Empagliflozin decreases the plasma concentration of plasminogen activator inhibitor-1 (PAI-1) in patients with type 2 diabetes: Association with improvement of fibrinolysis

Running title: Effect of empagliflozin on PAI-1 and fibrinolysis

Shintaro Sakurai, Teruo Jojima*, Toshie Iijima, Takuya Tomaru, Isao Usui, and Yoshimasa Aso*

Department of Endocrinology and Metabolism, Dokkyo Medical University,
Tochigi, 321-0293, Japan.

*Corresponding authors: T. Jojima or Y. Aso. E-mail: jojima@dokkyomed.ac.jp, or yaso@dokkyomed.ac.jp

Key words: plasminogen activator inhibitor-1, fibrinolysis, thrombosis, empagliflozin, leptin, adiponectin

Word count: 3373 Number of tables: 3 Number of figures: 3

Number of supplementary materials: 3

Abstract

Aims: Elevation of the plasma concentration of plasminogen activator inhibitor-1 (PAI-1), a rapid-acting inhibitor of fibrinolysis, is associated with development of vascular thrombotic diseases, including coronary artery disease and stroke. We investigated the effects of empagliflozin, a sodium-glucose co-transporter-2 (SGLT2) inhibitor, on the plasma concentration of PAI-1 and fibrinolytic activity in patients with type 2 diabetes.

Methods: In a randomized, active-controlled, open-label trial, 51 patients with type 2 diabetes were randomly allocated at a 2:1 ratio to receive empagliflozin (10 mg/day, n=31) or standard therapy (n=18) for 12 weeks. We measured the plasma concentrations of PAI-1 and plasmin-α2-antiplasmin complex (PAP) as indicators of fibrinolytic activity. Serum leptin and high-molecular weight (HMW) adiponectin were also measured.

Results: In 49 patients who completed the trial, baseline plasma PAI-1 showed a positive correlation with body weight, visceral fat area (VFA), γ-glutamyltranspeptidase (GGT), leptin, and the platelet count, while it showed a negative correlation with HDL cholesterol and PAP. Body weight and VFA decreased significantly in the empagliflozin group, but not in the control group. The serum level of GGT showed a significant decrease at 12 weeks in the empagliflozin group, while it was unchanged in the control group. Serum HMW adiponectin increased significantly in the empagliflozin group. Plasma PAI-1 decreased significantly by 25% in the empagliflozin group, but not in the control group. In the empagliflozin group, the change of plasma PAI-1 was positively correlated with the changes of body weight and leptin, but was negatively correlated with the change of PAP.

Conclusions: Empagliflozin reduced the plasma PAI-1 concentration through its synergistic actions of a glucose-lowering effect, VFA loss, and restoring the adipokine

balance. (Clinical trial registry: UMIN000025418)

Introduction

Plasminogen activator inhibitor-1 (PAI-1) is the major physiologic inhibitor of tissue-type plasminogen activator (t-PA) in plasma (1, 2). Elevation of the plasma concentration of PAI-1, a rapid-acting inhibitor of fibrinolysis, is associated with the development of vascular thrombotic diseases, including coronary artery disease (CAD) and stroke, in patients with type 2 diabetes (3). Elevated plasma PAI-1 is associated with the features of insulin resistance, which include glucose intolerance, hyperinsulinemia (insulin resistance), visceral obesity, and adipokines (1, 2, 4). The plasma concentration of PAI-1 reflects production by several sources, such as vascular endothelial cells, adipose tissue, and the liver (4). In persons with metabolic syndrome or in obese patients with type 2 diabetes, plasma PAI-1 is believed to be largely derived from visceral adipose tissues (5). The severity of non-alcoholic fatty liver disease (NAFLD) is also independently associated with an increase of the plasma PAI-1 level (6). Type 2 diabetes is associated with a high incidence of atherosclerosis and thrombosis, leading to increased morbidity and mortality from CAD, stroke, and peripheral vascular disease (1). Impaired fibrinolysis induced by elevation of PAI-1 may contribute to a high risk of cardiovascular disease (CVD) in type 2 diabetes (1). In a previous study (7), we demonstrated that because of elevated PAI-1 in the blood, fibrinolytic compensation for hypercoagulation is incomplete in obese patients with type 2 diabetes by utilizing measurements of plasmin-α2–anti-plasmin complex (PAP), a sensitive indicator of ongoing fibrinolysis, and thrombin-antithrombin III complex (TAT), a marker for activation of intravascular coagulation.

