The investigation of SGLT2-inhibitors on cardiometabolic health

Ph.D. Thesis Booklet

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List of abbreviations

AHA: American Heart Association

ALT: Alanine transaminase

AMPK: Adenosine Monophosphate-activated Protein Kinase

AST: Aspartate transaminase

AUC: Area under the curve

BMI: Body mass index

CD: Cluster of differentiation

CDC: Centers for Disease Control and Prevention

CHD: Coronary Heart Disease

CKD: Chronic kidney disease

CRP: C-reactive protein

CVD: Cardiovascular Disease

DGAT2: Diacylglycerol O-acyltransferase 2

ELISA: Enzyme-linked immunosorbent assay

FFA: Free fatty acid

FGF21: Fibroblast growth factor 21

GGT: Gamma glutamyl transferase

HbA1c: Hemoglobin A1c

HFpEF: Heart Failure with preserved Ejection Fraction

HFmrEF: Heart Failure with mildly reduced Ejection Fraction

HFrEF: Heart Failure with reduced Ejection Fraction

HDL: High-density lipoprotein

Lb: Large, buoyant

LDL: Low-density lipoprotein

mTOR: Mammalian target of rapamycin

PPARs: Peroxisome proliferator-activated receptors

ROC: Receiver operating characteristic

Sd: Small, dense

SGLT: Sodium-glucose cotransporter

SLC5: Solute carrier family 5

SPSS: Statistical Product and Service Solutions

T2DM: Type 2 diabetes mellitus

TC: Total cholesterol

TG: Triglycerides

TSH: Thyroid stimulating hormone

USA: United States of America

UTIs: Urinary tract infections

VAT: Visceral Adipose Tissue

WHO: World Health Organization

Introduction

Cardiovascular diseases (CVDs) are the leading cause of mortality worldwide with an estimated nearly 18 million deaths each year. More than 80% percent of CVD-associated mortalities are due to heart attacks and cerebrovascular strokes.

One of the most important risk factors of cardiovascular diseases include hypertension, dyslipidemia, diabetes mellitus, an unhealthy diet, physical inactivity, smoking and alcohol consumption. These can lead to elevated blood pressure, blood glucose level, blood lipid levels, overweight and obesity. These are also direct risk factors that lead to atherosclerotic processes.

Overweight and obesity are defined by the World Health Organization (WHO) as abnormal or excessive accumulation of fat, which presents a risk to health. Obesity is associated with numerous diseases, such as type 2 diabetes mellitus (T2DM), cardiovascular diseases, hypertension, dyslipidemia, specific types of cancer and even pneumological, nephrological, skeletal muscle, rheumatologic, dermatologic and neuropsychologic complications, that all result in premature mortality. The pathologic adipose tissue, especially the dysfunctional visceral adipose tissue (VAT) is a key component of many metabolic disorders, including insulin resistance, glucose intolerance and atherogenic dyslipidemia, which is characterized by high triglyceride and apolipoprotein B levels, increased proportion of small, dense low-density lipoprotein (LDL) -particles, and low levels of high-density lipoprotein (HDL) -cholesterol levels. These abnormalities are accompanied with low-grade systemic inflammation as well.

Obesity can produce chronic inflammation, as the excess fat tissue raises the levels of pro-inflammatory hormones and molecules. The exact mechanisms by which obesity induces inflammation are not fully understood, however it is known to involve numerous interrelated pathways. The trigger is the dysfunctional adipocyte, which produces pro-inflammatory cytokines, adipokines, that induce local and systemic inflammation, accompanied by the immune cell infiltration of the visceral fat tissue.

Adipokines, or adipocytokines, are cytokines produced by tha fat tissue. They regulate the appetite and the immune system, and they contribute in the development of the obesity-associated low-grade state of inflammation, and to the development of metabolic syndrome, type 2 diabetes mellitus and atherosclerosis as well. Leptin plays a crucial role in the regulation of body weight and energy balance. It acts on the hypothalamus, a region of the brain that controls appetite and metabolism, to suppress appetite and increase energy expenditure. In obesity the production of leptin is elevated, which originates from the inflammatory processes. Leptin's transcription is induced by hypoxia and inflammatory mediators. In contrast to the leptin's elevated levels, its

effect is deteriorated, which is called leptin resistance, in which the hypothalamus becomes less responsive to the effects of leptin. This leads to a dysregulation of appetite and energy balance, contributing to the development of obesity. The exact mechanism behind this is not fully understood, nevertheless several theories exist. According to the latest hypothesis it is due to a chronic inflammation as well, that deteriorates leptin's signaling pathways in the hypothalamus. Leptin resistance also plays a part in the develppment of insuline resistance and T2DM.

Sodium-glucose cotransporters (SGLTs) are a family of mammalian solute carrier family 5 (SLC5) membrane proteins that play a critical role in the regulation of glucose transport across the plasma membrane of cells. Sodium-glucose cotransporter 2, is a membrane protein that is primarily expressed in the S1 and S2 segments of the renal proximal tubules in the kidneys. SGLT2 uses the energy from sodium ions (Na^+) moving down their concentration gradient to transport glucose against its concentration gradient. Specifically, SGLT2 binds to both a sodium-ion and a glucose molecule at the outer surface of the renal proximal tubule cell membrane. This binding triggers a conformational change in SGLT2, which allows the protein to transport both sodium and glucose across the cell membrane and into the interior of the cell. Once inside the cell, glucose and sodium are transported out of the cell and back into the bloodstream by other transporters.

