

Empagliflozin's Ameliorating Effect on Plasma Triglycerides: Association with Endothelial Function Recovery in Diabetic Patients with Coronary Artery Disease

Takahiro Sawada¹, Kenzo Uzu¹, Naoko Hashimoto², Tetsuari Onishi¹, Tomofumi Takaya¹, Akira Shimane¹, Yasuyo Taniguchi¹, Yoshinori Yasaka¹, Takeshi Ohara² and Hiroya Kawai¹

¹Hyogo Prefectural Himeji Cardiovascular Center, Division of Cardiovascular Medicine, Hyogo, Japan

²Hyogo Prefectural Himeji Cardiovascular Center, Division of Diabetes and Endocrinology, Hyogo, Japan

Aim: So far, the mechanisms behind the cardiovascular benefits of sodium/glucose cotransporter 2 (SGLT2) inhibitors have not been fully clarified.

Methods: In order to evaluate the effects of SGLT2 inhibitors on systemic hemodynamics, glucose metabolism, lipid profile, and endothelial function, 50 diabetic patients with established coronary artery disease (CAD) were included in this analysis and were given empagliflozin 10 mg/d. Cookie meal testing (carbohydrates: 75 g, fats: 28.5 g), endothelial function testing using flow-mediated dilatation (FMD), and body composition evaluation were performed before and after six months of treatment. Changes in %FMD between the treatment periods and its association with metabolic biomarkers were evaluated.

Results: After six months of treatment, the body weight and body fat percentage decreased significantly, while the body muscle percentage increased significantly. The hemoglobin A1c level and fasting and postprandial plasma glucose levels were significantly decreased with treatment. Postprandial insulin secretion was also significantly suppressed and the insulin resistance index was significantly decreased. Furthermore, the fasting and postprandial triglyceride (TG) levels decreased significantly, while total ketone bodies increased significantly after the six-month treatment. While the plasma brain natriuretic peptide level was not changed, the C-reactive protein level was decreased and FMD was significantly improved after the six-month treatment. Multiple regression analysis showed that the strongest predictive factor of FMD improvement is change in the plasma TG levels.

Conclusion: SGLT2 inhibitors improve multiple metabolic parameters. Of these, a reduction in plasma TGs was strongly associated with endothelial function recovery in diabetic patients with CAD, and this reduction may be related to the cardiovascular benefits of SGLT2 inhibitors.

See editorial vol. 27: 637-638

Key words: Diabetes mellitus, Sodium/glucose transporter 2 inhibitors, Triglycerides, Endothelial function, Flow-mediated dilatation

Introduction

Type 2 diabetes mellitus (T2DM) is a prevalent disease that is recognized as a major risk factor for coronary artery disease (CAD)^{1, 2)}. The selective inhibitors of sodium/glucose cotransporter 2 (SGLT2) are glucose-lowering agents that target the kidney to reduce the reabsorption of glucose and promote uri-

nary glucose excretion, and they have been approved for T2DM treatment in numerous countries³⁾. In previous large studies, BI 10773 (Empagliflozin) Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME)⁴⁾ and the Canagliflozin Cardiovascular Assessment Study (CANVAS) trial⁵⁾, it has been demonstrated that SGLT2 inhibitors were the first glucose-lowering

Address for correspondence: Takahiro Sawada, Hyogo Prefectural Himeji Cardiovascular Center, Division of Cardiovascular Medicine Department of Internal Medicine 520, Saisho-Kou, Himeji, Hyogo, 670-0981, Japan E-mail: taktak319@yahoo.co.jp

Received: May 13, 2019 Accepted for publication: September 11, 2019

Copyright©2020 Japan Atherosclerosis Society

This article is distributed under the terms of the latest version of CC BY-NC-SA defined by the Creative Commons Attribution License.

agents to reduce both cardiovascular deaths and the high risk of cardiovascular diseases in patients with T2DM.

Because the EMPA-REG OUTCOME and CANVAS trials were not designed to determine the mechanism underlying their results, several explanations for the reduction in cardiovascular deaths with SGLT2 inhibitors have been proposed, including improved glycemic and lipid control, reduction in body weight, lowering of blood pressure, and amelioration of albuminuria⁶⁻⁸). However, the precise mechanisms behind the cardiovascular benefits of SGLT2 inhibitors have not been fully clarified.

Impaired endothelial function may play an important role in the initiation and progression of atherosclerosis⁹). Flow-mediated dilatation (FMD) in the brachial artery, which is a noninvasive parameter, is considered to be the current gold-standard surrogate marker of coronary endothelial function, and it has been demonstrated in a previous study that brachial FMD is a predictor of the severity of CAD, coronary plaque vulnerability, and cardiovascular events¹⁰⁻¹³).

Aim

In this study, we surrogated brachial FMD as the incidence of cardiovascular events and conducted an exploratory study to evaluate the effects of an SGLT2 inhibitor, empagliflozin, on systemic hemodynamics, glucose metabolism, lipid profile, and endothelial function.

Methods

Participants

This study was an open-label, single-arm, prospective observational study. It was approved by the Ethics Committee of Hyogo Prefectural Himeji Cardiovascular Center and complied with the Declaration of Helsinki. Informed consent was obtained from all eligible patients before participation.

A total of 56 Japanese patients with T2DM with documented chronic CAD were enrolled in this study from our outpatients between April 2017 and March 2018. The inclusion criteria were as follows: (1) SGLT2 inhibitor-naïve patients with T2DM who had been treated for more than 12 weeks with some anti-diabetic drugs or diet and exercise; (2) patients with a hemoglobin A1c (HbA1c) (National Glycohemoglobin Standardization Program, NGSP) level of <8.0%; and (3) males and females aged 40–90. CAD was defined as stenosis of >50% of the diameter of a coronary artery on angiography or on computed tomography coronary angiography, or a history of myocar-

dial infarction, percutaneous coronary intervention, or coronary artery bypass surgery. T2DM was diagnosed according to the Japan Diabetes Society criteria (HbA1c \geq 6.4%, fasting plasma glucose [PG] \geq 126 mg/dL, and PG \geq 200 mg/dL at 2 h after the oral glucose tolerance test).

The exclusion criteria were as follows: (1) patients who, within 12 weeks of enrollment, had started or had had the dose changed or had been on any concomitant drug, such as antidiabetic drugs, statins, or antihypertensive drugs; (2) patients treated with insulin or glucagon-like peptide-1 agonists; (3) patients with type 1 DM or secondary DM; (4) patients with systemic diseases, including hepatic diseases, renal diseases (serum creatinine level $>$ 2.0 mg/dL), collagen diseases, infections, malignancies, or acute coronary syndrome; and (5) patients with permanent implanted pacemakers, implanted cardiac defibrillators, or cardiac resynchronization therapy devices.

All patients met, at least once, with a dietician for nutritional guidance and were encouraged to start and maintain a low-calorie diet and mild-to-moderate exercise levels before enrollment in the study. At enrollment, we added empagliflozin 10 mg/d to the regimen, which was maintained for six months. At each visit, we asked the participants about any adverse events and medication compliance. In addition, all participants were prohibited from changing the dose of the concomitant drugs or adding any other drugs throughout the study period if no adverse effect occurred.

Measurements of the Body Composition

Body composition was measured using InBody 770 (InBody Co., Ltd., Seoul, South Korea) at enrollment and after six months of treatment. InBody 770 sends a very weak alternating current through the body, which is resisted by the body's tissues. This machine, InBody 770, divides the human body into five cylinders (right and left arms, right and left legs, and trunk) to increase the accuracy of the measurement and delivers currents of 50–1,000 kHz^{14,15}). Participants are asked to stand barefoot on the balance scale and to hold the handles of the machine. The output of the measurements, which takes approximately 15 s, is then printed.

Blood Biochemistry

Blood samples were collected from all patients after an overnight fast and were used to determine PG, HbA1c, 1,5-anhydroglucitol, immunoreactive insulin (IRI), total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein

cholesterol (LDL-C), triglyceride (TG), remnant-like particle cholesterol (RLP-C) as a marker of postprandial hyperlipidemia¹⁶, total ketone body fraction, brain natriuretic peptide (BNP) as a marker of heart failure, C-reactive protein (CRP) as an inflammatory marker, and plasminogen activator inhibitor-1 (PAI-1) as a marker of thrombotic diathesis.

