

ORIGINAL ARTICLE

Basal insulin secretion capacity predicts the initial response and maximum levels of beta-hydroxybutyrate during therapy with the sodium-glucose co-transporter-2 inhibitor tofogliflozin, in relation to weight loss

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Abstract

Aims: To investigate predictors of the initial response of beta-hydroxybutyrate (BHB) and maximum BHB (max-BHB) values during long-term therapy with the sodium-glucose co-transporter-2 inhibitor tofogliflozin (TOFO), and to explore the association of the initial elevation of BHB with subsequent clinical effects in people with type 2 diabetes mellitus.

Methods: We analysed 774 people receiving TOFO in phase 3 trials in two groups based on measurable BHB change at week 4 (initial response): the top quartile [$n = 194$] and the three lower quartiles [$n = 579$]. Multivariate analysis was used to determine baseline predictors of inclusion in the top quartile and the max-BHB values. To investigate the association of the initial response with subsequent clinical effects, adjusted changes in variables in the two groups were compared using an analysis of covariance model.

Results: Of the participants, 66% were men, and the mean age, glycated haemoglobin, body mass index and estimated glomerular filtration rate were 58.5 years, 8.1%, 25.6 kg/m² and 83.9 mL/min/1.73 m², respectively. Median changes in BHB at week 4 in the top quartile and lower three quartiles were +246.4* and +30.8* $\mu\text{mol/L}$, respectively (* $P < .001$ vs baseline). Lower baseline insulin secretion capacity predicted the inclusion in the top quartile and greater max-BHB levels. The top quartile was associated with greater weight loss following greater increases in free fatty acids and greater reductions in fasting C-peptide levels compared with the lower three quartiles.

Conclusions: Lower basal insulin secretion capacity might predict greater initial BHB elevations and max-BHB levels during long-term TOFO therapy. Greater weight loss through lipid use might be related to high initial BHB elevations.

KEYWORDS

beta-hydroxybutyrate, free fatty acid, insulin secretion, sodium-glucose co-transporter-2 inhibitor, tofogliflozin

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1 | INTRODUCTION

Sodium-glucose co-transporter-2 (SGLT2), located in the renal proximal tubule, reabsorbs the majority of filtered glucose; therefore, the inhibition of SGLT2 promotes glycaemic improvement via an increase in urinary glucose excretion (UGE).¹ Increased UGE causes an energy loss that can result in weight loss.^{2,3} In addition, striking reductions in the relative risk of cardiovascular and renal outcomes using SGLT2 inhibitors in people with type 2 diabetes mellitus (T2DM) were observed in the EMPA-REG OUTCOME trial, the CANVAS programme, DECLARE-TIMI 58, and the CREDENCE trial.⁴⁻⁸ In addition, however, SGLT2 inhibitors increase endogenous glucose production and decrease tissue glucose disposal, which is followed by increased lipid use.⁹ Subsequently, the use of lipids as fuel causes an increase in plasma ketone levels.¹⁰ Ketone elevation has been observed after 2 to 4 weeks (initial)^{9,10} and after 24 to 52 weeks (chronic)^{2,3} of SGLT2 inhibitor treatment.

Episodes of ketoacidosis have been reported recently among patients receiving SGLT2 inhibitors.¹¹ Case reports describing SGLT2 inhibitor-associated ketoacidosis report near-normal glucose values (euglycaemic ketoacidosis)¹² at presentation, leading to delays in diagnosis and management. SGLT2 inhibitor use is thought to reduce the threshold for the development of ketoacidosis in people with diabetes, and the presence of precipitating causes, such as infections, surgical stress, poor oral intake, dietary habits, insulin reduction or omission, or active complications of T2DM might further reduce the threshold for the development of ketoacidosis.¹³⁻¹⁶ However, clinical predictors, such as age, sex, duration of diabetes, glycaemic status (glycated haemoglobin [HbA1c] and fasting plasma glucose [FPG]), status of insulin action (insulin secretion capacity and insulin resistance) and estimated glomerular filtration rate (eGFR), of the response of ketones to SGLT2 inhibitor administration have not yet been clarified. In addition, the association of increased ketone levels with subsequent clinical effects induced by SGLT2 inhibitor treatment is not yet fully understood. The aim of the present study, therefore, was to clarify both baseline predictors for initial changes in beta-hydroxybutyrate (BHB) levels and the maximum BHB level (max-BHB) during long-term administration of the SGLT2 inhibitor, tofogliflozin (TOFO). We also explored the association of the initial BHB response with subsequent clinical effects in people with T2DM.

2 | RESEARCH DESIGN AND METHODS

A pooled analysis of two TOFO phase 3 studies (CSG004JP and CSG005JP, as shown in Table S1) was performed that enrolled patients with T2DM and compared various dosages of TOFO, either as monotherapy or as an add-on to other antidiabetic agents, over a period of 52 weeks. The CSG004JP study (TOFO 20 and 40 mg monotherapy) and CSG005JP study (TOFO 20 mg and 40 mg as an add-on to other oral antidiabetic agents) were both 52-week randomized controlled, open-label, phase 3 studies that had been designed to investigate the safety and efficacy of TOFO in people with T2DM.³ Details of these studies and their results, including patient inclusion

and exclusion criteria, were reported previously.³ Individual-level data from the 52-week core treatment periods in each study were used for this pooled analysis. Each of those studies was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The protocols were reviewed and approved by the institutional review board of each participating centre. All participants provided written informed consent prior to being enrolled.

