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

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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 post-prandial 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.

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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 albuminuria. 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 atherosclerosis. 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 events.

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-naive patients with T2DM who had been treated for more than 12 weeks with some antidiabetic 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 myocardial infarction, percutaneous coronary intervention, or coronary artery bypass surgery. T2DM was diagnosed according to the Japan Diabetes Society criteria (HbA1c ≥ 6.4%, fasting plasma glucose [PG] ≥126 mg/dL, and PG ≥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. 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. 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), and high-sensitivity C-reactive protein (hs-CRP).
cholesterol (LDL-C), triglyceride (TG), remnant-like particle cholesterol (RLP-C) as a marker of postprandial hyperlipidemia\(^\text{16}\), 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\(^\text{17, 18}\). 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 = \(\left(\frac{\text{baseline hemoglobin level}}{\text{hemoglobin baseline value after six months of treatment}}\right) \times (100 – \text{hematocrit value after six months of treatment})/(100 – \text{baseline hematocrit value})\) × 100\(^\text{19}\).

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). 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 × 2). In order to assess the postprandial TG change, we calculated incremental AUC-TG (AUC-TG – fasting TG × 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\(^\text{12, 21}\). 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\(^\text{12, 21, 22}\).

**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%\(^\text{18}\). Therefore, a minimum of 44 patients are required to yield 90% power to detect a significant difference between before and after treatment.
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 $P < 0.0001$, 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

### 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).

| Variables                                      | $n = 50$ |
|-----------------------------------------------|----------|
| Age (y.o)                                      | 66.5 ± 9.4 |
| Sex (Male, $n$, %)                             | 44 (88.0%) |
| Coronary risk factor                           |          |
| Hypertension ($n$, %)                          | 45 (90.0%) |
| Dyslipidemia ($n$, %)                          | 49 (98.0%) |
| Current smoker ($n$, %)                        | 4 (8.0%)  |
| Combined drugs                                 |          |
| Statins ($n$, %)                               | 49 (98.0%) |
| n-3 polyunsaturated fatty acids ($n$, %)       | 16 (32.0%) |
| Calcium channel blocker ($n$, %)               | 20 (40.0%) |
| Angiotensin converting enzyme inhibitors / angiotensin-receptor blocker ($n$, %) | 36 (72.0%) |
| Dipeptidyl peptidase 4 inhibitors ($n$, %)     | 26 (52.0%) |
| Metformin / thiazolidine ($n$, %)              | 10 (20.0%) |
| Sulfonylurea agent ($n$, %)                    | 6 (12.0%)  |
| Alpha glucosidase inhibitors ($n$, %)          | 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 $\chi^2$ 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.
| 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/m²) | 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 (%) | 60.1 ± 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 |
| Weight change (%) | -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 (× 10⁶/µL) | 61.6 ± 14.6 | 60.7 ± 15.4 | 0.67 |
| Red blood cell (× 10⁶/µL) | 476.2 ± 36.9 | 498.0 ± 38.4 | <0.0001 |
| Hemoglobin (g/dL) | 16.4 ± 1.1 | 15.0 ± 1.2 | <0.0001 |
| Hematocrit (%) | 43.3 ± 2.9 | 44.8 ± 3.0 | 0.0001 |
| Platelet (× 10⁹/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 (µmol/L/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
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-

| variable                                      | univariate | multivariate |
|----------------------------------------------|------------|--------------|
| 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         |
| Changes in 1,5-anhydroglucitol               | -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         |
| 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      |
| Changing in incremental AUC-TG               | -2.112     | 0.04         |
| 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         |

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

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 Δ%FMD group (Δ%FMD ≤ 0.7%, n=25 patients) and higher Δ%FMD group (Δ%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 Δ%FMD group was significantly lower than that in the lower Δ%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 Δ%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 Δ%FMD group (Fig.1). Furthermore, a decrease in the CRP level and a significant increment in total ketone bodies occurred only in the higher Δ%FMD group.