The change in plasma volume can be used for prognostication in acute decompensated heart failure, and whole body plasma volume loss may be one of the possible mechanisms for the beneficial effects of SGLT2 inhibitors for cardiovascular diseases^{17, 18}. The Strauss–Davis–Rosenbaum formula is an easily accessible marker of estimating the percentage change in plasma volume in the clinical setting. Therefore, we calculated the change in plasma volume according to the following formula: Change in plasma volume = $\left(\left[\frac{\text{baseline hemoglobin level}}{\text{hemoglobin level after six months of treatment}} \right] \times (100 - \text{hematocrit value after six months of treatment}) / (100 - \text{baseline hematocrit value}) - 1 \right) \times 100$ ¹⁹.

All biochemical analyzes were performed using a commercially available kit. HbA1c levels were measured using high-performance liquid chromatography. IRI and BNP concentrations were measured using a chemiluminescent enzyme immunoassay. RLP-C was measured using an assay kit (Japan Immunoresearch Laboratories Co., Ltd., Takasaki, Japan). The total ketone body fraction level was measured by enzyme circulating methods (Kinos Inc., Tokyo, Japan). PAI-1 was measured using a latex photometric immunoassay.

Cookie Meal Test

A cookie meal test (CMT) was performed before and six months after empagliflozin treatment to evaluate the changes in postprandial glucose, insulin, and fat metabolic dynamics. The cookie consisted of carbohydrates (75 g; 85% flour starch, 15% maltose), butter fat (28.5 g), and protein (8 g), with an overall caloric value of 592 kcal (Saraya Corp., Osaka, Japan). As previously reported, the CMT is sufficient for providing information regarding glucose intolerance and postprandial hypertriglyceridemia²⁰.

All participants completed a 12 h overnight fast before the CMT, as previously described¹⁷. Briefly, the cookie was ingested with water within a 20 min period, and blood samples for PG, IRI, and TG measurement were obtained at 0, 1, and 2 h after the participant had ingested half the volume of the cookie. These values were reported as PG-1h, PG-2h, IRI-1h, IRI-2h, TG-1h, and TG-2h, respectively.

The homeostatic model assessment ratio (HOMA-R = $\text{fasting IRI} \times \text{fasting PG} / 405$) was used as an index of insulin resistance.

We also calculated the area under the response curves for PG (AUC-PG), IRI (AUC-IRI), and TG (AUC-TG) using the trapezoid rule. As an index of postprandial hyperglycemia, we calculated the incremental AUC-PG (AUC-PG – fasting PG \times 2). In order to assess the postprandial TG change, we calculated incremental AUC-TG (AUC-TG – fasting TG \times 2).

Measurement of Endothelial Function

Before undergoing FMD testing, the participants were instructed to fast for >12 h and to abstain from any medications, smoking, alcohol, caffeine, and antioxidant vitamins during that time. All the participants rested for at least 15 min in a seated position in a quiet dark air-conditioned room (22–25 °C) before the FMD measurements, as previously described^{12, 21}. In brief, a longitudinal image of the right or left brachial artery was recorded at baseline using high-resolution ultrasonography and a 10 MHz linear array transducer probe (UNEX, Nagoya, Japan). A forearm cuff was inflated for 5 min at 50 mmHg above the systolic blood pressure just prior to the FMD measurement. After deflating the cuff, the diastolic diameter of the brachial artery was semiautomatically and continuously recorded for 2 min using a software-equipped instrument that can monitor the arterial diameter. The %FMD was estimated as the percent change in the vessel diameter, which corresponded to the maximum dilatation reached during reactive hyperemia divided by the baseline value. Because the %FMD value is highly dependent on the baseline diameter of the vessel, we compared the baseline diameters and absolute changes in the diameters of the brachial artery for each group. We have also previously confirmed that there is excellent intra- and interobserver agreement for the %FMD measurement^{12, 21, 22}.

Primary Endpoints

The primary endpoint of this study was the change in %FMD at enrollment and after six months of empagliflozin treatment. We also evaluated the association between changes in %FMD and changes in metabolic biomarkers.

Statistical Analysis

The sample size was determined by the paired samples t-test power analysis using previously reported data with the following assumptions: a type I error of 0.05 (two-tailed), a type II error of 0.1, a 1.0% difference in %FMD before and after empagliflozin treatment, and a standard deviation of 2.0%²³. Therefore, a minimum of 44 patients are required to yield 90% power to detect a significant difference between before and after treatment.

Table 1. Baseline characteristics

Variables	<i>n</i> = 50
Age (y.o)	66.5 ± 9.4
Sex (Male, <i>n</i> , %)	44 (88.0%)
Coronary risk factor	
Hypertension (<i>n</i> , %)	45 (90.0%)
Dyslipidemia (<i>n</i> , %)	49 (98.0%)
Current smoker (<i>n</i> , %)	4 (8.0%)
Combined drugs	
Statins (<i>n</i> , %)	49 (98.0%)
n-3 polyunsaturated fatty acids (<i>n</i> , %)	16 (32.0%)
Calcium channel blocker (<i>n</i> , %)	20 (40.0%)
Angiotensin converting enzyme inhibitors / angiotensin-receptor blocker (<i>n</i> , %)	36 (72.0%)
Dipeptidyl peptidase 4 inhibitors (<i>n</i> , %)	26 (52.0%)
Metformin / thiazolidine (<i>n</i> , %)	10 (20.0%)
Sulfonylurea agent (<i>n</i> , %)	6 (12.0%)
Alpha glucosidase inhibitors (<i>n</i> , %)	5 (10.0%)

y.o: years old

All statistical analyzes were performed using MedCalc (Version 9.3; MedCalc Software, Mariakerke, Belgium), and a two-tailed *P*-value of <0.05 was considered statistically significant.

Continuous variables with a normal distribution were reported as mean ± standard deviation, or otherwise as median and interquartile ranges. Intergroup comparisons of normally distributed data were performed using the unpaired Student's *t*-test. The Mann–Whitney *U* test was used if the variables were not normally distributed. Comparison of categorical variables was performed using the χ^2 test. Differences in normally distributed biochemical data before and after treatment were compared using the paired *t*-test; otherwise, the Wilcoxon rank-sum test was used. Multivariable regression analyzes were used to identify independently associated factors of changes in %FMD. Potential factors of interest with a univariate *P*-value of <0.05 were entered into the multivariate models.

Results

Study Population

A total of 56 diabetic patients with CAD were enrolled in this study. Of these, one patient had hypoglycemic attacks, one had a gastrointestinal disorder, and four withdrew their consent before the second CMT. Therefore, six patients were excluded from the final analyzes, leaving 50 patients.

Table 1 shows the baseline characteristics of the participants. The concomitant coronary risk factors were hypertension (90.0%), dyslipidemia (98.0%),

and smoking (8.0%). The combined medications were statins (98.0%), n-3 polyunsaturated fatty acids (32.0%), angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (72.0%), dipeptidyl peptidase-4 inhibitors (52.0%), metformin/thiazolidine (20.0%), sulfonylurea agent (12.0%), and alpha glucosidase inhibitors (10.0%). Notably, all patients had CAD and multiple coronary risk factors (two or more coronary risk factors).

Changes in Anthropometric Measurements and Body Composition

As shown in **Table 2**, the body weight and body mass index were significantly decreased after the six-month empagliflozin treatment ($P < 0.0001$ and $P < 0.001$, respectively). Although the total fat quantity, fat percentage, total muscle quantity, and whole body water volume ($P < 0.001$, $P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively) were also decreased significantly, the extracellular water volume did not change and the muscle percentage was significantly increased ($P < 0.0001$). Furthermore, the systolic and diastolic blood pressures were significantly decreased during the six months ($P = 0.03$, $P = 0.03$).

