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CLINICAL TRIAL

Effect of administration and withdrawal of the sodium-glucose cotransporter 2 inhibitor, tofogliflozin, on renal protection in individuals with type 2 diabetes mellitus and diabetic nephropathy: A multicenter, single-arm study (RESTORE-nephropathy study)

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Keywords
Diabetic nephropathy, Drug withdrawal, Renal protection, Tofogliflozin, Urinary albumin

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ABSTRACT

Aims/Introduction: The mechanisms of the renoprotective effects of sodium-glucose cotransporter 2 inhibitors are unknown. This study aimed to explore the effect and mechanism of tofogliflozin on urinary albumin by administration, withdrawal, and re-administration.

Materials and Methods: Individuals with type 2 diabetes mellitus and stage 2 or 3 diabetic nephropathy were enrolled. Tofogliflozin was administered for 24 weeks, withdrawn for 12 weeks (withdrawal period), and re-administered for 24 weeks. The primary endpoint was the change in urinary albumin/creatinine ratio (UACR). The secondary endpoints included hemoglobin A1c (HbA1c), hepatic biomarkers, lipid profiles, physical examinations, and blood counts.

Results: A total of 47 individuals were enrolled. UACR significantly decreased throughout the observation period. It also significantly decreased, increased, and again decreased during the period of the 1st administration, withdrawal, and re-administration, respectively. HbA1c, body weight, waist circumference, and systolic blood pressure also showed the same tendency. Aspartate aminotransferase and alanine aminotransferase significantly decreased throughout the observation period, but did not increase during the withdrawal period.

Conclusions: Urinary albumin improved during the administration of tofogliflozin and worsened during its withdrawal, suggesting the reversibility of its renoprotective effect. The administration of tofogliflozin should be continued to avoid the reversal of glycemic control, renoprotective effects, and other beneficial effects.

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INTRODUCTION

Diabetic nephropathy is the leading cause of new dialysis inductions, and its prevalence has been increasing over the past several years in Japan[1]. Individuals with diabetic nephropathy are known to be at high risk of cardiovascular diseases and have a poor prognosis after dialysis induction. Therefore, appropriate and early treatment is required to prevent the onset of cardiovascular diseases and progression to end-stage renal failure.

Treatment to prevent the progression of diabetic nephropathy through appropriate and comprehensive risk management is expected to prevent the onset and progression of diabetic nephropathy and also lead to remission of albuminuria. In particular, the Steno-2 study, a representative study of comprehensive treatment, compared intensive treatment with strict control and standard therapy in accordance with guidelines[2]. As a result, progression from early nephropathy to overt nephropathy was reduced by 61%, and the occurrence of cardiovascular diseases was reduced by 53% in the intensive treatment group compared with the standard treatment group. Moreover, the 5.5-year follow-up study after the intervention showed a 50% reduction in all-cause and cardiovascular mortality and suppression of nephropathy progression in the intensive treatment group[3]. Furthermore, the J-DOIT3 study also showed that intensive treatment in individuals with type 2 diabetes mellitus complicated by hypertension or dyslipidemia suppressed the onset of nephropathy[4]. Another study showed that intensive glycemic control suppressed the onset or progression of albuminuria or renal events, suggesting that hyperglycemia is a risk factor for the onset or progression of nephropathy[5].

Sodium-glucose cotransporter 2 inhibitors (SGLT2is) inhibit re-uptake of sodium and glucose from the primary urine and promote urinary excretion of glucose in urine, lowering blood glucose levels. Since their mode of action is insulin-independent, SGLT2is are known to have a relatively low risk of hypoglycemia and reduce body weight[6]. The EMPA-REG OUTCOME study and CANVAS Program also reported the preventive effects of SGLT2is on cardiovascular and renal events[7–9]. Regarding the renoprotective effects of SGLT2is, they have been reported to affect renal hemodynamics[10], suppress the reduction of estimated glomerular filtration rate (eGFR)[7], and reduce the excretion of urinary albumin[11, 12]. However, the mechanisms underlying the renal protective effects of SGLT2is, whether or not they are reversible due to renal hemodynamics or irreversible structural changes, are still unknown. Moreover, determining whether or not there is a difference in the albuminuria-reducing effect of SGLT2is between initial administration and re-administration may be important for determining the appropriateness of their withdrawal. To the best of our knowledge, there are few reports on the effect of withdrawal and re-administration of SGLT2is on renal function. One study reported that 2.6 years of empagliflozin administration and a month of withdrawal from it did not increase urinary albumin levels[11]. However, another study reported that 6-week administration and 6-week withdrawal of dapagliflozin reversed the urinary albumin level to baseline, and the degree of urinary albumin reduction during the first dapagliflozin administration period and the re-administration period were comparable[13].