SGLT2 inhibitors are a relatively new class of medications that have shown great promise in the treatment of type 2 diabetes. By inhibiting SGLT2, these drugs reduce the amount of glucose that is reabsorbed into the bloodstream, leading to increased glucose excretion in the urine and a decrease in blood glucose levels. This mechanism of action is independent of insulin and does not increase the risk of hypoglycemia (low blood sugar), which is a common complication of many other diabetes medications.

SGLT2 inhibition affects cardiometabolic conditions through several mechanisms and have been shown to reduce the risk of CV death, worsening of heart failure and to improve health status in patients with heart failure with both reduced and preserved ejection fraction (HFrEF, HFmrEF, HFpEF). Furthermore, SGLT2 inhibitors have renoprotective effects as well. Besides their beneficial effects on the conventional risk factors for kidney disease (such as blood pressure, hyperglycemia, and body weight), it has also been hypothesized that they reduce the intraglomerular pressure, change the activation of the renin-aldosterone-angiotensin system and shift renal fuel consumption towards ketone bodies. One potential drawback of SGLT2 inhibitors is their association with an increased risk of urinary tract infections (UTIs) and genital infections such as yeast infections and bacterial vaginosis. These side effects are thought to be due to the increased glucose content in the urine, which can promote the growth of bacteria and fungi. To

minimize the risk of these infections, patients taking SGLT2 inhibitors are advised to maintain good hygiene and to seek prompt medical attention if they experience any symptoms of UTIs or genital infections.

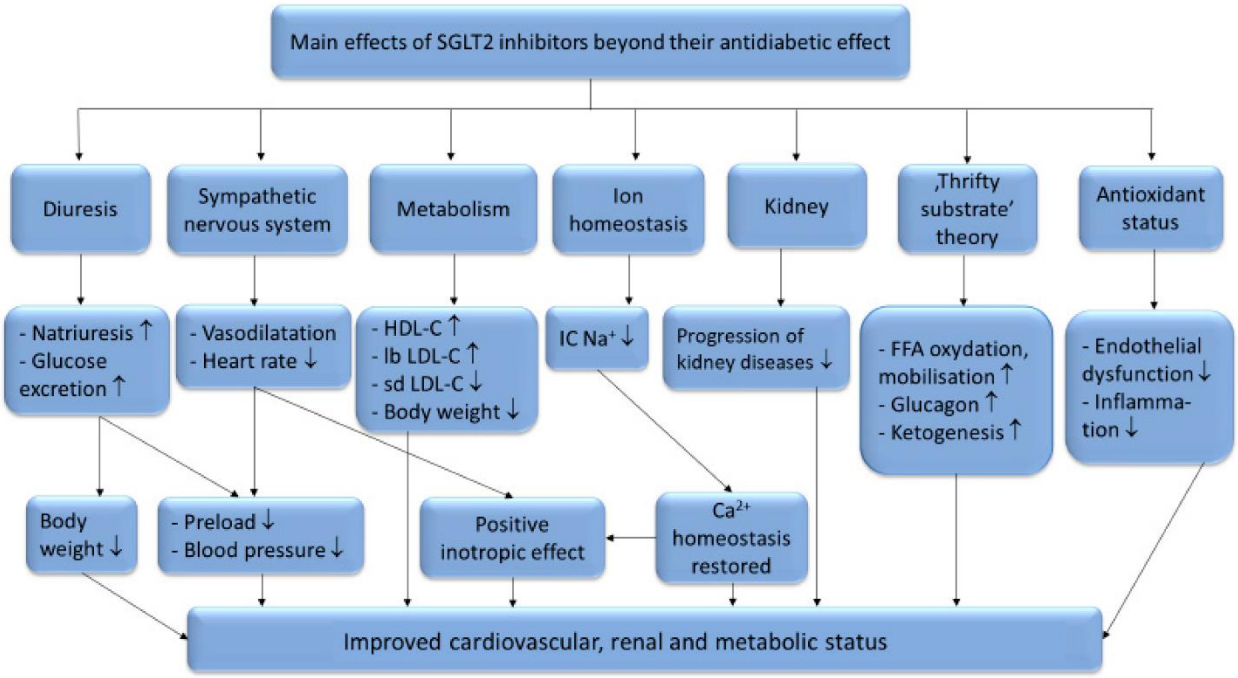

Figure 1. The systematic effects of SGLT2-inhibitors. HDL-C: high-density lipoprotein cholesterol; lb LDL-C: large buoyant low-density lipoprotein cholesterol; sd LDL-C: small dense low-density lipoprotein cholesterol; TG: triglycerides; IC Na+: intracellular sodium-ion; FFA: free fatty acids; ↑: increased amount; ↓: decreased amount[105].

Several studies reported lowered serum levels of total cholesterol (TC) and triglycerides (TG) as a result of SGLT2 inhibitor therapy, however there is a debate regarding the changes observed in the serum levels of HDL-cholesterol and LDL-cholesterol. The SGLT2 inhibitor therapy increases the production of ketone bodies through various pathways. The decreased level of glucose in the blood increases the production of glucagon, which promotes ketogenesis.

