arrest after a myocardial infarction during placebo treatment, and two participants exited the study because of adverse events (AEs), one because of a myocardial infarction and the other because of skin rash, both during dapagliflozin treatment. Furthermore, another participant was excluded from analysis because of investigator error in administration of study medication, and four participants were excluded because their proteomic analyses failed. Thus, the final analysis was performed on 32 participants (Supplementary Fig. 2). Adherence assessed by pill count was >80% for all included participants.

Baseline characteristics for the overall population, as well as divided by first treatment, are listed in Table 1. Briefly, 88% participants were men; the participants had high BMI (mean ± SD, 33.7 ± 5.4 kg/m$^2$), high $HbA_{1c}$ (mean ± SD, 73 ± 14 mmol/mol [8.8% ± 1.3%]), long diabetes duration (mean ± SD, 15.4 ± 4.5 years); and 28% of participants had macroalbuminuria.

### Outcomes

Treatment with dapagliflozin significantly lowered the CKD273 score compared with placebo after 12 weeks of treatment (−0.221; 95% CI −0.356, −0.087; *P* = 0.002) (Fig. 1*A*, Table 2). Likewise, dapagliflozin treatment led to a significant regression of a high-risk CKD273 pattern (>0.154) compared with placebo (*n* = 14 participants [44%] vs. 24 participants [75%]; end-to-end difference, *n* = −10, *P* = 0.021) (Fig. 1*B*, Table 2). In the sensitivity analysis including period, sequence, and treatment effects, and baseline covariates, the effect of dapagliflozin treatment on the CKD273 score was the same (−0.224; 95% CI −0.356, −0.091; *P* = 0.001). $HbA_{1c}$, UACR, measured GFR, 24-h systolic and diastolic BPs, weight, and BMI all decreased significantly during dapagliflozin treatment compared with placebo. The percent decline in UACR from baseline to end of the dapagliflozin and placebo treatment periods was also significantly greater after treatment with dapagliflozin ($P < 0.001$). No significant change was observed for eGFR, office systolic BP, or office diastolic BP (Table 2). Additional exploration of a possible sequence effect on the CKD273 classifier is illustrated in Supplementary Fig. 3. No immediate sequence effect was apparent. CKD273 scores after placebo treatment matched baseline levels

**Table 1—Baseline characteristics of the overall trial participants, and stratification by first treatment**

| | | First treatment | |
|---|---|---|---|
| Variable | Overall (*N* = 32) | Dapagliflozin (*n* = 17) | Placebo (*n* = 15) |
| Age (years) | 63.0 (8.3) | 63.7 (9.0) | 62.3 (7.8) |
| Male sex, *n* (%) | 28 (88) | 16 (94) | 12 (80) |
| Diabetes duration (years) | 15.4 (4.5) | 14.4 (3.7) | 16.4 (5.2) |
| Weight (kg) | 105.9 (19.7) | 104.2 (20.8) | 107.9 (18.8) |
| BMI (kg/m$^2$) | 33.7 (5.4) | 33.8 (5.6) | 33.6 (5.3) |
| $HbA_{1c}$ (mmol/mol) | 73 (14) | 70 (12) | 76 (16) |
| $HbA_{1c}$ (%) | 8.8 (1.3) | 8.6 (1.1) | 9.1 (1.5) |
| UACR (mg/g) | 154 (94, 329) | 157 (86, 402) | 150 (115, 251) |
| 24-h systolic BP (mmHg) | 147 (12) | 147 (11) | 146 (13) |
| 24-h diastolic BP (mmHg) | 83 (8) | 84 (10) | 82 (6) |
| Office systolic BP (mmHg) | 141 (15) | 143 (14) | 138 (16) |
| Office diastolic BP (mmHg) | 83 (10) | 82 (11) | 83 (10) |
| eGFR (mL/min/1.73 m$^2$) | 89 (19) | 89 (20) | 89 (18) |
| LDL cholesterol (mmol/L) | 1.62 (0.66) | 1.59 (0.74) | 1.66 (0.60) |
| ALT (U/L) | 40.3 (15.6) | 41.7 (15.8) | 38.8 (15.8) |
| Macroalbuminuria, *n* (%) | 9 (28) | 6 (35) | 3 (20) |
| ACE inhibitor use, *n* (%) | 18 (56) | 7 (41) | 11 (73) |
| ARB use, *n* (%) | 14 (44) | 10 (59) | 4 (27) |
| Insulin use, *n* (%) | 19 (59) | 9 (53) | 10 (67) |

Continuous variables are presented as mean (SD) and nonnormally distributed as median (interquartile range). Categorical variables are presented as *n* (%). ARB, angiotensin II receptor blocker.