Changes in Fasting and Postprandial Blood Chemistry Analysis and Endothelial Function

The fasting and postprandial blood chemistry data at baseline and at six months are also shown in **Table 2**. After six months of treatment, the HbA1c level, fasting PG, and IRI were significantly decreased; furthermore, a significant reduction in the insulin resistance index (HOMA-R) was observed, and a low

Table 2. Changes in anthropometric measurements and body compositions, %flow-mediated dilatation and blood chemistry data

Variable	Baseline	6-month	P-value
Body composition data and flow-mediated dilatation data			
Weight (kg)	68.6 ± 9.9	65.3 ± 9.7	< 0.0001
Body mass index (kg/m ²)	24.9 ± 3.0	23.7 ± 3.1	< 0.0001
Body fat quantity (kg)	18.3 ± 5.1	15.9 ± 5.2	< 0.0001
Body fat percentage (%)	26.9 ± 5.7	24.6 ± 6.3	< 0.0001
Muscle quantity (kg)	46.9 ± 7.2	45.8 ± 6.9	< 0.0001
Muscle percentage (%)	69.1 ± 5.5	71.3 ± 6.1	< 0.0001
Whole body water volume (L)	36.5 ± 5.9	35.7 ± 5.4	< 0.0001
Extracellular water volume (L)	13.9 ± 2.1	13.7 ± 2.0	0.17
%change plasma volume (%)	-6.9 (-11.0, -1.3)		
Systolic blood pressure (mmHg)	133.8 ± 15.1	130.6 ± 14.3	0.03
Diastolic blood pressure (mmHg)	80.3 ± 10.3	78.4 ± 9.5	0.03
%flow-mediated dilatation (%)	3.5 (2.4, 5.5)	4.7 (3.6, 5.6)	0.001
Absolute change in diameter (mm)	0.17 ± 0.08	0.19 ± 0.08	0.001
Blood chemistry data			
White blood cell (× 10 ² /μL)	61.6 ± 14.6	60.7 ± 15.4	0.67
Red blood cell (× 10 ⁴ /μL)	476.2 ± 36.9	498.0 ± 38.4	< 0.0001
Hemoglobin (g/dL)	14.6 ± 1.1	15.0 ± 1.2	< 0.0001
Hematocrit (%)	43.3 ± 2.9	44.8 ± 3.0	0.0001
Platelet (× 10 ⁴ /μL)	21.7 ± 4.6	20.5 ± 4.5	0.001
Plasma creatinine (mg/dL)	0.82 ± 0.16	0.84 ± 0.17	0.07
e-GFR (mg/dL)	73.4 ± 14.6	71.3 ± 14.0	0.06
Urine albumin (mg/dL)	16.8 (7.2, 52.7)	11.3 (7.4, 25.1)	0.01
Uric acid (mg/dL)	5.4 ± 1.3	4.7 ± 1.1	< 0.0001
Hemoglobin A1c (%)	7.0 ± 0.5	6.6 ± 0.4	< 0.0001
1,5-anhydroglucitol (μg/dL)	11.1 ± 5.7	2.1 ± 2.0	< 0.0001
Plasma glucose level (mg/dL)			
Fasting	129.4 ± 16.1	114.8 ± 18.7	< 0.0001
After 1 hour	238.7 ± 33.4	217.2 ± 33.6	< 0.0001
After 2 hours	235.9 ± 46.4	213.7 ± 51.1	0.001
Incremental AUC-PG (mg/dL/2 hr)	162.6 ± 46.4	151.9 ± 43.4	0.07
Immunoreactive insulin (μU/mL)			
Fasting	7.8 (4.6, 9.5)	5.0 (3.2, 8.5)	< 0.0001
After 1 hour	40.9 (25.0, 60.3)	33.5 (18.2, 59.8)	0.0005
After 2 hours	49.2 (28.3, 79.9)	43.0 (23.5, 60.5)	0.0006
Incremental AUC-IRI (μU/mL/2 hr)	54.7 (30.7, 88.7)	46.3 (25.8, 78.8)	0.0002
HOMA-R	2.4 (1.4, 3.2)	1.3 (0.9, 2.3)	< 0.0001
Total cholesterol (mg/dL)	164.9 ± 25.6	163.6 ± 29.7	0.62
LDL cholesterol (mg/dL)	90.6 ± 23.5	90.8 ± 24.1	0.92
HDL cholesterol (mg/dL)	51.1 ± 19.6	51.1 ± 9.4	0.99
Plasma triglyceride (mg/dL)			
Fasting	119.9 ± 54.9	95.7 ± 33.5	0.0002
After 1 hour	142.5 ± 53.5	122.6 ± 41.0	0.001
After 2 hours	184.3 ± 70.2	153.5 ± 52.8	< 0.0001
Incremental AUC-TG (mg/dL/2 hr)	54.7 ± 34.8	55.8 ± 32.6	0.80
RLP cholesterol (mg/dL)	5.0 (3.3, 7.0)	3.8 (2.4, 4.9)	< 0.0001
C reactive protein (mg/dL)	0.11 (0.03, 0.22)	0.07 (0.03, 0.13)	0.003
Brain natriuretic peptide (pg/mL)	31.3 (14.4, 58.6)	20.6 (9.2, 54.6)	0.37
PAI-1 (ng/mL)	18.6 ± 7.8	13.8 ± 5.0	< 0.0001
Total ketone body (μmol/L)	89.0 (61.0, 152.0)	145.5 (94.0, 218.0)	0.0002

e-GFR: estimate glomerular filtration rate, AUC-PG: area under the curve of plasma glucose, HOMA-R: homeostasis model assessment insulin resistance, AUC-IRI: area under the curve of immunoreactive insulin, LDL: low density lipoprotein, HDL: high density lipoprotein, AUC-TG: area under the curve of triglyceride, RLP: remnant like particle

Table 3. Regression analysis for predicting %flow-mediated dilatation improvement

variable	univariate		multivariate	
	t	p-value	t	p-value
Age	-0.119	0.91		
Sex	0.892	0.38		
Changes in weight	-0.154	0.88		
Changes in body fat percentage	0.593	0.56		
Changes in body muscle percentage	0.919	0.36		
Changes in whole body water volume	0.918	0.36		
%change plasma volume	0.007	0.99		
Changes in hematocrit	-0.036	0.97		
Changes in urine albumin	1.727	0.09		
Changes in e-GFR	0.125	0.90		
Changes in uric acid	-0.074	0.94		
Changes in hemoglobin A1c	-2.438	0.02	-1.568	0.12
Changes in 1,5-anhydro-glucitol	-0.615	0.54		
Changes in fasting plasma glucose	-0.214	0.83		
Changes in incremental AUC-PG	0.710	0.48		
Changes in fasting IRI	-2.433	0.02	-1.444	0.16
Changes in incremental AUC-IRI	-2.057	0.05		
Changes in HOMA-R	-1.769	0.08		
Changes in LDL cholesterol	-1.025	0.31		
Changes in HDL cholesterol	-0.107	0.92		
Changes in fasting triglyceride	-5.939	<0.0001	-5.703	<0.0001
Changing in incremental AUC-TG	-2.112	0.04	-1.667	0.10
Changes in RLP cholesterol	-4.971	<0.0001		
Changes in C-reactive protein	0.299	0.77		
Changes in BNP	1.618	0.11		
Changes in PAI-1	-0.428	0.67		
Changing total ketone body	2.057	0.04	1.267	0.21

e-GFR: estimate glomerular filtration rate, AUC-PG: area under the curve of plasma glucose, HOMA-R: homeostasis model assessment insulin resistance, AUC-IRI: area under the curve of immunoreactive insulin, LDL: low density lipoprotein, HDL: high density lipoprotein, AUC-TG; area under the curve of triglyceride, RLP: remnant like particle, BNP: brain natriuretic peptide, PAI-1: plasminogen activator inhibitor-1

1,5-anhydroglucitol level indicated a deleterious effect.

Regarding the lipid profiles, fasting TG and RLP-C exhibited a significant reduction, although there were no significant changes in total cholesterol, HDL-C, and LDL-C.

Postprandial PG (PG-1h, PG-2h, and incremental AUC-PG) and postprandial IRI (IRI-1h, IRI-2h, and incremental AUC-IRI) were all decreased significantly. In a similar fashion, a significant reduction in postprandial TG (TG-1h, TG-2h) was observed, but the incremental AUC-TG did not exhibit a significant change.