One participant from the CSG005JP study was excluded from the final analysis because the baseline insulin level recorded was suspected to be a non-fasting value. The following laboratory variables were measured at baseline: HbA1c, FPG, fasting insulin (IRI), C-peptide, glucagon, glucagon-to-insulin ratio, HDL cholesterol, LDL cholesterol, triglycerides (TG), free fatty acids (FFAs), FFA-to-IRI ratio, BHB, acetoacetate, adiponectin, C-peptide index ($100 \times \text{C-peptide} [\text{ng/mL}] / \text{FPG} [\text{mg/dL}]$), homeostatic model assessment of insulin resistance (HOMA-IR) score, homeostatic model assessment of β -cell function (HOMA- β), and the eGFR calculated from serum creatinine. Blood biochemical and laboratory variables were measured using standardized validated methods at an accredited central laboratory (Mitsubishi Chemical Medience Corp., Tokyo, Japan). Body weight and waist circumference were also measured at baseline. BHB was measured at baseline and every 4 weeks after the start of administration of TOFO; thus the initial response of BHB was defined as the change in BHB at week 4 after the initiation of TOFO treatment. Max-BHB was defined as the maximum BHB level during long-term TOFO therapy. The ketogenic index, $\log_{10}[\text{FFA}^* (1/\text{fasting IRI})^* (\text{baseline FPG}/\text{FPG at day 1 after starting treatment with empagliflozin})]$, was previously proposed to predict the risk of increases in plasma ketone concentrations after empagliflozin administration.¹⁷ In the phase 3 studies, FPG levels were not measured 1 day after the administration of TOFO; therefore, we examined the chronic ketogenic index-IRI, $\log_{10}[\text{FFA}^* (1/\text{fasting IRI})^* (\text{baseline FPG}/\text{FPG})]$. To determine the chronic ketogenic index, we used data on FFAs (mmol/L), fasting IRI (mmol/L) and FPG (mg/dL) at week 52 of treatment with TOFO. Also examined at week 52 was the chronic ketogenic index-C-peptide, $\log_{10}[\text{FFA}^* (1/\text{fasting C-peptide})^* (\text{baseline FPG}/\text{FPG})]$, which substituted fasting C-peptide for fasting IRI. Values for fasting C-peptide (mmol/L) obtained at week 52 during treatment with TOFO were examined.

A recent post hoc analysis indicated a time-course change in BHB during 52 weeks of TOFO treatment.¹⁸ In examining data from the phase 3 studies, we noted that, after 4 weeks of treatment with TOFO, BHB levels were significantly increased (median [interquartile range] +57.1 [+10.7 to +148.6] $\mu\text{mol/L}$). One person was excluded from this subgroup analysis because the BHB level at week 4 was not measured. Thus, to investigate the association of a greater initial response of BHB with subsequent clinical effects, we divided study participants into two groups according to the initial BHB response (top quartile, change in BHB from baseline to week 4 $\geq 148.6 \mu\text{mol/L}$; lower three quartiles, change in BHB from baseline to week 4 < 148.6). All of the variables (17 variables at baseline: dosage of TOFO, age, sex, duration of diabetes, HbA1c, FPG, eGFR, body mass index [BMI], waist circumference, HDL cholesterol, LDL cholesterol, TGs, FFAs, adiponectin, HOMA-IR, C-peptide index, and \log_{10} -

transformed BHB) were used in a logistic regression model, with the top quartile as the dependent variable. Also, to identify baseline clinical factors that might independently influence max-BHB levels, those variables were initially identified based on clinical considerations. The model was simplified using a stepwise method by removing variables that had a P value $>.05$.

To examine the difference between the two groups, the adjusted assessments of HbA1c, FPG, body weight, BMI, waist circumference, fasting IRI, C-peptide, glucagon, glucagon-to-insulin ratio, C-peptide index, HDL cholesterol, LDL cholesterol, TGs, FFAs, acetoacetate, adiponectin and eGFR were analysed using an analysis of covariance (ANCOVA) model with the groups, their baseline values, age, sex and eGFR as covariates.

We investigated the impact of baseline C-peptide index levels on differences in changes in fasting C-peptide and the C-peptide index between the top quartile group and the lower three quartiles group based on the initial BHB response. For this purpose we divided our study participants into four subgroups according to quartile of baseline C-peptide index levels (quartile 1, C-peptide index <0.59 ; quartile 2, $0.59 \leq$ C-peptide index <0.77 ; quartile 3, $0.77 \leq$ C-peptide index <1.06 ; quartile 4, $1.06 \leq$ C-peptide index). Then, to examine the difference between the top quartile and the lower three quartile groups based on the initial BHB response, the adjusted assessments of the C-peptide index and fasting C-peptide were analysed using an ANCOVA model with the two groups, their baseline values, age, sex and eGFR as covariates in each quartile subgroup based on baseline C-peptide index levels.

Correlations between \log_{10} transformed-BHB levels and ketogenic indices at week 52 were analysed by Pearson's product-moment correlation coefficients.

For each group, participants' demographics were summarized with appropriate descriptive statistics (means and SD for continuous variables and counts and percentages for categorical variables). Additionally, differences in the assessments across the groups were analysed using ANOVA, Wilcoxon rank-sum test and Fisher's exact test. Differences in BHB from baseline to weeks 4, 24 and 52 were analysed using the Wilcoxon signed-rank test. In this report, all HbA1c values are

presented using National Glycohaemoglobin Standardization Programme units. The (two-sided) significance level for each test was 0.05.

