The investigation of SGLT2-inhibitors on cardiometabolic health

Ph.D. Dissertation

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1. List of abbreviations

ACC: Acetyl-CoA carboxylase

AHA: American Heart Association

ALT: Alanine transaminase

AMPK: Adenosine Monophosphate-activated Protein Kinase

ANGPTL4: Angiopoietin-like protein 4

AST: Aspartate transaminase

ATP: Adenosine triphosphate

AUC: Area under the curve

BMI: Body mass index

CAD: Coronary artery disease

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

Elk-1: Erythroblast Transformation Specific Like-1 protein

ERK: Extracellular Signal-regulated Kinase

ESC: European Society of Cardiology

EU: European Union

FAT: Fatty acid translocase

FFA: Free fatty acid

FGF21: Fibroblast growth factor 21

GGT: Gamma glutamyl transferase

GLUT: Glucose Transporter

Grb2: Growth Factor Receptor-bound Protein 2

GSK3: Glycogen Synthase Kinase 3

HbA1c: Hemoglobin A1c

HFpEF: Heart Failure with preserved Ejection Fraction

HFrEF: Heart Failure with reduced Ejection Fraction

HDL: High-density lipoprotein

IL-1β: Interleukin-1β

IL-6: Interleukin-6

IRS-1: Insulin receptor substrate-1

Lb: Large, buoyant

LDL: Low-density lipoprotein

LHDL: Large high-density lipoprotein

LPL: Lipoprotein-lipase

mTOR: Mammalian target of rapamycin

PDK-1: Phosphoinositide-dependent Kinase-1

PI-3K: Phosphoinositide 3-kinase

PIP2: Phosphatidylinositol 4,5-bisphosphate

PIP3: Phosphatidylinositol 3,4,5-triphosphate

PKB: Protein Kinase B

PPARs: Peroxisome proliferator-activated receptors

PTEN: Phosphatase and Tensin Homolog

RAF-1: Rapidly Accelerated Fibrosarcoma-1

Ras: Rat sarcoma virus

ROC: Receiver operating characteristic

ROS: Reactive oxygen species

Sd: Small, dense

SGLT: Sodium-glucose cotransporter

SH: Src Homology

SLC5: Solute carrier family 5

SNP: Single nucleotide polymorphism

SPSS: Statistical Product and Service Solutions

T2DM: Type 2 diabetes mellitus

TC: Total cholesterol

TG: Triglycerides

TNF-α: Tumor necrosis factor- α

TSH: Thyroid stimulating hormone

USA: United States of America

UTIs: Urinary tract infections

VAT: Visceral Adipose Tissue

VLDL: Very low-density lipoprotein

WAT: White adipose tissue

WHO: World Health Organization

2. Introduction

2.1. Epidemiology of cardiovascular diseases

Cardiovascular diseases (CVDs) are the leading cause of mortality worldwide with an estimated nearly 18 million deaths each year. CVDs are diseases that affect the heart and the blood vessels, including coronary heart disease, cerebrovascular and peripheral artery diseases. More than 80% percent of CVD-associated mortalities are due to heart attacks and cerebrovascular strokes[1]. According to the latest data from the Centers for Disease Control and Prevention (CDC), one person dies every 33 seconds in the United States of America (USA) due to CVD, and about 695 000 people died in the United States because of heart disease in 2021. It is also worth mentioning, that heart disease costs the USA nearly 240 billion dollars each year, which includes health care services, treatment, and the lost productivity due to death[2]. According to the American Heart Association (AHA), the direct and indirect costs of total CVDs were roughly 407 billion dollars. Moreover, analyzing the CVDs, it is clear, that coronary heart disease (CHD) was the leading cause of mortality (41.2%) attributable to CVD in the USA, followed by cerebrovascular stroke (17.3%), other CVDs (16.8%), high blood pressure (12.9%), heart failure (9.2%) and artery diseases (2.6%)[3].