Although the white blood cell count remained unchanged, significant increases were observed in the red blood cell count, hemoglobin level, and hematocrit level, whereas the platelet count decreased slightly. Plasma uric acid and urinary albumin level decreased

significantly, whereas the serum creatinine level was slightly increased. No significant change was observed in the BNP.

The median baseline %FMD was 3.5%, and there was no significant association between the baseline %FMD and coronary risk factors and other baseline parameters. After six months of treatment, the %FMD, the primary endpoint of this study, improved significantly (median: 3.5–4.7%, $P < 0.0001$), whereas CRP and PAI-1 decreased significantly. Absolute change in brachial artery diameter was also increased significantly.

Regression Analysis of Factors Predicting %FMD Improvement

The univariate and multivariate regression analysis results are shown in **Table 3**. The univariate analy-

sis revealed that changes in the HbA1c level, fasting and postprandial IRI and TG levels, and plasma total ketone body level were associated with the changes in %FMD. The multiple regression analysis revealed that the strongest independent factor predicting FMD changes was change in the TG level.

Subgroup Analysis Based on the Patients Divided by the Median Values of Changes in %FMD

We divided the patients into two subgroups according to the median values of the changes in %FMD: lower $\Delta\%$ FMD group ($\Delta\%$ FMD $\leq 0.7\%$, $n=25$ patients) and higher $\Delta\%$ FMD group ($\Delta\%$ FMD $> 0.7\%$, $n=25$ patients).

As shown in **Table 4**, we noted significant differences in the baseline fasting and postprandial TG and RLP-C levels between the two groups. We also noted that the baseline %FMD in the higher $\Delta\%$ FMD group was significantly lower than that in the lower $\Delta\%$ FMD group.

After six months of treatment, both groups exhibited similar changes in anthropometric measurements and hemodynamic data. The %change plasma volume also exhibited a similar change.

Although both groups showed a significant reduction in HbA1c, fasting PG, and HOMA-R, the improvement of fasting and postprandial insulin secretion was significantly larger in the higher $\Delta\%$ FMD group (**Fig. 1**). Among the lipid profiles, fasting and postprandial TG (e.g., incremental AUC-TG and RLP-C) were also significantly decreased only in the higher $\Delta\%$ FMD group (**Fig. 1**). Furthermore, a decrease in the CRP level and a significant increment in total ketone bodies occurred only in the higher $\Delta\%$ FMD group.

Discussion

In this study, we noted that six months of treatment with empagliflozin improved not only the glyce-mic control, but also multiple coronary risk factors, including blood pressure, obesity, insulin resistance, hypertriglyceridemia, and hyperuricemia. In addition, it ameliorated several atherosclerotic markers, such as inflammation, thrombogenicity, and endothelial function. Among these factors, an improvement in hypertriglyceridemia was the strongest independent factor predicting an improvement in endothelial function in diabetic patients with CAD treated with empagliflozin.

T2DM is a major risk factor for the progression of atherosclerosis and development of cardiovascular diseases. Among various oral glucose-lowering agents with different mechanisms of action, SGLT2 inhibi-

tors are one of the few oral diabetes drugs that was able to achieve a significant reduction in major cardiovascular outcomes in large randomized controlled trials^{4, 5}. As shown in several previous studies, the present study also revealed multiple favorable effects^{6-8, 24-27}. However, the precise mechanisms underlying the cardiovascular benefits of SGLT2 inhibitors remain to be elucidated. We revealed that the reduction of both fasting and postprandial TG and insulin was significantly greater in the higher $\Delta\%$ FMD group compared to the lower $\Delta\%$ FMD group. In addition, the multivariate regression analysis revealed that an improvement in hypertriglyceridemia is the strongest independent factor predicting an improvement in endothelial function in diabetic patients with CAD. Similar to the present study, we previously demonstrated that α -glucosidase inhibitors, which have ameliorating effects on hyperglycemia without inducing of insulin secretion, improved hypertriglyceridemia and insulin resistance; these improvements were associated with the improvement of oxidative stress and endothelial function in diabetic patients with CAD²⁸. Based on these observations, we are of the opinion that the mechanisms behind the cardiovascular benefits of SGLT2 inhibitors may be associated with the improvement in hypertriglyceridemia and insulin resistance.

In patients with insulin-resistant T2DM, the lipoprotein lipase activity is decreased and lipolysis is impaired, and there are few surface remnants available to be incorporated into the HDL particles. Consequently, the chylomicron remnant levels, which are highly atherogenic and act by enhancing systemic inflammation, platelet activation, coagulation, thrombus formation, and macrophage foam cell formation, are often elevated in such patients^{29, 30}. Therefore, T2DM commonly demonstrates elevated TG, low HDL-C, and predominance of small dense LDL particles due to insulin resistance, and this type of dyslipidemia is considered to be atherogenic dyslipidemia^{31, 32}. Sone *et al.* demonstrated that the serum TG level is a leading predictor of CAD, which is comparable to LDL-C in Japanese patients with T2DM³³. On the other hand, since the report of Zilvermit³⁴, postprandial hypertriglyceridemia has also been noticed to be a risk factor for atherosclerosis, especially CAD. Therefore, researchers are currently focusing on fasting and postprandial hypertriglyceridemia as a residual risk factor for cardiovascular events in statin-treated diabetic patients. Although some studies failed to show a relationship between hypertriglyceridemia and endothelial dysfunction and some controversy persists^{35, 36}, there are many reports revealing that fasting and postprandial hypertriglyceridemia are closely associated with endothelial dysfunction and its amelioration link

Table 4. Comparison of changes in body composition, flow-mediated dilatation and blood chemistry between lower and higher Δ %FMD group