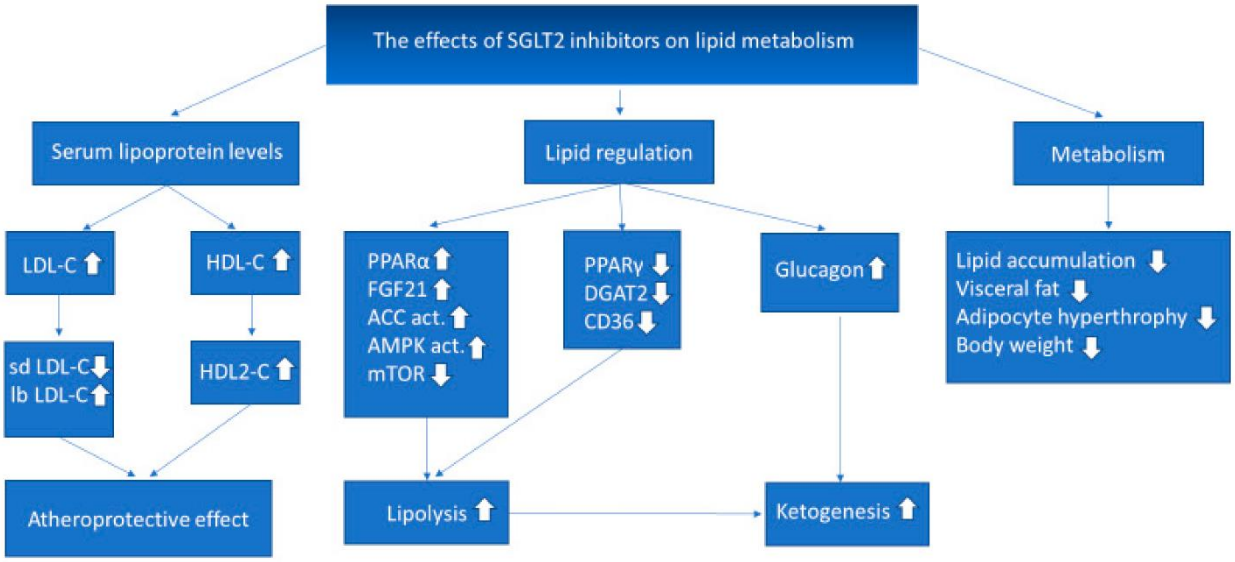

Figure 2. The effects of SGLT2 inhibitor therapy on lipid metabolism. LDL-C: low-density lipoprotein cholesterol; sd LDL-C: small dense low-density lipoprotein cholesterol; lb LDL-C: large buoyant low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; PPARα: peroxisome proliferator-activated receptor α; FGF21: fibroblast growth factor 21; ACC act.: acetyl-CoA carboxylase activation; AMPK act.: AMP-activated protein kinase activation; mTOR: mammalian target of rapamycin; PPARγ: peroxisome proliferator-activated receptor γ; DGAT2: diacylglycerol O-acyltransferase 2; CD36: cluster of differentiation 36; ↑: increased amount; ↓: decreased amount[105].

Focus and Aims of the present work

Clinical Study of Metabolic Parameters, Leptin and the SGLT2 Inhibitor Empagliflozin among Patients with Obesity and Type 2 Diabetes

The link between obesity and T2DM has long been recognized and explains the high prevalence of T2DM. Type 2 diabetes mellitus is associated with many vascular complications. Microvascular complications include diabetic kidney disease, retinopathy, and neuropathy, whereas macrovascular complications include coronary artery, cerebrovascular, and peripheral vascular diseases. The main goals of treatment in patients with T2DM are to achieve adequate glycemic control, reduce body weight and prevent vascular damage, and target organ damage. Novel antidiabetic therapies such as sodium-glucose co-transporter 2 inhibitors provide a new approach to preventing or ameliorating the complications that insulin resistance and hyperglycemia create. The aim of our study was to investigate certain laboratory parameters such as lipids, inflammatory markers, blood glucose level, glycated hemoglobin level, kidney function, leptin level, as well as body mass index, body fat and visceral fat percentage among patients afflicted with obesity and diabetes as well as to examine the effect of empagliflozin treatment.

Materials and methods

Subjects

102 patients (35 female, 67 male) were enrolled in our study between 2019 and 2022. Patients were recruited from different internal medicine and outpatient departments by various physicians from the 1st Department of Medicine, University of Pecs. They voluntarily agreed to participate in our study in which they signed an informed consent letter. Subgroup analysis was performed based on different metabolic states. Patients who did not have type 2 diabetes and were not obese were assigned to group C (20 patients), declared as the control group. Obese patients without diabetes were assigned to group O (obese), (20 patients). Non-obese patients with type 2 diabetes were selected into group D (diabetic), (19 patients). Obese and diabetic patients were assigned into group OD (obese and diabetic), (19 patients). Obese, diabetic patients receiving empagliflozin therapy for at least 3 months were assigned to group ODE (20 patients). Patients were considered obese if their BMI was 30.0 kg/m2 or higher. Antihypertensive, antidiabetic, and antihyperlipidemic therapies were recorded from the

patient's history as well as their comorbidities, such as diabetes mellitus, hypertension, and cardiovascular diseases. Exclusion criteria include the following: previous SGLT2 inhibitor therapy for groups C, O, D, OD; active cancer disease; and refusal to sign the consent form.

Anthropometric measurements

The patients' body composition was assessed using an Omron HBF-511 body composition scale (Omron HealthCare Co., Ltd., Kyoto, Japan). We measured weight, BMI, body fat percentage, and visceral fat percentage. Height was measured using a measuring tape.

Laboratory tests

Pre-prandial laboratory tests were performed on every patient. These include complete blood count (red and white blood cell count, platelet count, hemoglobin level, hematocrit), fibrinogen, basic metabolic panel (pre-prandial glucose, sodium, potassium, calcium, blood urea nitrogen, and creatinine levels), lipid panel (total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride levels), liver panel (aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT) levels), HbA1c level, and the thyroid stimulating hormone (TSH) level.

Immunoassay tests

Plasma leptin 1 levels were measured in duplicate using enzyme-linked immunosorbent assay (ELISA) kits (Cat. No. RD191001100). The blood samples were centrifuged at 2500× g for 10 min. The recovered plasma was stored at −70 °C in aliquots until assayed. The tests were performed in full accordance with the recommendations of the manufacturer, with a detection limit of 0.08 and 0.2 ng/mL, respectively. (BioVendor GmbH., Brno, Czech Republic).

