Renal effects of a sodium-glucose cotransporter 2 inhibitor, tofogliflozin, in relation to sodium intake and glycaemic status

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Abstract

Aims: Little is known about whether sodium intake is associated with the clinical effects of SGLT2 inhibitors (SGLT2is); however, SGLT2is may increase urinary sodium excretion. Thus, we investigated the impact of daily sodium intake on the estimated glomerular filtration rate (eGFR) via an SGLT2i, tofogliflozin (TOFO), in patients with type 2 diabetes (T2D).

Methods: Individual-level data on 775 T2D patients in TOFO Phase 3 trials were analysed. Adjusted changes in variables during 52 weeks of TOFO therapy were compared according to basal daily salt intake (DSI), which was measured based on estimated daily urinary sodium excretion using the Tanaka formula. Multivariable analysis was used to investigate the impact of basal DSI on changes in eGFR at Weeks 4 and 52.

Results: Sixty-six percent of participants were men; mean age, HbA1c, body mass index, eGFRMDRD and median DSI were 58.5 years, 8.0%, 25.6 kg/m², 83.9 mL/min/1.73 m² and 9.3 g/d, respectively. In all participants, eGFRMDRD sharply dipped during Week 4, and gradually increased by Week 52, showing a significant increase overall from baseline to Week 52. Multivariable analysis showed that basal DSI and HbA1c levels were independently correlated with eGFRMDRD changes at Weeks 4 and 52. Additionally, lower baseline HbA1c and DSI levels were significantly correlated with a greater increase in eGFRMDRD at Week 52.

Conclusions: Dietary salt intake, in addition to glycaemic control, correlates with changes in eGFRMDRD via TOFO. Thus, an appropriate dietary approach to therapy should be considered before treatment of T2D patients with an SGLT2i.

KEYWORDS
daily salt intake, glomerular filtration rate, sodium-glucose cotransporter 2 inhibitor, tofogliflozin
INTRODUCTION

Type 2 diabetes (T2D) is the leading cause of renal and cardiovascular disease in the world. Hyperglycaemia as the result of diabetes is thought to be exaggerated by hyper-reabsorption of renal glucose in the proximal tubule. However, the etiology of the increased glycosuria threshold in T2D is still unclear. One of the mechanisms of hyper-reabsorption of glucose is an increased expression of sodium/glucose cotransporter-2 (SGLT2) in patients with T2D and diabetic nephropathy. Increased SGLT2 induces increased proximal tubular reabsorption of, not only glucose, but also sodium, both of which are SGLT2 substrates. This reduces sodium chloride and fluid delivery from the proximal tubule to the downstream macula densa, causing glomerular hyperfiltration via impaired tubuloglomerular feedback (TGF). Finally, glomerular hyperfiltration exaggerates the work of glomerular hyperfiltration via impaired tubuloglomerular feedback from the proximal tubule to the downstream macula densa, causing SGLT2 substrates. This reduces sodium chloride and fluid delivery from the proximal tubule to the downstream macula densa, leading to subsequent kidney damage. Therefore, diabetes-induced glomerular hyperfiltration is one of the major risk factors for the subsequent development of diabetic kidney disease.

High intake of dietary sodium was associated with an elevated incidence of cardiovascular disease in patients with T2D, while urinary sodium excretion was nonlinearly associated with all-cause mortality and the cumulative incidence of ESRD. Lower urinary sodium excretion was reported to be associated with increased all-cause and cardiovascular mortality in T2D patients. Therefore, sodium intake may be associated with risks of cardiovascular and kidney disease. Also, dietary sodium intake was reported to influence renal haemodynamics. However, little is known about the mechanism of the effects of sodium intake on renal haemodynamics.

The SGLT2 reabsorbs, not only filtered glucose, but also sodium. In diabetes, increased expression and activity of SGLT2, and fully activated SGLT1, account for almost 50 g of sodium, which may represent over 10% of the filtered sodium load, may be reabsorbed via SGLT-dependent pathways. Although post meal urinary sodium excretion, in addition to urinary glucose excretion, was increased from baseline, both acutely and chronically, by administration of an SGLT2 inhibitor (SGLT2i), little is known about the association of sodium intake with the clinical effects of SGLT2is. Fundamental experiments indicated that genetic and pharmacological inhibition of SGLT2 attenuated primary proximal tubule hyper-reabsorption of sodium and glucose in diabetic models and, thereby, lowered glomerular hyperfiltration via TGF. Additionally, lowering of the glomerular filtration rate (GFR) via the SGLT2i, empagliflozin, was reported in patients with type 1 diabetes. Recently, striking reductions in the relative risk of, not only cardiovascular, but also renal, outcomes with use of SGLT2is in patients with T2D were observed in the EMPA-REG OUTCOME trial, the CANVAS Program and the DECLARE-TIMI 58 study. However, the renal effects, particularly those on the estimated glomerular filtration rate (eGFR) at different levels of baseline sodium intake estimated from urinary sodium excretion, have not been investigated. We therefore investigated the impact of basal salt intake on changes in the eGFR in patients with T2D using an SGLT2i, tofogliflozin (TOFO), focusing on early and chronic effects, as well as effects two weeks after the termination of treatment.