3 | RESULTS

The 774 participants who were receiving TOFO were included in this pooled analysis (Table S1). Of these participants, 66.0% were men, and the mean age, HbA1c, BMI and eGFR were 58.5 years, 8.1%, 25.6 kg/m² and 83.9 mL/min/1.73 m², respectively. The median changes in BHB levels in the top quartile and in the three lower quartiles, respectively, were +246.4* and +30.8* at week 4, +101.0* and +30.3* at week 24, and +68.6*, and +22.1* $\mu\text{mol/L}$ at week 52 (* $P < .001$ vs baseline; Figure 1B). BHB levels were maintained from week 24 to week 52 in both groups.

The proportion of men was greater and the duration of diabetes was longer in the top quartile (Table 1). Higher levels of HbA1c and FPG were also observed in the top quartile. Insulin secretion capacity indices, C-peptide index and HOMA- β were substantially lower in the top quartile, while the insulin resistance index, HOMA-IR, tended to be lower in the top quartile ($P = 0.078$). Max-BHB levels were higher in the top quartile (median +460.5 $\mu\text{mol/L}$) than in the lower three quartiles (221.0 $\mu\text{mol/L}$).

Levels of BHB were measured at baseline and every 4 weeks during 52 weeks of TOFO therapy. The proportion of participants with max-BHB levels was greatest at week 4; however, the proportions of participants experiencing their max-BHB level were similar from week 8 to week 52. The average period in which max-BHB was measured was 24.8 weeks during 52 weeks of TOFO therapy. In 33 participants, the max-BHB was $>1000 \mu\text{mol/L}$. In 32 of those 33 participants, baseline C-peptide index levels were < 1.0 (Figure S1).

From the results of the multivariate analyses, being male, and having lower basal insulin secretion capacity (C-peptide index), and higher FPG levels predicted participation in the top quartile (Table 2). Also, being male, having lower C-peptide index levels, and higher FPG levels were independent clinical factors that predicted higher max-BHB levels during the entire period of TOFO therapy (Table 2).

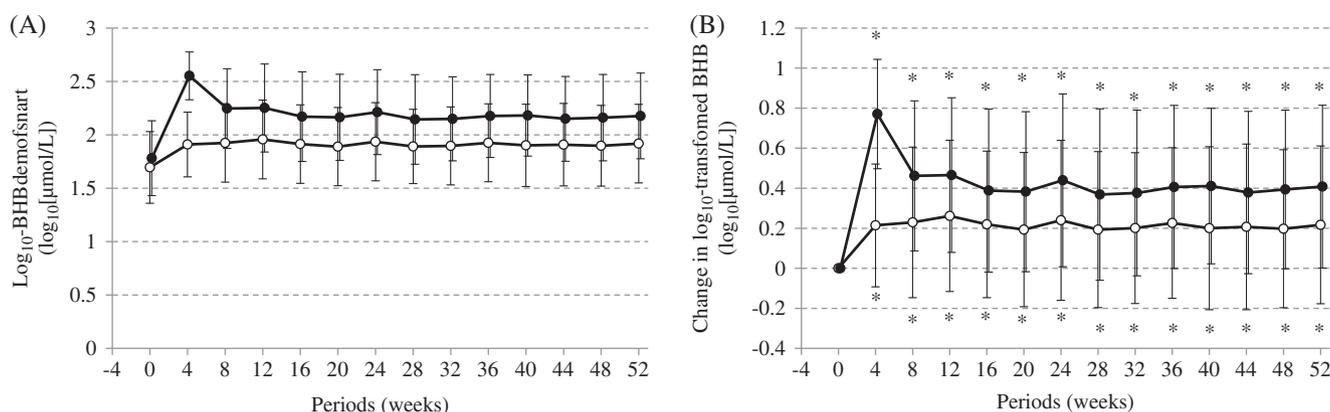


FIGURE 1 Time course of beta-hydroxybutyrate (BHB) values and changes in BHB beginning at week 4 in the top quartile and lower three quartiles according to changes in BHB. A, Time course of \log_{10} -transformed BHB levels (●, top quartile; ○, lower three quartiles). B, Time course of changes in \log_{10} -transformed BHB (●, top quartile; ○, lower three quartiles). Values are mean (SD). Paired t-test * $P < .001$ vs baseline

TABLE 1 Baseline characteristics of study participants according to the top quartile and lower three quartiles regarding change in beta-hydroxybutyrate at week 4