Sodium-glucose co-transporter-2 (SGLT2) inhibitors are a new class of oral antidiabetic drugs that reduce hyperglycemia independently of insulin secretion by

promoting urinary excretion of glucose (8). Patients treated with SGLT2 inhibitors show weight loss of around 2 to 3 kg (8). The majority of this weight loss induced by SGLT2 inhibitors is due to fat loss, with significantly greater reduction in the volume of abdominal visceral adipose tissue (VAT) compared with subcutaneous adipose tissue (9). The weight loss observed with SGLT2 inhibitors is primarily driven by glycosuria, which in turn leads to loss of energy and water via osmotic diuresis (8). We recently reported that the SGLT2 inhibitor dapagliflozin decreases hepatic steatosis as well as VAT in patients with type 2 diabetes and NAFLD, and attenuates liver fibrosis in patients with significant fibrosis (10).

There is robust evidence that SGLT2 inhibitors, including empagliflozin, reducing the risk of adverse CVD outcomes in people with type 2 diabetes who have either established CVD or are at risk of CVD (11). We hypothesized that SGLT2 inhibitors might decrease the plasma concentration of PAI-1 by reducing weight (visceral fat) and liver fat, which may enhance fibrinolytic activity, based on prevention of CV events observed in a large-scale clinical trial (EMPA-REG outcome) (11). However, no studies have examined the effects of SGLT2 inhibitors on the coagulation-fibrinolysis system or plasma PAI-1 level. Accordingly, we investigated the effects of empagliflozin, an SGLT2 inhibitor, on the plasma concentration of PAI-1, fibrinolytic activity evaluated by plasma concentration of PAP, and visceral adipose tissues in patients with type 2 diabetes.

Research design and Methods

Study design

This study was performed according to a prospective, randomized, open-label, blinded endpoint design. Patients were randomly allocated at a 2:1 ratio to receive either

empagliflozin or standard treatment without SGLT2 inhibitors. Participant randomization and allocation was performed using a central computer-generated randomization by a specialized center that was independent of the participating sites. Allocation factors included sex, age, and body weight. Each subject was followed for 12 weeks and was reviewed every month. The dose of empagliflozin was fixed at 10 mg/day. Each subject was given advice about appropriate nutrition and exercise programs and subjects were asked not to alter lifestyle, including diet, physical activity, and habit, during the study. Throughout the study, all patients receive standard care for type 2 diabetes and the investigators were encouraged to manage their patients according to local guidelines in order to achieve optimal glycemic control. The dose of the antihypertensive agents or statin were not changed from 12 weeks before the start to the end of study.

We studied 51 patients with type 2 diabetes who were referred to the diabetes outpatient clinic of Dokkyo Medical University. Eligible patients had type 2 diabetes, were at least 20 years old, and had a glycated hemoglobin (HbA1c) level of 6.0 to 12.0% on stable dosages of 1-3 oral antidiabetic agents with or without insulin for at least 3 months. Patients with type 1 diabetes, renal insufficiency (estimated glomerular filtration rate [eGFR] <45 ml/min/1.73m^2), or liver dysfunction (alanine aminotransferase > 3 times the institutional upper limit of normal) were excluded. Patients using anticoagulants that could affect the coagulation or fibrinolytic systems were also excluded from study.

All of the subjects gave informed consent to participation in this study and it was approved by the Institutional Review Board of Dokkyo Medical University (C-296-01). This study was registered with University Hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN000025418).

Measurements

Venous blood was obtained between 7:00 and 9:00 am after an overnight fast (a minimum of 10 h fasting) and collected in tubes containing 3.8% sodium citrate for separation of plasma. Plasma PAI-1 was measured with a latex photometric immunoassay (LPIA tPAI-1 test, IATRON Laboratories, Tokyo, Japan) that detected both active and latent PAI-1, as well as PAI-1 bound to t-PA. Intra-assay and inter-assay CVs were 2.01% and 2.38%, respectively (7). The plasma concentration of plasmin-α2–anti-plasmin complex (PAP) was determined by sandwich enzyme immunoassay (Enzygnost PAP Micro; Dade Behring, Marburg, Germany). Plasma thrombin-antithrombin III complex (TAT) was measured with a chemiluminescent EIA (CLEIATAT, LSI Co., Tokyo, Japan).