Tofogliflozin has the highest selectivity for SGLT2 among the existing SGLT2is and is known to have characteristic pharmacokinetics, such as a short half-life and a low protein binding rate, with a decreasing effect on blood glucose levels and body weight[14–16]. This study aimed to explore the effect and mechanism of tofogliflozin on urinary albumin levels.

MATERIALS AND METHODS

Participants

Individuals with type 2 diabetes mellitus and stage 2 or 3 diabetic nephropathy were enrolled in this study. Diabetic nephropathy stage was diagnosed according to the Japanese diagnostic criteria[17]: stage 2 (microalbuminuria), an urinary albumin to creatinine ratio (UACR) between 30 and 299 mg/g·Cre and eGFR of 30 mL/min/1.73 m^2 or higher; stage 3 (macroalbuminuria), an UACR of 300 mg/g·Cre or higher or urinary protein to creatinine ratio of 0.5 g/g·Cre or higher, and eGFR of 30 mL/min/1.73 m^2 or higher. The exclusion criteria included individuals with type 1 diabetes mellitus or secondary diabetes; individuals with a history of SGLT2i use; individuals who started to use or changed the dose of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers within 3 months before giving their consent. The detailed eligibility criteria are listed in Table S1.

Study design

This "*R*esearch to *E*xplore the effects of a *S*GLT2 inhibitor, *T*ofogliflozin, *O*n *R*enal protection *E*stimated by first administration and readministration in patients with stage 2–3 diabetic *nephropathy*" (RESTORE-nephropathy study) was a multicenter, single-arm study that was conducted at 10 medical institutions in Japan under the management of the Japan society for Patient Reported Outcome. Participant enrollment was conducted between January 2020 and September 2022. All study procedures were conducted in accordance with the ethical standards of the Declaration of Helsinki, the Clinical Trials Act, and other current legal regulations in Japan. It was approved (approval number: N20190001-1re) by the Certified Review Board of Keio. This study was registered in the Japan Registry of Clinical Trials (jRCT) (registration number: jRCTs031190185). Written informed consent was obtained prior to intervention from all enrolled individuals who met the eligibility criteria. To avoid bias and ensure quality, data collection, data management, monitoring, and audit were performed by third-party entities (Soiken Inc., Osaka, Japan).

Randomization, study intervention, and observation

Since this was a single-arm study, randomization was not performed. Tofogliflozin 20 mg/day was administered to all

enrolled individuals for 24 weeks (1st administration period). Then, tofogliflozin was withdrawn for 12 weeks (withdrawal period). After this, tofogliflozin 20 mg/day was again administered for 24 weeks (re-administration period). The total observation period was 60 weeks, with six observation points at baseline and weeks 12, 24, 36, 48, and 60. The study design is shown in Figure S1. Throughout the observation period, including the withdrawal period, the use of other SGLT2is was prohibited. If the HbA1c value exceeded that at baseline, a change, dose increase, or addition of other antidiabetic agents other than SGLT2is was allowed. If the HbA1c value exceeded that at baseline during the withdrawal period, tofogliflozin was re-administered in addition to the antidiabetic agents used at that point.

Outcomes

The primary endpoint was the change in UACR from baseline to week 60. Secondary endpoints included hemoglobin A1c (HbA1c), hepatic biomarkers, lipid profiles, physical examinations, blood counts, and the frequency of adverse events. The presence or absence of adverse events, including hypoglycemia or hyperglycemia, was investigated by medical interview.

Sample size

Based on the integrated data of phase 2/3 clinical trials about tofogliflozin[12], data points of individuals with a UACR of 30 mg/g·Cre or higher were extracted. Change in UACR from baseline to week 24 was calculated to be −0.64 (log-transformed, ln[mg/g·Cre]) (our unpublished data). Considering the study design in this study, we assumed the change in the log-transformed UACR from baseline to week 24 and from baseline to week 60 to be −0.64 with a standard deviation of 0.9. Under these assumptions, the minimum sample size required to achieve a significance of 0.05 from a two-sided test with a statistical power of 80% was determined to be 18 individuals. In addition, under the assumptions that the change in the log-transformed UACR from week 36 to week 60 was −0.4 with a standard deviation of 0.9, the minimum sample size required to achieve a significance of 0.05 from a two-sided test with a statistical power of 80% was determined to be 42 individuals. We estimated the dropout rate to be 10%; hence, the planned enrollment required to conduct the three tests in the closed testing procedure was set at 47 individuals.