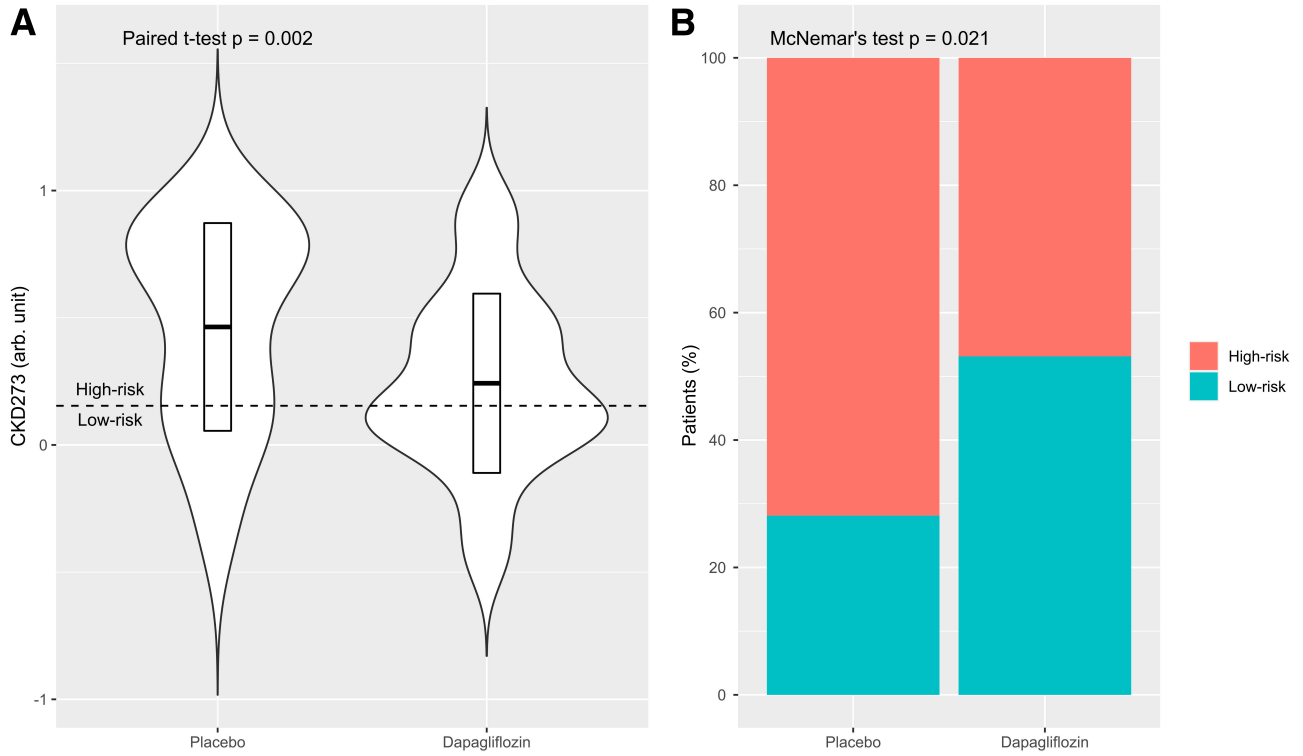


**Figure 1**—*A*: Violin plot illustrating urinary proteomic classifier CKD273 scores after treatment with placebo and after dapagliflozin. The violins illustrate the distribution of the participants, and the central bars represent the mean ± SD. The horizontal dashed line represents the high-/low-risk CKD273 cutoff value of 0.154. *B*: Bar plot illustrating the distribution of participants with a high- or low-risk value of CKD273 after placebo and after dapagliflozin. Arb, arbitrary.

well in both groups. Comparative analyses, using paired *t* tests, of end-to-end CKD273 scores after treatments, as well as comparisons to baseline levels, were all nonsignificant ($P > 0.150$) in both sequence groups.