N	Top quartile 194	Lower three quartiles 579	P (top quartile vs lower three quartiles)
Age, y	59.9 (10.5)	58.1 (10.4)	.034
Men/Women, n (%)	151 (77.8)/43 (22.2)	360 (62.2)/219 (37.8)	<.001
TOFO (20 mg/40 mg), n (%)	51 (26.3)/143 (73.7)	184 (31.8)/395 (68.2)	.176
Concomitant anti-diabetic drugs, n (%)	154 (79.4)	429 (74.1)	.149
Monotherapy/alpha-GI/ biguanides/DPP-4 inhibitors/ glinides/sulphonylureas/ thiazolidines, n (%)	40 (20.6)/33 (17.0) /17 (8.8) /26 (13.4)/5 (2.6)/40 (20.6)/33 (17.0)	150 (25.9)/63 (10.9)/81 (14.0)/77 (13.3)/17 (2.9)/125 (21.6)/66 (11.4)	-
Concomitant anti-hypertensive drugs, n (%)	81 (41.8)	285 (49.2)	.081
ARBs/ACE inhibitors/CCBs/ β-blockers/diuretics, n (%)	60 (30.9)/6 (3.1)/43 (22.2)/6 (3.1)/17 (8.8)	215 (37.1)/12 (2.1)/175 (30.2)/21 (3.6)/47 (8.1)	-
Duration of diabetes, y	8.3 (6.6)	6.8 (5.6)	.001
BMI, kg/m ²	25.0 (4.3)	25.8 (4.3)	.020
Body weight, kg	67.4 (13.3)	68.7 (14.3)	.276
Waist circumference, cm	88.1 (9.8)	90.0 (10.5)	.024
eGFR, mL/min/1.73 m ²	85.3 (18.9)	83.4 (18.2)	.207
HbA1c, mmol/mol	66.1 (11.0)	63.9 (9.5)	.007
HbA1c, %	8.2 (1.0)	8.0 (0.9)	.007
Fasting plasma glucose, mmol/L	9.5 (2.2)	8.7 (2.0)	<.001
Fasting plasma glucose, mg/dL	171.6 (39.4)	157.2 (35.3)	<.001
Fasting insulin, pmol/L	41.2 (27.4)	51.2 (35.4)	<.001
Fasting C-peptide, pmol/L	407.4 (165.7)	463.9 (206.7)	.001
Fasting glucagon, pmol/L	19.2 (4.9)	20.2 (5.0)	.015
HOMA-IR	3.0 (2.3)	3.3 (2.4)	.078
HOMA-β	24.9 (17.1)	36.4 (29.2)	<.001
C-peptide index	0.7 (0.3)	0.9 (0.4)	<.001
Fasting glucagon to insulin ratio	0.6 (0.4)	0.5 (0.3)	.003
Adiponectin, μg/mL	8.9 (5.6)	8.2 (5.2)	.142
FFAs, mmol/L	0.59 (0.22)	0.58 (0.22)	.770
FFA to fasting insulin ratio, mmol/ L/nmol/L	19.2 (12.2)	16.0 (11.8)	.001
HDL cholesterol, mg/dL	59.9 (17.3)	62.0 (15.9)	.126
LDL cholesterol, mg/dL	123.6 (29.0)	123.6 (29.5)	.978
TG, mg/dL	136.0 (89.9)	144.4 (96.7)	.288
BHB, μmol/L	55.7 (33.1-97.4)	43.5 (28.2-77.0)	.002
Acetoacetate, μmol/L	26.8 (17.1-44.0)	24.3 (16.3-39.7)	.070
Max-BHB, μmol/L	460.5 (328.0-711.0)	221.0 (142.0-371.0)	<.001
Change in BHB at week 4, μmol/L	246.4 (188.6-351.0)	30.8 (1.6-72.5)	<.001

Note: Data are expressed as mean (SD).

Note: Data are expressed as median (interquartile range) for ketone values.

Note: Analyses were performed by one-way analysis of variance and Fisher's exact test.

Note: Analyses for ketone values were performed by Wilcoxon rank sum test.

Abbreviations: α-GI, α-glucosidase inhibitor; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BHB, beta-hydroxybutyrate; BMI, body mass index; CCB, calcium channel blocker; C-peptide index, 100*C-peptide/fasting plasma glucose; DPP-4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; FFAs, free fatty acids; HOMA-β, homeostatic model assessment of β-cell function; HOMA-IR, homeostatic model assessment of insulin resistance; max-BHB, maximum BHB level during long-term TOFO therapy as measured in each participant; TG, triglycerides; TOFO, tofogliflozin.

TABLE 2 Clinical predictors of inclusion in the top quartile and maximum beta-hydroxybutyrate level

Predictors for the participants in top quartile	Odds ratio (95% CI)	P
Men (vs Women)	2.036 (1.380-3.003)	.0003
Fasting plasma glucose (higher 10 mg/dL)	1.054 (1.006-1.103)	.0255
C-peptide index (higher 0.1 unit)	0.885(0.838-0.936)	<.001
Predictors for log ₁₀ -transformed max-BHB	Regression coefficient	
Log ₁₀ -transformed BHB (log ₁₀ -transformed[μmol/L])	0.88	<.001
Men	0.32	<.001
TOFO 40 mg (vs 20 mg)	0.17	.0008
HDL cholesterol (higher 10 mg/dL)	0.04	.0087
Fasting plasma glucose (higher 10 mg/dL)	0.02	.0023
Adiponectin (higher 1 μg/mL)	0.01	.0426
C-peptide index (higher 0.1 unit)	-0.02	.0003

Note: Factors remained through stepwise variable selection with $P < .05$.

Note: Potential baseline predictors were dosage of TOFO (20 mg vs 40 mg), age, sex, duration of diabetes, HbA1c, FPG, eGFR, BMI, waist circumference, HDL cholesterol, LDL cholesterol, TG, FFAs, adiponectin, HOMA-IR, C-peptide index and log₁₀-transformed BHB. Abbreviations: BHB, beta-hydroxybutyrate; BMI, body mass index; C-peptide index, 100*C-peptide/fasting plasma glucose; eGFR, estimated glomerular filtration rate; FFAs, free fatty acids; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; max-BHB, maximum BHB level during long-term TOFO therapy as measured in each participant; TG, triglycerides; TOFO, tofogliflozin.

Mean changes in body weight at week 52 in the top quartile and the three lower quartiles, respectively, were -3.4 kg and -2.8 kg ($P < .001$ between two groups; Figure 2A). In addition, there was a greater increase in fasting FFA levels (Figure 2B) and a greater reduction in fasting C-peptide levels (Figure 2D) in the top quartile over the whole treatment period. In the top quartile, a weaker increase in the C-peptide index (Figure 2F) was observed at week 52. Also, a greater increase in the FFA-to-IRI ratio was observed in the top quartile over the whole treatment period (Figure 2G). The reduction in HbA1c levels at week 52 was similar between the groups (Table S2).

