

# Changes in Levels of Biomarkers Associated with Adipocyte Function and Insulin and Glucagon Kinetics During Treatment with Dapagliflozin Among Obese Type 2 Diabetes Mellitus Patients

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## Abstract

**Objectives** This study aimed to investigate changes in levels of biomarkers associated with adipocyte function and insulin and glucagon kinetics after a meal tolerance test (MTT) during treatment with dapagliflozin among obese type 2 diabetes mellitus (T2DM) patients.

**Methods** T2DM patients with hemoglobin A1c (HbA1c) levels >6.5 % and body mass index (BMI) >25 kg/m<sup>2</sup> were treated with dapagliflozin 5 mg/day for at least 12 weeks. HbA1c, body weight, ketone bodies, adiponectin, plasminogen activator inhibitor-1 (PAI-1), and C-reactive protein (CRP) were measured before and after treatment with dapagliflozin. A subset of patients underwent an MTT.

**Results** Of 27 participating patients (mean age 47.9 years; 17 males), five were drug-naïve and 22 were treated with other antidiabetic agents, including insulin and glucagon-like peptide-1 (GLP-1) receptor agonists. Following treatment with dapagliflozin, HbA1c levels significantly improved ( $7.44 \pm 0.56$  to  $6.70 \pm 0.057$  %;  $p < 0.01$ ), body weight significantly decreased ( $90.9 \pm 16.5$  to  $87.1 \pm 15.9$  kg;  $p < 0.01$ ), ketone bodies increased, adiponectin significantly

increased, and high-sensitivity CRP tended to decrease. During the MTT, blood glucose  $\Delta AUC_2$  significantly decreased, glucagon  $\Delta AUC_2$  increased, and immunoreactive insulin (IRI) did not change in 11 of 27 patients.

**Conclusion** Although ketone bodies increased significantly, adiponectin increased and high-sensitivity CRP decreased significantly. These findings suggest that sodium-glucose cotransporter-2 (SGLT2) inhibitors may potentially improve adipocyte function in treating obese T2DM patients.

## Key Points

Treatment of obese type 2 diabetes mellitus (T2DM) patients with dapagliflozin 5 mg once daily for 12 weeks significantly improved HbA1c levels and body weight to the same level as previous reports.

Although ketone bodies increased significantly, high-sensitivity C-reactive protein significantly decreased, while adiponectin significantly increased.

During the meal tolerance test, blood glucose  $\Delta$  area under the curve from time zero to 2 h ( $AUC_2$ ) reduced significantly, but glucagon  $\Delta AUC_2$  increased, while immunoreactive insulin did not differ.

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## 1 Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder associated with high blood glucose levels. Its pathology involves insulin resistance in adipocytes and skeletal

muscle, and insulin hyposecretion from pancreatic  $\beta$  cells [1]. Sustained hyperglycemia is a strong risk and accelerative factor for macroangiopathy and microangiopathy [2]. Both the American Diabetes Association and the Japanese Diabetes Society recommend achieving a hemoglobin A1c (HbA1c) level of  $<7\%$  as a therapeutic goal since the adequate control of blood glucose is associated with the prevention of micro- and macro-atherosclerotic complications [3, 4].

The treatment of T2DM typically involves the use of antidiabetic agents, as well as diet and exercise therapy. In 2013, the Japan Diabetes Clinical Data Management Study Group (JDDM) reported that, in Japan, the average HbA1c level was  $6.96\%$  in patients with T2DM [5], which suggests that half of the T2DM patients in Japan failed to achieve their therapeutic goal and needed to undergo further aggressive treatment. In particular, only one-third of patients treated with insulin or a glucagon-like peptide-1 (GLP-1) receptor agonist met the goal of an HbA1c level  $<7\%$  [5], suggesting that supplemental or add-on therapy might be needed to better manage T2DM.

Kidneys play a key role in maintaining blood glucose levels via glycogenesis, glucose filtration at glomeruli, and reuptake of glucose at the tubules [6]. Almost the entire amount of glucose filtered by the tubules is actively reabsorbed by sodium glucose transporters (SGLT) in the proximal tubule. Sodium-glucose cotransporter-2 (SGLT2) inhibitors decrease renal glucose reabsorption; the remaining filtered glucose is reabsorbed by SGLT1 at the descending proximal tubule [7, 8]. An increase in SGLT2 expression at the proximal tubule has been reported in T2DM patients [9], and this increased expression has been suggested to be causally associated with increased glucose levels in T2DM.