Correlation analysis between baseline UACR and CKD273 showed a significant positive relationship ($R = 0.345$; $P = 0.043$); however, no such relationship was found between either change in UACR and CKD273 after dapagliflozin treatment ($R = 0.148$; $P = 0.42$) or baseline UACR and change in CKD273 after dapagliflozin treatment ($R = 0.049$; $P = 0.79$) (Fig. 2). None of the changes in $HbA_{1c}$, measured GFR, 24-h systolic BP, weight, and BMI after dapagliflozin treatment

**Table 2—Levels of the main outcomes and investigated clinical variables after treatment with placebo and dapagliflozin**

| Variable | After placebo | After dapagliflozin | End vs. end | 95% CI | *P* |
|---|---|---|---|---|---|
| Main outcomes | | | | | |
| CKD273 score (arbitrary unit) | 0.462 | 0.231 | −0.221 | −0.356, −0.087 | 0.002 |
| CKD273 high-risk pattern, *n* (%) | 24 (75) | 14 (44) | −10 | — | 0.021 |
| Other clinical variables | | | | | |
| $HbA_{1c}$ (mmol/mol) | 73.4 (14.2) | 65.8 (13.3) | −7.6 | −11.0, −4.2 | <0.001 |
| $HbA_{1c}$ (%) | 8.9 (1.3) | 8.2 (1.2) | −0.7 | −1.0, −0.4 | <0.001 |
| UACR (mg/g) | 168 (75, 310) | 113 (67, 158) | −33 (−45, −22) | — | <0.001 |
| UACR change compared with baseline (%) | 3 (−27, 19) | −31 (−53, −14) | −32 (−48, −9) | — | <0.001 |
| 24-h systolic BP (mmHg) | 146 (11) | 144 (12) | −4.2 | −8.2, −0.1 | 0.044 |
| 24-h diastolic BP (mmHg) | 81 (6) | 79 (7) | −2.8 | −4.7, −0.9 | 0.006 |
| Office systolic BP (mmHg) | 139 (14) | 135 (14) | −3.6 | −7.8, 0.7 | 0.097 |
| Office diastolic BP (mmHg) | 81 (10) | 80 (9) | 0.9 | −1.8, 3.6 | 0.500 |
| GFR (mL/min/1.73 $m^2$) | 88 (25) | 77 (23) | −10.8 | −16.1, −5.5 | <0.001 |
| eGFR (mL/min/1.73 $m^2$) (CKD-EPI) | 88 (19) | 87 (19) | −1.5 | −3.8, 0.9 | 0.210 |
| Weight (kg) | 105.4 (19.9) | 104.5 (19.7) | −1.8 | −2.6, −1.0 | <0.001 |
| Body mass index (kg/$m^2$) | 33.5 (5.7) | 33.2 (5.6) | −0.61 | −0.86, −0.35 | <0.001 |
| LDL cholesterol (mmol/L) | 1.66 (0.75) | 1.67 (0.67) | 0.09 | −0.06, 0.24 | 0.228 |

Values are presented as mean (SD) or median (interquartile range) for nonnormally distributed variables. End-to-end estimates and *P* values are calculated from paired *t* tests for all variables except for the CKD273 high-risk pattern and UACR, which were calculated by the McNemar test and Mann-Whitney *U* test, respectively. CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

