Tofogliflozin decreases body fat mass and improves peripheral insulin resistance

Ren Matsuba MD1 | Ikuro Matsuba MD2 | Mototsugu Shimokawa PhD3 | Yoshio Nagai MD1 | Yasushi Tanaka MD1

1Department of Internal Medicine, Division of Metabolism and Endocrinology, St Marianna University School of Medicine, Kanagawa, Japan
2Matsuba Medical Clinic, Kanagawa, Japan
3Department of Cancer Information Research, National Kyushu Cancer Center, Clinical Research Institute, Fukuoka, Japan

Correspondence
Ren Matsuba MD, Department of Internal Medicine, Division of Metabolism and Endocrinology, St. Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki-shi, Kanagawa 216-8511, Japan.
Email: r2matsuba@marianna-u.ac.jp

Funding information
Kowa Company Ltd

INTRODUCTION

Tofogliflozin is a potent and highly selective sodium-glucose co-transporter-2 (SGLT2) inhibitor that promotes urinary glucose excretion and controls hyperglycaemia in rodents, without causing hypoglycaemia, as well as improving hyperglycaemia and reducing body weight (BW) in clinical studies.1 The efficacy of SGLT2 inhibitors, alone or combined with dipeptidyl peptidase-4 (DPP-4) inhibitors, has recently been examined in several studies.2–4 Prescription of SGLT2 inhibitors is increasing, and clinical investigation of a combination drug is ongoing.2,3 so combined use of SGLT2 inhibitors with DPP-4 inhibitors is expected to become more frequent. SGLT2 inhibitors have the potential to satisfy the unmet need for weight control in patients with diabetes. These drugs reduce BW through calorie loss via renal glucose excretion and increased lipolysis in adipose tissue, and may be useful for obese patients with type 2 diabetes mellitus (T2DM) and insulin resistance; however, few investigations have assessed whether SGLT2 inhibitors improve insulin resistance in patients with T2DM or explored the relationship between changes in insulin resistance and body composition variables.5

The aim of the present study, therefore was to study the effect of combined therapy with tofogliflozin and a DPP-4 inhibitor on insulin sensitivity (glucose infusion rate [GIR]) in patients with T2DM, including the relationship between changes in insulin sensitivity and body composition.

METHODS

2.1 Study design and participants

This single-arm, single-centre, randomized, open-label study was registered with the University Hospital Medical Information Network
Clinical Trials Registry (no.: UMIN000014425).

The participants were 16 patients with T2DM, receiving treatment with a DPP-4 inhibitor, who gave written informed consent, satisfied all of the inclusion criteria, and did not meet any of the exclusion criteria. Body composition and laboratory variables were compared between before administration of tofogliflozin (20 mg once daily) and after its administration for 12 weeks.

The 4-dimensional impedance method (T-Scan Plus) was used to measure body composition variables. The impact of tofogliflozin on peripheral glucose uptake (M value and M/I ratio) was determined using the hyperinsulinaemic-euglycaemic clamp method.

2.2 | Clinical laboratory tests

Fasting blood and urine samples were collected at each visit for laboratory tests. In weeks 0 and 12, samples were collected before administration of tofogliflozin and performance of the clamp test. Most measurements were carried out by LSI Medience Corporation (Tokyo, Japan) using standard methods.

2.3 | Hyperinsulinaemic-euglycaemic clamp

The peripheral glucose uptake rate was assessed using the combined hyperinsulinaemic-euglycaemic clamp technique, as reported elsewhere. The clamp test was performed in weeks 0 and 12. Both the insulin infusion rate and the GIR were controlled by an artificial pancreas (STG-55; Nikkiso Co., Ltd., Shizuoka, Japan). The plasma glucose level was determined at 1-minute intervals during the clamp test using the continuous glucose monitoring system of the artificial pancreas, with a blood sampling rate of 2 mL/h. A hyperinsulinaemic state was achieved by primed-constant infusion of insulin at 2.58 mU/kg/min (17.9 pmol/kg/min), corresponding to ~100 mU/m²/min (695 pmol/m²/min).