Statistical analysis

All tests were two-sided, and a *P*-value of less than 0.05 was considered statistically significant. Multiplicity was not adjusted for all endpoints. A statistical analysis plan was developed prior to the database lock. All statistical analyses were conducted by independent biostatisticians using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

Three analysis sets were defined in this study; the full analysis set (FAS) included all individuals who were registered. However, individuals with severe protocol violations were excluded from the FAS. The per-protocol set (PPS) excluded individuals with a protocol violation, such as a violation of eligibility criteria, use of prohibited concomitant treatments, or poor adherence to the study agent. The safety analysis set included all individuals who were registered in this study and who received at least one dose of the study agent. The analyses of the primary endpoint were performed with data from the FAS and PPS. The analyses of the secondary endpoints were performed with data from the FAS. Safety analysis was performed with the safety analysis set.

Participant characteristics at baseline are presented as frequencies and proportions for categorical data and summary statistics (number of individuals, mean, standard deviation, minimum, first quartile, median, third quartile, and maximum) for continuous data. The primary endpoint, change in UACR, was analyzed by the mixed effect model for repeated measures (MMRM) in the closed testing procedure in the following order: from baseline to week 24, from baseline to week 60, and change from week 36 to week 60. The MMRM was performed with an unstructured covariance structure, with time and value at baseline as fixed effects, and participants as random effects. If the calculation results did not converge, compound symmetry was used. Summary statistics for measurements and changes were calculated, and one-sample *t*-tests were performed as the sensitivity analyses. The secondary endpoints regarding the UACR were analyzed by the same methods as the primary endpoint. For other secondary endpoints, summary statistics for measurements and changes were calculated, and one-sample *t*-tests were performed. For the safety endpoints, summary statistics for the adverse events (number of individuals and proportion) were calculated.

RESULTS

Baseline participant characteristics

A flowchart of the study population is shown in Figure 1. A total of 345 individuals were evaluated for eligibility, and 47 were enrolled in the study as planned. Two participants dropped out before the initiation of the study intervention and were excluded from the analysis; hence, the safety analysis set comprised 45 individuals. After initiation of the study intervention, two participants were found to be ineligible and one withdrew consent; consequently, they were excluded from the FAS, resulting in the FAS comprising 42 individuals. The baseline characteristics of the participants are shown in Table 1.

Primary endpoint and renal biomarkers

UACR significantly decreased throughout the observation period from baseline (mean ± standard error (SE): 598.4 ± 192.7 mg/g·Cre) to week 60 (526.2 ± 175.0 mg/g·Cre) (change in adjusted log-transformed mean analyzed by MMRM: −0.35 ln(mg/g·Cre); 95% confidence interval [CI]: −0.59 to −0.10 ln(mg/g·Cre); $P = 0.006$). It also significantly decreased during the 1st administration period from baseline to

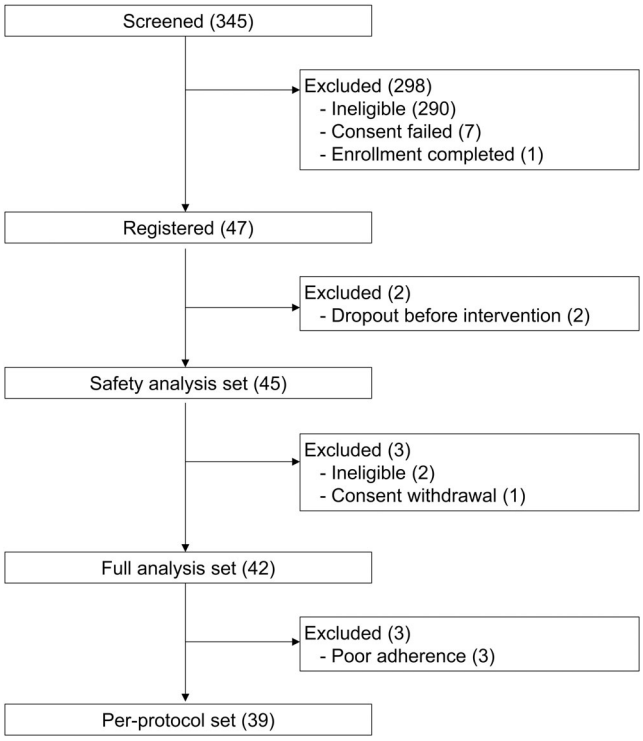

Figure 1 | Study flow chart showing patient enrollment and analysis.