Investigation of subgroups based on the quartiles of baseline CPI levels showed a greater reduction in fasting C-peptide at week 52 in the top quartile for the initial BHB response in the lower 3 subgroups based on the baseline CPI (Figure S2A). Also, in only the lowest subgroup based on the baseline CPI, a weaker increase in the CPI was observed in the top quartile for the initial BHB response (Figure S2B).

The log₁₀BHB level at week 52 was positively correlated with the ketogenic index-IRI ($r = 0.56$, $P < .001$; Figure S3A) and the ketogenic index-C-peptide ($r = 0.63$, $P < .001$; Figure S3B) in the entire study population.

4 | DISCUSSION

Results of multivariate analyses indicated that a lower basal insulin secretion capacity might predict which individuals with T2DM would have higher initial BHB elevations and greater max-BHB levels during long-term TOFO therapy. In addition, there was a larger increase in fasting FFA levels and a greater reduction in fasting C-peptide levels in those with higher initial BHB elevations. Based on these findings, the decrease in tissue glucose disposal and the shift to lipid use might be exaggerated by TOFO therapy because of insufficient basal insulin action and a further reduction in insulin secretion after initiation of TOFO treatment. Also, participants with higher initial BHB elevations subsequently had a greater weight loss, irrespective of improved HbA1c levels. To avoid the risk of the development of severe ketosis being induced by long-term SGLT2 inhibitor therapy the coexistence of precipitating causes of severe ketosis, such as basal endogenous insulin secretion capacity and the clinical process of weight loss caused by lipolysis, should be examined.

Several mechanisms may explain the enhanced ketosis associated with SGLT2 inhibitor use, including a greater reduction in insulin, stimulation of glucagon release, and decreased urinary ketone body excretion.^{9,19} In a recent study, after 2 weeks of treatment with the SGLT2 inhibitor dapagliflozin, a decrease in glucose oxidation and an increase in lipid oxidation during the insulin clamp compared with placebo were observed.¹⁰ Also, fasting ketone levels were increased fourfold. These results suggest that dapagliflozin might shift the oxidative pathway from glucose to fat, leading to increased β -oxidation in relation to the increased production of acetyl-CoA, which might then be converted to ketones. Plasma insulin and glucagon levels were suggested to be key components in determining the production of acetyl-CoA.²⁰ Glucagon stimulates the expression of hydroxymethylglutaryl-CoA synthase, which plays a role in the conversion of acetyl-CoA to ketones, while insulin suppresses its expression. Recent animal experiments examined the effects of insulin or a SGLT2 inhibitor on ketogenic enzymes and transporters in a diabetic mouse model.²¹ The SGLT2 inhibitor rather than insulin treatment increased BHB content in the liver, kidney and colon tissues, as well as in serum and urine, despite a similar decrease in serum glucose levels via those treatments. In SGLT2 inhibitor-treated mice, serum insulin levels were decreased compared with insulin-treated mice. Ketogenic enzymes and transporters were decreased in the insulin-treated mice, but were maintained or increased in the SGLT2 inhibitor-treated mice. Insulin is a potent inhibitor of peroxisome proliferator-activated receptor- α (PPAR α), which plays a role in the activation of ketogenic genes. In those experiments, an increased expression of PPAR α in the liver in the SGLT2 inhibitor-treated mice was observed. Our results showed a greater reduction in fasting IRI and increase in the fasting-glucagon-to-insulin ratio at week 52 in the top quartile of the initial response of BHB compared with the lower three quartiles; however, the change in glucagon was similar between the two groups. Thus, the further decrease in fasting IRI in the top quartile might contribute to the initial response of BHB and higher levels of max-BHB during TOFO therapy. However, since we