After the GIR became stable, the mean steady-state GIR for a 30-minute period was determined as an index of peripheral glucose uptake. Blood samples were collected before and after the start of insulin infusion to measure plasma glucose and insulin concentrations. The real rate of tissue glucose uptake during the insulin clamp test (M value) was calculated by subtracting urinary glucose excretion from glucose disposal to determine insulin-mediated tissue glucose uptake.5

2.4 | Statistical analysis

The M value and M/I ratio were compared between before and after tofogliflozin treatment using the paired t test. Pearson’s correlation coefficient analysis was used to assess associations between changes in the M value and changes in body composition or laboratory variables. All reported P values are two-sided. Analyses were performed using SAS version 9.4 (SAS Institute, Cary, North Carolina).

3 | RESULTS

Of the 16 patients with T2DM receiving basal DPP-4 inhibitor therapy who were candidates for 12 weeks of add-on tofogliflozin in this study, 2 were excluded at screening (Table S1).

Table 1 lists the laboratory variables that were measured at baseline and week 12 of add-on tofogliflozin treatment. The M value, an index of insulin resistance, showed a significant increase of 0.90 ± 1.28 in week 12 (P < .05). The M/I ratio also showed a significant increase of 0.49 ± 0.60 (P < .05).

Furthermore, there was significant improvement in the following markers of glucose metabolism: glycated haemoglobin decreased by 1.05 ± 0.47%, fasting plasma glucose decreased by 2.17 ± 1.70 mmol/L, glycoalbumin decreased by 4.21 ± 2.07% and homeostasis model assessment of β-cell function index increased by 13.35 ± 9.33 (all P < .001). There was no significant change in glucagon level (~0.04 ± 6.95 pmol/L [Sceti Medical Labo, Tokyo, Japan] or 0.38 ± 4.63 pmol/L [Cosmic Corporation, Tokyo, Japan]), and no significant change in either glucagon-like peptide-1 or blood ketone levels.

Liver function tests showed significant improvement, with alanine aminotransferase decreasing by 8.57 ± 10.95 IU/L (P < .05) and γ-glutamyl transpeptidase declining by 17.93 ± 27.09 IU/L (P < .05).

Among serum lipid levels, HDL cholesterol showed a significant increase of 0.09 ± 0.15 mmol/L (P < .05).

Among inflammatory markers, there was a significant increase in tumour necrosis factor-α by 0.20 ± 0.58 ng/L (from 1.48 ± 0.32 to 1.68 ± 0.61 ng/L; P < .001).

Significant weight loss and reduction of body mass index were observed after 12 weeks of add-on tofogliflozin treatment, with BW decreasing by 2.87 ± 1.48 kg (P < .001) and body mass index declining by 1.11 ± 0.57 kg/m² (P < .001). Body composition variables generally showed significant decreases, including reduction of body fat mass by 1.33 ± 0.99 kg, lean body mass by 1.54 ± 0.77 kg, body water mass by 1.11 ± 0.55 kg, and muscle mass by 1.37 ± 0.72 kg (all P < .001).

Comparing the M value and body composition variables, there was a significant negative correlation between changes in the M value and body fat mass (r = −0.67, P = .012; Table 2), but there was no correlation with the decrease in muscle mass or body water mass. Regarding adverse events, five patients (31.3%) reported pollakiuria and one patient (6.3%) had a urinary tract infection, but sexually transmitted infections, hypoglycaemia, ketosis and pancreatitis were not observed.

4 | DISCUSSION

Both basic and clinical studies have shown that insulin resistance is improved by the direct hypoglycaemic effect of SGLT2 inhibitors, as well as by alleviation of glucotoxicity and weight loss (reduction of BW and body fat). Thus, it is clinically important to evaluate insulin resistance during SGLT2 inhibitor treatment.

In pair-fed mice with or without tofogliflozin treatment for 8 weeks, the hyperinsulinaemic-euglycaemic clamp test showed a significant increase in GIR in the tofogliflozin group and improvement in systemic insulin resistance, with muscle glucose uptake also increasing significantly in the tofogliflozin group, indicating a decrease in fat stores and improvement in peripheral insulin resistance. In the present study, the M value and M/I ratio both increased significantly...
SGLT2 inhibitors improve insulin resistance in patients with T2DM.5

One patient was excluded from M value analysis because of failure to fast before blood collection, leaving 13 patients. This patient was also excluded from analysis of the M/I ratio along with another patient due to hemolysis of the blood sample, leaving 12 patients.