week 24 (change: −0.38; 95% CI: −0.63 to −0.14; $P = 0.002$). Then, it significantly increased during the withdrawal period from week 24 to week 36 (change: 0.35; 95% CI: 0.11–0.59; $P = 0.005$). It again significantly decreased during the re-administration period from week 36 to week 60 (change: −0.31; 95% CI: −0.55 to −0.07; $P = 0.012$) (Figure 2a). One-sample *t*-tests as the sensitivity analyses also showed the same results that the UACR significantly decreased throughout the observation period ($P = 0.024$), during the 1st administration period from baseline to week 24 ($P = 0.003$), and during the re-administration period from week 36 to week 60 ($P = 0.034$), whereas it significantly increased during the withdrawal period from week 24 to week 36 ($P = 0.008$). A post-hoc analysis showed that there was no statistically significant change from baseline to week 36 ($P = 0.72$).

In contrast, the serum creatinine and blood urea nitrogen (BUN) significantly increased throughout the observation period (Figure 2b,c), and the eGFR significantly decreased throughout the observation period (Figure 2d). The serum creatinine significantly increased both during the 1st administration period and the re-administration period, and numerically (but not significantly) decreased during the withdrawal period. The BUN significantly increased during the 1st administration period and significantly decreased during the withdrawal period. It also numerically (but not significantly) increased during the re-administration period. The eGFR significantly decreased during the 1st administration period and significantly increased during the withdrawal period. It also numerically (but not significantly) decreased during the tofogliflozin re-administration period.

Table 1 | Participant characteristics

Characteristic	Total ($n = 42$)
Age (years)	64.5 ± 8.9
Women (*n* [%])	9 (21.4)
BMI (kg/m^2)	26.1 ± 4.0
Diabetes duration (years)[†]	16.5 ± 11.2
Drinking habit	24 (57.1)
Current smoking	12 (28.6)
History of cardio-/cerebrovascular disease	2 (4.8)
Microvascular complication	42 (100.0)
Diabetic retinopathy[‡]	15 (39.5)
Diabetic nephropathy	42 (100.0)
Stage 2	26 (61.9)
Stage 3	16 (38.1)
Diabetic neuropathy[§]	16 (59.3)
Other complication	41 (97.6)
Renal disease except diabetic nephropathy	2 (4.8)
Hepatic disease	14 (33.3)
Cardio-/cerebrovascular disease	2 (4.8)
Hypertension	37 (88.1)
Use of anti-diabetic agent	40 (95.2)
SGLT2 inhibitor	0 (0.0)
Sulfonylurea	11 (26.2)
α-Glucosidase inhibitor	6 (14.3)
Biguanide	26 (61.9)
Thiazolidine	0 (0.0)
Glinide	2 (4.8)
DPP-4 inhibitor	29 (69.0)
GLP-1 receptor agonist	6 (14.3)
Insulin	14 (33.3)
Antihypertensive agent	35 (83.3)
Diuretic	7 (16.7)
Calcium antagonist	25 (59.5)
Angiotensin receptor blocker	30 (71.4)
Angiotensin-converting enzyme inhibitor	1 (2.4)
Beta-antagonist	2 (4.8)
Alpha/beta-antagonist	1 (2.4)
Direct renin inhibitor	1 (2.4)
Mineralocorticoid receptor antagonist	1 (2.4)
Angiotensin receptor neprilysin inhibitor	0 (0.0)
Others	0 (0.0)
Hypolipidemic agent	24 (57.1)
Antiplatelet agent	6 (14.3)
Antihyperuricemic agent	5 (11.9)

Data are presented as means ± standard deviations for continuous variables and as the number of individuals (%) for categorical variables. [†]$n = 41$. [‡]$n = 38$. [§]$n = 27$. BMI, body mass index; DPP-4, dipeptidyl peptidase 4; GLP-1, glucagon-like peptide 1; SGLT2, sodium-glucose cotransporter 2.