Given the foregoing, compounds that inhibit SGLT2 have been targeted and developed as agents to treat T2DM. Dapagliflozin, one such SGLT2 inhibitor, was introduced into clinical practice in Japan in 2014. SGLT2 inhibitors reduce blood glucose levels by increasing the amount of glucose excreted into urine [10]. It is anticipated that these inhibitors will become one of the basic tools for treating T2DM since hypoglycemia occurs less often when compared with using existing antidiabetic agents, and their mechanisms of action also differ. Despite this, few studies have examined the effects of SGLT2 inhibitors, including their influence on insulin and glucagon kinetics and biomarkers associated with adipocyte function in clinical settings in Japan.

To this end, we investigated changes in levels of biomarkers associated with adipocyte function and insulin and glucagon kinetics after a meal tolerance test (MTT) during treatment with dapagliflozin.

## 2 Subjects and Methods

### 2.1 Subjects

Outpatients with T2DM who visited a medical clinic (Okamoto Medical Clinic) that specializes in diabetes treatment in Tokyo, Japan, from June 2014 to December 2014, were screened. On a monthly basis, approximately 980 outpatients visited the medical clinic, and approximately  $92\%$  were diabetic patients. Mean age and HbA1c were 57.4 years and  $7.4\%$ , respectively, and proportions of diabetic retinopathy and nephropathy were 21.7 and  $32.6\%$ , respectively.

Among these patients, those who satisfied the following inclusion criteria were eligible for enrollment: (1) HbA1c level  $\geq 6.5\%$ , despite treatment with only diet and exercise therapy or treatment with a combination of antidiabetic agents, including insulin and GLP-1 receptor antagonists, and diet and exercise therapy for at least 3 months prior to the study period; (2) body mass index (BMI)  $>25\text{ kg/m}^2$ ; and (3) age  $\geq 20$  years. Patients with type 1 diabetes mellitus, severe cardiovascular diseases, renal disease with an estimated glomerular filtration rate (eGFR) of  $<60\text{ mL/min/1.73 m}^2$ , severe liver disorder, dementia, and/or a malignant tumor were excluded from the study.

### 2.2 Study Design

This was a single-arm interventional study. In addition with present treatment, dapagliflozin 5 mg (Forxiga<sup>®</sup>; ONO Pharmaceutical Co., Ltd, Osaka, Japan) was administered orally once daily in the morning for at least 12 weeks. The dosages of concomitant agents did not change during treatment with dapagliflozin. Blood samples were collected at fasting in the morning to measure levels of HbA1c, serum creatinine, ketone bodies (acetoacetic acid, 3-hydroxybutyric acid, and total ketone), high-sensitivity C-reactive protein (hs-CRP), adiponectin, and plasminogen activator inhibitor-1 (PAI-1). Physical characteristics and body weight were also assessed.

In a subset of patients, the MTT was performed before and after treatment with dapagliflozin. The test meal (total calories, 491.5 kcal; carbohydrate, 75 g; fat, 11.5 g; protein, 11.5 g) was prepared by a meal delivery company (Seven-Eleven Japan Co., Ltd, Tokyo, Japan) according to recommendations of the Japanese Diabetes Society. The MTT was initiated at 9:00 am, and patients ate the test meal within 15 min. Blood samples were collected immediately before and 1 and 2 h after finishing the test meal, for simultaneous measurement of blood glucose, immunoreactive insulin (IRI), and glucagon levels.

### 2.3 Assessments

The primary endpoint was change in HbA1c level at 12 weeks after treatment with dapagliflozin, measured using National Glycohemoglobin Standardization Program (NGSP) values, and the secondary endpoint was change in adiponectin and PAI-1 levels from baseline at 12 weeks after treatment with dapagliflozin. In addition to these assessments, changes in glucagon and insulin levels after the MTT were also investigated as a secondary endpoint. Homeostasis model assessment–insulin resistance (HOMA–IR) and homeostasis model assessment– $\beta$ -cell function (HOMA– $\beta$ ) were calculated using the following equations:  $\text{HOMA–IR} = [\text{fasting IRI } (\mu\text{U/mL}) \times \text{fasting blood glucose level (mg/dL)}] / 405$ ;  $\text{HOMA–}\beta = [360 \times \text{fasting IRI } (\mu\text{U/mL})] / [\text{fasting blood glucose level (mg/dL)}] - 63$  [11]. eGFR was calculated using the Japanese GFR equation:  $\text{eGFR (ml/min/1.73 m}^2) = 194 \times \text{Cr} - 1.094 \times \text{age} - 0.287$  ( $\times 0.739$  if female).