Variable	Lower Δ %FMD group; <i>n</i> = 25 (Δ %FMD of \leq 0.7%)		Higher Δ %FMD group; <i>n</i> = 25 (Δ %FMD of $>$ 0.7%)		<i>P</i> -value
	Baseline	6-month	Baseline	6-month	
Body composition data					
Weight (kg)	68.5 \pm 9.7	65.2 \pm 9.2*	68.7 \pm 10.2	65.4 \pm 10.2*	0.94
Absolute Δ	-3.3 (-4.6, -1.9)		-2.7 (-4.4, -2.1)		0.75
Body mass index (kg/m ²)	24.9 \pm 2.4	23.7 \pm 2.5*	24.9 \pm 3.6	23.7 \pm 3.7*	0.97
Absolute Δ	-1.1 (-1.6, -0.7)		-1.1 (-1.7, -0.7)		0.72
Body fat quantity (kg)	18.0 \pm 4.3	15.7 \pm 4.2*	18.6 \pm 5.9	16.2 \pm 6.3*	0.72
Absolute Δ	-1.7 (-3.8, -0.6)		-2.1 (-3.0, -1.4)		0.54
Body fat percentage (%)	26.6 \pm 5.6	24.3 \pm 5.8**	27.3 \pm 6.0	24.9 \pm 7.0*	0.66
Absolute Δ	-1.7 (-4.0, -0.3)		-2.5 (-3.3, -0.8)		0.72
Muscle quantity (kg)	47.5 \pm 8.2	46.5 \pm 7.8**	46.2 \pm 6.0	45.1 \pm 6.0**	0.55
Absolute Δ	-1.0 (-1.8, 0.2)		-1.1 (-1.5, -0.7)		0.81
Muscle percentage (%)	69.4 \pm 5.3	71.5 \pm 5.6**	68.7 \pm 5.7	70.9 \pm 6.7*	0.67
Absolute Δ	1.6 (0.3, 3.8)		2.4 (0.7, 3.1)		0.82
Whole body water volume (L)	37.0 \pm 6.4	36.3 \pm 6.0**	36.0 \pm 4.6	35.2 \pm 4.6**	0.55
Absolute Δ	-0.6 (-1.4, 0.2)		-0.9 (-1.1, -0.5)		0.89
Extracellular water volume (L)	14.0 \pm 2.5	13.9 \pm 2.2	13.8 \pm 1.6	13.5 \pm 1.7	0.79
Absolute Δ	-0.3 (-0.5, 0.2)		-0.3 (-0.4, -0.1)		0.81
%change plasma volume (%)		-6.99 (-10.59, -1.67)		-6.88 (-11.46, -1.22)	0.83
Systolic blood pressure (mmHg)	130.1 \pm 13.0	127.1 \pm 11.7	136.9 \pm 16.7	134.1 \pm 15.9	0.15
Absolute Δ		-3.0 (-8.0, 2.3)		-4.0 (-9.0, 5.3)	0.96
Diastolic blood pressure (mmHg)	80.1 \pm 10.7	78.2 \pm 9.8	80.1 \pm 10.1	78.6 \pm 9.3***	0.88
Absolute Δ		-1.0 (-6.0, 3.3)		-2.0 (-5.3, 2.0)	0.56
%flow-mediated dilatation (%)	5.5 (3.4, 6.0)	4.7 (2.8, 5.8)	2.6 (1.9, 3.6)	4.6 (3.8, 5.1)*	0.0001
Absolute Δ		-0.3 (-0.6, -0.1)		1.7 (1.0, 2.4)	< 0.0001
Absolute change in diameter (mm)	0.19 (0.15, 0.26)	0.18 (0.12, 0.24)	0.11 (0.09, 0.16)	0.19 (0.16, 0.24)*	< 0.0001
Absolute Δ		-0.02 (-0.03, 0.00)		0.07 (0.04, 0.11)	< 0.0001
Blood chemistry data					
White blood cell ($\times 10^2/\mu$ L)	60.1 \pm 15.0	55.8 \pm 12.9	63.0 \pm 14.4	65.7 \pm 16.2	0.50
Absolute Δ		0.0 (-10.5, 62.5)		20.0 (-50.0, 90.0)	0.14
Red blood cell ($\times 10^4/\mu$ L)	472.7 \pm 42.1	491.0 \pm 43.1*	479.6 \pm 31.2	505.0 \pm 32.6*	0.52
Absolute Δ		15.0 (6.0, 30.0)		21.0 (9.0, 45.0)	0.47
Hemoglobin (g/dL)	14.4 \pm 1.0	14.9 \pm 1.0**	14.5 \pm 1.3	15.1 \pm 1.4**	0.86
Absolute Δ		0.5 (0.2, 0.9)		0.5 (0.2, 1.1)	0.75
Hematocrit (%)	43.0 \pm 2.6	44.5 \pm 2.4**	43.6 \pm 3.2	45.1 \pm 3.5***	0.48
Absolute Δ		1.7 (0.2, 2.6)		1.6 (0.0, 3.3)	0.84
Platelet ($\times 10^4/\mu$ L)	21.0 \pm 4.0	19.7 \pm 3.9***	22.3 \pm 5.2	21.3 \pm 5.0***	0.32
Absolute Δ		-1.4 (-2.8, 0.6)		-1.3 (-2.5, 0.5)	0.79
Plasma creatinine (mg/dL)	0.79 \pm 0.12	0.81 \pm 0.16	0.84 \pm 0.16	0.87 \pm 0.17	0.31
Absolute Δ		0.02 (-0.04, 0.06)		0.02 (-0.04, 0.06)	1.00
e-GFR (mg/dL)	75.1 \pm 15.4	73.1 \pm 13.4	71.6 \pm 13.9	69.4 \pm 14.5	0.40
Absolute Δ		-2.2 (-7.1, 4.6)		-1.3 (-5.9, 1.1)	0.95
Urine albumin (mg/dL)	13.8 (6.2, 62.8)	10.8 (6.0, 23.6)	18.0 (10.1, 46.6)	12.4 (8.6, 26.9)	0.72
Absolute Δ		-2.1 (-17.4, 1.1)		-4.3 (-19.8, 2.8)	0.79
Uric acid (mg/dL)	5.3 \pm 1.4	4.5 \pm 1.0**	5.9 \pm 1.2	4.8 \pm 1.2**	0.46
Absolute Δ		-0.6 (-1.3, -0.2)		-0.8 (-1.5, -0.4)	0.51
HemoglobinA1c (%)	7.2 \pm 0.5	6.8 \pm 0.4*	6.8 \pm 0.3	6.4 \pm 0.4**	0.11
Absolute Δ		-0.4 (-1.0, -0.2)		-0.3 (-0.5, -0.1)	0.26
1,5-anhydroglucitol (μ g/dL)	10.9 \pm 6.2	2.5 \pm 2.7*	11.3 \pm 5.4	1.8 \pm 0.7*	0.83
Absolute Δ		-7.6 (-9.8, -4.2)		-9.7 (-12.2, -5.6)	0.25

(Cont. Table 4)

Variable	Lower $\Delta\%$ FMD group; $n = 25$ ($\Delta\%$ FMD of $\leq 0.7\%$)		Higher $\Delta\%$ FMD group; $n = 25$ ($\Delta\%$ FMD of $> 0.7\%$)		P-value
	Baseline	6-month	Baseline	6-month	
Plasma glucose level (mg/dL)					
Fasting	130.1 \pm 16.6	114.0 \pm 12.7*	128.2 \pm 15.8	115.6 \pm 23.4*	0.61
Absolute Δ		-15.0 (-29.8, -6.8)		-16.0 (-23.3, -7.3)	0.66
After 1 hour	242.7 \pm 34.1	212.8 \pm 28.3*	234.7 \pm 32.9	221.6 \pm 34.4	0.40
Absolute Δ		-32.0 (-53.5, -10.8)		-13.0 (-40.3, 1.5)	0.16
After 2 hours	238.7 \pm 51.0	204.8 \pm 45.6**	233.0 \pm 42.2	222.7 \pm 55.4	0.67
Absolute Δ		-36.0 (-69.0, -1.0)		-13.0 (-45.5, 16.0)	0.07
Incremental AUC-PG (mg/dL/2hr)	166.2 \pm 46.0	144.1 \pm 42.3*	158.9 \pm 41.3	159.6 \pm 43.8	0.56
Absolute Δ		-20.5 (-45.0, 0.9)		-0.5 (-24.8, 28.8)	0.09
Immunoreactive insulin (μ U/mL)					
Fasting	6.3 (4.1, 9.1)	5.4 (3.5, 8.5)	7.9 (4.8, 9.9)	4.4 (3.0, 8.7)*	0.35
Absolute Δ		-0.7 (-2.3, 1.1)		-2.3 (-3.5, -0.5)	0.03
After 1 hour	40.7 (22.9, 54.3)	37.6 (18.1, 58.9)	41.1 (25.7, 69.3)	27.0 (19.9, 60.4)**	0.51
Absolute Δ		-1.9 (-8.0, 3.4)		-7.5 (-16.8, -3.8)	0.01
After 2 hours	47.9 (27.4, 65.9)	42.8 (22.6, 60.0)	50.7 (32.1, 93.5)	43.2 (27.0, 70.3)	0.14
Absolute Δ		-5.1 (-16.4, 2.9)		-8.0 (-31.0, -2.7)	0.12
Incremental AUC-IRI (μ U/mL/2 hr)	53.7 (30.6, 75.1)	48.0 (23.7, 75.1)	57.6 (35.8, 100.0)	41.0 (29.3, 79.5)**	0.31
Absolute Δ		-6.6 (-9.2, 2.1)		-13.9 (-21.8, -0.1)	0.04
HOMA-R	2.4 (1.4, 3.1)	1.7 (1.4, 2.2)***	2.6 (1.5, 3.2)	1.3 (0.8, 2.7)**	0.53
Absolute Δ		-0.5 (-1.2, 0.1)		-0.9 (-1.4, -0.3)	0.21
Total cholesterol (m/dL)					
Fasting	157.6 \pm 21.0	161.4 \pm 22.6	172.2 \pm 28.1	165.8 \pm 38.8	0.06
Absolute Δ		4.0 (-9.2, 17.2)		-6.0 (-16.2, 3.8)	0.09
LDL cholesterol (mg/dL)	88.1 \pm 18.7	89.4 \pm 17.8	93.1 \pm 27.7	92.2 \pm 29.4	0.46
Absolute Δ		3.0 (-8.0, 11.0)		-3.0 (-10.2, 6.3)	0.34
HDL cholesterol (mg/dL)	48.4 \pm 8.8	50.9 \pm 9.8	53.8 \pm 26.4	51.2 \pm 9.2	0.33
Absolute Δ		0.0 (-3.0, 10.0)		1.0 (-3.0, 8.3)	0.97
Plasma triglyceride (mg/dL)					
Fasting	99.0 \pm 33.7	96.2 \pm 33.3	140.8 \pm 64.0	95.1 \pm 34.4*	0.006
Absolute Δ		-9.0 (-19.5, 16.8)		-36.0 (-67.0, -15.8)	0.0002
After 1 hour	119.8 \pm 32.9	124.2 \pm 43.7	165.2 \pm 60.7	121.0 \pm 38.9*	0.002
Absolute Δ		0.0 (-10.3, 20.8)		-38.0 (-56.5, -20.7)	< 0.0001
After 2 hours	155.6 \pm 49.5	154.4 \pm 57.9	212.9 \pm 76.9	152.5 \pm 48.3*	0.003
Absolute Δ		4.0 (-20.5, 18.0)		-47.0 (-80.8, -34.8)	< 0.0001
Incremental AUC-TG (mg/dL/2 hr)	49.1 \pm 30.1	57.1 \pm 36.2	60.4 \pm 38.8	52.5 \pm 29.1*	0.25
Absolute Δ		5.5 (-14.5, 22.8)		-9.5 (-15.9, 3.1)	0.05
RLP cholesterol (mg/dL)					
Fasting	4.1 (2.6, 6.0)	4.1 (2.6, 5.1)	6.1 (4.2, 9.8)	3.7 (2.4, 4.7)*	0.01
Absolute Δ		-0.4 (-1.2, 0.9)		-1.9 (-4.4, -1.0)	0.0003
C reactive protein (mg/dL)					
Fasting	0.07 (0.03, 0.18)	0.06 (0.03, 0.11)	0.12 (0.04, 0.29)	0.10 (0.04, 0.20)***	0.22
Absolute Δ		-0.01 (-0.08, 0.02)		-0.02 (-0.07, 0.003)	0.87
Brain natriuretic peptide (pg/dL)	31.4 (14.0, 59.2)	19.4 (9.1, 39.7)	28.8 (14.2, 59.0)	22.0 (10.8, 58.0)	0.93
Absolute Δ		-3.1 (-17.8, 0.7)		2.1 (-10.7, 9.4)	0.17
PAI-1 (ng/mL)	16.9 \pm 6.5	12.9 \pm 4.8**	20.3 \pm 8.6	14.7 \pm 5.1*	0.13
Absolute Δ		-4.0 (-8.3, 0.0)		-3.0 (-8.8, -2.0)	0.44
Total ketone body (μ mol/L)					
Fasting	95.0 (65.5, 143.0)	134.0 (86.0, 152.5)	80.0 (50.0, 138.5)	191.0 (107.0, 336.0)*	0.36
Absolute Δ		23.0 (-37.0, 76.0)		82.5 (15.0, 170.5)	0.02

