Sodium–glucose cotransporter 2 inhibitor, tofogliflozin, shows better improvements of blood glucose and insulin secretion in patients with high insulin levels at baseline

Kazuyuki Tobe[1]* , Hideki Suganami[2], Kohei Kaku[3]

[1]First Department of Internal Medicine, Faculty of Medicine, University of Toyama, Toyama, [2]Clinical Data Science Department, Kowa Company, Ltd., Tokyo, and [3]Department of Internal Medicine, Kawasaki Medical School, Okayama, Japan

ABSTRACT

Aims/Introduction: Sodium glucose cotransporter 2 (SGLT2) inhibitors are a new class of drugs for the treatment of type 2 diabetes mellitus that improve control of plasma glucose and bodyweight, giving great hope for the clinical utility of these agents. However, it is unclear for which patients SGLT2 inhibitors will be useful.

Materials and Methods: We analyzed data from long-term tofogliflozin monotherapy in an open-label, randomized controlled trial in Japanese patients with type 2 diabetes mellitus. Patients were divided into tertiles by baseline insulin level: group low (L): insulin ≤5.6 μU/mL, group medium (M): 5.6< insulin ≤10 μU/mL and group high (H): insulin >10 μU/mL.

Results: Glycated hemoglobin and fasting plasma glucose levels, along with bodyweight, were significantly reduced from the baseline in all groups. The changes in levels of plasma glucose area under the curve for 2 h, C-peptide index area under the curve for 2 h during the meal tolerance tests and the insulin secretion index were the largest in the H group. The incidence of drug-related adverse events was not different among the three groups.

Discussion: Although tofogliflozin was effective regardless of baseline insulin level, it showed the highest efficacy in the H group.

INTRODUCTION

Tofogliflozin is a sodium–glucose cotransporter 2 (SGLT2) inhibitor that was developed in Japan. Tofogliflozin is highly selective for SGLT2 vs SGLT1 and SGLT6[1]. Previous studies showed that tofogliflozin was well tolerated, and significantly reduced serum glycated hemoglobin (HbA1c) levels and bodyweight in Japanese patients with type 2 diabetes mellitus[2,3].

In the treatment of type 2 diabetes, it is important to control both fasting and postprandial glucose levels, without causing hypoglycemia. In addition, in order to maintain glycemic control over a long period, weight management is of extreme importance.

Sodium glucose cotransporter 2 inhibitors are a new class of drugs for the treatment of type 2 diabetes mellitus, and improve the control of both plasma glucose levels and bodyweight, resulting in much anticipation for their use in the clinic. However, it has not yet been determined what patient characteristics could indicate a maximum utility of SGLT2 inhibitors.

In the course of the onset and progression of type 2 diabetes mellitus, the first changes are the occurrence of postprandial hyperglycemia, caused by a decrease of the initial postprandial insulin secretion, and subsequently, delayed insulin hypersecretion occurring in response to postprandial hyperglycemia[4]. In addition, insulin affects anabolism in adipose cells as compared with skeletal muscle cells[5]. Thus, the excessive insulin leads to an accumulation of fat in adipose cells, resulting in obesity, and the serum insulin level is correlated with the degree of obesity[6]. As aforementioned, serum insulin levels are related to both the

pathology of diabetes and the degree of obesity; however, it has not been studied whether or not serum insulin levels could affect the efficacy and safety of SGLT2 inhibitors.

Therefore, we examined the efficacy and safety of tofogliflozin in a subgroup analysis, in which patients were classified according to their baseline insulin levels, using the data from a 52-week, monotherapy, open-label, randomized controlled trial in Japanese patients with type 2 diabetes mellitus.

METHODS

Study design

The present analysis was a subanalysis of a multicenter, open-label, 52-week, randomized controlled trial of tofogliflozin as monotherapy in Japanese patients with type 2 diabetes mellitus. This trial was carried out at Japanese clinics and hospitals in accordance with the Declaration of Helsinki and Good Clinical Practice. The protocols were reviewed and approved by the institutional review boards of each participating center. All study patients provided written informed consent.