**TABLE 1** Insulin sensitivity and laboratory variables before and after add-on tofogliflozin therapy

|                           | N  | Baseline      | 12 weeks      | Change         |
|---------------------------|----|---------------|---------------|----------------|
| Insulin sensitivity (M value) | 13 | 4.85 ± 1.46   | 5.75 ± 1.22   | 0.90 ± 1.28*   |
| M/I ratio                 | 12 | 2.32 ± 0.94   | 2.81 ± 0.85   | 0.49 ± 0.60*   |
| HbA1c, %                  | 14 | 8.24 ± 0.75   | 7.19 ± 0.67   | -1.05 ± 0.47***|
| Fasting plasma glucose, mmol/L | 14 | 10.12 ± 1.60   | 7.95 ± 0.99   | -2.17 ± 1.70***|
| Insulin, pmol/L           | 14 | 61.74 ± 21.46 | 64.17 ± 28.06 | 2.43 ± 14.24   |
| Glycoalbumin, %           | 14 | 21.67 ± 3.12  | 17.46 ± 2.25  | -4.21 ± 2.07***|
| HOMA-IR                   | 14 | 4.01 ± 1.55   | 3.31 ± 1.63   | -0.71 ± 1.46   |
| HOMA-β                    | 14 | 28.66 ± 15.37 | 42.01 ± 15.67 | 13.35 ± 9.33***|
| QUICKI                    | 14 | 0.30 ± 0.00   | 0.31 ± 0.03   | 0.01 ± 0.03    |
| Total cholesterol, mmol/L | 14 | 4.89 ± 0.87   | 4.90 ± 0.80   | 0.01 ± 0.50    |
| LDL cholesterol, mmol/L   | 14 | 2.67 ± 0.82   | 2.69 ± 0.93   | 0.02 ± 0.70    |
| HDL cholesterol, mmol/L   | 14 | 1.25 ± 0.30   | 1.35 ± 0.31   | 0.09 ± 0.15*   |
| Triglycerides, mmol/L     | 14 | 2.10 ± 1.64   | 1.88 ± 2.14   | -0.22 ± 1.27   |
| Non-HDL-cholesterol, mmol/L| 14 | 3.63 ± 1.01   | 3.55 ± 0.99   | -0.08 ± 0.41   |
| Adiponectin, mg/L         | 14 | 7.24 ± 3.84   | 7.69 ± 3.63   | 0.45 ± 1.15    |
| IL-6, ng/L                | 14 | 1.30 ± 0.57   | 1.29 ± 0.61   | -0.01 ± 0.62   |
| TNF-α, ng/L               | 14 | 1.48 ± 0.32   | 1.68 ± 0.61   | 0.20 ± 0.58*** |
| hsCRP, mg/L               | 14 | 0.90 ± 1.25   | 0.60 ± 0.51   | -0.30 ± 1.30   |
| C-peptide, nmol/L         | 14 | 0.62 ± 0.13   | 0.65 ± 0.22   | 0.03 ± 0.13    |
| Glucagon (RIA), pmol/L    | 14 | 38.86 ± 9.33  | 38.82 ± 6.74  | -0.04 ± 6.95   |
| Glucagon (sandwich ELISA), pmol/L | 14 | 17.58 ± 8.07 | 17.96 ± 8.60 | 0.38 ± 4.63 |
| Active GLP-1, pmol/L      | 14 | 6.54 ± 4.22   | 6.38 ± 3.58   | -0.17 ± 3.71   |
| Blood ketone bodies, μmol/L| 14 | 200.00 ± 96.08| 290.00 ± 177.44| 90.00 ± 177.44 |
| AST, IU/L                 | 14 | 23.43 ± 9.91  | 20.36 ± 6.22  | -3.07 ± 5.41   |
| ALT, IU/L                 | 14 | 31.43 ± 16.22 | 22.86 ± 8.46  | -8.57 ± 10.95*|
| ALP, IU/L                 | 14 | 215.93 ± 40.82| 199.64 ± 44.22| -16.29 ± 37.63|
| γ-GTP, IU/L               | 14 | 56.07 ± 54.32 | 38.14 ± 27.74 | -17.93 ± 27.09*|
| Scr, μmol/L               | 14 | 56.32 ± 12.64 | 57.08 ± 13.01 | 0.76 ± 5.71    |
| eGFR, ml/min/1.73 m²      | 14 | 92.71 ± 15.67 | 91.11 ± 13.92 | -1.61 ± 8.36   |