Statistical analysis

IBM SPSS statistics, version 28.0.0. (SPSS, Chicago, IL, USA, 2022); software for statistical; was used to conduct descriptive analyses and to describe the sample. Data are shown as means ± standard deviation.

Differences in the continuous variables were evaluated using a one-way repeated ANOVA

statistical test (Tamhane post-hoc test) following the administering of the Kolmogorov–Smirnov test to check the normality of the data distribution. The continuous variables did not differ from the normal distribution.

In the case of categorical variables, data are shown as percentages and incidence (absolute number compared to total number). Differences were evaluated by using chi-square test analyses.

Multivariate linear regression and stepwise analyses of the data were performed regarding the leptin values for HbA1c, LDL, triglyceride, creatinine, hemoglobin, and visceral fat.

Multiple regression analysis with various models including leptin, HbA1c, and visceral fat considering the principle of multicollinearity was performed to reveal which factors predict the occurrence of diabetes and obesity.

The diagnostic power of variables was assessed using the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. The predicted probabilities were calculated from the variables produced by binary logistic regression analysis, in which $p \leq 0.05$ was considered statistically significant.

Results

Body Mass Index, Body Fat, and Visceral Fat

BMI was significantly lower in the control group when compared to the obese ($p < 0.001$), to the obese and diabetic ($p < 0.001$), and to the empagliflozin treated group ($p < 0.001$). It was also significantly lower in the diabetic group when compared to the obese ($p < 0.001$), to the obese and diabetic ($p < 0.001$), and to the empagliflozin-treated group ($p < 0.001$). BMI was significantly lower in the empagliflozin-treated group when compared to the obese and diabetic group ($p < 0.001$). There was no significant difference between the other groups.

Body fat was significantly lower in the control group, when compared to the obese ($p < 0.001$), and to the obese and diabetic ($p < 0.001$) groups. It was also significantly lower in the diabetic group when compared to the obese ($p = 0.001$) and to the obese and diabetic ($p = 0.001$) groups. Body fat was significantly lower in the empagliflozin-treated group when compared to the obese ($p < 0.001$) and to the obese and diabetic group ($p = 0.002$). There were no significant differences between the other groups.

Visceral fat was significantly lower in the control group when compared to the obese ($p < 0.001$), to the obese and diabetic ($p < 0.001$), and to the empagliflozin-treated group ($p < 0.001$). It was

also significantly lower in the diabetic group when compared to the obese ($p < 0.001$), to the obese and diabetic ($p < 0.001$), and to the empagliflozin-treated group ($p < 0.001$). Visceral fat was significantly lower in the empagliflozin-treated group when compared to the obese and diabetic group ($p < 0.014$). There were no significant differences between the other groups.

Hemoglobin Levels

Hemoglobin levels were significantly higher in the empagliflozin-treated group when compared to the diabetic, and obese and diabetic groups ($p = 0.004$ and $p < 0.001$, respectively). There was no significant difference between the diabetic and the obese and diabetic groups ($p = 0.850$). The obese group had a significantly higher hemoglobin when compared to the obese and diabetic group and a significantly lower level when compared to the empagliflozin-treated obese group ($p = 0.033$ and $p = 0.007$ respectively) (Table 1).

Table 1. Laboratory parameters in the different groups. C = control group, n = number of patients, O = obese group, D = diabetic group, OD = obese diabetic group, ODE = obese diabetic group treated with empagliflozin, g = gram, L = liter, mmol = millimole, mL = milliliter, CRP = C-reactive protein, mg = milligram, µmol = micromole, Total chol. = total cholesterol, HDL = High-density lipoprotein cholesterol, LDL = Low-density lipoprotein cholesterol, ng = nanogram, G = giga, T = terra, mIU = milli-international unit, # = statistically significant difference when compared to the C group, ## = statistically significant difference when compared to the O group, *= statistically significant difference when compared to the D group, ** = statistically significant difference when compared to the OD group, *** = statistically significant difference when compared to the ODE group.