### 2.4 Statistical Analysis

Data are presented as mean  $\pm$  standard deviation (SD) for continuous variables, and number and percentage for categorical variables. To assess potential factors that modify adipocyte function, univariate linear regression analysis was performed among changes in continuous variables, at 12 weeks after treatment from baseline. Multivariate linear regression analysis was also performed for changes in adiponectin or PAI-1 as the response variable, and changes in other parameters (HbA1c, body weight, eGFR, total ketone, and hs-CRP) as predictor variables. The area under the curve from time zero to 2 h ( $\text{AUC}_2$ ) during the MTT was calculated to evaluate changes in blood glucose, IRI, and glucagon levels. For paired analysis, the Wilcoxon signed-rank test was used for continuous variables. All statistical analyses were performed using the Statistical Package for JMP 10 (SAS Institute Inc., Cary, NC, USA). All reported  $p$  values were two-sided (significance level 0.05).

The study protocol was reviewed and approved by the Council of Okamoto Medical Clinic, and all procedures followed were in accordance with ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and 2008. Informed consent was obtained from all patients in the study.

## 3 Results

During the study period, 27 patients were treated with dapagliflozin for 12 weeks and had blood samples taken. Patient characteristics at baseline are summarized in

Table 1. Mean age was  $47.9 \pm 8.8$  years, and 17 patients (63.0 %) were male. Mean BMI at baseline was  $32.7 \pm 6.5$   $\text{kg/m}^2$ . Five patients (18.5 %) were being treated with only diet and exercise therapy, and 22 patients had been previously treated with other antidiabetic agents. The mean number of coadministered agents was  $2.5 \pm 1.3$  (range 1–5), and four patients had been receiving diuretics during the study period. Serum potassium did not show any significant change, from  $4.2 \pm 0.4$  to  $4.2 \pm 0.4$  mEq/L ( $p = 0.87$ ), and serum sodium slightly, but significantly, decreased from  $142.0 \pm 2.1$  to  $141.0 \pm 2.3$  ( $p < 0.05$ ) (Table 2).

Changes in the assessed parameters are shown in Table 2. Mean HbA1c and mean body weight significantly decreased by  $0.75 \pm 0.38$  % ( $p < 0.01$ ) and  $3.8 \pm 3.2$  kg ( $p < 0.01$ ), respectively, after treatment with dapagliflozin. While eGFR did not significantly change, levels of ketone bodies significantly increased after treatment with dapagliflozin. Moreover, hs-CRP significantly decreased and PAI-1 showed no significant change after treatment with dapagliflozin. Interestingly, adiponectin slightly, but significantly, increased after treatment.

In the univariate linear regression analysis, inverse correlations were found between changes in HbA1c level/body weight and change in adiponectin level, and positive correlations were found between changes in HbA1c level/body weight and change in adiponectin level (Table 3). Multivariate linear regression analysis revealed inverse change in HbA1c level and body weight to be strong contributors to change in adiponectin level (Table 4).

Among the 11 patients who underwent the MTT (male proportion 72.7 %, mean age  $49.1 \pm 7.8$  years), mean HbA1c level decreased from  $7.28 \pm 0.46$  to  $6.52 \pm 0.45$  %, but HOMA–IR and HOMA– $\beta$  did not significantly change, after treatment with dapagliflozin

**Table 1** Patient characteristics at baseline ( $N = 27$ )

	$N$ (%) or mean $\pm$ SD
Sex, men	17 (63.0)
Age, years	$49.7 \pm 8.8$
Body mass index, $\text{kg/m}^2$	$32.7 \pm 6.5$
Oral hypoglycemic agents, yes	22 (81.5)
DPP-4 inhibitor	15 (55.6)
Sulfonylurea	5 (18.5)
Metformin	18 (66.7)
Thiazolidine	6 (22.2)
Insulin	8 (29.6)
GLP-1 receptor agonist	3 (11.1)