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; γ-GTP, γ-glutamyl transpeptidase; GLP-1, glucagon-like peptide 1; HbA1c, glycated haemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β-cell function; hsCRP, high-sensitivity C-reactive protein; IL-6, Interleukin-6; QUICKI, quantitative insulin sensitivity check index; RIA, radioimmunoassay; Scr, serum creatinine; TNF-α, tumour necrosis factor-α. Data are presented as the mean ± SD. *P < .05, **P < .01, ***P < .001 vs baseline (one-sample t test). Two patients were excluded at screening. One patient was excluded from M value analysis because of failure to fast before blood collection, leaving 13 patients. This patient was also excluded from analysis of the M/I ratio along with another patient due to hemolysis of the blood sample, leaving 12 patients.

(P < .05) in patients receiving add-on tofogliflozin therapy. There was a significant negative correlation between changes in the M value and body fat mass (r = −0.67, P = .012; Figure S1), but no correlation of the M value with the decrease in muscle mass, suggesting that alteration of muscle mass had a negligible influence on insulin resistance.

Only a few studies have evaluated the mechanism by which SGLT2 inhibitors improve insulin resistance in patients with T2DM.5,11 DeFronzo et al.11 performed a glucose clamp test in 18 patients with T2DM after 2 weeks of SGLT2 inhibitor or placebo administration. They found a significant increase in muscle glucose uptake in the SGLT2 inhibitor group, suggesting improvement in peripheral insulin resistance. In the present study, we evaluated insulin resistance by the hyperinsulinaemic-euglycaemic clamp method and observed significant improvement in the M value and M/I ratio (P < .05), along with a significant decrease in body fat mass. There was a negative correlation between the decrease in body fat mass and improvement in the M value, indicating that reduction of body fat was related to elevation of muscle glucose uptake by add-on tofogliflozin therapy.

The present study assessed the add-on effect of tofogliflozin in patients with T2DM who were refractory to DPP-4 inhibitors, but the changes detected were similar to those previously reported in patients on tofogliflozin monotherapy. In previous studies performed in humans and mice, improvement in systemic insulin resistance has generally been observed despite elevation of endogenous glycogenesis.5,11 Increased glycogenesis is believed to occur secondary to an increase in the glucagon/insulin ratio, rather than being attributable to exacerbation of hepatic insulin resistance.5 DPP-4 inhibitors reduce endogenous glucose production through promotion of insulin secretion by β cells and suppression of glucagon secretion by α cells.12,13 Two recent studies revealed reduction of the glucagon level after combined therapy with an SGLT2 inhibitor and a DPP-4 inhibitor.14,15 Glucagon has multiple effects on cardiac function,16 and a
large-scale clinical trial showed a decrease in cardiovascular mortality and hospitalization for heart failure after treatment with empagliflozin.\textsuperscript{17} In the present study, glucagon did not increase after initiation of add-on SGLT2 inhibitor therapy in patients with T2DM already using DPP-4 inhibitors. The absence of an increase in the glucagon/insulin ratio might have prevented elevation of endogenous glucose production, but it was not evaluated in the present study and further investigation is warranted. Combining a DPP-4 inhibitor with an SGLT2 inhibitor seems to be a reasonable therapeutic option because glycemic control is improved while elevation of glucagon is suppressed.

The limitations of the present study were the lack of a placebo group and the small number of participants (because of the burden of the clamp test); however, our results are consistent with those of a previous clinical study in terms of the main effects of tofogliflozin on BW and blood glucose. Further investigation will be required to better define the influence on body composition variables when an SGLT2 inhibitor is added to a DPP-4 inhibitor.

In conclusion, glucose clamp studies showed an increase in peripheral glucose uptake (by muscle and fat) and reduction of body fat mass when tofogliflozin was added to basal therapy with a DPP-4 inhibitor.