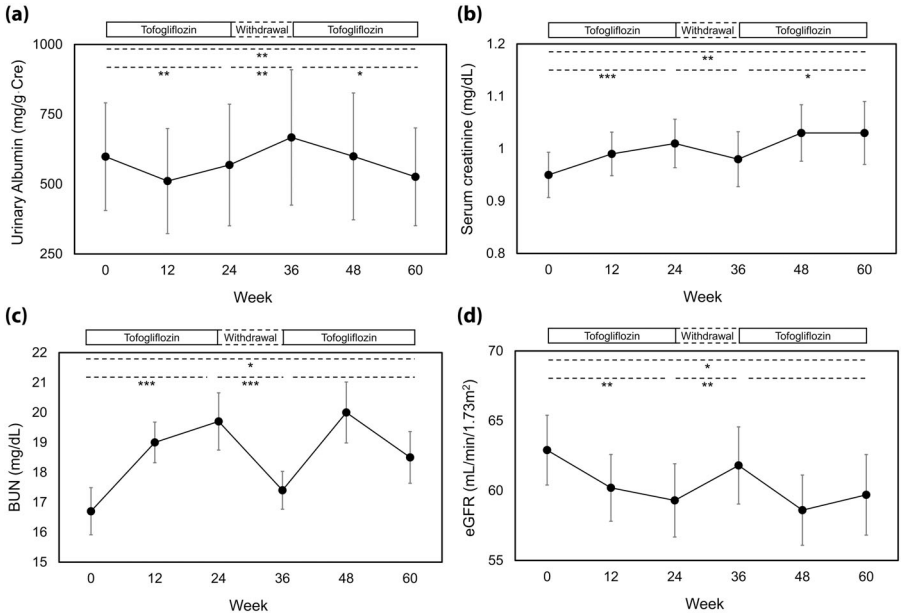

Figure 2 | Change in renal biomarkers. Data are presented as means ± standard errors. *, **, and ***Represent $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, for intragroup comparisons. For the intragroup comparison of urinary albumin (a), the MMRM was performed with an unstructured covariance structure, with time and value at baseline as fixed effects, and participants as random effects. If the calculation results did not converge, compound symmetry was used. For the intragroup comparisons of serum creatinine (b), BUN (c), and eGFR (d), one-sample *t* tests were performed. BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; MMRM, mixed effect model for repeated measures.

Biomarkers of glucose metabolism, hepatic function, and lipid metabolism

The HbA1c value significantly decreased, increased, and again decreased during the 1st administration period, withdrawal period, and re-administration period, respectively. It also significantly decreased throughout the observation period (Figure 3a). Both AST and ALT levels significantly decreased throughout the observation period; however, they did not increase during the withdrawal period (Figure 3b,c). The TC and LDL-C levels increased during the 1st administration (significantly for TC) and re-administration periods, and significantly decreased during the withdrawal period (Figure 3d,e). The HDL-C level significantly increased during the 1st administration period; however, it numerically decreased during the withdrawal period (Figure 3f).

Physical examinations

Body weight, waist circumference, and systolic blood pressure significantly decreased, increased, and again decreased during the 1st administration period, withdrawal period, and re-administration period, respectively (Figure 4a–c). Although the decrease in diastolic blood pressure during the 1st administration period and the increase during the withdrawal period were not significant, it significantly decreased during the re-administration period and throughout the observation period (Figure 4d).

Blood counts

The RBC, hemoglobin, and hematocrit significantly increased, decreased, and again increased during the 1st administration period, withdrawal period, and re-administration period, respectively, and increased throughout the observation period (Figure S2a,c,d); however, the WBC did not change significantly in any period (Figure S2b). The platelet counts significantly decreased throughout the observation period; however, the change in each period was not significant (Figure S2e).

Safety

Adverse events occurred in 27 individuals (60%) throughout the observation period. Frequent adverse events included hypoglycemia (7 individuals, 15.6%) and hyperglycemia (6 individuals, 13.3%). No deaths were reported, and three serious adverse events (aortic stenosis [1 patient, 2.2%], hemorrhagic intestinal diverticulum [1 patient, 2.2%], and pyomyositis [1 patient, 2.2%]) were reported (Table S2).

DISCUSSION

This study showed that tofogliflozin improved urinary albumin levels in individuals with type 2 diabetes mellitus throughout the observation period. However, this effect worsened during the withdrawal period and again improved during the re-administration period, suggesting reversibility of the effect of tofogliflozin on renal function. Due to its reversibility, the