Serum HMW adiponectin was measured with our sandwich ELISA employing a monoclonal antibody targeting human HMW adiponectin, as described previously (12). Serum leptin was measured with a radioimmunoassay (Human leptin RIA Kit, EMD Millipore Co., Billerica, MA 01821). Because serum adiponectin and leptin are known to be closely associated with plasma PAI-1 as well as coagulation/platelet function (1-3), we selected these two adipokines for the study.

Serum remnant like lipoprotein (RLP)-cholesterol, a triglyceride-rich lipoprotein, was measured using a mixed immunoaffinity gel containing monoclonal anti-human apoA-I (H-12) and anti-human apoB-100 (JI-H) antibodies (Japan Immunoresearch Laboratories, Takasaki, Japan).

The visceral fat area (VFA) was defined as the area under curve in the umbilical region on bioelectrical impedance analysis (Dual Scan®, Omron Healthcare Company, Kyoto, Japan). VFA detected by the Dual Scan® is equivalent to that determined by

abdominal computed tomography, which is the gold standard for measurement of visceral adipose tissue (13).

Outcomes

The primary endpoint of this study was the change of plasma PAI-1 from baseline to 12 weeks of treatment. Secondary endpoints were the changes of PAP, TAT, liver enzymes (ALT and GGT), VAT, leptin, and HMW adiponectin from baseline to 12 weeks.

Statistical analysis

Results are expressed as the mean ± SD or as the median with interquartile range. Differences between groups were analyzed by Student's paired *t*-test or the unpaired *t*-test, while between-group differences in non-parametric data were analyzed by Wilcoxon's matched-pairs test or the Mann-Whitney U test. Correlations were determined by linear regression analysis or Spearman's rank correlation analysis. Multivariate analysis was performed to identify independent determinants of the baseline plasma PAI-1 level. Differences in prevalence of each drug use between groups were assessed by the chi-squared test. Logarithmic transformation of the data on leptin, PAP, and TAT was done to normalize the distribution for parametric tests. Statistical analyses were carried out by using GraphPad Prism 7 software **(**GraphPad Software, Inc**.**, La Jolla, CA), and $P < 0.05$ was accepted as indicating statistical significance.

We calculated that for a 2:1 ratio of the empagliflozin group to the standard treatment group, a sample of 57 participants (38 empagliflozin, 19 control) was required for 90% power at a significance of 0.05 to detect a difference in the mean of PAI-1 of 10.5,

assuming an SD of 13.0 (14).

Results

A total of 51 participants were screened and underwent randomization to receive empagliflozin (n=32) or standard treatment (n=19; supplemental Fig. 1). In the empagliflozin group, one participant was discontinued due to stroke, then 31/32 participants completed the trial, while 18/19 participants completed it in the standard treatment group.

At baseline, the two groups were well balanced with respect to demographic characteristics and laboratory data (Table 1). HbA1c was comparable between the two groups, but FPG was lower in the control group than in the empagliflozin group. Table 1 also provides information on the use of antidiabetic drugs or antihypertensive agents. Angiotensin converting enzyme inhibitor (ACEI), which may affect plasma level of PAI-1(15), was prescribed for two patients in the empagliflozin group and for one patient in the standard therapy group.

In the 49 patients who completed the study, the baseline plasma PAI-1 level showed a positive correlation with body weight, BMI, waist circumference, VFA, triglycerides, RLP-C, GGT, platelet count, leptin, and TAT, while it was negatively correlated with age and PAP (Table 2). According to multivariate analysis, in a model that explained 86.5% of the variation of plasma PAI-1, triglycerides, platelet count, and TAT were independent determinants of the baseline plasma PAI-1 level in the 49 patients (Table 2).

In the empagliflozin group, body weight, BMI, WC, and VFA all showed a significant decrease at the end of the treatment period, while no changes of these parameters were found in the standard treatment group (Table 3). HbA1c decreased significantly from 8.0±0.9% at baseline to 7.2±0.6% after 12 weeks of empagliflozin treatment, while it did not change in the standard treatment group. After 12 weeks, there was a significant decrease of GGT in the empagliflozin group, while there were no changes of liver enzymes in the standard treatment group (Table 3). In agreement with previous studies (8-10), empagliflozin trreatment reduced the uric acid level and increased the hematocrit at 12 weeks.