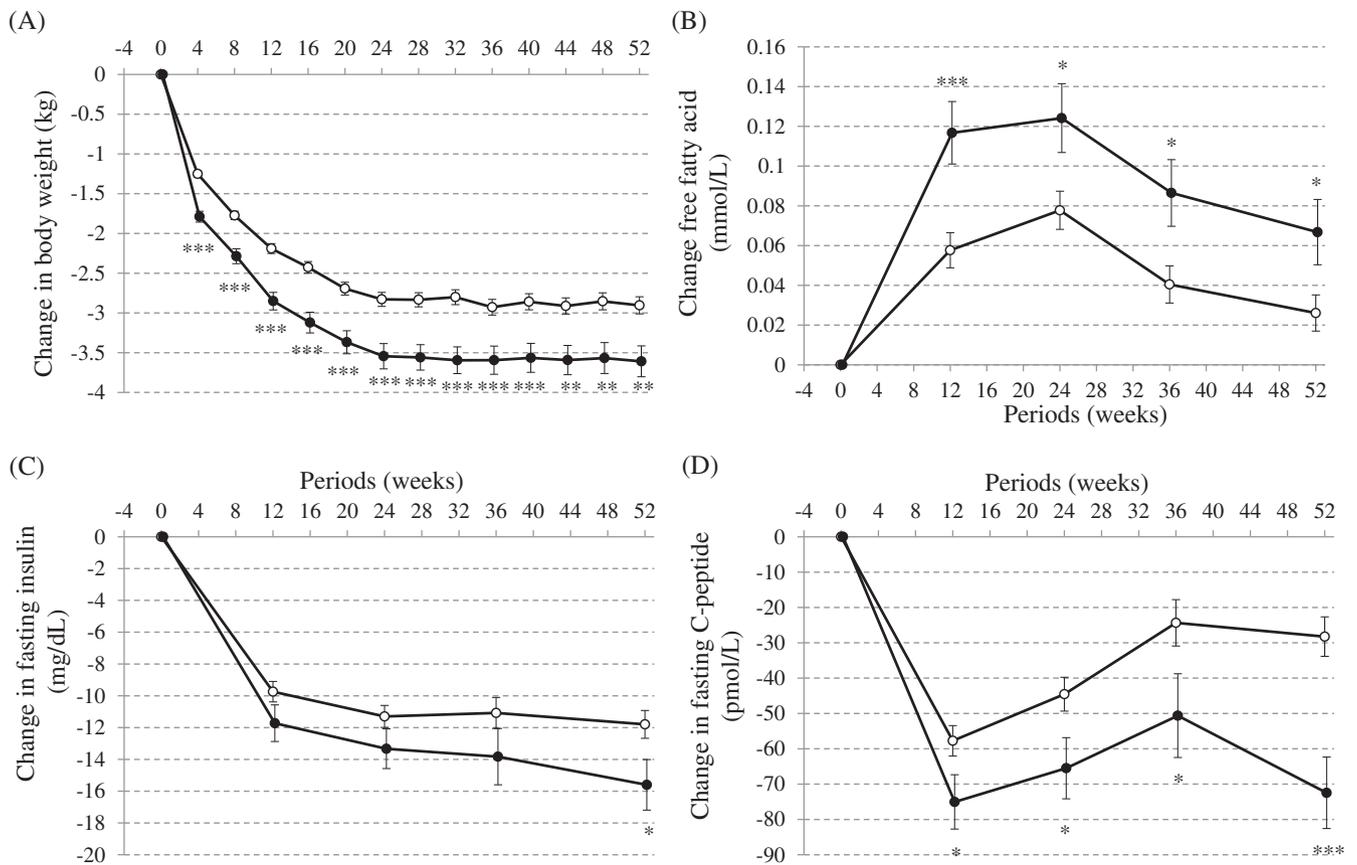


FIGURE 2 Comparison of the time course of variables between the top quartile and lower three quartiles of changes in beta-hydroxybutyrate (BHB) values beginning at week 4. A, Changes in body weight (●, top quartile; ○, lower three quartiles). B, Changes in free fatty acids (●, top quartile; ○, lower three quartiles). C, Changes in fasting insulin (●, top quartile; ○, lower three quartiles). D, Changes in fasting C-peptide (●, top quartile; ○, lower three quartiles). E, Changes in fasting plasma glucose (●, top quartile; ○, lower three quartiles). F, Changes in C-peptide index (●, top quartile; ○, lower three quartiles). G, Changes in fasting free fatty acid to fasting insulin ratio (●, top quartile; ○, lower three quartiles). Least squares mean (SE) adjusted by their baseline values, age, sex and baseline estimated glomerular filtration rate and to compare differences between the top quartile group and the lower three quartiles group. * $P < .05$, ** $P < .01$, *** $P < .001$ top quartile vs lower three quartiles. C-peptide index = $100 \times \text{C-peptide} / \text{fasting plasma glucose}$

did not evaluate tissue and urine levels of BHB, further study is needed to explore the determinant hormonal factors related to increased serum BHB levels in people with T2DM.

We observed that a lower basal insulin secretion capacity was one predictor of a greater initial elevation in BHB and higher max-BHB levels during long-term TOFO therapy, a result that was similar to a previous report.²² In addition, a greater reduction in fasting C-peptide during the entire treatment period was observed in participants in the top quartile compared with those in the lower three quartiles. The additional subgroup analysis based on quartile of baseline C-peptide index levels showed a greater reduction in fasting C-peptide in the top quartile of the initial response of BHB compared with the lower three quartiles, with the exception of the highest quartile of baseline C-peptide index levels. Thus, baseline insulin secretion capacity might differently influence changes in fasting C-peptide according to the initial response of BHB after long-term treatment with TOFO. These results suggest that the reduction in insulin secretion might influence maintenance of the increase in BHB levels. Also, the close positive correlations between the ketogenic indices using fasting IRI or C-peptide

and the BHB values at week 52 suggested that there was a close association of insulin secretion with the chronic increase in BHB after long-term TOFO treatment. In addition, SGLT2 inhibitors improve hyperglycaemia independently of insulin action and, recently, improved hyperglycaemia via SGLT2 inhibitors was shown to result in an improvement in β -cell function using glucose clamp methods.^{23,24} Our results also showed significant increases in the C-peptide index from baseline in both groups; however, the increase tended to be weaker in the top quartile. In addition, a weaker increase in the C-peptide index in the top quartile group compared with the lower three quartiles group according to the initial response of BHB was only shown in the lowest subgroup of the baseline C-peptide index; therefore, a lower basal insulin secretion capacity might be associated with not only the greater reduction in insulin secretion but also with the weaker recovery of β -cell function via TOFO, leading to higher elevations of BHB after both initial and chronic treatment.