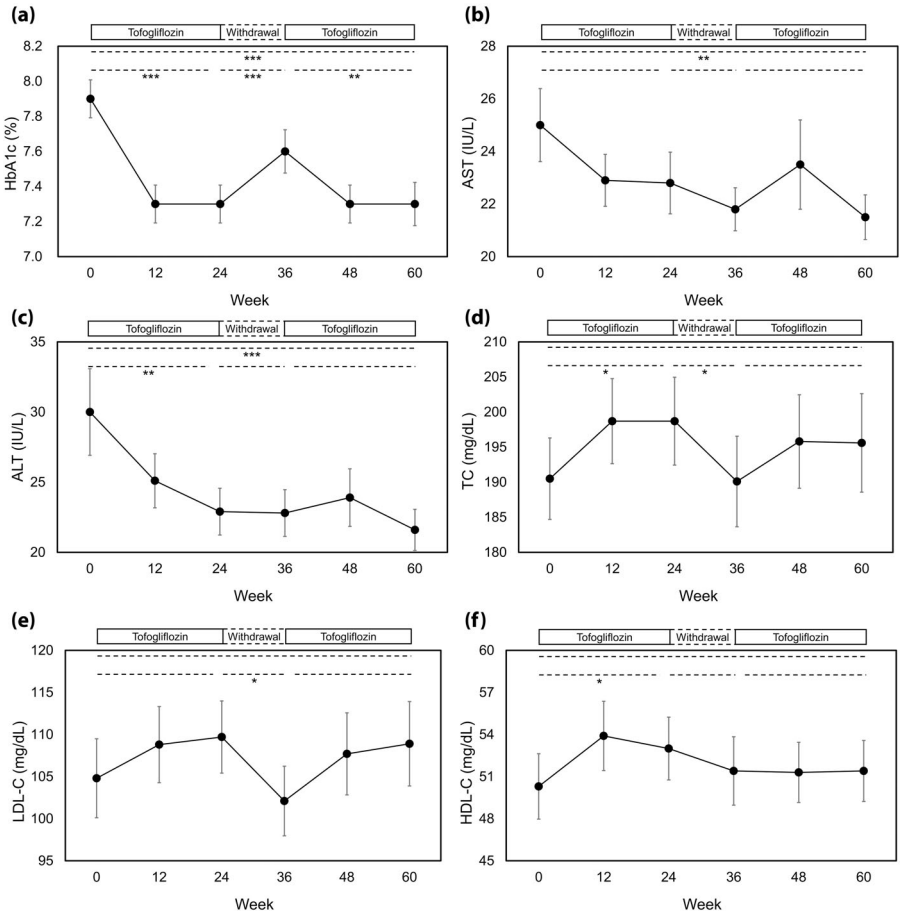

Figure 3 | Change in clinical laboratory tests. Data are presented as means ± standard errors. *, **, and ***Represent $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, for intragroup comparisons. For the intragroup comparisons, one-sample *t* tests were performed. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol.

administration of tofogliflozin should be continued not only for glycemic control but also for its renoprotective effect. In this study, tofogliflozin could not provide a sustainable renoprotective effect despite its administration for at least half a year. Regarding the mechanism of the action of tofogliflozin on renal function, one possibility may be the normalization of tubuloglomerular feedback (TGF)[18]. Since SGLT2is suppress the reabsorption of sodium and glucose in the proximal tubules[19], TGF may be normalized, attenuating the hyperfiltration in the renal glomeruli and reducing blood pressure and glomerular hypertension. In contrast, a sub-analysis of the EMPA-REG OUTCOME trial demonstrated that 2.6 years of treatment with empagliflozin reduced the UACR and maintained the lowered UACR after approximately 1 month of withdrawal[11]. The difference between the results in the EMPA-REG OUTCOME trial and this study may suggest that a half-year intervention with tofogliflozin may not have been enough to obtain a persistent "legacy effect" on renal function, or that 3 months' withdrawal of tofogliflozin was too long to maintain its beneficial effect on renal function. Since at least to our knowledge, there have still been no reports of the legacy effect by tofogliflozin, we could not judge whether the cause of the difference in the legacy effect is the specific effect of empagliflozin or tofogliflozin, or by the difference in the duration of SGLT2is administration and withdrawal. Further studies with longer intervention periods or varied withdrawal periods with different SGLT2is are required. Despite the improvement of urinary albumin, serum creatinine increased during the 1st administration and re-administration periods and throughout the observation period; moreover, the eGFR decreased during the 1st administration period and throughout the observation period, and inversely increased during the withdrawal period. The reason for the opposite tendency between urinary albumin, serum creatinine, and eGFR is not clear; however, it is known that eGFR tends to temporarily decrease during the early phase of SGLT2i administration (so called "initial dip")[8, 20, 21], and that