In the empagliflozin group, plasma PAI-1 decreased significantly from 32.5 (22.0, 53.0) ng/ml at baseline to 26.0 (22.0, 33.0) ng/ml at 12 weeks of treatment (P=0.0014), while it did not decrease in the control group (Fig. 1A; Table 3). The magnitude of both the absolute reduction and percent reduction of PAI-1 were significantly greater in the empagliflozin group (-9 [-23.3, -0.1] ng/ml) than the control group (8.1 [-0.3, 25.5] ng/ml; P<0.0001; Fig. 1B). In addition, serum HMW adiponectin showed a significant increase at 12 weeks in the empagliflozin group, but not in the standard treatment group (Table 3).

We investigated the relationship between changes of PAI-1 and clinical variables in the empagliflozin group. The change of PAI-1 after treatment with empagliflozin showed a significant positive correlation with those of body weight (Fig. 2A), RLP-C (Fig. 2B), or leptin (Fig. 2C). We also found a significant negative correlation between the changes of PAI-1 and PAP in the empagliflozin group (Figure 3), suggesting that reduction of plasma PAI-1 by empagliflozin may be associated with enhanced fibrinolysis in patients with type 2 diabetes.

We divided the 31 patients who completed empagliflozin treatment into two subgroups, a PAI-1 decrease subgroup (N=20) and a PAI-1 increase subgroup (N=10), which were stratified according to the difference of PAI-1 at 12 weeks from that at baseline. One subject was excluded from analysis, because its subtraction of plasma PAI-1 at baseline from plasma PAI-1 at 12 weeks after empagliflozin was just zero, which was neither a decrease nor an increase.

At baseline, plasma PAI-1 was significantly higher in the PAI-1 decrease subgroup than in the PAI-1 increase subgroup, while plasma PAP was lower in the PAI-1 decrease subgroup (supplemental Table 2). The percent increase of PAP was significantly greater in the PAI-1 decrease subgroup than in the PAI-1 increase subgroup (supplementary Fig. 2).

Discussion

This is the first study to demonstrate a significant reduction of plasma PAI-1 by 25% after 12 weeks of treatment with the SGLT2 inhibitor empagliflozin compared with baseline, while there was no change in the control group. We also found that the percent reduction of PAI-1 from baseline to 12 weeks was significantly larger in the empagliflozin group than in the control group. PAI-1 inhibits plasminogen activators, including t-PA, thereby limiting dissolution of fibrin clots (1-4). High plasma PAI-1 levels may impair fibrin clearance and thereby augment the risk of thrombosis. Thus, hypofibrinolysis (low plasma fibrinolytic activity), which can be induced by an increase of PAI-1 activity, may be associated with a higher risk of CVD (1-4). An imbalance between coagulation and fibrinolysis, partly caused by high plasma concentrations of PAI-1, may be associated with the increased risk of atherothrombotic diseases in obese patients with type 2 diabetes (7). A

meta-analysis has demonstrated that elevated plasma PAI-1 levels are associated with major adverse cardiovascular events (16). A number of studies, including ours, have shown that plasma PAI-1is significantly higher in patients with type 2 diabetes than healthy subjects (7, 17, 18) because of multiple factors, including hyperglycemia, central (visceral) obesity, insulin resistance, and dyslipidemia (low HDL-C and high triglyceride). Therefore, enhancement of fibrinolysis via reduction of PAI-1 by empagliflozin may be a therapeutic strategy for prevention of CVD in patients with type 2 diabetes. Previous studies have also shown that PAI-1 decreases in patients with type 2 diabetes after treatment with SGLT2 inhibitors, but the changes were not significant (19, 20). Unlike our study, however, these studies did not look at changes of fibrinolytic activity in addition to PAI-1.

Although we found no change of plasma PAP, a marker of fibrinolytic activity, in all 31 patients treated with empagliflozin, the change of PAP after treatment was significantly larger in the PAI-1 decrease subgroup (n=20) than in the PAI-1 increase subgroup (n=10). Furthermore, changes of PAP after treatment showed a significant negative correlation with those of PAI-1 in the empagliflozin group. Plasmin is a key enzyme for fibrinolysis, and activated blood coagulation results in the increased formation of plasmin. Plasmin and α2-antiplasmin form a stoichiometric 1:1 complex, which produces the plasmin‐α2‐antiplasmin complex (PAP) and neutralizes plasmin activity. The presence of PAP in plasma is therefore a good indicator of the in vivo activity of plasmin and reflects a hyperfibrinolytic state (21). These reports suggest that lowering plasma PAI-1 by empagliflozin may be associated with enhancement of fibrinolysis in patients with type 2 diabetes, leading to a reduced risk of atherothrombotic diseases.