The same trend is observable in Europe: European Heart Network reported that 3.9 million deaths in Europe and over 1.8 million deaths in the European Union (EU) are attributable to CVDs. Over the past 25 years, the absolute number of CVD cases has only increased both in Europe and in the EU[4]. The data presented by the Hungarian Central Statistical Office reports that 42 710 deaths occurred to CVDs in 2021, which is more than 27 percent of the number of all deaths that year[5].

2.2. Risk factors and prevention of CVDs

One of the most important risk factors of cardiovascular diseases include hypertension, dyslipidemia, diabetes mellitus, an unhealthy diet, physical inactivity, smoking and alcohol consumption[6]. These can lead to elevated blood pressure, blood glucose level, blood lipid levels, overweight and obesity[7]. These are also direct risk factors that lead to atherosclerotic processes. This stage can be identified by the primary health care system and thus, with proper attention, escalation can be slowed or even stopped with firm intervention[7]. These actions include education regarding the importance of eliminating the harmful lifestyle choices that led to this stage: changing the diet (eating more vegetables and fruit, reduction of salt, avoiding frequent use of

alcohol), regular physical activity, and the cessation of smoking. Naturally, proper health policies are also necessary, which create conducive environments for making these lifestyle choices not only available, but affordable as well. These are all essential to motivate people in sustaining health[8].

The relationship between hypertension and other cardiovascular diseases have been descripted by a large number of studies. Blood pressure is an important risk factor in the development of numerous diseases, such as myocardial infarction, stroke, peripheral artery disease and heart failure among others. Hypertension has been described as systolic blood pressure values ≥140 mmHg and/or diastolic blood pressure values ≥90 mmHg. Its prevalence in European countries is around 30-45% of the general population[9]. Lifestyle interventions are indicated for all patients with high-normal blood pressure or hypertension because they can delay the need for drug treatment or complement the blood pressure-lowering effect of the drug treatment. Dietary habits influence CV risk through various factors, for example lipids, blood pressure, body weight and diabetes mellitus. Dietary Approaches to Stop Hypertension (DASH) trial revealed that there is a dose-response relationship between sodium reduction and blood pressure reduction. Even though the recommended maximal intake of salt is 5 g/day, in most Western countries it exceeds this limit (~9-10 g/day). Furthermore, the optimal intake might be even lower, around 3 g/day. The necessary salt intake reduction can be achieved by consuming less processed foods and by reformulating the foods by lowering their salt content[10].

The treatment of hypertension involves lifestyle interventions for all patients and drug therapy for most patients. The recommended ultimate systolic blood pressure treatment target range for younger patients (18-69 years) is 120-130 mmHg. The ultimate target systolic blood pressure for patients aged >70 years is <140 mmHg and down to 130 mmHg if tolerated[10].

Dyslipidemia is a prevalent metabolic disorder characterized by abnormal levels of lipids in the bloodstream. It is also one of the key risk factors in the development of cardiovascular diseases. It commonly manifests as increased levels of low-density lipoprotein- (LDL) cholesterol and triglycerides, along with decreased levels of high-density lipoprotein- (HDL) cholesterol[11]. The condition often occurs in obese patients due to the disrupted lipid metabolism caused by excess white adipose tissue. The increased storage of triglycerides in adipocytes leads to an imbalance in lipid metabolism. This results in the secretion of larger and more numerous very low-density lipoprotein (VLDL) particles from the liver, which are rich in triglycerides and cholesterol. Simultaneously, the clearance of triglyceride-rich lipoproteins is impaired, contributing to the elevated levels of LDL-cholesterol and triglycerides observed in

dyslipidemia[12].

The decrease in HDL-cholesterol observed in dyslipidemia can be attributed to alterations in the metabolism and transport of HDL particles. In obesity, there is a reduction in the production and secretion of HDL-cholesterol from the liver, coupled with increased clearance of HDL particles from the circulation. This disruption in HDL-cholesterol metabolism leads to decreased levels of HDL-cholesterol, which is crucial for reverse cholesterol transport: a process that removes excess cholesterol from peripheral tissues and transports it back to the liver for excretion[13].