Patients

The full eligibility criteria are described in the original report[3]. Mainly, eligibility criteria were patients who were aged at least 20 years with type 2 diabetes and whose HbA1c levels were 6.8–10.3% with diet and exercise alone for at least 8 weeks. Patients were randomly assigned to receive tofogliflozin, 20 or 40 mg, administered once daily, orally, for 52 weeks. For the analyses, patients were divided into three groups, based on the tertile allocation of baseline insulin values: group low (L), insulin ≤5.6 μU/mL; group medium (M), 5.6< insulin ≤10 μU/mL; and group high (H), insulin >10 μU/mL.

Measurement

In the original report[3], laboratory tests were carried out at 4-week intervals during the treatment period. The meal tolerance tests (MTT) were also carried out on the baseline visit and the visit at 52 weeks. In the present study, we used the data of the laboratory test and MTT on baseline and 52 weeks or the final visit.

Statistical analysis

Analyses were carried out in the full analyses set ($n = 190$), which included all randomized patients with type 2 diabetes who received at least one dose of trial medication, and who had at least one evaluable measurement after the initiation of therapy with the study drug.

Baseline characteristics were summarized descriptively. Categorical variables were expressed as frequencies and percentages. Continuous variables were expressed as mean ± standard deviation. Comparisons of continuous and categorical variables among the three groups were carried out using analysis of variance (ANOVA) and Fisher's exact tests, respectively.

The changes in bodyweight, percent change of bodyweight, insulin levels and C-peptide levels from baseline to week 52 were shown as mean ± standard deviation, and were analyzed using one-sample t-tests. The changes in other measurements are shown as the least squares mean ± standard error, and were analyzed by analysis of covariance (ANCOVA).

We used general linear models to assess the relationships between the changes in bodyweight and serum insulin levels.

To assess the relationships between the change in weight and changes in variables, such as HbA1c, area under the curve (AUC) of C-peptide index after MTT or fasting serum insulin, Pearson's correlation coefficient was calculated. A stepwise multiple regression analysis was carried out to determine independent predictors of efficacy on glucose metabolism and insulin secretion with tofogliflozin treatment.

In the safety analysis, adverse events were coded and classified into preferred terms and system organ classes, using the Medical Dictionary for Regulatory Activities, version 13.1. All data were analyzed using SAS System Release 9.3 (SAS Institute, Cary, NC, USA). All reported P-values are two sided, and determined significant when $P < 0.05$.

RESULTS

Baseline patient characteristics, according to their insulin level at baseline, are summarized in Table 1. The 190 patients were divided into the low-insulin group (L group; $n = 66$), medium-insulin group (M group; $n = 60$) and the high-insulin group (H group; $n = 64$).

Among the three groups, the mean age, and the average duration of diabetes, weight, body mass index, insulin level, C-peptide immunoreactivity (CPR) and CPR index (CPI) at baseline were significantly different. In contrast, the serum HbA1c level, fasting plasma glucose, estimated glomerular filtration rate and medications for hypertension or dyslipidemia at baseline were similar across all of the groups (Table 1).

When the clinical parameters of each group were compared, patients in the H group were relatively younger and had a shorter duration of diabetes, higher bodyweights and higher body mass indexes compared with those in other groups (Table 1).

Changes in laboratory data after tofogliflozin treatment are summarized in Table 2. HbA1c, fasting plasma glucose and 2-h postprandial glucose levels decreased significantly from baseline in all groups. In addition, compared with baseline, both bodyweights and basal insulin levels significantly decreased, and urinary glucose significantly increased in all groups.

When the change of each group was compared, regarding the degree of reduction of HbA1c and 2-h postprandial glucose levels, there were significant differences between the L group and the M or H group (Table 2). In addition, the H group showed a significant reduction in fasting plasma glucose than the L group (Table 2).

Based on the data of the MTT, we compared the effects of tofogliflozin treatment on the AUC of blood glucose for 2 h (Figure 1). The reduction of the AUC of blood glucose was greater in patients in the H group, as compared with those in