Groups	C (n = 20)	O (n = 20)	D (n = 19)	OD (n = 19)	ODE (n = 20)
Hemoglobin, g/L	141.85±20.16	139.85±11.85**,***	133.79±18.20***	126.32±14.72$^{\#,***}$	152.90±10.56$^{\#,*,**}$
Blood glucose, mmol/L	5.48±0.85*,**,***	5.62±1.17*,**,***	6.79±1.95$^{\#,\#\#}$	6.20±1.53$^{\#,\#\#}$	7.01±1.61$^{\#,\#\#}$
HbA1c, %	5.48±0.08*,**,***	5.67±0.93*,**,***	6.72±0.34$^{\#,\#\#}$	6.39±0.15$^{\#,\#\#}$	7.68±0.33$^{\#,\#\#}$
CRP, mg/L	1.93±0.43	6.81±2.08	3.83±1.08	4.55±1.61	3.94±0.60
Urea nitrogen, mmol/L	6.30±0.75	5.22±0.32**	6.34±0.42	9.69±0.28$^{\#\#,***}$	5.71±0.31**
Creatinine, µmol/L	83±3.99	81.55±3.42^{od}	94.68±3.75	120.26±9.75$^{\#\#,***}$	81.71±3.87**
Total chol., mmol/L	4.73±0.25	4.44±0.26	3.79±0.24	4.12±0.36	4.12±0.28
HDL, mmol/L	1.33±0.08	1.25±0.06	1.11±0.05	1.10±0.08	1.06±0.05
LDL, mmol/L	3.15±0.25	2.58±0.21	2.21±0.22	2.38±0.31	2.21±0.23
Triglycerides, mmol/L	1.76±0.38	1.77±0.27	1.81±0.19	1.78±0.22	1.88±0.16
Leptin, ng/mL	5.97±0.70	19.42±3.06	10.33±2.21	29.86±3.61	17.43±2.99
White blood cell count, G/L	6.87±2.12	7.25±2.28	6.90±1.77	7.93±1.76	8.39±1.83
Red blood cell count, T/L	4.58±0.43	4.68±0.64	4.74±0.67	4.32±0.50	5.07±0.36
Platelet count, G/L	232.55±66.39	256.20±85.41	244.63±63.50	222.42±54.44	226.00±55.79
Fibrinogen, g/L	2.63±0.65	3.50±0.66	3.02±0.52	3.27±0.99	2.95±0.52
Uric acid, µmol/L	347.94±138.52	331.85±68.29	330.78±99.75	332.79±92.27	314.71±52.85
Sodium, mmol/L	140.40±2.62	140.90±1.45	140.89±2.16	141.79±2.78	141.05±2.26
Potassium, mmol/L	4.35±0.35	4.29±0.37	4.34±0.40	4.62±0.57	4.35±0.45
Thyroid-stimulating hormone, mIU/L	2.29±1.29	2.32±1.24	2.64±1.85	2.28±1.46	1.82±0.75

Blood glucose levels and HbA1c Levels

Blood glucose and HbA1c levels were significantly lower in the control group when compared with the diabetic, the obese and diabetic, and the empagliflozin-treated obese and diabetic groups ($p = 0.029$, $p = 0.005$, and $p < 0.001$, respectively). Blood glucose and HbA1c levels were significantly lower in the obese group when compared with the diabetic, the obese and diabetic, and the empagliflozin-treated obese and diabetic groups ($p = 0.015$, $p = 0.008$, and $p < 0.001$, respectively). There were no significant differences between the other groups regarding blood glucose and HbA1c levels.

Renal Parameters

Urea nitrogen level increases significantly with the appearance of diabetes in obesity (O vs. OD) ($p = 0.002$). In the empagliflozin-treated group, the urea nitrogen level was significantly lower when compared to the obese and diabetic group ($p = 0.008$) (Table 1).

Creatinine significantly increases with the appearance of diabetes in the obese groups (O vs. OD) ($p = 0.011$). In the empagliflozin-treated group, the creatinine level was significantly lower when compared to the obese and diabetic group ($p = 0.012$).

Leptin Levels

Leptin levels were significantly higher with the appearance of obesity ($p = 0.003$) even if obesity was present with diabetes ($p < 0.001$) when compared to the control group. It was also significantly higher in diabetic patients when compared with the control group ($p = 0.029$). Obese and diabetic patients had a significantly higher level of leptin when compared to diabetic yet not obese patients ($p = 0.001$). In the empagliflozin-treated group, the leptin level was significantly lower when compared to the obese and diabetic group ($p = 0.048$).

Diagnostic power of leptin for composit endpoints

ROC analysis was carried out with HgbA1c and leptin to test the diagnostic power for the composit endpoint diabetes. The analysis of HgbA1c indicated a cut-off point of 5.48 for the endpoint with an AUC of 0.88 (p=0.001) (sensitivity = 94,8%; specificity = 63,2%). The analysis of leptin indicated a cut-off point of 5.68 with an AUC of 0.60 (p=0.001) (sensitivity = 74.1%; specificity = 71.1%)

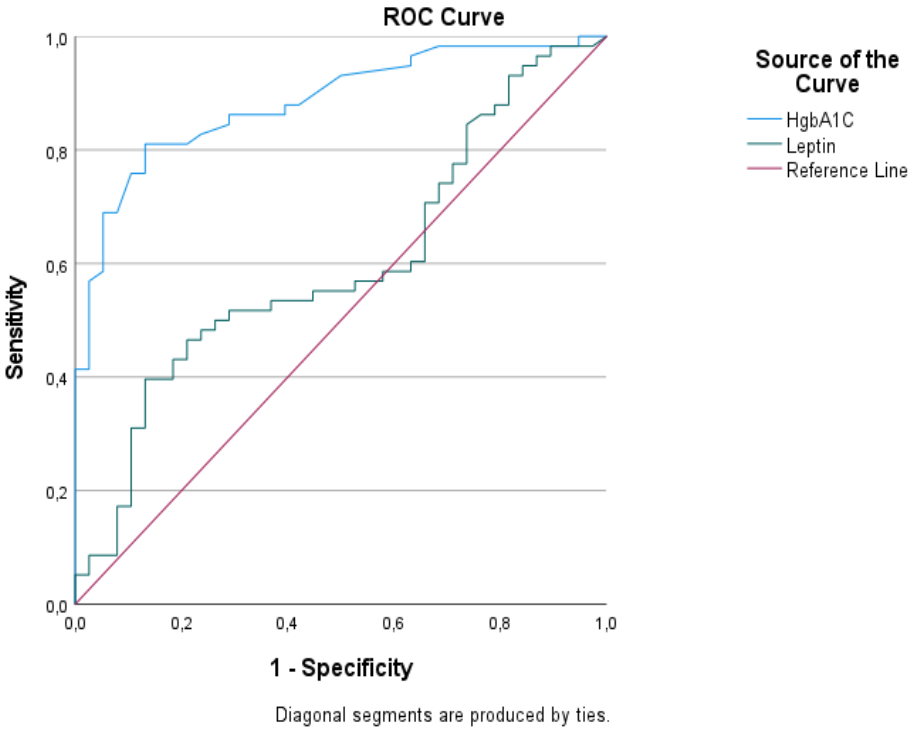

Figure 3. ROC analysis of HgbA1C and leptin to test the diagnostic power for composit endpoint diabetes.