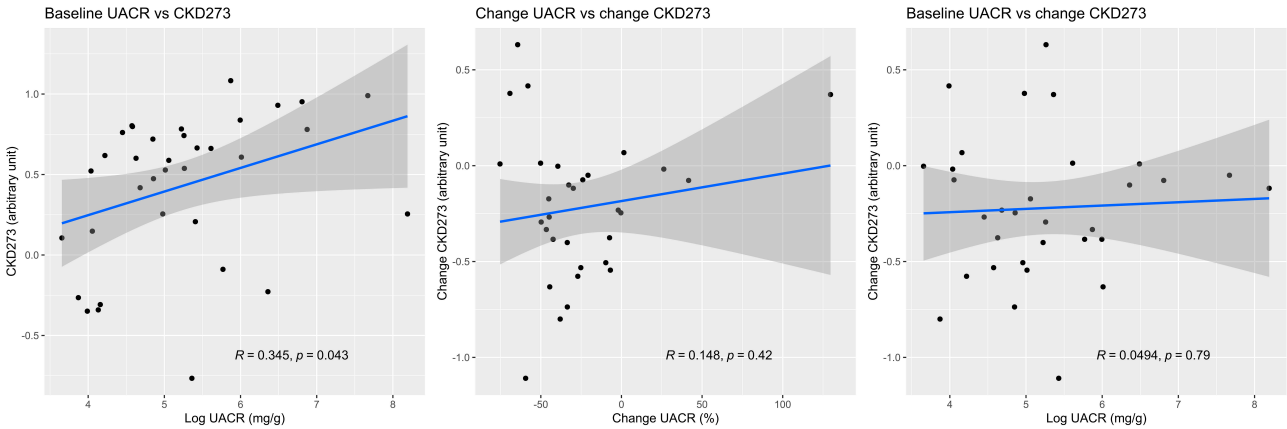


**Figure 2**—Scatterplots illustrating (from left to right) the relationship between *1*) baseline UACR and baseline CKD273 classifier score; *2*) change in UACR and change in CKD273 classifier score after dapagliflozin treatment; and *3*) baseline UACR and change in CKD273 classifier score after dapagliflozin treatment.

were correlated with the changes in CKD273.

After treatment with dapagliflozin, single-peptide-fragment analysis showed significant change in 12 peptide fragments of the 273 included in the complete classifier (false discovery rate adjusted $P < 0.05$). A significant reduction was observed for two peptides from α-1-antitrypsin, one from α-1B-glycoprotein, and seven from serum albumin. A significant increase was detected in one peptide from collagen α-1(III) and one from polymeric-immunoglobulin receptor. Additionally, we observed a trend of four peptides from collagen α-1(III) and two from collagen α-1(I) being increased ($P < 0.1$) (Supplementary Table 2).

### Safety

A total of 44 AEs and 6 serious adverse events (SAEs) were recorded for all 40 randomized participants. During treatment with dapagliflozin, 26 AEs and 5 SAEs were recorded in 29 participants, and during the placebo phase, 18 AEs occurred and 1 SAE in 19 participants (Supplementary Table 3). There were six occurrences of genital fungal infection in four participants during dapagliflozin treatment and three occurrences in three participants receiving placebo; one instance of urinary tract infection during dapagliflozin treatment and one during placebo treatment occurred throughout the trial. No ketoacidosis was recorded during either dapagliflozin or placebo treatment.

## CONCLUSIONS

We have demonstrated that treatment with dapagliflozin reduced CKD273 scores significantly compared with placebo in individuals with T2D and albuminuria. Likewise, we have shown that dapagliflozin treatment leads to a significant reduction in high-risk pattern prevalence in this population of 32 participants with T2D and albuminuria. This change was independent of baseline UACR and was not correlated with the albuminuria-lowering effect of dapagliflozin. Finally, the analysis of the single peptides indicated that the classifier change was mediated by a reduction in urinary inflammatory and fibrotic markers. This suggests that dapagliflozin may exert a modulating effect on processes affecting kidney tissue structure and damage.

Several cross-sectional studies have shown a marked relationship between CKD273 score and prevalent kidney disease (2). In addition, observational studies have implied an ability of the CKD273 classifier to aid in predicting future kidney damage and kidney disease (24). The prospective PRIORITY study investigated this relationship and demonstrated that a high-risk pattern of CKD273 predicts onset of albuminuria and development of CKD stage III in normoalbuminuric T2D, thus preceding the most commonly used screening tool, to date, for assessing kidney risk in diabetes. In the nested randomized controlled trial of high-risk participants in the PRIORITY study, there was no significant effect of spironolactone treatment compared with placebo in preventing microalbuminuria and kidney function decline. However, despite the optimistic results, relevant concerns regarding the CKD273 classifier's sensitivity in discriminating development of kidney disease, and its usefulness in clinical practice, have been voiced (25); therefore, results should be interpreted with caution.

Interestingly, in the present study, we demonstrated that baseline UACR was indeed correlated with baseline CKD273 scores in the participants, but no correlation was found either between the dapagliflozin-induced changes in the markers or between baseline UACR and change in CKD273. Reductions in albuminuria have been associated with improved renal and cardiovascular prognosis (26). Although UACR and CKD273 are both markers of kidney function decline, the results from this trial may indicate a physiological divide between the two that needs further investigation.