The mechanisms responsible for reduction of plasma PAI-1 by empagliflozin remain to be determined. The plasma concentration of PAI-1 reflects production from several sources, such as vascular endothelial cells, adipose tissue, and the liver (4). Platelets also store large amounts of PAI-1 (22). In subjects with the metabolic syndrome and type 2 diabetes, plasma PAI-1 is strongly correlated with components of the metabolic syndrome, including the BMI, visceral fat, blood pressure, plasma insulin, triglycerides, and HDL cholesterol (1-4, 22). Accordingly, obesity and insulin resistance are the main determinants of plasma PAI-1 in type 2 diabetes (23).

One possible explanation for reduction of PAI-1 by empagliflozin is weight loss, because glucosuria-induced energy loss caused by SGLT2 inhibitors leads to weight loss that appears to be sustained over time. Weight loss (mainly visceral adipose tissue loss) induced by empagliflozin may be associated with a decrease of plasma PAI-1 because we found a positive correlation between changes of plasma PAI-1 and body weight after treatment with empagliflozin. The Finnish Diabetes Prevention study demonstrated that plasma PAI-1 was significantly decreased in the intensive lifestyle intervention group of subjects with impaired glucose tolerance compared with the control group (24), suggesting that the decrease of PAI-1 was mainly explained by weight loss after intensive intervention. Above all, VAT loss may contribute to a reduction in PAI-1 after treatment with empagliflozin, since VAT is a main source of PAI-1 in people with metabolic syndrome ot type 2 diabetes (1, 2)

Another possible explanation is that empagliflozin may improve adipocyte dysfunction in visceral adipose tissue (VAT), resulting in reduction of PAI-1 (an inflammatory adiopokine). VAT dysfunction, characterized by changes of various immune

cell populations, is associated with altered production of inflammatory adipokines that sustain chronic low-grade inflammation (25). VAT may also increase the thrombotic tendency through platelet dysfunction, hypercoagulation, and impaired fibrinolysis (26). The present study demonstrated a significant reduction of VFA in the empagliflozin group, but not in the control group. We also showed that empagliflozin significantly increased serum HMW adiponectin in patients with type 2 diabetes, in agreement with previous studies of other SGLT2 inhibitors (10, 27, 28). In previous studies, we found that both canagliflozin and dapagliflozin significantly increased serum HMW adiponectin by 10~15% (10, 23). Similar to a previous report (29), this study also identified a significant positive correlation between changes of PAI-1 and leptin in the empagliflozin group. Leptin is a hormone mainly secreted by adipocytes that is involved in regulation of food intake via its action on the hypothalamus, leading to suppression of appetite (30). However, obesity is characterized by hyperleptinemia due to development of leptin resistance (30). Therefore we speculate that improvement of adipocyte dysfunction along with visceral fat reduction by empagliflozin contributes to a favorable adipokine profile in patients with type 2 diabetes, leading to a decrease of PAI-1, which is one of the inflammatory adipokines. A third possible explanation is that empagliflozin improves liver dysfunction, because the liver is a source of PAI-1. NAFLD is associated with an increase of plasma PAI-1 (6). In the present study, liver enzymes were also significantly decreased after treatment with empagliflozin, in agreement with our previous investigation (10). A fourth possible explanation is that an improvement in glycemic control may contribute to a reduction in plasma PAI-1 after treatment with empagliflozin, because a reduction in HbA1c after treatment was greater in the empagliflozin group than in the standard therapy

group. Several reports have shown chronic hyperglycemia is associated with high plasma levels of PAI-1 in people with type 2 diabetes or women with gestational diabetes (31, 32). However, unexpectedly, we found a negative correlation between changes in PAI-1 and HbA1c in the empagliflozin group, suggesting that chronic hyperinsulinemia (insulin resistance) rather than chronic hyperglycemia may be the primary determinant of elevated plasma PAI-1 in people with type 2 diabetes (1, 2, 15).