RESEARCH DESIGN AND METHODS

A pooled analysis was conducted on two Phase 3 studies (Table S1) of administration of TOFO to patients with T2D. Various doses of TOFO, either as monotherapy or as an adjuvant antidiabetic agent, were compared. The CSG004JP study (TOFO, 20 and 40 mg monotherapy) and the CSG005JP study (TOFO, 20 and 40 mg as add-on to other oral antidiabetic agents) were both 52-week, randomized, controlled, open-label, Phase 3 studies. Individual-level data from the 52-week core treatment and 2-week termination of treatment periods of each study were used for analysis. Each included study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Protocols were reviewed and approved by the institutional review boards of each participating center. All patients provided written informed consent prior to enrollment.

The following laboratory variables were measured at baseline: HbA1c, fasting plasma glucose (FPG), sodium, potassium, uric acid, brain natriuretic peptide (BNP), urine creatinine, urine sodium, urine potassium, urine albumin-to-creatinine ratio (ACR), creatinine and cystatin C. The eGFR of creatinine (eGFRMDRD) was estimated using the Modification of the Diet in Renal Disease (MDRD) formula for the Japanese population and the eGFRCKD-EPI was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula for the Japanese population. In addition, the eGFRCRE + CYS from the Japanese equation was derived from both serum creatinine and cystatin C values. The other variables measured were systolic blood pressure (SBP), diastolic blood pressure (DBP) and body weight. To determine predicted 24-hour urinary sodium and potassium at baseline, we used the Tanaka formula:

\[ 24\text{-hour urinary sodium (Na)} \times \text{mmol/d} = 21.98 \times (\text{Naspot} \times \text{Spot urinary creatinine} \times \text{PrUCr24h})^{0.431}, \]

where PrUCr24h is predicted 24-hour urinary creatinine (mmol/d).

To assess the effects of basal DSI on changes in metabolic variables during the use of TOFO, participants were divided into four groups according to quartiles of basal DSI (1st quartile, DSI < 7.9; 2nd quartile, 7.9 to < 9.3; 3rd quartile, 9.3 to < 11.0; 4th quartile, ≥ 11.0). Adjusted assessments of HbA1c, FPG, sodium, potassium, body weight, SBP, DBP, eGFRMDRD, BNP and ACR were analysed using an analysis of covariance (ANCOVA) model, with the baseline values age, sex and eGFRMDRD as covariates to determine changes across quartiles. In the evaluation of adjusted assessments of cystatin C, eGFRCKD-EPI and eGFRCRE + CYS, changes were analysed using the ANCOVA model, with baseline values, age and sex as covariates. The adjusted assessment of creatinine was also analysed, using the...
Changes in eGFR\textsubscript{MDRD} were assessed in participants receiving, and in those not receiving, renin-angiotensin system (RAS) inhibition drugs (ARB and/or ACE inhibitor [ACEI]) as concomitant antihypertensive therapy and in participants with or without hyperfiltration. Participants were divided into two groups according to whether they received RAS inhibition drugs and into another two groups according to basal eGFR\textsubscript{MDRD} (without hyperfiltration [eGFR\textsubscript{MDRD} < 120 mL/min/1.73 m\textsuperscript{2}]; with hyperfiltration [eGFR\textsubscript{MDRD} > 120]).

Correlations between change in eGFR\textsubscript{MDRD} at Weeks 4 and 52 and baseline variables were analysed by Pearson's product-moment correlation coefficients. Correlations between changes in eGFR\textsubscript{MDRD} and blood pressure at Weeks 4 and 52 and baseline DSI were investigated in the entire participant population and in those using or not using RAS inhibition drugs as concomitant antihypertensive therapy. Correlations between changes in eGFR\textsubscript{MDRD} and blood pressure and body weight at Week 4 were also analysed in the entire participant population and in each quartile group according to DSI. Finally, correlations between changes in eGFR\textsubscript{MDRD} and changes in HbA1c from Week 4 to Week 52 were investigated.