Table 1 | Patient characteristics according to insulin level at baseline

	Group L (n = 66)	Group M (n = 60)	Group H (n = 64)	P-values
Male/female (n)	47/19	40/20	39/25	0.4620[‡]
Age (years)	61.6 ± 9.2	59.1 ± 11.3	53.5 ± 10.5	<0.0001[†]
Duration of diabetes (years)	6.6 ± 5.7	6.0 ± 5.4	3.8 ± 3.0	0.0030[†]
Weight (kg)	59.5 ± 8.9	66.4 ± 10.8	80.3 ± 15.9	<0.0001[†]
BMI (kg/m^2)	22.4 ± 2.4	24.9 ± 2.9	29.5 ± 4.5	<0.0001[†]
HbA1c (%)	7.95 ± 1.07	7.80 ± 0.83	7.74 ± 0.77	0.4029[†]
Fasting plasma glucose (mg/dL)	155.2 ± 42.0	154.4 ± 32.6	158.6 ± 38.2	0.8038[†]
eGFR (mL/min/1.73 m^2)	81.0 ± 14.2	83.3 ± 19.0	84.9 ± 21.0	0.4779[†]
Insulin (μU/mL)	3.8 ± 1.2	7.4 ± 1.2	16.7 ± 8.5	<0.0001[†]
C-peptide (ng/mL)	0.93 ± 0.25	1.28 ± 0.27	2.14 ± 0.74	<0.0001[†]
C-peptide index	0.63 ± 0.18	0.85 ± 0.23	1.40 ± 0.58	<0.0001[†]
Concomitant drugs				
Antihypertensive medication (n)	23	20	31	0.1709[‡]
Hyperlipidemic medication (n)	28	29	25	0.5901[‡]

P-values are calculated with [†]ANOVA or [‡]Fisher's exact test. BMI, body mass index; eGFR, estimated glomerular filtration rate; Group H, the high-insulin group (>10 μU/mL); Group L, the low-insulin group (insulin ≤5.6 μU/mL); Group M, the medium-insulin group (5.6< insulin ≤10 μU/mL); HbA1c, glycated hemoglobin.

the L or M groups (L vs H, $P < 0.0001$; M vs H, $P < 0.0484$; Figure 1). Additionally, there was a significant difference between the M group and L group (L vs M, $P < 0.0091$).

To investigate the effects of tofogliflozin treatment on insulin secretion, we estimated the homeostatic model assessment of β-cell function, secretory units of islets in transplantation and CPI using the values of fasting insulin, blood glucose and CPR. The CPI was calculated from the ratio of CPR to blood glucose ×100 at each time-point before and after the meal test. The change from baseline in the secretory units of islets in transplantation and CPI values was greater in the H group, as compared with the other two groups (Table 2). However, changes in homeostatic model assessment of β-cell function were not different among the three groups. The insulinogenic index, which represents the immediate response of β-cells after 30 min of a meal test, increased only in the H group (Figure 2). Using CPI values after the meal tests, we evaluated the change in the CPI AUC for 2 h after the meal test. In the H and M groups, the CPI AUC increased significantly from baseline (Table 2). The H group showed a greater magnitude of change in the CPI AUC than the other two groups. These data show that patients with higher fasting insulin values at baseline, when taking SGLT2 inhibitor, received beneficial changes in postprandial blood glucose and insulin secretion after meals.

Reductions in bodyweight and fat volume with SGLT2 inhibitor therapy can also contribute to the improvement of blood glucose. However, there are some patients whose bodyweight reduced while the blood glucose remained or even became elevated. Thus, we tried to find the group in which bodyweight reduction is correlated with improved glucose control. Changes in the blood glucose AUC for 2 h after the meal test and bodyweight were positively correlated only in the H group (data not

shown). Although the percent reductions of HbA1c and bodyweight were correlated significantly positively in the total study population, when stratified by baseline insulin level, they showed a significant, positive correlation in the M and H groups (Figure 3). We speculate that the significant correlation between weight reduction and improved glucose control observed in the H group might be caused by the recovery of insulin secretion in the H group. Thus, we investigated the relationship between changes in bodyweight and the summation of the CPI changes from baseline. Divided into groups based on the baseline insulin levels, the significant correlation between reduced bodyweight and increased summation of CPI changes from baseline was observed only in the H group (Fig. S1). These data showed that improvements in both bodyweight and blood glucose or CPI after meals were concurrently observed in patients with higher insulin levels at baseline.

As fasting insulin levels reflect insulin resistance, we next examined the association of the changes in bodyweight reduction and those in fasting insulin levels among the three groups. In the total study population, decreased bodyweight was correlated with changes in fasting insulin values. Divided into three groups, based on the baseline insulin levels, the same correlation was observed in the M and H groups (Fig. S2). These data showed that bodyweight changes are associated with an improvement in glucose control, the summation of CPI after a meal test and reduction of fasting insulin levels in the H group.