ROC analysis was carried out with HgbA1c, leptin and visceral fat to test the diagnostic power for the composit endpoint diabetes and obesity. The analysis of HgbA1c indicated a cut-off point of 5.61 for the endpoint with an AUC of 0.61 (p = 0.001) (sensitivity = 89.5%; specificity = 64.9%). The analysis of leptin indicated a cut-off point of 5.69 for the endpoint with an AUC of 0.791 (p = 0.001) (sensitivity = 84.2%; specificity = 68.8%). The analysis of visceral fat indicated a cut-off point of 7.5 for the endpoint with an AUC of 0.756 (p = 0.001) (sensitivity = 94.7%; specificity = 93.5%).

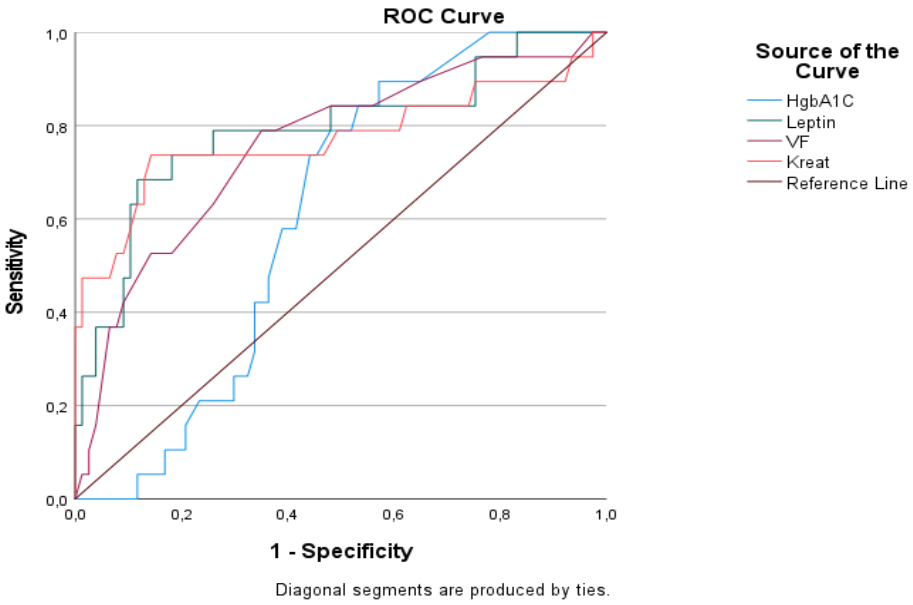

Figure 4. ROC analysis of HgbA1C, leptin, visceral fat (VF) and creatinine (Kreat) to test the diagnostic power for composit endpoint diabetes and obesity.

Other Measured Parameters

There was no significant difference in body muscle percentage, white blood cell count, red blood cell count, platelet count, fibrinogen levels, uric acid, triglyceride, sodium and potassium levels, and thyroid-stimulating hormone levels among the groups.

There was no significant difference in the cholesterol levels among the groups. It bears mentioning, cholesterol levels were strongly affected by the antihyperlipidemic agents.

The continuous variables did not differ from the normal distribution. Data are shown as means ± standard deviation in Table 1.

Discussion

Leptin was the first identified adipokine in the 1990s known to suppress food intake through the suppression of appetite and mediate energy homeostasis including glucose and lipid metabolism. The serum level of leptin is elevated paradoxically in obesity, and this high level of leptin may induce leptin resistance and result in altered glucose metabolism and insulin resistance. Hyperleptinemia has also been associated with increased inflammation, oxidative stress, endothelial dysfunction, atherogenesis, and thrombosis. Based on these effects, leptin is attributed to a significant role in the development of cardiovascular diseases. Additionally, patients with T2DM scored a higher percentage of hypertension, obesity, metabolic syndrome, and endothelial dysfunction if they had elevated leptin levels.

In our study, we examined metabolic and inflammatory parameters, kidney function, and leptin levels among patients afflicted with hypertension, obesity, T2DM, and cardiovascular diseases. The aim of our study was to detect the severity of the metabolic state among these patients and to examine a subgroup of patients treated with empagliflozin. In our study, we found empagliflozin-treated obese, diabetic patients had significantly lower BMI, body fat, and visceral fat values as well as lower serum creatinine and leptin levels when compared to patients with obesity and type 2 diabetes treated with usual antidiabetics (such as biguanides and sulfonylureas). Leptin levels were already higher among patients with T2DM even with normal BMI and were significantly higher in obese non-diabetic patients and were the highest in obese patients with T2DM. Furthermore, we discovered that increased visceral fat and leptin levels predicted diabetes similarly to HbA1c.

The underlying hypothetic mechanisms of SGLT2 inhibitors beyond their antidiabetic effects are summarized in Figure 5.