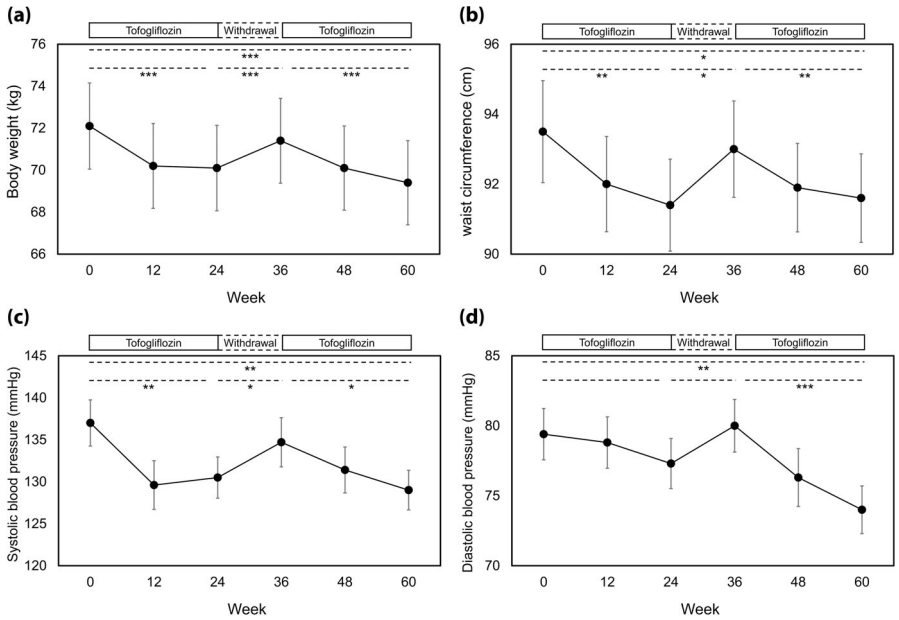

Figure 4 | Change in physical examinations. Data are presented as means ± standard errors. *, **, and ***Represent $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, for intragroup comparisons. For the intragroup comparisons, one-sample *t* tests were performed.

it is maintained or inversely increases thereafter. Therefore, it may be possible that the increase in serum creatinine and decrease in eGFR from the administration and re-administration in this study was not due to the worsening of renal function but rather the temporary effect of tofogliflozin, suggesting that the TGF did not fully normalize within the short period of half a year. Prolonged administration of tofogliflozin may recover serum creatinine levels and eGFR to those at baseline, or further improve them thereafter. Since the temporary withdrawal of tofogliflozin caused only the initial dip without long-term beneficial effects on renal function, the administration of tofogliflozin should be continued.

Because of the short period of tofogliflozin administration and withdrawal in this study, it is not likely that the improvement in glycemic control promoted the improvement in renal function. The prespecified correlation analysis between the change in UACR and change in HbA1c in each period as continuous variables showed a statistically significant correlation only during the 1st tofogliflozin administration period (0–24 weeks) ($P = 0.002$) and no other statistically significant correlation between them was found. We also analyzed the correlations between the worsening (increasing)/improvement (decreasing) of UACR and worsening (increasing)/improvement (decreasing) of HbA1c as categorical variables post-hoc, and again the statistically significant correlation was only during the 1st administration period ($P < 0.001$) and no other statistically significant correlation between them was found. These results may also suggest that the worsening of UACR during the tofogliflozin withdrawal period and re-improvement during the 2nd administration period are not directly caused by the changes in glycemic control. This study also showed the reversibility of clinical laboratory test values during the administration period and the withdrawal period, including glycemic control, lipid profiles, physical examinations, and blood counts. Nevertheless, post-hoc correlation analyses showed no statistically significant correlation during the tofogliflozin withdrawal period between the increasing of UACR and that of any clinical laboratory test items except BUN. Therefore, the changes of UACR by the administration or the withdrawal of tofogliflozin seemed not to be directly caused by the changes of these factors. A recent study of luseogliflozin also demonstrated its overall efficacy on HbA1c levels, body weight, eGFR, systolic blood pressure, hepatic biomarkers, lipid profile, and hematological tests[22]. The results in this study were almost consistent with this report. Interestingly, although AST and ALT levels also improved in this study, they did not inversely increase during the withdrawal period. Being different from the other effects, the effect of tofogliflozin on hepatic function may have a "legacy effect." A study of ipragliflozin with a 4-week administration and 1-week withdrawal period also showed that ALT levels decreased during the administration period and were sustained even during the withdrawal period[23]. This is consistent with our study results with a further longer period. It has also been reported that SGLT2is improved or attenuated steatosis and steatohepatitis[24, 25]. Improvement in insulin resistance through attenuation of steatosis or steatohepatitis could be a possible mechanism for the improvement in hepatic function; however, this study did not evaluate the insulin resistance or steatosis/