To identify baseline clinical factors that might independently influence changes in eGFR\textsubscript{MDRD} and creatinine level at weeks 4 and 52, 14 variables at baseline (dosage of TOFO, use of RAS inhibition drugs as concomitant antihypertensive therapy, age, sex, duration of diabetes, HbA1c, DBP, BNP, BMI, uric acid, eGFR\textsubscript{MDRD}, creatinine, DSI and ACR <30 mg/g Cre [vs ACR ≥30 mg/g Cre]) were initially identified based on clinical considerations. Finally, correlations between changes in eGFR\textsubscript{MDRD} and creatinine were adjusted for each other. Moreover, to identify clinical factors that might independently influence changes in eGFR\textsubscript{MDRD} from Week 4 to Week 52, 12 variables (dosage of TOFO, use of RAS inhibition drugs as concomitant antihypertensive therapy, age, sex, duration of diabetes, DSI, ACR <30 mg/g Cre [vs ACR ≥30 mg/g Cre], and variables at week 4 [HbA1c, DBP, BMI, uric acid, and eGFR\textsubscript{MDRD}]) were initially identified based on clinical considerations. Finally, to identify clinical factors that might independently influence changes in eGFR\textsubscript{MDRD} from Week 52 to Week 54, 13 variables (dosage of TOFO, use of RAS inhibition drugs as concomitant antihypertensive therapy, age, sex, duration of diabetes, DSI, ACR <30 mg/g Cre [vs ACR ≥30 mg/g Cre]) were initially identified based on clinical considerations. Finally, differences in BNP level and urine ACR were insignificant across quartiles at Week 52. On the contrary, at Week 4, the reductions in eGFR\textsubscript{MDRD} and eGFR\textsubscript{CKD-EPI} were higher, based on increased basal DSI, while glycemic status and blood pressure were consistent among all quartiles. Changes in variables, glycemic status, body weight and blood pressure, at all weeks 4 and 52 were consistent across quartiles (Table S2). Moreover, differences in BNP level and urine ACR were insignificant across quartiles at Week 52. On the contrary, at Week 4, the reductions in eGFR\textsubscript{MDRD} and eGFR\textsubscript{CKD-EPI} were higher, based on increased basal DSI. At Week 52, the increase in eGFR\textsubscript{MDRD} and eGFR\textsubscript{CKD-EPI} tended to be smaller, according to the increased basal DSI, while the reduction in eGFR\textsubscript{CRE+CYS} and increase in cystatin C were greater, based on increased basal DSI.

At Week 4, baseline HbA1c, fasting plasma glucose, SBP, BNP, urinary sodium and potassium excretion, and DSI were significantly correlated with changes in eGFR\textsubscript{MDRD} (Table S3). There were significant differences in the change in eGFR\textsubscript{MDRD} at Week 4 according to dosage of TOFO, use of concomitant antihypertensive drugs including ARB and RAS inhibition drugs, and ACR category (<30 mg/g Cre or ≥30) (Table S4). At Week 52, age, baseline HbA1c, fasting plasma glucose, cystatin C and urinary sodium excretion, and DSI were correlated with change in eGFR\textsubscript{MDRD} (Table S3). At Week 52, there were significant differences in the change in eGFR\textsubscript{MDRD}, according to the use of concomitant antihypertensive drugs, including the use of ARB (Table S4).

Change in eGFR\textsubscript{MDRD} at Week 4 was not correlated with change in DBP (r = 0.01; P = 0.780) in the entire participant group, while change in eGFR\textsubscript{MDRD} at Week 4 was significantly correlated with change in SBP (r = 0.10; P = 0.004) and with change in body weight (r = 0.20; P < 0.001). There was not a significant correlation between change in eGFR\textsubscript{MDRD} from Week 4 to Week 52 and change in HbA1c (r = 0.05; P = 0.163).