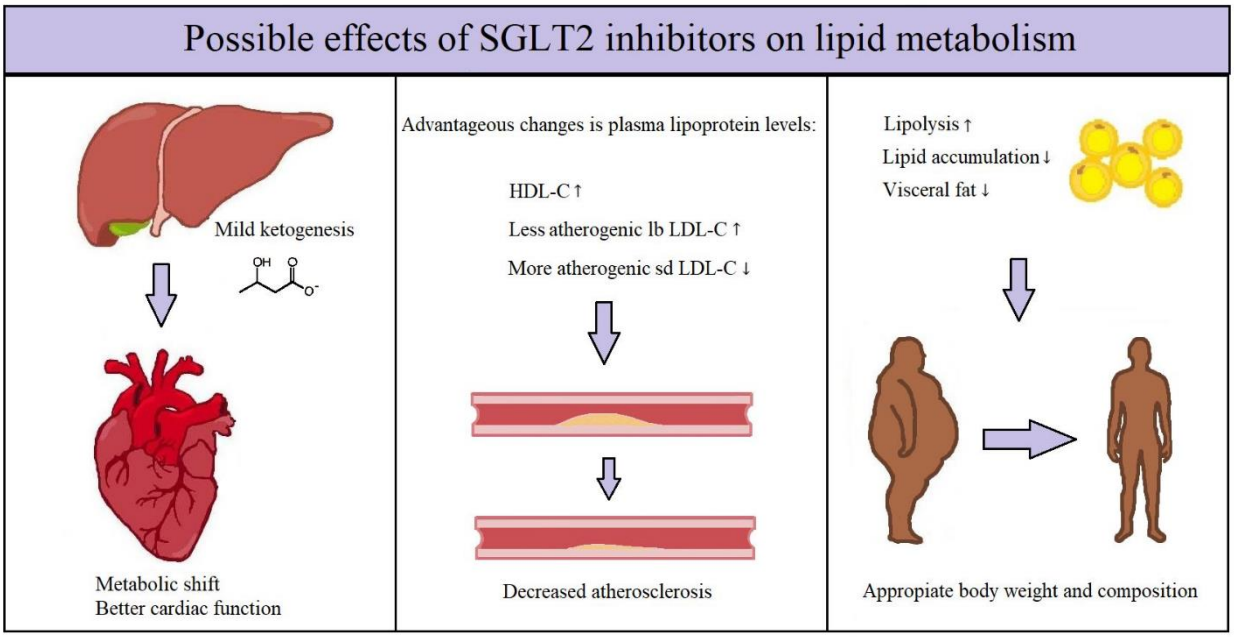

Figure 5. Possible effects of SGLT2 inhibitors on lipid metabolism[105].

In our study, we examined metabolic and inflammatory parameters, kidney function, and leptin levels among patients afflicted with hypertension, obesity, T2DM, and cardiovascular diseases. The aim of our study was to detect the severity of the metabolic state among these patients and to examine a subgroup of patients treated with empagliflozin. In our study, we found empagliflozin-treated obese, diabetic patients had significantly lower BMI, body fat, and visceral fat values as well as lower serum creatinine and leptin levels when compared to patients with obesity and type 2 diabetes treated with usual antidiabetics (such as biguanides and sulfonylureas). Leptin levels were already higher among patients with T2DM even with normal BMI and were significantly higher in obese non-diabetic patients and were the highest in obese patients with T2DM. Furthermore, we discovered that increased visceral fat and leptin levels predicted diabetes similarly to HbA1c.

Excess visceral adiposity is a major risk factor for metabolic and cardiovascular disorders. It plays a crucial role in the development of a diabetogenic and atherogenic metabolic profile inducing insulin resistance and increased cardiometabolic risk. In our study, BMI, body fat, and visceral fat percentage were the highest among patients with obesity and T2DM (Group OD). In the empagliflozin-treated obese, diabetic patients (Group ODE), BMI, body fat, and visceral fat were significantly lower when compared with obese and diabetic patients (OD) treated with usual antidiabetics.

Dysregulated production of adipocytokines is involved in the development of obesity-related diseases. An increased leptin level is associated with insulin resistance and T2DM

development. In T2DM, a link has also been reported between high leptin concentrations and increased cardiovascular risk, including the presence of microvascular complications and cardiac autonomic dysfunction. Furthermore, obesity, hypertension, metabolic syndrome, and endothelial dysfunction are more frequent in T2DM patients with increased leptin levels. In our study, the leptin level was already higher among patients with type 2 diabetes even with normal BMI (Group D), was significantly higher in obese non-diabetic patients (Group O) and was the highest in obese patients with type 2 diabetes (Group OD) when compared to the control group. Also, the leptin level was significantly lower in the empagliflozin-treated obese and type 2 diabetic patients when compared to the obese, diabetic patients treated with other antidiabetics. To the best of our knowledge, this is the first time the beneficial effect of empagliflozin on the leptin level has been demonstrated in a clinical setting.

A link between increased plasma leptin concentrations and chronic kidney disease (CKD) has been reported, which is possibly due to reduced renal clearance. Leptin concentrations gradually increased with the severity of CKD. In CKD patients, plasma leptin levels have been inversely associated with glomerular filtration rate and directly associated with urinary albumin levels as well as age and obesity markers (BMI and waist circumference). Among the empagliflozin-treated obese and T2DM patients, the creatinine level was significantly lower eliciting improved renal function.

HbA1c is a well-known screening and diagnostic tool in detecting diabetes. A score higher than 5.7 % value implies prediabetes, and consequently, higher than 6.5 % confirms diabetes. Our ROC analysis has proven the recommended 5.7 % cut-off value effectively predicted altered glucose homeostasis with very high sensitivity and acceptable specificity. In the same analysis, leptin was found to be similar in the prediction of diabetes. This is congruent with previous observations stating elevated leptin levels are associated with insulin resistance and T2DM development.

The second ROC analysis with the composite endpoint diabetes and obesity showed, in addition to HgA1c, leptin, and visceral fat may have a role in the diagnosis of diabetes among obese adults. These findings emphasize patients with increased visceral fat, which is easily measured using a smart weight scale, are prime candidates to be screened for insulin resistance or diabetes with HbA1c and fasting glucose value.