Multivariate analysis was followed by stepwise model selection with P values <0.05 to determine the baseline factors influencing reduction of glucose AUC during the MTT. After adjusting with the parameters in Table 1, the baseline insulin, sex and the baseline of the glucose AUC were independently related to high efficacy on AUC of glucose during the MTT

Table 2 | Changes in laboratory data

|		Group L	Group M	Group H	P-value L vs M	P-value L vs H	P-value M vs H
HbA1c (%)	LS mean	−0.37	−0.70	−0.65	0.0156	0.0348	0.7322
	(95% CI)	(−0.55, −0.19)	(−0.89, −0.51)	(−0.83, −0.47)			
	n	66	60	64			
FPG (mg/dL)	LS mean	−22.6	−28.2	−29.8	0.1180	0.0443	0.6758
	(95% CI)	(−27.5, −17.7)	(−33.4 −23.1)	(−34.7, −24.8)			
	n	66	60	64			
2-h PPG (mg/dL)	LS mean	−44.6	−60.3	−71.8	0.0180	<0.0001	0.0751
	(95% CI)	(−54.0, −35.3)	(−69.2, −51.3)	(−80.9, −62.8)			
	n	53	58	57			
Glucose $AUC_{0-2\,h}$ (mg/dL)	LS mean	−73.5	−97.9	−116.1	0.0091	<0.0001	0.0484
	(95% CI)	(−86.7, −60.2)	(−110.5, −85.3)	(−128.9, −103.3)			
	n	53	58	57			
C-peptide (ng/mL)	Mean ± SD	−0.041 ± 0.263	−0.202 ± 0.300***	−0.367 ± 0.472***	0.0123	<0.0001	0.0110
	n	66	60	63			
C-peptide $AUC_{0-2\,h}$ (ng/mL)	LS mean	−0.428	−0.375	0.335	0.7951	0.0028	0.0019
	(95% CI)	(−0.740, −0.116)	(−0.651, −0.100)	(0.011, 0.658)			
	n	53	58	57			
C-peptide index	LS mean	−0.014	−0.025	0.132	0.7844	0.0046	0.0008
	(95% CI)	(−0.073, 0.046)	(−0.081, 0.032)	(0.066, 0.198)			
	n	66	60	63			
C-peptide index $AUC_{0-2\,h}$	LS mean	0.311	0.486	1.126	0.2146	<0.0001	<0.0001
	(95% CI)	(0.098 0.523)	(0.295 0.677)	(0.910 1.342)			
	n	53	58	57			
HOMA-β	LS mean	−2.432	−2.349	0.796	0.9775	0.3586	0.3372
	(95% CI)	(−6.690, 1.826)	(−6.483, 1.784)	(−3.850, 5.440)			
	n	66	60	63			
SUIT	LS mean	0.825	2.712	11.382	0.2963	<0.0001	<0.0001
	(95% CI)	(−1.737, 3.388)	(0.143, 5.280)	(8.635, 14.129)			
	n	66	60	63			
Urinary glucose (g/2 h)	LS mean	10.4	9.7	10.5	0.4806	0.9382	0.4339
	(95% CI)	(9.0, 11.8)	(8.3, 11.1)	(9.2, 11.9)			
	n	53	54	57			
Bodyweight (kg)	Mean ± SD	−2.53 ± 2.17***	−3.36 ± 2.27***	−3.85 ± 2.54***	0.0467	0.0014	0.2391
	n	66	60	64			
Percent change of bodyweight (%)	Mean ± SD	−4.26 ± 3.62***	−5.08 ± 3.31***	−4.94 ± 3.32***	0.1825	0.2604	0.8213
	n	66	60	64			
Insulin (μU/mL)	Mean ± SD	−0.50 ± 1.38**	−2.18 ± 2.11***	−6.07 ± 5.34***	0.0063	<0.0001	<0.0001
	n	66	60	63			

**P < 0.01 vs baseline, ***P < 0.001 vs baseline. P-values are calculated with the paired t-test. 2-h PPG, 2-h postprandial glucose; $AUC_{1-2\,h}$, area under the curve for 2 h; FPG, fasting plasma glucose; Group H, the high-insulin group (>10 μU/mL); Group L, the low-insulin group (insulin ≤5.6 μU/mL); Group M, the medium-insulin group (5.6< insulin ≤10 μU/mL); HbA1c, glycated hemoglobin; HOMA-β, homeostatic model assessment of β-cell function; LS, least squares; SD, standard deviation; SUIT, secretory units of islets in transplantation.