SGLT2 inhibitors exert multiple metabolic benefits, including reduction of glycated hemoglobin (HbA1c), improvement of glycemic control (fasting and postprandial), body weight loss (visceral adipose tissue), reduction of the systolic and diastolic blood pressure, and elevation of HDL cholesterol (33). The EMPA-REG OUTCOME® trial demonstrated that adding empagliflozin to standard care reduced the risk of CV death by 38% ($p < 0.001$), heart failure (HF) hospitalization by 35% ($p < 0.001$) and a composite endpoint of incident/worsening nephropathy by 39% ($p < 0.001$) compared with placebo (11). Assessment of the cardiovascular safety profile of anti-diabetic drugs has uncovered robust evidence that SGLT2 inhibitors (empagliflozin, canagliflozin, and dapagliflozin) reduce the risk of adverse CVD outcomes in T2DM with either established CVD or a risk of developing CVD (34, 35). In the present study, the observed effects of empagliflozin on adipokines, including PAI-1 reduction and elevation of HMW adiponectin, may partly account for the cardioprotective effects of empagliflozin. Reduction of plasma PAI-1 by empagliflozin may be a novel effect of SGLT2 inhibitors that promotes cardiovascular protection.

The present study had several limitations, including the small number of subjects. Therefore, a larger study needs to be performed to confirm our findings. The second

limitation is that our standard therapy group cannot serve enough as a real control group, because the standard group is a heterogeneous population. The third limitation is its correlation analysis that does not allow proof of a causal relationship between plasma PAI-1 and clinical variables. The fourth limitation is that plasma PAP does not necessarily directly reflect real fibrinolytic activity. In addition, unfortunately, we did not measure plasma t-PA, which is directly relevant to ongoing fibrinolysis in blood.

In conclusion, empagliflozin reduced the plasma PAI-1 concentration through its glucose-lowering effect, VFA loss, and restoring the adipokine balance.

Acknowledgements

We wish to thank Dr. T. Tomotsune, Dr. K Yanagi, and Ms. A. Kezuka from Dokkyo Medical University for their kind support.

Contributions

Teruo Jojima (T.J.), Shintaro Sakurai (S.S), and Yoshimasa Aso (Y.A.) designed and performed the experiments, and wrote the manuscript. T.J., S.S, and T.I. performed the clinical trial. Y.A and S.S. also analyzed the data. T.I., T.T. and I.U. gave valuable advice and opinions.

References

1. Aso Y. Plasminogen activator inhibitor (PAI)-1 in vascular inflammation and thrombosis. Front Biosci. 2007; 12:2957-66.
2. Schneider DJ, Sobel BE. PAI-1 and diabetes: a journey from the bench to the bedside. Diabetes Care. 2012; 35:1961-7.
3. Kearney K, Tomlinson D, Smith K, Ajjan R. Hypofibrinolysis in diabetes: a therapeutic target for the reduction of cardiovascular risk. Cardiovasc Diabetol. 2017; 16(1):34.
4. Vaughan DE. PAI-1 and atherothrombosis. J Thromb Haemost. 2005; 3:1879-83
5. Alessi MC, Peiretti F, Morange P, Henry M, Nalbone G, Juhan-Vague I. Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease. Diabetes. 1997; 46:860-7.
6. Ajmera V, Perito ER, Bass NM, Terrault NA, Yates KP, Gill R, Loomba R, Diehl AM, Aouizerat BE; NASH Clinical Research Network. Novel plasma biomarkers associated with liver disease severity in adults with nonalcoholic fatty liver disease. Hepatology. 2017; 65:65-77.
7. Aso Y, Matsumoto S, Fujiwara Y, Tayama K, Inukai T, Takemura Y. Impaired fibrinolytic compensation for hypercoagulability in obese patients with type 2 diabetes: association with increased plasminogen activator inhibitor-1. Metabolism. 2002; 51:471-6.
8. Tahrani AA, Barnett AH, Bailey CJ. SGLT inhibitors in management of diabetes. Lancet Diabetes Endocrinol. 2013; 1:140-51.
9. Bolinder J, Ljunggren Ö, Kullberg J, Johansson L, Wilding J, Langkilde AM, Sugg J, Parikh S. Effects of dapagliflozin on body weight, total fat mass, and regional adipose

tissue distribution in patients with type 2 diabetes mellitus with inadequate glycemic control on metformin. J Clin Endocrinol Metab. 2012; 97:1020-31