A maintained reduction in fasting IRI levels and a biphasic change in the reduced fasting C-peptide levels were observed from time-course evaluations in the present study. Equimolar amounts of IRI and

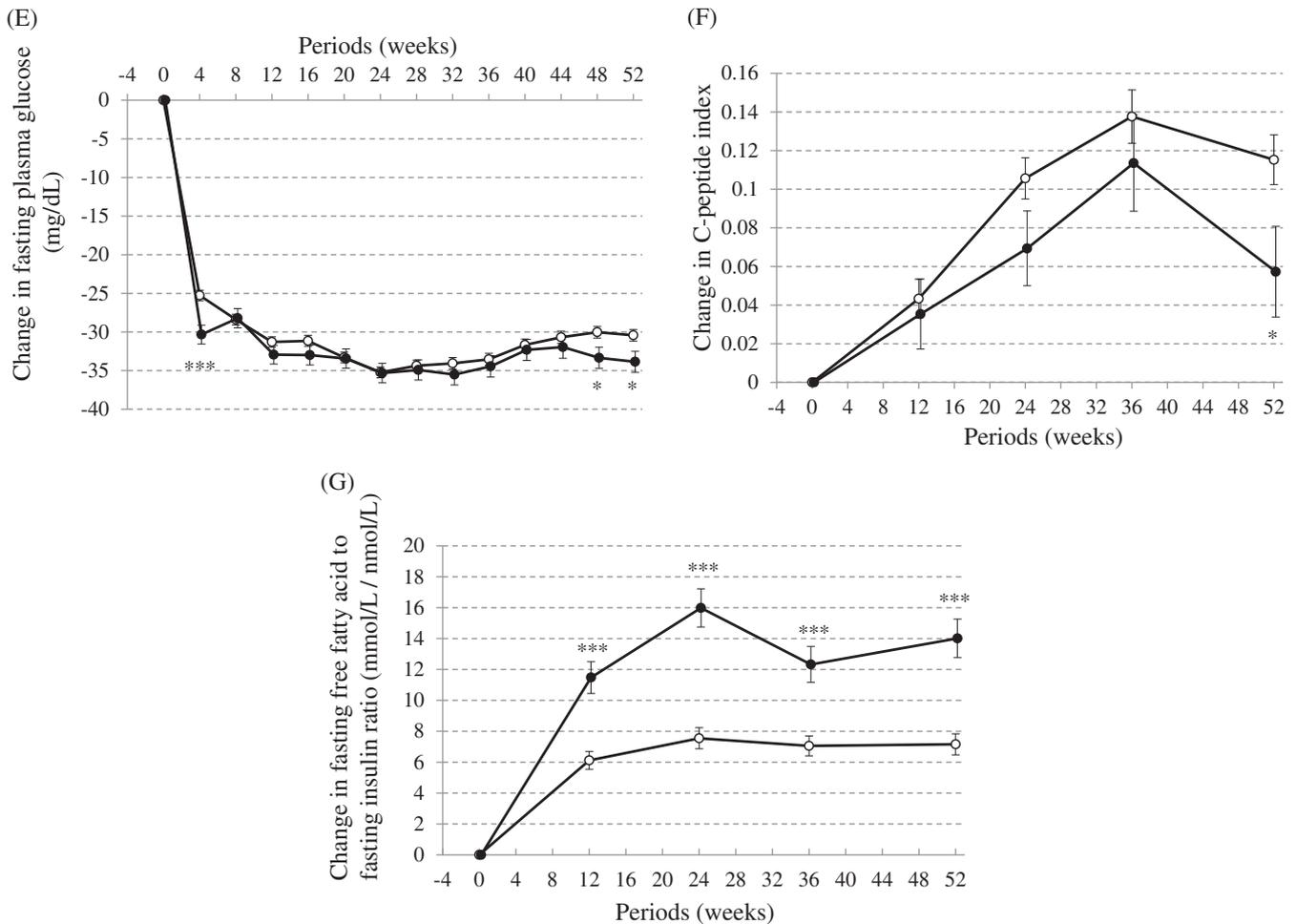


FIGURE 2 (Continued)

C-peptide are secreted from β cells. First, they are delivered to the liver, and then C-peptide is not degraded in the liver, while IRI is, after which the diminished IRI levels are evident in peripheral blood. Thus, the insulin levels that can be monitored in peripheral blood have been influenced by their hepatic clearance status in liver. In a previous report, an SGLT2 inhibitor reduced IRI levels to a greater degree than C-peptide levels, suggesting that the agent might enhance hepatic insulin clearance.²⁵ Also, the single administration of another SGLT2 inhibitor was reported to increase the insulin metabolic clearance rate estimated by the glucose-clamp method.⁹ Taken together, the reduced IRI levels might be influenced by changes in hepatic insulin clearance during long-term treatment with TOFO. Further, an increase in the ratio of fasting C-peptide to IRI levels, suggesting hepatic insulin clearance, was reported to be correlated with the improved peripheral insulin sensitivity after an intervention with a 4-month hypocaloric diet.²⁶ In addition, the ratio of fasting C-peptide to IRI was positively correlated with insulin sensitivity indices in people with T2DM.²⁷ The changed insulin clearance via TOFO-induced weight loss, leading to improved insulin sensitivity, might therefore contribute to the reduced insulin levels. With regard to fasting C-peptide levels, the correction of hyperglycaemia via an SGLT2 inhibitor was reported to improve insulin secretion capacity,²⁴ and insulin secretion, calculated according to glucose and C-peptide levels after a

meal tolerance test, was reported to be improved by an SGLT2 inhibitor.²³ The observed biphasic changes in fasting C-peptide levels suggest an improved insulin secretion capacity, which might be supported by the increase in the C-peptide index after long-term treatment with TOFO. Finally, C-peptide is metabolized in the kidney and, in a previous clinical report, the metabolism of C-peptide in the kidney in healthy participants was not influenced by the SGLT2 inhibitor.²⁸ To our knowledge, our results are the first to indicate the dissociation of the time course of fasting IRI and C-peptide levels during long-term treatment with SGLT2 inhibitors. Further study will be needed to elucidate the mechanism of differences in changes in IRI and C-peptide levels via SGLT2 inhibitors.