(Table S1). In the case of insulinogenic index, the baseline insulin was also an independent factor related to improvement of the insulinogenic index (Table S2).

DISCUSSION

In the present subanalysis, tofogliflozin had the greatest beneficial effect on postprandial blood glucose levels in patients with high fasting insulin levels at baseline. Both the insulinogenic index and the summation of CPI changes

from baseline improved significantly in the H group, although these did not improve in the L or M groups. These changes in insulin secretion likely contribute to the observed beneficial changes in blood glucose in the H group. Previously, it was shown that SGLT2 inhibitors lead to immediate amelioration of pancreatic β-cell dysfunction[7]. Together with these results, those changes on β-cell function will occur not only acutely, but chronically as well, during SGLT2 inhibitor administration.
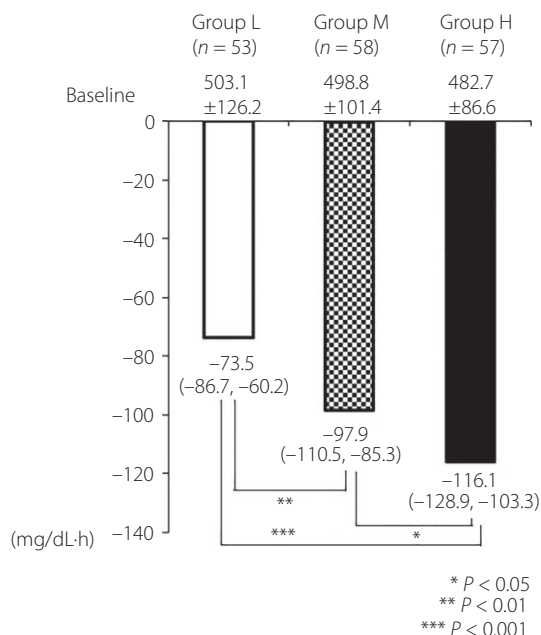
Figure 1 | Change in glucose area under the curve for 2 h during the meal tolerance test. The meal tolerance test was carried out before and after 52 weeks of tofogliflozin treatment. Changes in glucose area under the curve for 2 h during the MTT are shown. Data are expressed as least squares mean (95% confidence interval). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ among the groups. Group H, the high-insulin group (>10 µU/mL); Group L, the low-insulin group (insulin ≤5.6 µU/mL); Group M, the medium-insulin group (5.6< insulin ≤10 µU/mL).
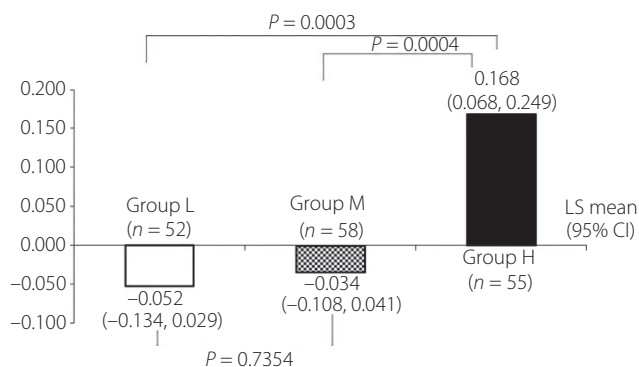
Figure 2 | Changes in the insulinogenic index during the meal tolerance test (MTT). The insulinogenic index was calculated with following formula. Insulinogenic index = (insulin level 30 min after MTT) − (fasting insulin)/(glucose level 30 min after MTT) − (fasting glucose). Data are expressed as least squares (LS) mean (95% confidence interval [95% CI]).

One possible mechanism for the differences in improvements of insulin secretion among those three groups might come from those with the ability to recover from impaired insulin secretion as a result of glucotoxicity. Prolonged high serum glucose levels can cause β-cell apoptosis through generation of reactive oxygen species, or other stressors[8]. Conversely, insulin has pro-survival effects on β-cells[9]. Patients in the H group might have the highest ability to recover from impaired insulin secretion as a result of glucotoxicity, as compared with patients in other groups. This was likely further contributed to by the younger age and shorter duration of diabetes mellitus for patients in the H group[10].