Multivariable analysis demonstrated that higher basal DSI and HbA1c levels, and use of RAS inhibition drugs, were negatively correlated with changes in eGFR\textsubscript{MDRD} at Week 4 (Table 2), but were positively correlated with changes in creatinine levels (Table S5). At Week
| TABLE 1  | Baseline characteristics according to quartiles of basal estimated daily salt intake |
|----------|----------------------------------------------------------------------------------|
|          | Baseline estimated daily salt intake                                           |
|          | ALL  | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | P Across quartiles |
| N        | 775  | 193        | 194        | 194        | 194        | 0.004              |
| Age (y)  | 58.5 (10.5) | 59.5 (10.5) | 59.1 (10.4) | 59.2 (9.6) | 56.1 (11.2) | 0.004              |
| Sex, men / women, n (%) | 512 (66.1) / 263 (33.9) | 124 (64.2) / 69 (35.8) | 135 (69.6) / 59 (30.4) | 134 (69.1) / 60 (30.9) | 119 (61.3) / 75 (38.7) | 0.254              |
| TOFOa 20 mg / 40 mg, n (%) | 235 (30.3) / 540 (69.7) | 60 (31.1) / 133 (68.9) | 58 (29.9) / 136 (70.1) | 137 (70.6) / 57 (29.4) | 60 (30.9) / 134 (69.1) | 0.980              |
| Body weight (kg) | 68.4 (14.2) | 66.9 (14.4) | 66.6 (13.3) | 67.8 (13.8) | 72.5 (14.6) | <0.001              |
| Body mass index (kg/m2) | 25.6 (4.3) | 25.3 (4.5) | 25.1 (3.7) | 25.3 (4.2) | 26.7 (4.8) | <0.001              |
| HbA1c (mmol/mol) | 6.45 (9.9) | 6.45 (10.5) | 6.51 (10.5) | 6.31 (9.4) | 6.53 (9.1) | 0.120              |
| Fasting plasma glucose (mmol/L) | 8.9 (2.1) | 9.1 (2.1) | 8.9 (2.1) | 8.7 (1.9) | 9.0 (2.1) | 0.396              |
| Fasting plasma glucose (mg/dL) | 160.9 (37.0) | 163.3 (37.5) | 160.7 (38.3) | 157.3 (34.6) | 162.3 (37.2) | 0.396              |
| Sodium (meq/L) | 139.3 (1.9) | 139.4 (1.9) | 139.2 (1.9) | 139.3 (2.1) | 139.2 (1.8) | 0.627              |
| Potassium (meq/L) | 4.2 (0.3) | 4.2 (0.3) | 4.2 (0.3) | 4.1 (0.3) | 4.2 (0.3) | 0.034              |
| Systolic blood pressure (mm Hg) | 130.3 (14.1) | 128.7 (14.5) | 130.1 (13.7) | 130.8 (13.7) | 131.8 (14.2) | 0.188              |
| Diastolic blood pressure (mm Hg) | 77.3 (10.2) | 76.8 (10.2) | 76.3 (11.0) | 77.3 (10.0) | 78.9 (9.5) | 0.069              |
| Concomitant antihypertensive drugs (%) | 366 (47.2) | 90 (46.6) | 83 (42.8) | 96 (49.5) | 97 (50.0) | 0.465              |
| ARB, n (%) | 275 (35.5) | 65 (33.7) | 64 (33.0) | 76 (39.2) | 70 (36.1) | 0.577              |
| ACEI, n (%) | 18 (2.3) | 3 (1.6) | 6 (3.1) | 4 (2.1) | 5 (2.6) | 0.767              |
| ARB and or ACEI, n (%) | 291 (37.6) | 67 (34.7) | 70 (36.1) | 79 (40.7) | 75 (38.7) | 0.6209             |
| CCB, n (%) | 218 (28.1) | 51 (26.4) | 46 (23.7) | 52 (26.8) | 69 (35.6) | 0.059              |
| Beta-blockers, n (%) | 27 (3.5) | 10 (5.2) | 7 (3.6) | 6 (3.1) | 4 (2.1) | 0.406              |
| Diuretics, n (%) | 64 (8.3) | 22 (11.4) | 13 (6.7) | 13 (6.7) | 16 (8.3) | 0.309              |
| Duration of diabetes mellitus (y) | 7.1 (5.9) | 7.1 (6.1) | 6.9 (5.9) | 7.8 (6.3) | 6.8 (5.4) | 0.389              |
| Creatinine (mg/dL) | 0.71 (0.16) | 0.73 (0.15) | 0.74 (0.16) | 0.71 (0.17) | 0.66 (0.17) | <0.001              |
| Cystatin C (mg/L) | 0.72 (0.15) | 0.74 (0.14) | 0.74 (0.16) | 0.71 (0.13) | 0.70 (0.16) | 0.005              |
| eGFRMDRD (mL/min/1.73 m2) | 83.9 (18.4) | 79.5 (16.7) | 81.2 (17.5) | 83.9 (16.9) | 91.0 (20.1) | <0.001              |
| eGFRCKD-EPI (mL/min/1.73 m2) | 84.5 (11.5) | 83.1 (10.2) | 82.8 (11.7) | 83.9 (11.1) | 88.3 (12.3) | <0.001              |
| eGFRCRE+CYS (mL/min/1.73 m2) | 97.7 (15.0) | 94.9 (14.1) | 95.8 (16.1) | 98.3 (12.8) | 102.0 (15.8) | <0.001              |
| eGFRMDRD (mg/dL) | 30 (3.9) | 3 (1.6) | 6 (3.1) | 7 (3.4) | 14 (7.2) | 0.0365              |
| Uric acid (mg/dL) | 5.1 (1.3) | 5.3 (1.3) | 5.1 (1.4) | 5.1 (1.2) | 4.8 (1.3) | 0.012              |
| BNP (pg/mL) | 15.6 (17.4) | 132 (12.2) | 14.4 (16.7) | 15.3 (13.8) | 19.6 (23.8) | 0.002              |
| ACR (mg/g Cre) | 16.4 (9.0–46.0) | 142 (8.5–37.1) | 16.2 (8.8–47.1) | 15.5 (9.3–43.2) | 21.5 (9.5–63.5) | 0.028              |
| ACR > 30 mg/g Cre, n (%) | 255 (32.9) | 52 (26.9) | 63 (32.5) | 60 (30.9) | 80 (41.2) | 0.023              |
higher basal DSI and HbA1c levels, and use of RAS inhibition, drugs were negatively correlated with changes in eGFRMDRD, but were positively correlated with changes in creatinine levels. Both lower glycaemic status and DSI at baseline were correlated with the greater increase in eGFRMDRD at Week 52 (Figure 2).