Discussion

In this study, we noted that six months of treatment with empagliflozin improved not only the glycemic 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 inhibitors 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. As shown in several previous studies, the present study also revealed multiple favorable effects. 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 Δ%FMD group compared to the lower Δ%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 CAD. 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. 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. 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 T2DM. On the other hand, since the report of Zilversmit, 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, 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; n = 25 (Δ%FMD of ≤ 0.7%) | Higher Δ%FMD group; n = 25 (Δ%FMD of > 0.7%) | P-value |
|---------------------------------------|---------------------------------------------|---------------------------------------------|---------|
|                                       | Baseline 6-month                             | Baseline 6-month                             |         |
| Body composition data                 |                                             |                                             |         |
| Weight (kg)                           | 68.5 ± 9.7                                  | 68.7 ± 10.2                                 | 0.94    |
| Absolute Δ                            | -3.3 (-4.6, -1.9)                           | -2.7 (-4.4, -2.1)                           | 0.75    |
| Body mass index (kg/m²)               | 24.9 ± 2.4                                  | 24.9 ± 3.6                                 | 0.97    |
| Absolute Δ                            | -1.1 (-1.6, -0.7)                           | -1.1 (-1.7, -0.7)                           | 0.72    |
| Body fat quantity (kg)                | 18.0 ± 4.3                                  | 18.6 ± 5.9                                 | 0.72    |
| Absolute Δ                            | -1.7 (-3.8, -0.6)                           | -2.1 (-3.0, -1.4)                           | 0.54    |
| Body fat percentage (%)               | 26.6 ± 5.6                                  | 27.3 ± 6.0                                 | 0.66    |
| Absolute Δ                            | -1.7 (-4.0, -0.3)                           | -2.5 (-3.3, -0.8)                           | 0.72    |
| Muscle quantity (kg)                  | 47.5 ± 8.2                                  | 46.2 ± 6.0                                 | 0.55    |
| Absolute Δ                            | -1.0 (-1.8, 0.2)                            | -1.1 (-1.5, -0.7)                           | 0.81    |
| Muscle percentage (%)                 | 69.4 ± 5.3                                  | 68.7 ± 5.7                                 | 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 ± 6.4                                  | 36.0 ± 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 ± 2.5                                  | 13.8 ± 1.6                                 | 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 ± 13.0                                | 136.9 ± 16.7                               | 0.15    |
| Absolute Δ                            | -3.0 (-8.0, 2.3)                            | -4.0 (-9.0, 5.3)                           | 0.96    |
| Diastolic blood pressure (mmHg)       | 80.1 ± 10.7                                 | 80.1 ± 10.1                                | 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)                              | 2.6 (1.9, 3.6)                             | 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)                           | 0.11 (0.09, 0.16)                          | < 0.0001|
| Absolute Δ                            | -0.02 (-0.03, 0.00)                         | 0.07 (0.04, 0.11)                          | < 0.0001|
| Blood chemistry data                  |                                             |                                             |         |
| White blood cell (× 10^4/µL)          | 60.1 ± 15.0                                 | 63.0 ± 14.4                                | 0.50    |
| Absolute Δ                            | 0.0 (-10.5, 62.5)                           | 20.0 (-50.0, 90.0)                         | 0.14    |
| Red blood cell (× 10^4/µL)            | 472.7 ± 42.1                                | 479.6 ± 31.2                               | 0.52    |
| Absolute Δ                            | 15.0 (6.0, 30.0)                            | 21.0 (9.0, 45.0)                           | 0.47    |
| Hemoglobin (g/dL)                     | 14.4 ± 1.0                                  | 14.5 ± 1.3                                 | 0.86    |
| Absolute Δ                            | 0.5 (0.2, 0.9)                              | 0.5 (0.2, 1.1)                             | 0.75    |
| Hematocrit (%)                        | 43.0 ± 2.6                                  | 43.6 ± 3.2                                 | 0.48    |
| Absolute Δ                            | 1.7 (0.2, 2.6)                              | 1.6 (0.0, 3.3)                             | 0.84    |
| Platelet (× 10^4/µL)                  | 21.0 ± 4.0                                  | 22.3 ± 5.2                                 | 0.32    |
| Absolute Δ                            | -1.4 (-2.8, 0.6)                            | -1.3 (-2.5, 0.5)                           | 0.79    |
| Plasma creatinine (mg/dL)             | 0.79 ± 0.12                                 | 0.84 ± 0.16                                | 0.31    |
| Absolute Δ                            | 0.02 (-0.04, 0.06)                          | 0.02 (-0.04, 0.06)                         | 1.00    |
| e-GFR (mg/dL)                         | 75.1 ± 15.4                                 | 71.6 ± 13.9                                | 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)                            | 18.0 (10.1, 46.6)                          | 0.72    |
| Absolute Δ                            | -2.1 (-17.4, 1.1)                           | -4.3 (-19.8, 2.8)                          | 0.79    |
| Uric acid (mg/dL)                     | 5.3 ± 1.4                                   | 5.9 ± 1.2                                  | 0.46    |
| Absolute Δ                            | -0.6 (-1.3, -0.2)                           | -0.8 (-1.5, -0.4)                          | 0.51    |
| Hemoglobin A1c (%)                    | 7.2 ± 0.5                                   | 6.8 ± 0.4                                 | 0.11    |
| Absolute Δ                            | -0.4 (-1.0, -0.2)                           | -0.3 (-0.5, -0.1)                          | 0.26    |
| 1,5-anhydroglucitol (µg/dL)           | 10.9 ± 6.2                                  | 11.3 ± 5.4                                 | 0.83    |
| Absolute Δ                            | -7.6 (-9.8, -4.2)                           | -9.7 (-12.2, -5.6)                         | 0.25    |
(Cont. Table 4)