SD standard deviation, DPP-4 dipeptidyl peptidase 4, GLP-1 glucagon-like peptide-1

**Table 2** Comparison of measurements between baseline and 12 weeks after treatment with dapagliflozin ( $N = 27$ )

	Baseline	12 weeks	Change	$p$ value
HbA1c (%)	7.44 $\pm$ 0.56	6.70 $\pm$ 0.57	-0.75 $\pm$ 0.38	<0.01
Body weight (kg)	90.9 $\pm$ 16.5	87.1 $\pm$ 15.9	-3.8 $\pm$ 3.2	<0.01
eGFR (mL/min/1.73 m <sup>2</sup> )	91.6 $\pm$ 22.3	89.5 $\pm$ 21.9	-2.1 $\pm$ 8.1	0.19
Sodium (mEq/L)	142.0 $\pm$ 2.1	141.0 $\pm$ 2.3	-1.0 $\pm$ 0.2	<0.05
Potassium (mEq/L)	4.2 $\pm$ 0.4	4.2 $\pm$ 0.4	0 $\pm$ 0	0.87
Acetoacetic acid ( $\mu$ mol/mL)	22.1 $\pm$ 9.6	35.5 $\pm$ 26.9	13.4 $\pm$ 29.61	<0.01
3-hydroxybutyric acid ( $\mu$ mol/mL)	38.2 $\pm$ 14.4	73.9 $\pm$ 69.0	35.7 $\pm$ 68.3	<0.01
Total ketone bodies ( $\mu$ mol/mL)	60.3 $\pm$ 20.8	109.4 $\pm$ 94.7	49.1 $\pm$ 94.1	<0.01
High-sensitivity CRP (ng/mL)	2410 $\pm$ 2814	1607 $\pm$ 1960	-803 $\pm$ 1080	<0.01
Adiponectin ( $\mu$ g/mL)	5.1 $\pm$ 2.3	6.7 $\pm$ 4.2	1.7 $\pm$ 2.7	<0.01
PAI-1 (mg/mL)	30.0 $\pm$ 16.8	26.8 $\pm$ 30.1	-3.2 $\pm$ 32.5	0.07

Data are expressed as mean  $\pm$  standard deviation

*HbA1c* hemoglobin A1c, *eGFR* estimated glomerular filtration rate, *CRP* C-reactive protein, *PAI-1* plasminogen activator inhibitor-1

**Table 3** Correlation coefficients by univariate linear regression analysis ( $N = 27$ )

	$\Delta$ Adiponectin	$\Delta$ Plasminogen activator inhibitor-1
$\Delta$ HbA1c	-0.787 <sup>a</sup>	0.382 <sup>a</sup>
$\Delta$ Body weight	-0.696 <sup>a</sup>	0.435 <sup>a</sup>
$\Delta$ eGFR	0.206	0.039
$\Delta$ Acetoacetic acid	-0.107	-0.113
$\Delta$ 3-hydroxybutyric acid	-0.157	-0.063
$\Delta$ Total ketone bodies	-0.145	-0.078
$\Delta$ High-sensitivity CRP	0.106	-0.066
$\Delta$ Adiponectin	-	-0.326

$\Delta$  Difference from baseline to endpoint (12 weeks after treatment with dapagliflozin)

*HbA1c* hemoglobin A1c, *eGFR* estimated glomerular filtration rate, *CRP* C-reactive protein

<sup>a</sup>  $p < 0.05$

**Table 4** Coefficients by multivariate linear regression analysis ( $N = 27$ )

	$\Delta$ Adiponectin ( $R^2 = 0.714$ )	$\Delta$ Plasminogen activator inhibitor-1 ( $R^2 = 0.243$ )
$\Delta$ HbA1c	-0.5085 <sup>a</sup>	1.7003
$\Delta$ Body weight	-2.2729 <sup>a</sup>	33.1321
$\Delta$ eGFR	0.0470	0.3167
$\Delta$ Total ketone bodies	-0.0003	-0.0539
$\Delta$ High-sensitivity CRP	-0.0001	0.0019

$\Delta$  Difference from baseline to endpoint (12 weeks after treatment with dapagliflozin)