4 | DISCUSSION

The present study is the first to show an independent correlation between basal DSI and changes in eGFRMDRD at both Weeks 4 and 52 during treatment with the SGLT2i, TOFO, in patients with T2D. We also found that basal DSI did not correlate with improved hyperglycaemia, decreased body weight and blood pressure, and changes in ACR. Moreover, participants with both lower basal HbA1c and DSI experienced greater increases in eGFRMDRD as the result of TOFO indicated the attenuated glomerular relative-hyperfiltration and subsequent improved renal function, which might be attributed to the handling of reabsorption of, not only urinary glucose, but also sodium, through SGLT2 in the proximal tubule.

eGFRMDRD levels initially dipped at Week 4, which is consistent with results of previous studies. However, the clinical factors that influenced the initial reduction in eGFRMDRD remain to be elucidated. In our participants, the basal eGFRMDRD increased with higher levels of basal DSI, suggesting that the proportion of participants with glomerular relative-hyperfiltration might be higher following increases in DSI across quartiles. Multivariable analyses also indicated that basal DSI was correlated with eGFRMDRD and changes in creatinine levels, independent of basal eGFRMDRD, creatinine level, HbA1c level and use of RAS inhibition drugs. Therefore, these differences in the degree of initial dip in eGFRMDRD as the result of TOFO was attributed to the correction of TGF action via SGLT2i. These findings suggested that salt intake-induced glomerular relative-hyperfiltration might be caused by SGLT2 in the proximal tubule and that the inhibition of SGLT2 might attenuate it in patients with T2D.

Our results also indicated that the change in eGFRMDRD at Week 4 was not correlated with changes in DBP, but was significantly correlated with changes in SBP in the entire participant population. However, those correlations were weak. Further, the change in eGFRMDRD was not correlated with changes in blood pressure, according to results in each of the DSI quartile groups. Therefore, the contribution of changes in blood pressure to the change in eGFRMDRD at Week 4 might be small. We further indicated that baseline BNP might be a predictor of the change in eGFRMDRD at Week 4, according to results of multivariable analysis, although this observation might not apply to the change in eGFRMDRD at Week 52. Higher BNP levels, suggesting fluid retention, might cause the greater reduction in eGFRMDRD as the result of TOFO at Week 4. Thus, fluid loss soon after TOFO treatment might contribute, in part, to the reduction in eGFRMDRD at Week 4. That change in eGFRMDRD at Week 4 was significantly correlated with change in body weight provides support that fluid loss
might be associated, in part, with the reduction in eGFR_{MDRD} at Week 4.