P-values represent comparison of each values between groups at 6 months except for absolute Δ .

As for absolute Δ of each values, P-values represent comparison between the two groups.

Values are presented as means \pm standard deviation or medians and interquartile ranges, as indicated.

* $P < 0.0001$ vs baseline, ** $P < 0.001$ vs baseline, *** $P < 0.05$ vs baseline

e-GFR; estimated glomerular filtration rate, AUC; area under the response curve, IRI; immunoreactive insulin, HOMA-R; homeostasis model assessment ratio, LDL; low density lipoprotein, HDL; high density lipoprotein, TG; triglyceride, RLP; remnant-like particle, PAI-1; plasminogen activator inhibitor type 1

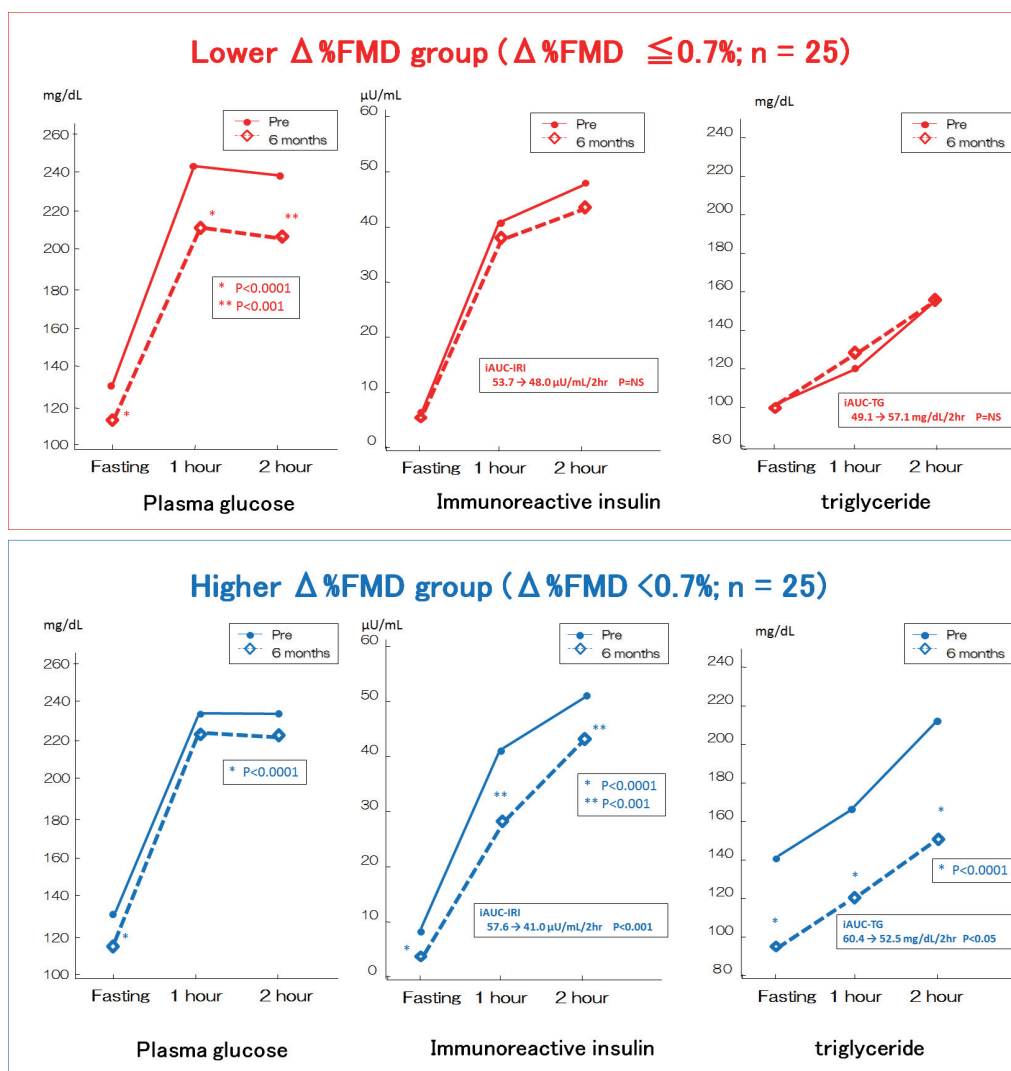


Fig. 1. PG, IRI, and TG levels during the CMT at baseline and after six months

* $P < 0.0001$ versus baseline. ** $P < 0.001$ versus baseline. Comparisons of data before and after treatment were performed using the paired t -test.

to the endothelial dysfunction recovery^{37, 38}). Our data completely support these facts and suggest that the SGLT2 inhibitor empagliflozin may be a promising antiatherosclerotic drug; hence, it may contribute to cardiovascular benefits.