| Variable | Lower Δ%FMD group; n = 25  | Higher Δ%FMD group; n = 25  | P-value |
|----------|---------------------------|----------------------------|---------|
|          | (Δ%FMD of ≤ 0.7%)         | (Δ%FMD of >0.7%)            |         |
|          | Baseline | 6-month       | Baseline | 6-month       |       |
| Plasma glucose level (mg/dL) | | | | | |
| Fasting | 130.1 ± 16.6 | 114.0 ± 12.7* | 128.2 ± 15.8 | 115.6 ± 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 ± 34.1 | 212.8 ± 28.3* | 234.7 ± 32.9 | 221.6 ± 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 ± 51.0 | 204.8 ± 45.6** | 233.0 ± 42.2 | 222.7 ± 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 ± 46.0 | 144.1 ± 42.3* | 158.9 ± 41.3 | 159.6 ± 43.8 | 0.56 |
| Absolute Δ | -20.5 (-45.0, 0.9) | -0.5 (-24.8, 28.8) | | | 0.09 |
| Immunoreactive insulin (μU/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 (μU/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) | 157.6 ± 21.0 | 161.4 ± 22.6 | 172.2 ± 28.1 | 165.8 ± 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 ± 18.7 | 89.4 ± 17.8 | 93.1 ± 27.7 | 92.2 ± 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 ± 8.8 | 50.9 ± 9.8 | 53.8 ± 26.4 | 51.2 ± 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 ± 33.7 | 96.2 ± 33.3 | 140.8 ± 64.0 | 95.1 ± 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 ± 32.9 | 124.2 ± 43.7 | 165.2 ± 60.7 | 121.0 ± 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 ± 49.5 | 154.4 ± 57.9 | 212.9 ± 76.9 | 152.5 ± 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 ± 30.1 | 57.1 ± 36.2 | 60.4 ± 38.8 | 52.5 ± 29.1* | 0.25 |
| Absolute Δ | 5.5 (-14.5, 22.8) | -9.5 (-15.9, 3.1) | | | 0.05 |
| RLP cholesterol (mg/dL) | 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) | 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 ± 6.5 | 12.9 ± 4.8** | 20.3 ± 8.6 | 14.7 ± 5.1* | 0.13 |
| Absolute Δ | -4.0 (-8.3, 0.0) | -3.0 (-8.8, -2.0) | | | 0.44 |
| Total ketone body (µmol/L) | 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 ± 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
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 Δ%FMD group were similar to those in the lower Δ%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 to the endothelial dysfunction recovery37, 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 failure4, 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 Δ%FMD group were similar to those in the lower Δ%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

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.
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 function. 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 models. 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 months. 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. 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 subjects, 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.

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