10. Shimizu M, Suzuki K, Kato K, Jojima T, Iijima T, Murohisa T, Iijima M, Takekawa H, Usui I, Hiraishi H, Aso Y. Evaluation of the effects of dapagliflozin, a sodium-glucose co-transporter-2 inhibitor, on hepatic steatosis and fibrosis using transient elastography in patients with type 2 diabetes and non-alcoholic fatty liver disease. Diabetes Obes Metab. 2019; 21:285-292
11. 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(22):2117-28.
12. Aso Y, Yamamoto R, Wakabayashi S, Uchida T, Takayanagi K, Takebayashi K, Okuno T, Inoue T, Node K, Tobe T, Inukai T, Nakano Y. Comparison of serum high-molecular weight (HMW) adiponectin with total adiponectin concentrations in type 2 diabetic patients with coronary artery disease using a novel enzyme-linked immunosorbent assay to detect HMW adiponectin. Diabetes. 2006; 55:1954-60.
13. Ida M, Hirata M, Odori S, Mori E, Kondo E, Fujikura J, Kusakabe T, Ebihara K, Hosoda K, Nakao K. Early changes of abdominal adiposity detected with weekly dual bioelectrical impedance analysis during calorie restriction. Obesity (Silver Spring). 2013;21(9):E350-3
14. Hadigan C, Meigs JB, Rabe J, D'Agostino RB, Wilson PW, Lipinska I, Tofler GH, Grinspoon SS; Framingham Heart Study. Increased PAI-1 and tPA antigen levels are

reduced with metformin therapy in HIV-infected patients with fat redistribution and insulin resistance. J Clin Endocrinol Metab. 2001; 86:939-43.

15. Vaughan DE, Rai R, Khan SS, Eren M, Ghosh AK. Plasminogen Activator Inhibitor-1 Is a Marker and a Mediator of Senescence. Arterioscler Thromb Vasc Biol. 2017;37(8):1446-1452.
16. Jung RG, Motazedian P, Ramirez FD, Simard T, Di Santo P, Visintini S, Faraz MA, Labinaz A, Jung Y, Hibbert B. Association between plasminogen activator inhibitor-1 and cardiovascular events: a systematic review and meta-analysis. Thromb J. 2018; 16:12.
17. Nagi DK, Mohamed Ali V, Jain SK, Walji S, Yudkin JS. Plasminogen activator inhibitor (PAI-1) activity is elevated in Asian and Caucasian subjects with non-insulin-dependent (type 2) diabetes but not in those with impaired glucose tolerance (IGT) or non-diabetic Asians. Diabet Med. 1996; 13:59-64.
18. Yarmolinsky J, Bordin Barbieri N, Weinmann T, Ziegelmann PK, Duncan BB, Inês Schmidt M. Plasminogen activator inhibitor-1 and type 2 diabetes: a systematic review and meta-analysis of observational studies. Sci Rep. 2016; 6:17714.
19. Okamoto A, Yokokawa H, Sanada H, Naito T. Changes in Levels of Biomarkers Associated with Adipocyte Function and Insulin and Glucagon Kinetics During Treatment with Dapagliflozin Among Obese Type 2 Diabetes Mellitus Patients. Drugs R D. 2016;16:255-261.
20. Sato T, Aizawa Y, Yuasa S, Kishi S, Fuse K, Fujita S, Ikeda Y, Kitazawa H, Takahashi M, Sato M, Okabe M. The effect of dapagliflozin treatment on epicardial adipose tissue volume. Cardiovasc Diabetol. 2018 Jan 4;17(1):6.

21. Montes R, Páramo JA, Anglès-Cano E, Rocha E. Development and clinical application of a new ELISA assay to determine plasmin-alpha2-antiplasmin complexes in plasma. Br J Haematol. 1996;92:979-85.
22. Booth NA, Simpson AJ, Croll A, Bennett B, MacGregor IR. Plasminogen activator inhibitor (PAI-1) in plasma and platelets. Br J Haematol 1998; 70: 327–333.
23. Aso Y, Wakabayashi S, Yamamoto R, Matsutomo R, Takebayashi K, Inukai T. Metabolic syndrome accompanied by hypercholesterolemia is strongly associated with proinflammatory state and impairment of fibrinolysis in patients with type 2 diabetes: synergistic effects of plasminogen activator inhibitor-1 and thrombin-activatable fibrinolysis inhibitor. Diabetes Care. 2005; 28:2211-6.
24. Hämäläinen H, Rönnemaa T, Virtanen A, Lindström J, Eriksson JG, Valle TT, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Rastas M, Aunola S, Uusitupa M, Tuomilehto J; Finnish Diabetes Prevention Study Group. Improved fibrinolysis by an intensive lifestyle intervention in subjects with impaired glucose tolerance. The Finnish Diabetes Prevention Study. Diabetologia. 2005; 48:2248-53.
25. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AWJr, Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003; 112:1796–1808.
26. Vilahur G, Ben-Aicha S, Badimon L. New insights into the role of adipose tissue in thrombosis. Cardiovasc Res. 2017; 113:1046-1054.
27. Matsumura M, Nakatani Y, Tanka S, Aoki C, Sagara M, Yanagi K, Suzuki K, Aso Y. Efficacy of Additional Canagliflozin Administration to Type 2 Diabetes Patients