The physiological mechanisms behind the kidney protective effect of SGLT2 inhibitors are still not fully understood. Several possible mechanisms have been proposed, including, hemodynamic change (27), as well as possible amelioration of renal hypoxia, as demonstrated by Laursen et al. (28), but conclusive results remain to be presented. To our knowledge, this is the first randomized human trial to investigate the antifibrotic effect of SGLT2 inhibitors. Studies have found SGLT2 inhibitors normalize histological kidney fibrosis in diabetic and nondiabetic mouse models (29,30). In humans, a post hoc analysis of a single-arm, open-label study of 40 individuals with noncomplicated type 1 diabetes showed no effect after treatment with 25 mg/d empagliflozin on CKD273 scores after 8 weeks (31), but all participants included in that study exhibited a low-risk CKD273 pattern at baseline, which would very likely have influenced results. In comparison, a post hoc analysis

of 44 participants with T2D and microalbuminuria included in the IRMA-2 trial assessing the effect of irbesartan, an angiotensin II blocker, on CKD273 showed a significant reduction in CKD273 score from 0.721 to 0.277 between baseline and follow-up, however no comparison with placebo was performed (8). Nevertheless, these results are highly relevant because the IRMA-2 trial was one of the defining trials cementing the kidney-protective potential of angiotensin II blockers (32), thus demonstrating that another kidney-protective drug may also lower CDK273 levels.

### Limitations

To our knowledge, our trial is the first to be specifically designed to investigate the role of SGLT2 inhibitors on the CKD273 classifier. However, due to the, by definition, exploratory nature of the trial, a sample-size calculation was not performed. Likewise, only participants with elevated UACR and $HbA_{1c}$ were included, limiting the generalizability of our results. Finally, given that UACR normalizes already after 2–4 weeks, we did not include a washout period at crossover; although a sensitivity analysis was performed showing no carry-over effect, we cannot conclusively rule out that this may have influenced results. Finally, this was a small trial including only 32 participants across a treatment period of 12 weeks. Therefore, the results should be interpreted with caution, and larger trials are warranted in which the long-term effect of CKD273 reduction on kidney outcomes is studied.

### Conclusion

We have demonstrated that treatment with dapagliflozin markedly improved the urinary proteomics-based kidney-risk classifier CKD273 compared with placebo in 32 participants with T2D and albuminuria. Furthermore, treatment with dapagliflozin lowered the frequency of a high-risk CKD273 pattern compared with placebo and suggested reduction in fibrosis and inflammation. Our results are an important first step for additional investigation of the CKD273 classifier as a modifiable treatment marker in DKD.

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**Duality of Interest.** This study was investigator initiated. Steno Diabetes Center Copenhagen received grants from AstraZeneca AB to conduct the study. The study medication was provided by AstraZeneca AB. P.R. has served as consultant, on advisory boards or as educator, for AstraZeneca, Astellas, AbbVie, Novo Nordisk, Boehringer Ingelheim, Eli Lilly, Merck, and Bayer (with all honoraria given to the institution); has equity interest in Novo Nordisk; and has received research grants to Steno Diabetes Center Copenhagen from Novo Nordisk and AstraZeneca. H.M. is the CEO and founder of Mosaiques Diagnostics and Therapeutics AG. F.P. has served as consultant, on advisory boards or as educator, for AstraZeneca, Novo Nordisk, Sanofi, Mundipharma, MSD, Boehringer Ingelheim, Novartis, and Amgen, and has received research grants to Mosaiques Diagnostics and Therapeutics AG from Novo Nordisk, Amgen, and AstraZeneca. No other potential conflicts of interest relevant to this article were reported.

**Author Contributions.** V.R.C., M.K.E., M.F.-M., T.W.H., P.R., T.S.A., and F.P conceived and designed the research. M.K.E. collected study data. V.R.C., M.K.E., T.R., M.F.-M., T.W.H., H.M., P.R., T.S.A., and F.P. analyzed and interpreted the data. V.R.C., T.R., and T.S.A. performed the statistical analysis. V.R.C. wrote the manuscript. All authors critically revised the manuscript for key intellectual content and approved the final version. V.R.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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