These dysregulated lipid profiles seen in dyslipidemia are significant risk factors for the development of CVDs, including atherosclerosis, coronary artery disease, and stroke. Elevated LDL-cholesterol levels can promote the accumulation of cholesterol in the arterial walls, initiating the formation of atherosclerotic plaques. Meanwhile, increased triglyceride levels contribute to the development of small, dense LDL particles that are more susceptible to oxidation and are associated with increased inflammation and endothelial dysfunction. The decrease in HDL-cholesterol levels compromises its ability to remove excess cholesterol, impairing the protective mechanisms against atherosclerosis[14].

Furthermore, as obesity is often accompanied by insulin resistance, it is important to assess their interactions. Insulin resistance affects lipid metabolism by reducing the ability of adipose tissue to take up and store fatty acids, leading to increased release of free fatty acids into the bloodstream. These free fatty acids are then taken up by the liver, where they contribute to the production of VLDL particles. Additionally, insulin resistance promotes the activity of enzymes involved in cholesterol synthesis, further contributing to elevated LDL-cholesterol levels[15].

Management strategies for primary dyslipidemia often include lifestyle modifications such as dietary changes, increased physical activity, and weight loss. In some cases, pharmacological interventions such as statins, fibrates, or other lipid-lowering medications may be prescribed to help normalize lipid levels and reduce cardiovascular risk. Treatment goals differ according to both the age of the patients and their known diseases. Apparently, healthy persons' prevention goal blood LDL-cholesterol concentration is <2.6 mmol/L (100 mg/dL), while patients with established atherosclerotic cardiovascular disease, severe target organ damage with type 2 diabetes mellitus, chronic kidney failure (CKD) or familiar hypercholesterinemia should achieve a blood LDL-cholesterol level that is <1.8 mmol/L (70 mg/dL). Additional prevention goals for apparently healthy persons include a blood LDL-C level <1.8 mmol/L (70 mg/dL) and 50% or more reduction in very-high-risk patients or a blood LDL-C level <1.4 mmol/L (55 mg/dL) and

50% or reduction in very-high-risk patients. Intensified prevention goals for patients with CKD or familiar hypercholesterinemia include a blood LDL-cholesterol level <1.8 mmol/L (70 mg/dL) in high risk, while >1.4 mmol/L (55 mg/dL) in very-high risk patients. Patients with T2DM without established atherosclerotic cardiovascular disease or target organ damage should achieve a blood LDL-cholesterol level <1.8 mmol/L (70 mg/dL), if either persists, a blood LDL-cholesterol level <1.4 mmol/L (55 mg/dL) for the additional prevention goal. Intensified prevention goal for patients with established atherosclerotic cardiovascular disease is a blood LDL-cholesterol <1.4 mmol/L (55 mg/dL)[16].

Dietary interventions include higher consumption of fruit, non-starchy vegetables, nuts, legumes, fish, vegetable oils, yogurt, and whole grains, along with a lower intake of processed and red meats, and foods higher in refined carbohydrates and salt. These changes lead to a lower incidence of cardiovascular events. The replacement of animal fats with vegetable sources also decreases the risk of atherosclerotic cardiovascular disease[16].

Lipid-lowering drugs include statins, fibrates, bile acid sequestrants and selective cholesterol absorption inhibitors as well as the novel proprotein convertase subtilisin/kexin type 9 inhibitors[16].

By addressing dyslipidemia effectively, individuals can mitigate the risk of cardiovascular diseases and improve overall health[17].