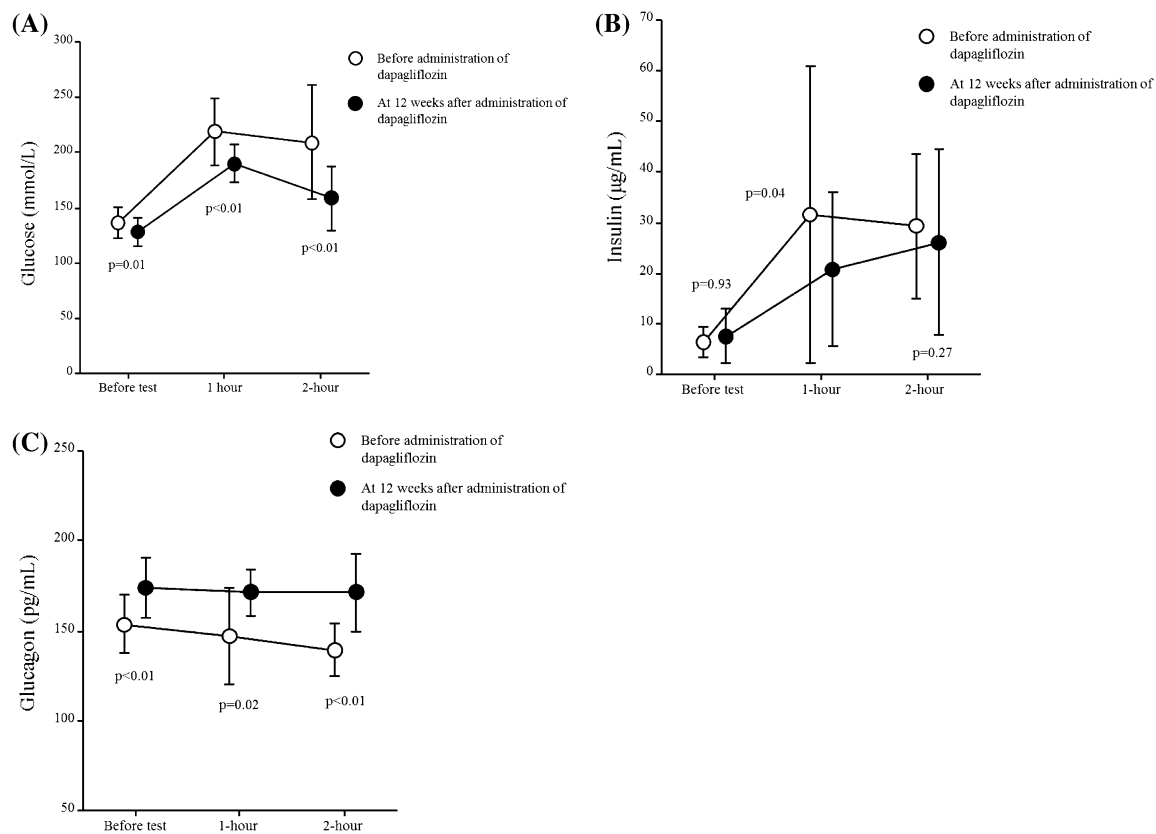
*HbA1c* hemoglobin A1c, *eGFR* estimated glomerular filtration rate, *CRP* C-reactive protein

<sup>a</sup>  $p < 0.05$

(2.53  $\pm$  1.68 to 2.33  $\pm$  1.52,  $p = 0.68$ , and 38.4  $\pm$  25.4 to 21.0  $\pm$  14.6,  $p = 0.06$ , respectively). Figure 1 shows the time-dependent changes in blood glucose, IRI, and glucagon levels after the MTT. Mean blood glucose at 2 h after the MTT reduced significantly compared with baseline,

whereas a significant increase was observed for glucagon. IRI did not change after the MTT compared with baseline.