We also observed a gradual increase in eGFR_{MDRD} from Week 4 to Week 52, irrespective of basal DSI. This recovery of eGFR_{MDRD} from the initial decrease was consistent with that shown in previous studies.\textsuperscript{18,27} However, in this study, a significant increase in eGFR_{MDRD} from baseline to Week 52 was observed. Results of multivariable analysis also indicated that basal DSI was not correlated, but HbA1c levels at Week 4 were negatively correlated with recovery of eGFR_{MDRD} (Table S6), suggesting that the lower levels of HbA1c soon after TOFO treatment might have contributed to that recovery. Further, change in eGFR_{MDRD} from Week 4 to Week 52 was not correlated with change in HbA1c. These results suggested that, not only the correction of salt intake-induced glomerular relative-hyperfiltration, but also the well-controlled hyperglycaemia before and soon after TOFO administration, might contribute to the recovery of and increase in eGFR_{MDRD} after the initial dip. Prolonged hyperglycaemia directly induces mesangial expansion and injury,\textsuperscript{28} and also causes cellular dysfunction in the proximal tubular cells as the result of hyperglycemia-induced excessive glucose reabsorption.\textsuperscript{29} Thus, the recovery of eGFR_{MDRD} from the initial decrease might be attributed to, not only attenuation of glomerular relative-hyperfiltration, but also the baseline renal status induced by a sustained well-controlled glycaemic status.

We observed changes in eGFR_{MDRD} during TOFO treatment, according to whether participants used RAS inhibition drugs (Figure S3). There were significant differences in the change in eGFR_{MDRD} at both Week 4 and Week 52, according to use of RAS inhibition drugs. Results of multivariable analysis indicated that the use of RAS inhibition drugs as concomitant antihypertensive medication might be negatively correlated with change in eGFR_{MDRD} at Weeks 4 and 52. Therefore, the use of RAS inhibition drugs as concomitant antihypertensive medication might contribute to the effects of TOFO treatment on eGFR_{MDRD} levels, independent of basal DSI. The preferential effect of SGLT2 inhibition on afferent renal arteriolar resistance was reported to be important in reducing intraglomerular pressure,\textsuperscript{30} while the effects of RAS inhibition on efferent renal arteriolar resistance might be thought to be important also in reducing intraglomerular pressure. Therefore, the combination of SGLT2 inhibition drugs and RAS inhibition drugs might contribute to further
TABLE 2  Baseline predictors for influencing the change in eGFRMDRD at week 4 and week 52

| Factors                                      | Regression coefficient | P    |
|----------------------------------------------|------------------------|------|
| BNP (higher 1 pg/mL)                        | −0.04                  | 0.0099|
| eGFR (higher 1 mL/min/1.73 m²)              | −0.08                  | <0.001|
| Estimated daily salt intake (higher 1 g/d)  | −0.47                  | <0.001|
| HbA1c (higher 1%)                           | −0.59                  | 0.0320|
| Tofogliflozin 40 mg (vs. 20 mg)             | −1.07                  | 0.0464|
| Use of RAS inhibition drugs (yes)           | −1.22                  | 0.0176|

Change in eGFRMDRD at week 52

| Factors                                      | Regression coefficient | P    |
|----------------------------------------------|------------------------|------|
| Age (higher 1 year)                         | −0.07                  | 0.0373|
| Estimated daily salt intake (higher 1 g/d)  | −0.40                  | 0.0104|
| HbA1c (higher 1%)                           | −1.69                  | <0.001|
| Use of RAS inhibition drugs (yes)           | −2.25                  | 0.0034|

Note: Factors remained through stepwise variable selection with P < 0.05. Potential baseline predictors were dosage of tofogliflozin, use of RAS inhibition drugs (ARB and/or ACEI), age, sex, duration of diabetes, HbA1c, DBP, BNP, BMI, uric acid, eGFRMDRD, DSI levels, and ACR <30 mg/g Cre vs. ACR = >30 mg/g Cre.

FIGURE 2  Changes in eGFRMDRD at Week 52 according to median values of baseline HbA1c and estimated daily salt intake. aP < 0.01, bP < 0.001 vs baseline, cP < 0.05, dP < 0.01, eP < 0.001 vs HbA1c ≥7.8% and DSI ≥9.3 g. One sample t test

correction of increased intraglomerular pressure, leading to a delay in the development and progression of diabetic kidney disease.30,31 Our results suggested that the use of RAS inhibition drugs might independently contribute to a further reduction in eGFRMDRD, followed by decreased intraglomerular pressure. On the contrary, our findings also supported the association of basal DSI with change in eGFRMDRD at Weeks 4 and 52, independent of the use of RAS inhibition drugs. However, the monitored period of TOFO treatment was limited and histological confirmation was not performed to evaluate structural changes in the kidney. Therefore, further investigation will be needed to evaluate the effects of RAS inhibition drugs on changes in eGFR as the result of long-term SGLT2i treatment.