Conflict of interest

This research received financial support from Kowa Company Ltd, but was planned and designed by the investigators. The company was not involved in the study design, patient enrolment, data aggregation and analysis, data interpretation, or preparation of this report.

Author contributions

R.M., I.M., Y.N. and Y.T. conceived the research design. R.M. and M.-S. performed acquisition and analysis of data. All authors contributed to interpretation of the data. R.M. wrote the manuscript. I.M., Y.N. and Y.T. performed critical revision of the manuscript for important intellectual content. All authors approved the final version of the manuscript.

ORCID

Ren Matsuba https://orcid.org/0000-0001-5041-6344

REFERENCES

1. 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. Nutr Diabetes. 2014;4:e125.
2. Garnero-Jones KP. Saxagliptin/dapagliflozin: a review in type 2 diabetes mellitus. Drugs. 2017;77(3):319-330.
3. Kadowaki T, Inagaki N, Kondo K, et al. Efficacy and safety of canagliflozin as add-on therapy to teneligliptin in Japanese patients with type 2 diabetes mellitus: results of a 24-week, randomized, double-blind, placebo-controlled trial. Diabetes Obes Metab. 2017;19(6):874-882.
4. Sharma MD. Potential for combination of dipeptidyl peptidase-4 inhibitors and sodium-glucose co-transporter-2 inhibitors for the treatment of type 2 diabetes. Diabetes Obes Metab. 2015;17(7):616-621.
5. Merovci A, Solis-Herrera C, Daniele G, et al. Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. J Clin Invest. 2014;124(2):509-514.
6. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol. 1979;237(3):E214-E223.
7. Emoto M, Morioka T, Yokoyama H, et al. Evaluation of insulin resistance in diabetes: standard protocol for a euglycemic-hyperinsulinemic clamp using an artificial pancreas. In: Inaba M, ed. Musculoskeletal Disease Associated with Diabetes Mellitus. Tokyo, Japan: Springer; 2016:219-235.
8. Ferrannini E. Sodium-glucose co-transporters and their inhibition: clinical physiology. Cell Metab. 2017;26(1):27-38.
9. Liao X, Wang X, Li H, et al. Sodium-glucose cotransporter 2 (SGLT2) inhibitor increases circulating zinc-A2-glycoprotein levels in patients with type 2 diabetes. Sci Rep. 2016;6:32887.
10. Seino Y, Yabe D, Sasaki T, et al. Sodium-glucose cotransporter-2 inhibitor luseogliflozin added to glucagon-like peptide 1 receptor agonist lirolaglutide improves glycemic control with bodyweight and fat mass reductions in Japanese patients with type 2 diabetes: a 52-week, open-label, single-arm study. J Diabetes Investig. 2017. https://doi.org/10.1111/jdi.12694 [Epub ahead of print].
11. 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(3):1029-1042.
12. Balas B, Baig MR, Watson C, et al. The dipeptidyl peptidase IV inhibitor vildagliptin suppresses endogenous glucose production and enhances islet function after single-dose administration in type 2 diabetic patients. J Clin Endocrinol Metab. 2007;92(4):1249-1255.
13. Duez H, Smith AC, Xiao C, et al. Acute dipeptidyl peptidase-4 inhibition rapidly enhances insulin-mediated suppression of endogenous glucose production in mice. Endocrinology. 2009;150(1):56-62.
14. Forst T, Falk A, Andersen G, et al. Effects on α- and β-cell function of sequentially adding empagliflozin and lirolaglutin to therapy in people with type 2 diabetes previously receiving metformin: an exploratory mechanistic study. Diabetes Obes Metab. 2017;19(4):489-495.
15. Wilding JP, Rajeev SP, DeFronzo RA. Positioning SGLT2 inhibitors/incretin-based therapies in the treatment algorithm. Diabetes Care. 2016;39(suppl 2):S154-S164.
16. Ceriello A, Genovesi S, Mannucci E, Gronda E. Glucagon and heart in type 2 diabetes: new perspectives. Cardiovasc Diabetol. 2016;15(1):123.
17. 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.

**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the supporting information tab for this article.