Hemoglobin values were the highest in the empagliflozin-treated group, which, may imply a slight hemoconcentration, and may be related to the osmotic diuretic effect of empagliflozin treatment.

There was no significant difference in CRP levels among the examined groups; however,

some differences were detected. The CRP level was the lowest in the non-obese, non-diabetic group. Although many factors can influence the CRP level, it may be important that it was higher among obese and diabetic patients, which may indicate a low level of inflammation and corresponds to previous observations. Among patients receiving empagliflozin treatment, the CRP level was lower when compared to the obese and diabetic group, which may reflect lower inflammation status, likely due to the empagliflozin treatment.

Notably, there was no significant difference in LDL-cholesterol levels. This may be due to the fact in which LDL-cholesterol levels were greatly influenced by antihyperlipidemic drugs. Previous literature data indicated a moderate increase in LDL level can be detected with SGLT2 inhibitor treatment. In our study, we did not observe higher LDL values in the empagliflozin-treated group when compared to the other groups. Additionally, in our study, CV disease incidence was provided primarily to describe the patient population. Although it was lower in the empagliflozin-treated group, it was not intended to examine this correlation.

The main strength of our clinical study is that, to the best of our knowledge, this is the first examination that has demonstrated that empagliflozin treatment has a beneficial effect on serum leptin levels under clinical conditions.

Limitations

It is worth mentioning, that our study has some limitations as well. Our study was conducted on a relatively small number of patients, so further studies on a larger patient population are needed to confirm our results. Also, there was a significant difference between the groups regarding their age, thus a more normalized study group is needed to verify our observations.

Summary of new findings

7.1. Among patients receiving empagliflozin treatment, the CRP level was lower when compared to the obese and diabetic group, which may reflect lower inflammation status, likely due to the empagliflozin treatment. It has been previously reported, that empagliflozin reduced renal inflammation and oxidative stress in spontaneously hypertensive rats.

7.2. The effects of empagliflozin on adipocytokines were examined in an animal study conducted on obese rats. Empagliflozin dose-dependently reduced body weight, body fat, adiponectin, and leptin following the 28-day treatment. In our study, the leptin level was significantly lower in the empagliflozin-treated obese and type 2 diabetic patients when compared to the obese, diabetic patients treated with other antidiabetics. The main strength of our study is that, to the best of our knowledge, this is the first examination that has demonstrated that empagliflozin treatment has a beneficial effect on serum leptin levels under clinical conditions.

7.3. The second ROC analysis with the composite endpoint diabetes and obesity showed, in addition to HgA1c, leptin, and visceral fat may have a role in the diagnosis of diabetes among obese adults. These findings emphasize that patients with increased visceral fat, which is easily measured using a smart weight scale, are prime candidates to be screened for insulin resistance or diabetes with HbA1c and fasting glucose value. Leptin levels were also notably higher in patients with T2DM without obesity, which is an interesting and new finding.

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Publications of the author

10.1. Topic-related journal articles

Zs. Szekeres, T. Stolcz, E. Szabados. A nátrium-glükóz-kotranszporter-2-gátlók a diabetes mellitus kezelésén túl. Cardiologia Hungarica 2020; 50: 372–377. DOI: 10.26430/CHUNGARICA.2020.50.5.372

Zs. Szekeres, K. Toth, E. Szabados. The effects of SGLT-2 inhibitors on lipid metabolism. Metabolites, 2021, DOI: 10.3390/metabo11020087.

Quartile Ranking: Q2 Impact Factor: 4.1 (2022)

Zs. Szekeres, B. Sandor, Z. Bognar, F.H.J: Ramadan, A. Palfi, B. Bodis, K. Toth, E. Szabados. Clinical Study of Metabolic Parameters, Leptin and the SGLT2 Inhibitor Empagliflozin among Patients with Obesity and Type 2 Diabetes. Int. J. Mol. Sci. 2023, 24(5), 4405; https://doi.org/10.3390/ijms24054405

Quartile Ranking: Q1 Impact Factor: 5.6 (2022)

10.2. Other articles

P. Petrovics, B. Sandor, A. Palfi, **Zs. Szekeres**, T. Atlasz, K. Toth, E. Szabados. Association between Obesity and Overweight and Cardiorespiratory and Muscle Performance in Adolescents. International Journal of Environmental Research and Public Health. 2021; 18(1):134. https://doi.org/10.3390/ijerph18010134

Quartile Ranking: Q1 Impact Factor: 4.614 (2021)

P. Petrovics, P, A. Nagy, B. Sandor, A., **Zs. Szekeres**, K. Toth, E. Szabados. Examination of Self-Esteem, Body Image, Eating Attitudes and Cardiorespiratory Performance in Adolescents. Int. J. Environ. Res. Public Health. 2021 Dec 14;18(24):13172. doi: 10.3390/ijerph182413172.

Quartile Ranking: Q1 IF: 4.614 (2021)

A. Palfi, **Zs. Szekeres**, B. Sandor, E. Szabados. Az elhízás és a COVID-19-infekció. Cardiologia Hungarica 2021; 51: 336-341. DOI: 10.26430/CHUNGARICA.2021.51.5.336

M. Hock, M. Jaromi, V. Premusz, **Zs. Szekeres**, P. Acs, B. Szilagyi, Z. Wang, A. Makai. Disease-Specific Knowledge, Physical Activity, and Physical Functioning Examination among Patients with Chronic Non-Specific Low Back Pain. Int. J. Environ. Res. Public Health 2022, 19(19), 12024; DOI: 10.3390/ijerph191912024

Quartile Ranking: Q1 Impact Factor: 4.53 (2022)