From our results, we could not explain the association of the Na+/H+-exchanger 3(NHE3), which contributed to reabsorption of urinary sodium, with the effects of long-term TOFO treatment on eGFRMDRD. SGLT2 is co-expressed with NHE3, which reabsorbs approximately 30% of filtered sodium. Recent studies provide evidence that SGLT2 may be functionally linked to NHE3, such that SGLT2 inhibition may also inhibit NHE3 in the proximal tubule.4,32,33 Our study indicated that baseline DSI, which was estimated from urinary sodium excretion, might independently influence changes in eGFRMDRD during long-term TOFO treatment. The interaction between SGLT2 and NHE3, and change in the reabsorption of sodium leading to increased delivery of sodium to the macula densa after SGLT2 inhibition, could be relevant in explaining the effect of SGLT2i on eGFR. Further investigations are required to clarify those interactions and their effect on eGFR as the result of SGLT2i treatment.

Two weeks after termination of treatment with TOFO, eGFRMDRD levels had further increased from Week 52, which was consistent with previous reports.18 These results supported the opinion that, with long-term administration of SGLT2i, the attenuation of glomerular relative-hyperfiltration might be maintained. Recently, Cherney et al. reported that, 30 days after termination of treatment with empagliflozin, a greater reduction in urine ACR was maintained with empagliflozin than with a placebo.34 In our study, the positive correlation between change in eGFRMDRD and urine ACR from Week 52 to Week 54 was observed in participants with albuminuria (Figure S4). This result suggested that attenuation of glomerular relative-hyperfiltration might contribute, in part, to the reduction in urine ACR. The report by Cherney et al. also suggested that long-term administration of empagliflozin might contribute to functional improvement of the kidney.34 Results of our correlation analysis indicated that increased eGFRMDRD during the termination period was not significantly correlated with the initial decrease, which might be caused by the attenuation of glomerular relative-hyperfiltration (Figure S5). Moreover, DSI was not significantly associated with increased eGFRMDRD during the termination period, based on results of multivariable analysis (Table S7). These findings suggested that increased eGFRMDRD after the termination of TOFO treatment might be caused, not only by the released attenuation of glomerular relative-hyperfiltration, but possibly by other factors as the result of long-term use of the medication, such as functional changes in the kidney.

Although study participants had a mean eGFRMDRD value at baseline of 83.9 mL/min/1.73m², 30 of these participants had eGFRMDRD levels ≥120 mL/min/1.73m² (hyperlfiltration). The proportion of participants with hyperfiltration tended to increase in association with increased basal DSI. We further investigated the effects on eGFRMDRD, according to whether participants had hyperfiltration during the study period (Figure S6). Reduction in eGFRMDRD at Week 4 was greater in participants with hyperfiltration than in those without
hyperfiltration, while changes in eGFR\textsubscript{MDRD} from Week 4 to Week 52, from baseline to Week 52, and from Week 52 to Week 54 were identical between participants with and without hyperfiltration. Changes in eGFR\textsubscript{MDRD} during TOFO treatment were similar between participants with and without hyperfiltration, with the exception of the initial dip in eGFR\textsubscript{MDRD}. However, further study involving a large number of participants with hyperfiltration is needed to clarify the effects of basal DSI on eGFR as the result of SGLT2i treatment.

Current approaches to preventing kidney complications in patients with diabetes have focused on lowering blood pressure, HbA1c, body weight, albuminuria and cholesterol, which have been shown to reduce the risk of cardiovascular disease and deterioration of kidney function.\textsuperscript{35,36} Our analysis also revealed decreased blood pressure, HbA1c and body weight at Weeks 4 and 52. The reductions in BP according to basal DSI quartiles were similar, regardless of the use of RAS inhibition drugs as concomitant antihypertensive medication. Therefore, these results might not indicate a clear association between baseline DSI and reductions in blood pressure after long-term TOFO treatment. TOFO was previously reported to reduce urine ACR and the N-acetyl-beta-D-glucosaminidase/creatinine ratio in patients with T2D with micro- and macroalbuminuria.\textsuperscript{37} In this study, less-controlled glycaemic status at baseline was associated with subsequent increase in eGFR\textsubscript{MDRD} as the result of TOFO treatment. Interestingly, independent of glycaemic status, lower salt intake at baseline was also associated with subsequent increase in eGFR\textsubscript{MDRD}.