steatohepatitis. Further studies are required to investigate the effect of tofogliflozin on insulin resistance or steatosis/steatohepatitis. Nevertheless, despite the improvement and legacy effect on ALT and AST levels, the TC and LDL-C levels did not improve but rather increased by the administration of tofogliflozin in this study. The aforementioned luseogliflozin study[22] and a systematic review[26] also showed an improvement in ALT, AST, and γ-GTP levels, but not in LDL-C and TG levels. These inconsistencies among hepatic function biomarkers and lipid profiles may suggest that the mechanism of improvement in hepatic function biomarkers by SGLT2is is not fully through the attenuation of steatosis or steatohepatitis. Several clinical studies showed the anti-inflammatory beneficial effects of SGLT2is[27–29]. These anti-inflammatory effects may contribute to the improvement in hepatic function biomarkers from the administration of tofogliflozin. Our study also showed another inconsistency in which both HDL-C and LDL-C levels increased. However, these results are consistent with previous reports[30, 31], including a meta-analysis[32]. One possible mechanism for the increase in LDL-C levels by SGLT2is may be that the SGLT2is shifted substrate utilization from carbohydrates to lipids, increasing the production of lipids in the liver, decreasing catabolism, and resulting in changes in the lipid profile[33]. Together with the fact that despite the increase of LDL-C levels, the proportion of atherogenic small dense LDL-C decreased, and less atherogenic large buoyant LDL-C increased[30, 31]; hence, the increase in LDL-C levels by SGLT2is may not mean a worsening of the lipid profile.

Regarding the safety of tofogliflozin, although the enrolled individuals were relatively high risk due to a relatively high body mass index, a long duration of diabetes, and age, serious adverse events were limited. This suggests the safety of tofogliflozin in individuals with stage 2 or 3 diabetic nephropathy. Since information on SGLT2i safety in individuals with diabetic kidney disease is limited[34], the results of this study may be worth considering when administering or ceasing SGLT2is in individuals with diabetic nephropathy.

This study had several limitations. First, this was an open-label trial that lacked blinding; hence, this may have resulted in some bias. Nonetheless, this study employed objectively measurable endpoints such as urinary albumin; therefore, we believe that the open-label study design minimally biased the study results. Second, this was a single-arm trial without a control group. Due to the design of this study with administration, withdrawal, and re-administration of tofogliflozin, it was difficult to prepare a control group. However, in other words, this study had three interventions in each participant, like a crossover study. Although a crossover study design generally requires an enough washout period and assumes that there is no carryover effect, this study rather investigated the carryover effect during the withdrawal period. Although the lack of the control group was a limitation of this study, comparability between the intervention periods in the same participants was rather a strength of this study. Third, this study was conducted only at medical institutions in Japan, and all participants were Japanese. Therefore, the generalizability of our results to other countries or individuals of other ethnicities remains unknown, and further international studies are required. Fourth, this study enrolled a relatively small number of participants. Nevertheless, changes in the UACR were comparable with the assumptions in the sample size calculation, and significant changes were detected. However, although some observation items showed numerical changes during the tofogliflozin administration period and the opposite responses during the withdrawal period, no significant changes were detected, probably because of insufficient power. Fifth, this study did not investigate the calorie intake and daily activities that may affect glucose metabolism and clinical parameters. Sixth, since wed did not compare different SGLT2is, we could not conclude that the findings in this study are the class effect of SGLT2is or specific to tofogliflozin. However, because of the consistency with previous reports of tofogliflozin or other SGLT2is, it seems to be possible that they are the class effect of SGLT2is.

In conclusion, urinary albumin improved during the administration of tofogliflozin and worsened during its withdrawal, suggesting the reversibility of its renoprotective effect. HbA1c levels, body weight, waist circumference, and systolic blood pressure also showed the same tendency. This suggests that the administration of tofogliflozin should be continued so as not to reverse its glycemic control, renoprotective effects, and other beneficial effects.

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Approval of the research protocol: The protocol of this study was approved (approval number: N20190001-1re) by the Certified Review Board of Keio (CRB3180017).

Informed consent: Written informed consent was obtained prior to intervention from all enrolled individuals who met the eligibility criteria.

Registry and the registration no. of the study/trial: This study was registered in the Japan Registry of Clinical Trials at January 14, 2020 (registration number: jRCTs031190185).

Animal studies: N/A.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are not publicly available due to the lack of a statement in the study protocol enabling data sharing with a third party after the end of the study and in the informed consent documents, as well as the lack of approval for data sharing by the ethics review board.

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SUPPORTING INFORMATION

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

Table S1 | Eligibility criteria.

Table S2 | Adverse event.

Figure S1 | Study design.

Figure S2 | Change in blood counts.