Diabetes mellitus is a chronic metabolic disorder characterized by high levels of glucose in the blood. This occurs when either the pancreas is not able to produce sufficient quantities of insulin (type 1 diabetes mellitus), or when the body is unable to effectively use the insulin it produces (T2DM). Type 1 diabetes mellitus typically develops in childhood or adolescence and is caused by the destruction of pancreatic beta cells that would produce insulin. T2DM usually develops in adulthood and its primary causes are insulin resistance and impaired insulin secretion. Since insulin is crucial in the transportation of glucose from the bloodstream into the cells its importance is self-evident in the carbohydrate metabolism and energy balance. Since the molecule is not able to enter the cells, it is associated with high levels of blood glucose, known as hyperglycemia. The long-term persistence of hyperglycemia results in various metabolic injuries, where almost every type of tissue is affected[18].

It is a major public health issue, as it affects millions of people worldwide and is associated with several health complications, such as cardiovascular diseases, kidney injury and vascular diseases. The prevalence of diabetes has been increasing globally over the past few decades, it is now one of the leading causes of mortality worldwide. The International Diabetes Federation

estimated more than 463 million adults living with diabetes in 2019, and this number is projected to double by 2045. This rise in prevalence is attributed to the same lifestyle changes that result in obesity as well: unhealthy diets and physical inactivity. Diabetes is more common in high-income countries, but it is rising in both low- and middle-income countries as well, especially in urban settings[19].

The management of diabetes is a complex issue, as it requires a multidisciplinary approach, including lifestyle modifications, pharmacological interventions, and regular monitoring of blood glucose levels. The goal of diabetes management is to maintain blood glucose levels within a target range to prevent or delay the onset of diabetes-related complications. Despite advancements in treatment, diabetes remains a significant burden on individuals, families, and healthcare systems worldwide. Therefore, there is a critical need for continued research to better understand the pathophysiology of diabetes, develop new and improved treatments, and prevent its complications[20]. Sodium-glucose cotransporter (SGLT) 2 inhibitors represent new possibilities in the treatment of diabetes. They have been shown to improve glycemic control and reduce the risk of cardiovascular events in people with T2DM[21].

Patients with type 2 diabetes mellitus have an increased cardiovascular disease risk compared to those without diabetes. This is likely due to a multifactorial origin. Hyperglycemia can lead to micro- and macrovascular complications through various molecular mechanisms including advanced glycation end-products and reactive oxygen species. Thus, proper glycemic control is of key importance in reducing cardiovascular disease risk[22].

Lifestyle management is a priority in cardiovascular disease and diabetes mellitus prevention and management. Since most type 2 diabetes mellitus patients are obese, weight control is a key factor. The predominance of fruits, vegetables, wholegrain cereals, and low-fat protein sources is more crucial than the proportions of the total energy provided by the main macronutrients. As mentioned before, salt intake should also be limited. There are even more recommendations, which include limiting the saturated and trans fats and alcohol intake, the monitoring of carbohydrate consumption, and increasing the quantity of dietary fibre. Furthermore, a Mediterranean-type diet is protective against cardiovascular diseases, which consists of fat sources that are derived primarily from monosaturated oils. The lifestyle management also includes proper training. A combination of aerobic and resistance training is an effective way of the prevention of the type 2 diabetes mellitus' progression, as well as for the glycemic control. Smoking is also an important factor in the development of atherosclerosis and hypertension[10].

The usage of tobacco products elevates the risk of CVDs, as proven by several studies[23]. Smoking causes direct damage to the endothelium, leading to the formation of fatty deposits, called plaques in the blood vessels' walls[24]. This results in restricted blood flow to the vital organs, increasing the risk of heart attack, cerebrovascular stroke, and peripheral artery disease. The chemicals in tobacco smoke damage the walls of vessels, thus they become less elastic and more prone to vasoconstriction. These effects raise blood pressure levels, thus leading to hypertension[25]. Smoking also reduces the amount of oxygen bound to hemoglobin by replacing oxygen particles with carbon monoxide. As a result, tissues may not receive adequate oxygen supply, damaging the organs globally. Smoking also increases the formation of blood clots, by altering blood clotting factors and platelet function. These obstruct the blood flow in the vessels and can lead to thrombosis or embolism. Tobacco usage also decreases the levels of high-density lipoprotein cholesterol and increases the levels of low-density lipoprotein cholesterol. This leads to an imbalance in the lipid profile, increasing the risk of atherosclerosis. Smoking also triggers an inflammatory response, thus contributing to oxidative stress. Chronic inflammation and oxidative stress damage not only the blood vessels, but in turn, vital organs as well[25].