On the other hand, Inzucchi *et al.* demonstrated that changes in markers such as the plasma volume, hematocrit, and hemoglobin are the most important mediator of a lower cardiovascular event rate¹⁸). Indeed, in the EMPA-REG OUTCOME and CANVAS trials, the greatest benefit of SGLT2 inhibitors was the reduction of hospitalization for heart failure^{4, 5}). Our data also revealed a significantly decreased whole body water volume and significantly increased red blood cell count, hemoglobin level, and hematocrit

level, along with a plasma volume reduction. Thus, the mitigating effect of SGLT2 inhibitors on volume overload probably contributed to the reduction of heart failure risks and might be one of the potential mechanisms for the reduction of cardiovascular events with SGLT2 inhibitors. However, the Hisayama study showed that both elevated and decreased hematocrit levels were associated with an increased incidence of cardiovascular diseases³⁹). However, in our study, we observed that the hematocrit and hemoglobin levels in the higher $\Delta\%FMD$ group were similar to those in the lower $\Delta\%FMD$ group. Furthermore, the BNP level exhibited no changes in both groups. Therefore, we could not conclude that the only volume reduction effect of SGLT2 inhibitors was the most plausible

mechanism of cardiovascular benefits of SGLT2 inhibitors.

Intriguingly, although the strongest independent factor of %FMD improvement was the reduction of plasma TG levels, the regression analysis also revealed that the total ketone body increment was associated with an improvement in endothelial function. Some researchers suggested that, under a mild hyperketonemia condition caused by SGLT2 inhibitors, the heart preferentially metabolizes ketone bodies instead of glucose, as an alternative fuel source, thereby improving the myocardial work efficiency and function⁴⁰). In fact, Aubert *et al.* indicated that a hypertrophied and failing myocardium shifts to ketone bodies as a significant fuel source for oxidative adenosine triphosphate production in mouse models⁴¹). Our data also support this hypothesis; however, further large-sized, randomized, controlled study is warranted.

Limitations

Several limitations in the present study warrant consideration. First, this was a study with a single-arm design and no control group. Therefore, changes that might be observed without SGLT2 inhibitor treatment could not be compared in this study. However, in our previous study, we observed a not so significant change in %FMD among patients who underwent just dietary and exercise therapy for six months²¹). Thus, we believe that SGLT2 inhibitor therapy may have an additive beneficial influence on endothelial dysfunction in comparison with non-SGLT2-inhibitor therapy. Second, although we calculated the required sample size according to a previous study, the number of participants required to detect a possible association between changes in FMD and those of several biochemical markers was relatively small. Third, several concomitant drugs (e.g., statins, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers) were used, which had the potency of improving the endothelial function; therefore, some bias is likely to have occurred in our results. However, the participants were prohibited from changing the dose of concomitant drugs or adding any other drugs within 12 weeks of enrollment and throughout the study to minimize the effects of factors other than empagliflozin. Fourth, the plasma TG level gradually increases after a meal and reaches a peak at about 4 h after the meal, but we did not assess the plasma TG level 4 h after the cookie load in this study^{35, 36}). This was due to difficulties associated with more multiple times blood sample obtainment for each outpatient. Therefore, the possibility of an inaccurate assessment of postprandial hypertriglyceridemia cannot be ruled

out. Because Sato *et al.* demonstrated that RLP-C in the fasting period may be suitable for detecting postprandial hyperlipidemic subjects¹⁶), we used RLP-C as an alternative marker of postprandial hypertriglyceridemia. In addition, we calculated the incremental AUC-TG to assess the postprandial change of plasma TGs. We believe that we can evaluate postprandial hypertriglyceridemia meaningfully using these multiple markers.

Conclusions

In conclusion, empagliflozin improved not only glycemic control, but also insulin resistance and hypertriglyceridemia. These ameliorations of metabolic abnormalities were strongly associated with improvements in concomitant endothelial function among diabetic patients with CAD. Although the mechanism of the cardiovascular benefits of SGLT2 inhibitors might be multifactorial, reduction of plasma TGs may be mostly related to the cardiovascular benefits of SGLT2 inhibitors.

Acknowledgements

None.

Conflicts of Interest

None.

References

- 1) Mak KH, Moliterno DJ, Granger CB, Miller DP, White HD, Wilcox RG, Califf RM, Topol EJ. Influence of diabetes mellitus on clinical outcome in the thrombolytic era of acute myocardial infarction, GUSTO-I Investigators. Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries. *J Am Coll Cardiol*, 1997; 30: 171-179
- 2) Malmberg K, Yusuf S, Gerstein HC, Brown J, Zhao F, Hunt D, Piegas L, Calvin J, Keltai M, Budaj A. Impact of diabetes on long-term prognosis in patients with unstable angina and non-Q wave myocardial infarction: results of the OASIS (Organization to Assess Strategies for Ischemic Syndrome) Registry. *Circulation*, 2000; 102: 1014-1019
- 3) Vallon V. The mechanisms and therapeutic potential of SGLT2 inhibitors in diabetes mellitus. *Annu Rev Med*, 2015; 66: 255-270
- 4) Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE; EMPA-REG OUTCOME Investigators. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med*, 2015; 373: 2117-2128
- 5) Mahaffey KW, Neal B, Perkovic V, de Zeeuw D, Fulcher G, Erond N, Shaw W, Fabbrini E, Sun T, Li Q, Desai M,