Receiving Insulin Therapy: Examination of Diurnal Glycemic Patterns Using Continuous Glucose Monitoring (CGM). Diabetes Ther. 2017; 8:821-827.

28. Garvey WT, Van Gaal L, Leiter LA, Vijapurkar U, List J, Cuddihy R, Ren J, Davies MJ. Effects of canagliflozin versus glimepiride on adipokines and inflammatory biomarkers in type 2 diabetes. Metabolism. 2018; 85:32-37.
29. Netto BD, Bettini SC, Clemente AP, Ferreira JP, Boritza K, Souza Sde F, Von der Heyde ME, Earthman CP, Dâmaso AR. Roux-en-Y gastric bypass decreases pro-inflammatory and thrombotic biomarkers in individuals with extreme obesity. Obes Surg. 2015; 25:1010-8.
30. Friedman J. 20 years of leptin: leptin at 20: an overview. J Endocrinol. 2014 ; 223(1):T1-8.
31. Lyon CJ, Hsueh WA. Effect of Plasminogen Activator inhibitor-1 in Diabetes Mellitus and Cardiovascular Disease. Am J Med. 2003;115 Suppl 8A:62S-68S.
32. Teliga-Czajkowska J, Sienko J, Zareba-Szczudlik J, Malinowska-Polubiec A, Romejko-Wolniewicz E, Czajkowski K. Influence of Glycemic Control on Coagulation and Lipid Metabolism in Pregnancies Complicated by Pregestational and Gestational Diabetes Mellitus. Adv Exp Med Biol. 2019;1176:81-88.
33. Inzucchi SE, Zinman B, Wanner C, Ferrari R, Fitchett D, Hantel S, Espadero RM, Woerle HJ, Broedl UC, Johansen OE. SGLT-2 inhibitors and cardiovascular risk: proposed pathways and review of ongoing outcome trials. Diab Vasc Dis Res. 2015; 12:90-100.
34. Zelniker TA, Wiviott SD, Raz I, Im K, Goodrich EL, Bonaca MP, Mosenzon O, Kato ET, Cahn A, Furtado RHM, Bhatt DL, Leiter LA, McGuire DK, Wilding JPH, Sabatine

MS. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. Lancet. 2019; 393(10166):31-39.

35. Ghosh-Swaby OR, Goodman SG, Leiter LA, Cheng A, Connelly KA, Fitchett D, Jüni P, Farkouh ME, Udell JA. Glucose-lowering drugs or strategies, atherosclerotic cardiovascular events, and heart failure in people with or at risk of type 2 diabetes: an updated systematic review and meta-analysis of randomised cardiovascular outcome trials. Lancet Diabetes Endocrinol. 2020;8(5):418-435.

Figure legends

Fig. 1

A. Change in plasma PAI-1 over time in the empagliflozin group and the standard treatment group. Values are presented as mean±SEM.

B. Comparison of changes in plasma levels of PAI-1 from baseline to 12 weeks of treatment with the standard therapy (control) and empagliflozin (10 mg/day).

Fig. 2

A. Correlation between changes in plasma PAI-1 and body weight (BW) after 12 weeks of treatment with empagliflozin (10 mg/day) in patients with type 2 diabetes.

B. Correlation between changes in plasma PAI-1 and serum leptin after 12 weeks of treatment with empagliflozin (10 mg/day) in patients with type 2 diabetes.

C. Correlation between changes in plasma PAI-1 and remnant like lipoprotein (RLP)-cholesterol after 12 weeks of treatment with empagliflozin (10 mg/day) in patients with type 2 diabetes.

Fig. 3

Correlation between changes in plasma PAI-1 and PAP after 12 weeks of treatment with empagliflozin (10 mg/day) in patients with type 2 diabetes.