Another important feature of tofogliflozin is bodyweight loss. The mean percent reduction in bodyweight was not different among the three groups. This finding showed that tofogliflozin causes bodyweight reduction, regardless of the baseline insulin levels. SGLT2 inhibitors reduce both blood glucose and bodyweight in most patients taking the drugs[11]. However, we sometimes encounter patients whose bodyweight reduced while the blood glucose remained or even became elevated after initiation of SGLT2 inhibitor therapy. In the present analysis, we found that changes in HbA1c levels and bodyweight strongly correlated in patients with higher insulin levels at baseline. This suggests that tofogliflozin shows stable effects in patients with high levels of insulin. In contrast, there was no significant correlation between changes in blood glucose and bodyweight in patients with low insulin levels at baseline. Therefore, in the L group, there might be patients who had reduced bodyweight, but unchanged or elevated blood glucose.

In the H group, bodyweight reduction after administration of tofogliflozin for 52 weeks was significantly associated with improvement in insulin secretion after a meal test (Fig. S1) and the reduction of fasting insulin levels (Fig. S2). High serum insulin levels lead to insulin resistance in skeletal muscles through reduced expression of IRS-1[12]. Thus, reduced insulin levels in the fasting state by tofogliflozin treatment will lead to an improvement in insulin resistance. In addition, improvement in postprandial insulin secretion promotes the uptake of glucose into muscle. Therefore, both reduction in fasting insulin levels and improvement in postprandial insulin secretion might contribute to the improved glucose uptake in skeletal muscle[13]. Consistent with this, Sano *et al.*[14] reported an increase in handgrip strength after administration of an SGLT2 inhibitor. Under the condition of SGLT2 inhibition, the volume of the adipose tissues depends on the balance of the uptake of glucose after a meal and lipolysis during fasting periods. Previous reports have shown that SGLT2 inhibitors contribute to the reduction of fat weight and triglyceride content in adipose tissue[13,15–17]. Lipolysis due to the reduction in basal insulin levels contributes to a reduction of bodyweight after tofogliflozin treatment. Improvement in the muscle tissue response to insulin along with reduction of the fat mass will provide further improvement of insulin resistance, and more beneficial effects are expected.

The beneficial effect of SGLT2 inhibitor therapy on the improvement of postprandial glucose levels was limited in the L group, although SGLT2 inhibitor treatment can lower them irrespective of basal insulin levels. This might result from the reduced effect of the alleviation from impaired insulin secretion
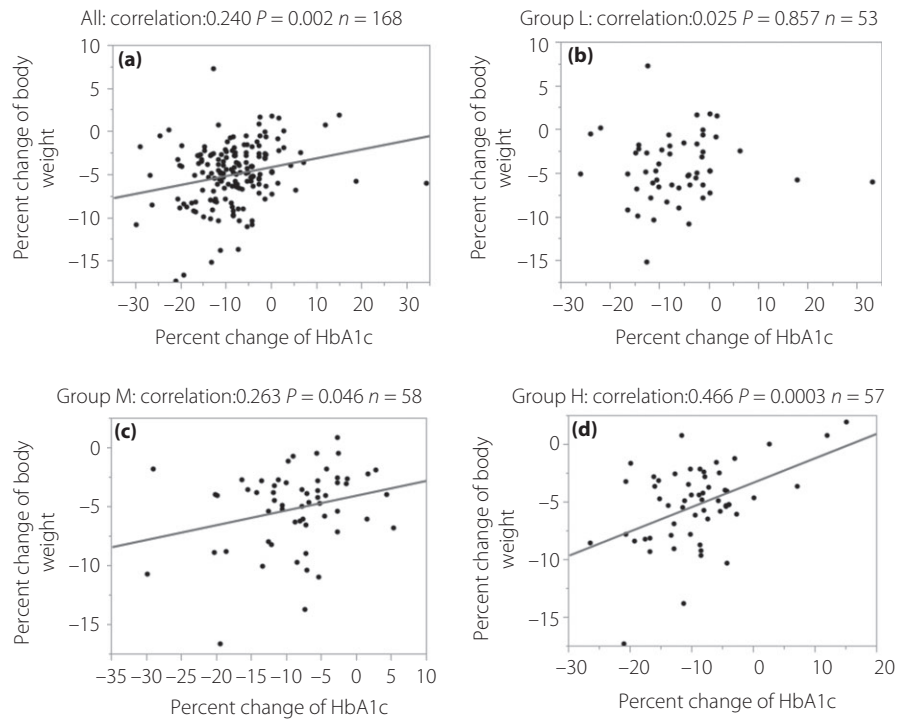
Figure 3 | Correlation between changes in bodyweight and glycated hemoglobin (HbA1c). The correlation between bodyweight and HbA1c was investigated (a) in all patients, (b) the low-insulin group (Group L; insulin ≤5.6 μU/mL), (c) the medium-insulin group (Group M; 5.6< insulin ≤10 μU/mL) and (d) the high-insulin group (Group H; >10 μU/mL). The correlation values are expressed as Pearson's correlation coefficients.