- Matthews DR; CANVAS Program Collaborative Group. Canagliflozin for primary and secondary prevention of cardiovascular events: Results from the CANVAS Program (Canagliflozin Cardiovascular Assessment Study). *Circulation*, 2018; 137: 323-334
- 6) Heerspink HJ, Perkins BA, Fitchett DH, Husain M, Cherney DZ. Sodium glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: cardiovascular and kidney effects, potential mechanisms, and clinical applications. *Circulation*, 2016; 134: 752-772
 - 7) Nauck MA, Del Prato S, Meier JJ, Durán-García S, Rohwedder K, Elze M, Parikh SJ. Dapagliflozin versus glipizide as add-on therapy in patients with type 2 diabetes who have inadequate glycemic control with metformin: a randomized, 52-week, double-blind, active-controlled noninferiority trial. *Diabetes Care*, 2011; 34: 2015-2022
 - 8) Sattar N, McLaren J, Kristensen SL, Preiss D, McMurray JJ. SGLT2 inhibition and cardiovascular events: Why did EMPA-REG Outcomes surprise and what were the likely mechanisms? *Diabetologia*, 2016; 59: 1333-1339
 - 9) Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC. Close relation of endothelial function in the human coronary and peripheral circulation. *J Am Coll Cardiol*, 1995; 26: 1235-1241
 - 10) Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*, 1992; 340: 1111-1115
 - 11) Neunteufl T, Heher S, Katzenschlager R, Wöfl G, Kostner K, Maurer G, Weidinger F. Late prognostic value of flow-mediated dilatation in the brachial artery of patients with chest pain. *Am J Cardiol*, 2000; 86: 207-210
 - 12) Sawada T, Emoto T, Motoji Y, Hashimoto M, Kageyama H, Terashita D, Mizoguchi T, Mizuguchi T, Iwasaki M, Taira K, Okamoto H, Matsuo Y, Kim SK, Takarada A, Yokoyama M. Possible association between noninvasive parameter of flow-mediated dilatation in brachial artery and whole coronary plaque vulnerability in patients with coronary artery disease. *Int J Cardiol*, 2013; 166: 613-620
 - 13) Neunteufl T, Katzenschlager R, Hassan A, Klaar U, Schwarzacher S, Glogar D, Bauer P, Weidinger F. Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. *Atherosclerosis*, 1997; 129: 111-118
 - 14) Cha K, Chertow GM, Gonzalez J, Lazarus JM, Wilmore DW. Multifrequency bioelectrical impedance estimates the distribution of body water. *J Appl Physiol*, 1995; 79: 1316-1319
 - 15) Kurinami N, Sugiyama S, Nishimura H, Morita A, Yoshida A, Hieshima K, Miyamoto F, Kajiwara K, Jinnouchi K, Jinnouchi T, Jinnouchi H. Clinical factors associated with initial decrease in body-fat percentage induced by add-on sodium-glucose co-transporter 2 inhibitors in patient with type 2 diabetes mellitus. *Clin Drug Investig*, 2018; 38: 19-27
 - 16) Sato I, Ishikawa Y, Ishimoto A, Katsura S, Toyokawa A, Hayashi F, Kawano S, Fujioka Y, Yamashita S, Kumagai S. Significance of measuring serum concentrations of remnant lipoproteins and Apolipoprotein B-48 in fasting period. *J Atheroscler Thromb*, 2009; 16: 12-20
 - 17) Hudson SR, Chan D, Ng LL. Change in plasma volume and prognosis in acute decompensated heart failure: an observational cohort study. *J R Soc Med*, 2016; 109: 337-346
 - 18) Inzucchi SE, Zinman B, Fitchett D, Wanner C, Ferranini E, Schumacher M, Schmoor C, Ohneberg K, Johansen OE, George JT, Hantel S, Bluhmki E, Lachin JM. How does empagliflozin reduce cardiovascular mortality? Insights from a medication analysis of the EMPA-REG OUTCOME Trial. *Diabetes Care*, 2018; 41: 356-363
 - 19) Strauss M, Davis R, Rosenbaum J, Rossmesl EC. Water diuresis produced during recumbency by the intravenous infusion of isotonic saline solution. *J Clin Invest*, 1951; 30: 862-868
 - 20) Harano Y, Miyawaki T, Nabiki J, Shibachi M, Adachi T, Ikeda M, Ueda F, Nakano T. Development of cookie test for the simultaneous determination of glucose intolerance, hyperinsulinemia, insulin resistance and postprandial dyslipidemia. *Endocr J*, 2006; 53: 173-180
 - 21) Sawada T, Tsubata H, Hashimoto N, Takabe M, Miyata T, Aoki K, Yamashita S, Oishi S, Osue T, Yokoi K, Tsukishiro Y, Onishi T, Shimane A, Taniguchi Y, Yasaka Y, Ohara T, Kawai H, Yokoyama M. Effects of 6-month eicosapentaenoic acid on postprandial hypertriglyceridemia, hyperlipidemia, insulin secretion ability, and concomitant endothelial dysfunction among newly-diagnosed impaired glucose metabolism patients with coronary artery disease. An open label, single blinded, prospective randomized controlled trial. *Cardiovasc Diabetol*, 2016; 15: 121
 - 22) Emoto T, Sawada T, Hashimoto M, Kageyama H, Terashita D, Mizoguchi T, Mizuguchi T, Motodi Y, Iwasaki M, Taira K, Okamoto H, Matsuo Y, Kim SK, Takarada A, Yokoyama M. Effect of 3-month repeated administration of miglitol on vascular endothelial function in patients with diabetes mellitus and coronary artery disease. *Am J Cardiol*, 2012; 109: 42-46
 - 23) Shigiyama F, Kumashiro N, Miyagi M, Ikehara K, Kanda E, Uchino H, Hirose T. Effectiveness of dapagliflozin on vascular endothelial function and glycemic control in patients with early-stage type 2 diabetes mellitus: DEFENCE study. *Cardiovasc Diabetol*, 2017; 16: 84
 - 24) Abdul-Ghani MA, Norton L, Defrozo RA. Role of sodium-glucose cotransporter type 2 (SGLT2) inhibitors for the treatment of type 2 diabetes. *Endocr Rev*, 2011; 32: 515-531
 - 25) Cai X, Ji L, Chen Y, Yang W, Zhou L, Han X, Zhang S, Ji L. Comparisons of weight changes between sodium-glucose cotransporter 2 inhibitors treatment and glucagon-like peptide-1 analogs treatment in type 2 diabetes patients: a meta-analysis. *J Diabetes Investig*, 2017; 8: 510-517
 - 26) Mazidi M, Rezaie P, Gao HK, Kengne AP. Effect of sodium-glucose cotransporter-2 inhibitors on blood pressure in people with type 2 diabetes mellitus: A systematic review and meta-analysis of 43 randomized control trials with 225228 patients. *J Am Heart Assoc*, 2017; 6: e005686
 - 27) Zhao Y, Xu L, Tian D, Xia P, Zheng H, Wang L, Chen L. Effects of sodium-glucose cotransporter 2 (SGLT2) inhib-

- itors on serum uric acid level: a meta-analysis of randomized controlled trials. *Diabetes Obes Metab*, 2018; 20: 458-462
- 28) Sawada T, Shiotani H, Terashita D, Nagasawa Y, Kim SS, Koide M, Yokoyama M. Comparison of effects of alpha-glucosidase inhibitors and glinide drugs on endothelial dysfunction in diabetic patients with coronary artery disease. *Circ J*, 2014; 78: 248-255
- 29) Masuda D, Yamashita S. Postprandial Hyperlipidemia and Remnant Lipoprotein. *J Atheroscler Thromb*, 2017; 24: 95-109
- 30) Fujioka Y, Ishikawa Y. Remnant lipoproteins as strong key particles to atherogenesis. *J Atheroscler Thromb*, 2009; 16: 145-154
- 31) Fruchart JC, Sacks F, Hermans MP, Assmann G, Brown WV, Ceska R, Chapman MJ, Dodson PM, Fioretto P, Ginsberg HN, Kadowaki T, Lablanche JM, Marx N, Plutzky J, Reiner Z, Rosenson RS, Staels B, Stock JK, Sy R, Wanner C, Zambon A, Zimmet P. The Residual Risk Reduction Initiative: a call to action to reduce residual vascular risk in patients with dyslipidemia. *Am J Cardiol*, 2008; 102: 1K-34K
- 32) Wu L, Parhofer KG. Diabetic dyslipidemia. *Metabolism*, 2014; 63: 1469-1479
- 33) Sone H, Tanaka S, Tanaka S, Iimuro S, Oida K, Yamasaki Y, Oikawa S, Ishibashi S, Katayama S, Ohashi Y, Akanuma Y, Yamada N; Japan Diabetes Complications Study Group. Serum level of triglycerides is a potent risk factor comparable to LDL cholesterol for coronary heart disease in Japanese patients with type 2 diabetes: subanalysis of the Japan Diabetes Complication Study (JDCS). *J Clin Endocrinol Metab*, 2011; 96: 3448-3456
- 34) Zilversmit DB. Atherosclerosis: a postprandial phenomenon. *Circulation*, 1979; 60: 473-485
- 35) Chowienczyk PJ, Watts GF, Wierzbicki AS, Cockcroft JR, Brett SE, Ritter JM. Preserved endothelial function in patients with severe hypertriglyceridemia and low functional lipoprotein lipase activity. *J Am Coll Cardiol*, 1997; 29: 964-968
- 36) Schnell GB, Robertson A, Houston D, Malley L, Anderson TJ. Impaired brachial artery endothelial function is not predicted by elevated triglycerides. *J Am Coll Cardiol*, 1999; 33: 2038-2043
- 37) Yunoki K, Nakamura K, Miyoshi T, Enko K, Kubo M, Murakami M, Hata Y, Kohno K, Morita H, Kusano KF, Ito H. Impact of hypertriglyceridemia on endothelial dysfunction during Statin ± Ezetimibe therapy in patients with coronary heart disease. *Am J Cardiol*, 2011; 108: 333-339
- 38) Noda Y, Miyoshi T, Oe H, Ohno Y, Nakamura K, Toh N, Kohno K, Morita H, Kusano K, Ito H. Alogliptin ameliorates postprandial lipemia and postprandial endothelial dysfunction in non-diabetic subjects: a preliminary report. *Cardiovasc Diabetol*, 2013; 12: 8. doi: 10.1186/1475-2840-12-8
- 39) Gotoh S, Hata J, Ninomiya T, Hirakawa Y, Nagata M, Mukai N, Fukuhara M, Ikeda F, Ago T, Kitazono T, Kiyohara Y. Hematocrit and the risk of cardiovascular disease in a Japanese community: The Hisayama Study. *Atherosclerosis*, 2015; 242: 199-204
- 40) Mudalier S, Alloju S, Henry RR. Can a shift in fuel energetics explain the beneficial cardioneral outcomes in the EMPA-REG OUTCOME study? A unifying hypothesis. *Diabetes Care*, 2016; 39: 1115-1122
- 41) Aubert G, Martin OJ, Horton JL, Lai L, Vega RB, Leone TC, Koves T, Gardell SJ, Krüger M, Hoppel CL, Lewandowski ED, Crawford PA, Muoio DM, Kelly DP. The failing heart relies on ketone bodies as a fuel. *Circulation*, 2016; 133: 698-705