Our findings suggested that basal DSI, in addition to correlation with glycaemic control, correlates with eGFR\textsubscript{MDRD} as the result of long-term TOFO treatment in participants with T2D, independent of the use of RAS inhibition drugs as concomitant antihypertensive treatment. Thus, an appropriate dietary approach to therapy should be considered before initiation of treatment with SGLT2is. On the contrary, glomerular relative-hyperfiltration induced by higher DSI might be attenuated by TOFO treatment, leading to a further reduction in eGFR\textsubscript{MDRD} levels. More studies are required to clarify whether these differences in results regarding the changes in eGFR\textsubscript{MDRD} that are influenced by basal DSI may contribute to clinically meaningful reductions in the development and progression of diabetic kidney diseases in patients with T2D. Unfortunately, we could not monitor changes in urinary sodium excretion in order to estimate changes in salt intake during TOFO treatment. Sustained lower-controlled salt intake may contribute to the further increase in eGFR during SGLT2i treatment, but additional investigations will be needed. SGLT2i inhibits the reabsorption of, not only urine glucose, but also sodium, which may have multifactorial effects on the risk factors for cardiovascular disease and deterioration of kidney function. However, dietary approaches may be needed, particularly in monitoring salt intake, to maximize the renal effects of SGLT2i.

We recognize that the estimation of DSI in our study is a serious limitation. Although we performed the analyses according to quartiles of basal DSI, SBP and DBP levels at baseline did not differ among the quartiles of DSI, nor did the proportion of concomitant antihypertensive drugs at baseline. Of note, the use of diuretics coincided with increases in basal DSI, although the use of diuretics might be clinically effective in controlling blood pressure in salt-sensitive hypertensive participants. Our study was based on pooled analyses from two TOFO studies that had been designed to evaluate the safety and efficacy of TOFO as monotherapy or as an add-on to other oral antidiabetic agents in T2D patients. Thus, the prescribed antihypertensive drugs might not have been selected according to basal DSI. Among study participants, 47.2% received antihypertensive drugs and 8.3% were received diuretics. We found that the proportion of participants with ACR levels ≥30 mg/g Cre was significantly increased, based on increased basal DSI. In Japanese T2D patients with microalbuminuria, the prevalence of hypertension that was salt sensitive was greater than that in patients without microalbuminuria,\textsuperscript{38} which provides support that our basal DSI levels were correctly estimated. Also, the formula for estimating DSI levels was calculated according to participant age, body weight, and urinary sodium secretion. Higher basal DSI values were more prevalent in younger participants and in those with higher body weight. It can be considered that the formula for estimating DSI in our study is applicable to clinical practice for patients receiving concomitant diuretics.\textsuperscript{39} Of course, further investigation of the effects of DSI on eGFR as the result of SGLT2i treatment, according to the use of concomitant hypertensive drugs, especially diuretics, will be needed. Importantly, measuring and monitoring actual daily urinary sodium excretion and investigating its association with the effect of long-term SGLT2i treatment would be desirable.

This study has several other limitations. We could not perform a comparison between a placebo and TOFO, and the baseline eGFR\textsubscript{MDRD} level in almost all participants was less than 120 mL/min/1.73m\textsuperscript{2}. We could not measure the actual GFR and molecular actions and pharmacological factors are required to confirm conclusions based on the results of this study.

In conclusion, basal DSI was independently correlated with changes in eGFR\textsubscript{MDRD} after long-term treatment with an SGLT2i, TOFO, in patients with T2D. Moreover, both low basal HbA1c and DSI levels were associated with a greater increase in eGFR\textsubscript{MDRD} at Week 52. The effects of the SGLT2i, TOFO, on eGFR might be associated with the handling of, not only glucose, but also sodium, through SGLT2 in the proximal tubule.
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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

All authors participated in writing the manuscript. K. N. and Y. S. wrote the draft of the manuscript and contributed to the discussion. K. K. contributed to the discussion and reviewed/editied the manuscript. A. Y. researched data and reviewed the manuscript. H. S. had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analyses. K. K. is a director of data collection. H. S. and K. K. are the guarantors of this work.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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