as a result of glucotoxicity in the L group. In contrast, the recently reported Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients-Removing Excess Glucose trial showed no difference in the benefits of SGLT2 inhibitor on cardiovascular disease prevention between the patients with insulin therapy and those without insulin therapy[18]. Thus, insulin injection combined with SGLT2 inhibitors for those patients is then considered from the perspective of efficacy and safety[19,20], although the insulin secretory capacity of those patients might be lower. Before prescribing the SGLT2 inhibitor, checking the insulin requirement[21] of those patients seems to be necessary.

Sodium glucose cotransporter 2 inhibitors showed the reduction of blood glucose and bodyweight, regardless of the patients' insulin levels. However, to utilize the potency of the SGLT2 inhibitors fully, it is reasonable to prescribe them to patients of a younger age, with shorter duration of diabetes mellitus and whose ability of insulin secretion is preserved.

In regard to limitations of the present study, there were some differences in the patients' demographics among the groups at baseline, and this might affect the study results. We need a prospective study, with patients matching in terms of baseline demographics other than insulin secretion. Furthermore, we should investigate the changes in gluconeogenesis, and other body components, during SGLT2 inhibitor therapy. Finally, it

needs to be clarified whether some of the differences we found among the three groups in the present study influence cardiovascular events, or other complications.

REFERENCES

1. Suzuki M, Honda K, Fukazawa M, et al. Tofogliflozin, a potent and highly specific sodium/glucose cotransporter 2

inhibitor, improves glycemic control in diabetic rats and mice. *J Pharmacol Exp Ther* 2012; 341: 692–701.

2. Kaku K, Watada H, Iwamoto Y, *et al.* Efficacy and safety of monotherapy with the novel sodium/glucose cotransporter-2 inhibitor tofogliflozin in Japanese patients with type 2 diabetes mellitus: a combined Phase 2 and 3 randomized, placebo-controlled, double-blind, parallel-group comparative study. *Cardiovasc Diabetol* 2014; 13: 65.

3. Tanizawa Y, Kaku K, Araki E, *et al.* Long-term safety and efficacy of tofogliflozin, a selective inhibitor of sodium-glucose cotransporter 2, as monotherapy or in combination with other oral antidiabetic agents in Japanese patients with type 2 diabetes mellitus: multicenter, open-label, randomized controlled trials. *Expert Opin Pharmacother* 2014; 15: 749–766.

4. DeFronzo RA. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* 2009; 58: 773–795.

5. Virtanen KA, Iozzo P, Hallsten K, *et al.* Increased fat mass compensates for insulin resistance in abdominal obesity and type 2 diabetes a positron-emitting tomography study. *Diabetes* 2006; 54: 2720–2726.

6. Arauz-Pacheco C, Ramirez LC, Schnurr-Breen L, *et al.* Relationship between insulin sensitivity and degree of obesity in mild hypertension. *Am J Med Sci* 1992; 304: 225–230.

7. Takahara M, Shiraiwa T, Matsuoka T, *et al.* Ameliorated pancreatic β cell dysfunction in type 2 diabetic patients treated with a sodium-glucose cotransporter 2 inhibitor ipragliflozin. *Endocr J* 2015; 62: 77–86.

8. Leibowitz G, Kaiser N, Cerasi E. b-Cell failure in type 2 diabetes. *J Diabetes Investig* 2011; 2: 82–90.

9. Malakar P, Chartarifsky L, Hija A, *et al.* Insulin receptor alternative splicing is regulated by insulin signaling and modulates beta cell survival. *Sci Rep* 2016; 6: 31222. https://doi.org/10.1038/srep31222.