Figure 1 shows changes in blood glucose, IRI, and glucagon levels after the MTT, before and 12 weeks after treatment with dapagliflozin. Blood glucose at baseline and



**Fig. 1** Changes in **a** glucose, **b** insulin, and **c** glucagon levels after the meal tolerance test

1 and 2 h after the test meal reduced significantly with dapagliflozin treatment [ $7.57 \pm 0.69$  vs.  $7.05 \pm 0.70$  mmol/L ( $136.2 \pm 12.4$  vs.  $126.9 \pm 12.7$  mg/dL),  $p < 0.05$ ;  $12.17 \pm 1.62$  vs.  $10.51 \pm 0.93$  mmol/L ( $219.1 \pm 29.1$  vs.  $189.1 \pm 16.7$  mg/dL),  $p < 0.01$ ;  $11.53 \pm 2.59$  vs.  $8.79 \pm 1.42$  mmol/L ( $207.5 \pm 46.6$  vs.  $158.3 \pm 25.6$  mg/dL),  $p < 0.01$ , respectively]. There was no difference in IRI at baseline and 2 h after the test meal, although it was reduced by 1 h after the test meal. Glucagon significantly increased with dapagliflozin treatment at baseline and 1 and 2 h after the test meal ( $149.5 \pm 28.6$  vs.  $173.7 \pm 22.5$  pg/mL,  $p < 0.01$ ;  $144.0 \pm 33.7$  vs.  $173.1 \pm 16.4$  pg/mL,  $p < 0.05$ ;  $136.2 \pm 27.8$  vs.  $170.0 \pm 26.3$  pg/mL,  $p < 0.01$ , respectively).

Blood glucose  $AUC_2$  decreased significantly during the MTT ( $391 \pm 51$  to  $287 \pm 28$  mg/dL·h,  $p = 0.01$ ), but IRI  $AUC_2$  did not change ( $49.5 \pm 35.2$  to  $36.9 \pm 24.4$  µU/mL·h,  $p = 0.85$ ). On the other hand, glucagon  $AUC_2$  increased significantly ( $287 \pm 58$  to  $345 \pm 38$  pg/dL·h,  $p = 0.04$ ).

No severe adverse reactions (e.g. hypoglycemia, renal dysfunction, urinary tract infection, dehydration, and ketoacidosis) were observed in patients after dapagliflozin treatment.

## 4 Discussion

Treatment of obese T2DM patients with dapagliflozin 5 mg once daily for 12 weeks significantly improved diabetic control and reduced body weight to the same level as previous reports. Although ketone bodies significantly increased, hs-CRP significantly decreased and adiponectin significantly increased. During the MTT, blood glucose  $\Delta AUC_2$  reduced significantly, but glucagon  $\Delta AUC_2$  increased, and that of IRI did not change. To the best of our knowledge, this is the first report to estimate changes in biomarkers associated with adipocyte function and insulin and glucagon kinetics using the MTT after treatment with dapagliflozin.

The glucose-lowering effects of dapagliflozin have been confirmed in several clinical trials conducted in Japan and overseas. In a dose determination study of drug naive Japanese patients with T2DM, dapagliflozin 5 and 10 mg/day reduced HbA1c level by 0.41 and 0.45 %, respectively, from baseline at 24 weeks after treatment [12]. In another study in Japan that evaluated the add-on effect of dapagliflozin to an existing antidiabetic agent, dapagliflozin 5 mg/day for 12 weeks reduced HbA1c level by approximately 0.6 % [13]. In our survey site, the HbA1c

values (average 7.4 %) where dapagliflozin was not added were not changed in the same survey period. Our results showed that adding dapagliflozin improved glycemic control at the same level of previous reports.

Insulin resistance and obesity are closely linked, and obesity is known to be a risk factor for inadequate glycemic control. Therefore, the importance of maintaining ideal body weight is emphasized in the treatment of diabetes mellitus. Among antidiabetic agents, GLP-1 receptor agonists reduce body weight, but several antidiabetic agents increase body weight while reducing glucose levels. In general, SGLT2 inhibitors promote weight loss and, indeed, dapagliflozin decreased body weight by 3.9 kg in this study. Consistent with this, previous studies have reported that dapagliflozin decreased body weight by approximately 2 kg [12, 13]. Since glucose in urine is cotransported with sodium ions via SGLT2, the inhibition of SGLT2 suppresses reabsorption of water with sodium ions, as well as the reabsorption of glucose. Water loss leads to weight loss in the early phases of treatment with SGLT2 inhibitors; however, weight loss during long-term treatment with SGLT2 inhibitors appears to be related to a loss of calories resulting from a reduction in available glucose [14]. Recently, the incidence of diabetic ketoacidosis (DKA) occurring in conjunction with SGLT-2 inhibitor therapy has risen, with our results showing elevated ketone bodies [15]. We need to pay attention to dehydration in order to prevent DKA.