Participants in the top quartile had a greater subsequent weight loss at week 52 than those in the lower three quartiles. Previous animal experimental results suggest that the SGLT2 inhibitor-reduced IRI levels might be associated with lipolysis in adipose tissue and subsequently with improvement in obesity and insulin resistance.²⁹ In these experiments, in addition to the reduced IRI levels with blood glucose reduction, serum levels of non-esterified fatty acid and ketones were increased in TOFO-treated mice, suggesting the acceleration of lipolysis in the white adipose tissue and β -oxidation. Also, phosphorylation of hormone-sensitive lipase and adipose triglyceride lipase protein levels in white adipose tissue were significantly increased. In the

present study, greater reductions in waist circumference and fasting IRI and an increase in fasting FFA levels at week 52 were observed in the top quartile compared with the lower three quartiles. The increase in the FFA-to-IRI ratio was greater in the top quartile throughout treatment with TOFO. That greater increase in fasting FFAs might be a reflection of the abundant lipid use, followed by the production of ketones. Therefore, the greater initial elevation of BHB was associated with subsequent clinical effects, weight loss and possibly reduction of fat mass caused by the reduced IRI-induced lipolysis, irrespective of the improvement in HbA1c levels.

The increases in BHB levels were maximized at week 4, then slowly weakened toward week 24, and finally were maintained to a similar degree until week 52. In particular, in the top quartile, the increases in BHB levels from week 4 to week 24 were attenuated. Recently, both dehydration and insulinopaenia were reported to be associated with euglycaemic ketoacidosis in SGLT2 inhibitor-treated rats.³⁰ That report showed that volume loss early after SGLT2 inhibitor treatment was associated with increases in plasma epinephrine and corticosterone to enhance lipolysis. In the top quartile, a greater reduction in body weight at week 4 was observed, which might include not only loss of fat mass but also of lean mass, as well as fluid loss. Consequently, the increases in BHB might have peaked at week 4 then weakened toward week 24 due to the attenuation of volume loss, especially in the top quartile. However, the proportion of administration of concomitant diuretics that might influence fluid loss was similar between the top quartile and the lower three quartiles in the present study. As we did not investigate body composition in the present study, more studies are needed to clarify the mechanism of the time course of BHB during long-term SGLT2 inhibitor treatment.

We measured BHB levels at a total of 9453 points in the whole study population after starting the medication. The highest number of participants (almost 15%) who experienced their max-BHB values was seen at week 4. From weeks 24 to 52 there was a constant increase in BHB, and throughout that period similar numbers of participants experienced their max-BHB values. A recent report showed that shortly after initiation of SGLT2 inhibitor treatment there was approximately twice the risk of diabetic ketoacidosis compared to that with dipeptidyl peptidase-4 inhibitors.³¹ Based on the present study, BHB levels should be monitored not only early after the initiation of SGLT2 inhibitor treatment, but also during the chronic phase, particularly with the coexistence of precipitating causes such as infections, surgical stress, poor oral intake, unfavourable dietary habits, insulin reduction or omission, or active complications of T2DM. In addition, being male, having lower C-peptide index levels, and higher FPG, which were similar clinical predictors for inclusion in the top quartile in the present analysis, predicted greater max-BHB levels during the entire period of TOFO therapy. In particular, almost all the participants whose max-BHB values >1000 $\mu\text{mol/L}$ had C-peptide index levels <1.0. Further, at the higher dosage of TOFO, higher levels of BHB were observed by multivariate analysis as predictors of greater max-BHB values. Therefore, to avoid the risks of ketosis, the predictors of both an initial elevation of BHB and max-BHB should be evaluated. Additionally, body weight values initially and

during the chronic phase after administration of an SGLT2 inhibitor should be considered in clinical settings.

This study had several limitations. It was a post hoc analysis of two TOFO studies; however, we did not perform a comparison between placebo and TOFO. Blood gas analyses were not performed in this study; thus, whether the elevation of ketones might be accompanied by a change in plasma pH levels has not been clarified. The time course of changes in BHB under similar diet and exercise guidance was analysed in our previous study in people with T2DM receiving treatment with antidiabetic drugs,¹⁷ and it was found that BHB was influenced by dietary conditions. The fact that we did not have data on the results of changes in diet is a limitation of the present study; however, we investigated the association of insulin secretion capacity estimated by FPG and fasting C-peptide with the change in BHB during long-term treatment with TOFO. Further study will be needed to evaluate insulin secretion and resistance using the glucose clamp technique. Finally, we did not investigate body composition, which could indicate both fat and fat-free mass. The effects of concomitant medications, including anti-hyperglycaemic and anti-hypertensive drugs that might influence fat mass and fluid were not evaluated, although we did determine that their administration was unchanged during the study period.

In people with T2DM receiving the SGLT2 inhibitor, TOFO, lower basal insulin secretion capacity might predict which the participants would experience a TOFO-induced greater initial BHB elevation and the max-BHB values in the whole participants during long-term TOFO therapy. Moreover, a subsequent greater weight loss probably caused by lipolysis might be suggested in the participants with higher initial elevations of BHB.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

All authors participated in writing the manuscript. Y.S. and K.N. wrote the draft and contributed to the discussion. K.K. contributed to the discussion and reviewed/edited the manuscript. A.Y. researched data and reviewed the manuscript. H.S. had full access to all of the 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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