10. Yagihashi S, Inaba W, Mizukami H. Dynamic pathology of islet endocrine cells in type 2 diabetes: b-cell growth, death, regeneration and their clinical implications. *J Diabetes Investig* 2016; 7: 155–165.

11. Vasilakou D, Karagiannis T, Athanasiadou E, *et al.* Sodium-glucose cotransporter 2 inhibitors for type 2 diabetes: a systematic review and meta-analysis. *Ann Intern Med* 2013; 159: 262–274.

12. Kubota T, Kubota N, Kumagai H, *et al.* Impaired insulin signaling in endothelial cells reduces insulin-induced glucose uptake by skeletal muscle. *Cell Metab* 2011; 13: 294–307.

13. Obata A, Kubota N, Kubota T, *et al.* Tofogliflozin improves insulin resistance in skeletal muscle and accelerates lipolysis in adipose tissue in male mice. *Endocrinology* 2016; 157: 1029–1042.

14. Sano M, Meguro S, Kawai T, *et al.* Increased grip strength with sodium-glucose cotransporter 2. *J Diabetes* 2016; 8: 736–737.

15. Bolinder J, Ljunggren O, Kullberg J, *et al.* Effects of dapagliflozin on body weight, total fat mass, and regional adipose tissue distribution in patients with type 2 diabetes mellitus with inadequate glycemic control on metformin. *J Clin Endocrinol Metab* 2012; 97: 1020–1031.

16. Bolinder J, Ljunggren O, Johansson L, *et al.* Dapagliflozin maintains glycaemic control while reducing weight and body fat mass over 2 years in patients with type 2 diabetes mellitus inadequately controlled on metformin. *Diabetes Obes Metab* 2014; 16: 159–169.

17. Suzuki M, Takeda M, Kito A, *et al.* Tofogliflozin, a sodium/glucose cotransporter 2 inhibitor, attenuates body weight gain and fat accumulation in diabetic and obese animal models. *Nutrition & Diabetes* 2014; 4: e125.

18. Zinman B, Wanner C, Lachin JM, *et al.* Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med* 2015; 373: 2117–2128.

19. Suzuki K, Mitsuma Y, Sato T, *et al.* Comparison of combined tofogliflozin and glargine, tofogliflozin added to insulin, and insulin dose-increase therapy in uncontrolled type 2 diabetes. *J Clin Med Res* 2016; 8: 805–814.

20. Araki E, Onishi Y, Asano M, *et al.* Efficacy and safety of dapagliflozin in addition to insulin therapy in Japanese patients with type 2 diabetes: results of the interim analysis of 16-week double-blind treatment period. *J Diabetes Investig* 2016; 7: 555–564.

21. Iwata M, Matsushita Y, Fukuda K, *et al.* Secretory units of islet in transplantation index is a useful predictor of insulin requirement in Japanese type 2 diabetic patients. *J Diabetes Investig* 2014; 5: 570–580.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 | Correlation between changes in body weight and the C-peptide immunoreactivity index (CPI) area under the curve (AUC). Correlation between percent change of bodyweight and the CPI AUC was investigated in all patients (left upper panel), the low-insulin group (L group; insulin ≤5.6 μU/mL, right upper panel), the medium-insulin group (M group; 5.6< insulin ≤10 μU/mL, left under panel) and the high-insulin group (H group; >10 μU/mL, right under panel). The correlation values were expressed as Pearson's correlation coefficients.

Figure S2 | Correlation between changes in bodyweight and fasting serum insulin levels. The correlation between the percent change in bodyweight and fasting serum insulin levels was investigated in all patients (left upper panel), the low-insulin group (L group; insulin ≤5.6 μU/mL, right upper panel), the medium-insulin group (M group; 5.6< insulin ≤10 μU/mL, left under panel) and the high-insulin group (H group; >10 μU/mL, right under panel). The correlation values were expressed as Pearson's correlation coefficients.

Table S1 | Multiple regression of parameters associated with change of glucose area under the curve. Multivariate analysis was followed by stepwise model selection with *P*-values <0.05 to determine the baseline factors in Table 1 influencing reduction of glucose area under the curve (AUC) during the meal tolerance test.

Table S2 | Multiple regression of parameters associated with change of insulinogenic indexMultivariate analysis was followed by stepwise model selection with *P*-values <0.05 to determine the factors in Table 1 influencing the degree of insulinogenic index improvement.