Reduction of abdominal fat volume is considered an essential element for weight loss therapy in obese patients. Bolinder et al. evaluated changes in body composition 24 weeks after treatment with dapagliflozin 10 mg by using dual energy X-ray absorptiometry, and reported a 2.08 kg reduction in weight, which was accompanied by a decrease in total fat volume, abdominal fat volume, and subcutaneous fat volume [16]. In another study, weight loss with diet and exercise therapy in obese patients significantly increased adiponectin levels and decreased PAI-1 levels [17, 18]. Given these findings from previous studies, although body composition was not assessed in the present study, it may be involved in the increase in adiponectin levels and decrease in PAI-1 levels. Since adiponectin was significantly associated with changes in HbA1c and body weight, SGLT2 inhibitors may improve adipocyte function.

Adipocyte function is closely related to insulin resistance. Thus, dapagliflozin may have mitigated insulin resistance by improving adipocyte function. HOMA-IR decreased slightly with dapagliflozin treatment, although the change was not significant. Postprandial glucose levels significantly decreased, but insulin release did not significantly change after treatment with dapagliflozin. These postprandial changes appear reasonable when considering

that the glucose-lowering effects of SGLT2 inhibitors do not depend on the amount of insulin secretion [19, 20].

We found that glucagon levels increased during the fasting phase, as well as after the MTT. Bonner et al. reported that dapagliflozin promotes glucagon secretion and hepatic gluconeogenesis in healthy mice [21], and also found that SGLT2 inhibition by dapagliflozin increases glucagon secretion, thereby mimicking the effects of lowering glucose levels, possibly through  $K_{ATP}$  channel activation and membrane repolarization, as reported by Zhang et al. [22]. Little evidence is available to determine whether treatment with SGLT2 inhibitors is associated with increased glucagon secretion, and further studies will be needed to address this issue.

From the physiological perspective, the mechanism by which SGLT2 inhibitors lower glucose levels differs from that achieved by restricting carbohydrate intake by diet. Restricting carbohydrate intake by diet reduces the amount of glucose absorbed by the digestive tract into the portal vein. In contrast, SGLT2 inhibitors increase the amount of glucose in urine by inhibiting the reuptake of glucose in the renal tubule. It is unclear how these effects of SGLT2 inhibitors provide feedback to glucose metabolism. When considering the physiological mechanism, since the source of metabolism may shift from glucose to fat due to reduction of glucose as an energy source, the level of ketone bodies may increase [23]. Consistent with this, we found an increase in ketone levels in our patients, although there were no harmful effects from this increase. A previous study reported that an increase in 3-hydroxybutyric acid was associated with tolerability to oxidative stress [24]. Further studies will be needed to determine the significance of increased ketone bodies induced by SGLT2 inhibitors.

#### 4.1 Limitations

This study has several limitations worth noting. First, there may have been selection bias given the small sample size and the fact that patients were from one medical institution specializing in diabetes treatment. In addition, the study lacked a control group and participants received heterogeneity of concomitant glucose-lowering drugs; therefore, application to actual clinical settings may be limited. A large-scale, multicenter, controlled study will be needed to better compare our data with data from other medical settings. Second, important factors such as health behavior were not evaluated. Such factors should also be evaluated in future studies. Finally, 12 weeks is a relatively short follow-up period. As a next step, cohort studies with longer follow-up periods should be conducted in order to assess long-term outcomes, including glycemic control.

## 5 Conclusions

In this study, we found that treatment of obese T2DM patients with dapagliflozin 5 mg once daily for 12 weeks significantly reduced HbA1c and body weight to the same level as previous reports. Although ketone bodies increased significantly, CRP significantly decreased, and adiponectin significantly increased. During the MTT, blood glucose  $\Delta\text{AUC}_2$  reduced significantly, but glucagon  $\Delta\text{AUC}_2$  increased, and that of IRI did not differ. These findings suggest that SGLT2 inhibitors may potentially improve adipocyte function in treating obese T2DM patients.

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### Compliance with Ethical Standards

The study has not received any funding. A.O., H.Y., H.S., and N.T. declare no conflict of interest. The study protocol was reviewed and approved by the Council of Okamoto Medical Clinic. All procedures followed were in accordance with ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and 2008. Informed consent was obtained from all patients in the study.

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