A healthy diet has been associated with a reduced risk of cardiovascular diseases and their complications. The American Heart Association and the European Society of Cardiology (ESC) have recently released updated guidelines regarding the importance of dietary interventions in the management of CVD risk. According to the AHA, a healthy dietary habit should primarily focus on the consumption of fruits, vegetables, whole grains, lean proteins, and low-fat dairy products, while limiting the intake of both saturated and trans fats, sodium, added sugar, and processed foods. The significance of portion control is also pivotal to maintaining a healthy body weight and structure, while reducing the risk of obesity, which is a major contributor to cardiovascular diseases[2]6. Similarly, the ESC propagates a Mediterranean-style diet to prevent cardiovascular diseases. This diet is characterized by the consumption of fruits, vegetables, whole grains, nuts, and olive oil, with the moderate consumption of fish, poultry, and dairy products. Processed and red meats, as well as sugary beverages and sweets, should rarely be consumed[27]. It is also important to note that both guidelines highlight the importance of personalized dietary recommendations that take individual preferences and socioeconomic factors also into account. Multidisciplinary approaches involving multiple healthcare professionals, including dietitians and nutritionists as well as physicists are required to provide guidance for individuals in adopting and maintaining a healthy dietary habit. This is essential to maintain an appropriate energy balance, where the energy intake and expenditure are in balance. It can also regulate blood glucose levels,

as the consumption of complex carbohydrates, such as whole grains, fruits and vegetables provide a steady glucose release into the bloodstream, preventing rapid spikes and crashes in the blood sugar. This also results in a stable insulin level and can reduce the risk of insulin resistance and type 2 diabetes mellitus[28]. A proper diet can positively impact lipid profiles with the consumption of healthy fats and limiting saturated and trans fats. The intake of omega-3 fatty acids and monosaturated fats can increase the levels of HDL-cholesterol, while lowering the amount of LDL-cholesterol, thus reducing the risk of cardiovascular diseases[29].

Physical activity is also recognized as a pivotal component in the prevention and management of cardiovascular diseases. Both the AHA and the ESC emphasize the importance of regular physical activity in maintaining cardiovascular health. AHA recommends at least 150 minutes of moderate-intensity aerobic exercise or 75 minutes of vigorous-intensity aerobic exercise per week for adults[30]. This can include activities like cycling, swimming, or brisk walking. Furthermore muscle-strengthening activities are also important twice every week, targeting major muscle groups. This involves weightlifting or bodyweight exercises as well. ESC also recommends and highlights the importance and positive impact of regular aerobic exercises of moderate intensity[31]. It is also important to note, that individualized physical activity is crucial, which is based on the person's fitness level, health conditions and personal preferences. Factors like age, body weight and overall health status should also be taken into consideration, when suggesting exercise regimens[32]. Gradual progression and the inclusion of activities, which the person enjoys are also required to develop a sustainable habit for the patient. Incorporating physical activity into daily routines is one of the most effective ways of creating these healthy habits[33]. On the other hand, reducing sedentary behavior is also important in maintaining cardiovascular health. Prolonged sitting or lack of movement throughout the day has been associated with an increased risk of cardiovascular diseases[34]. It is important to break up prolonged periods of sitting with light physical activities that include standing up and walking during breaks for a short period of time. Healthcare professionals play a crucial role in providing both guidance and support for individuals to adopt and maintain an active lifestyle. This includes counseling, personalized goal-setting and ongoing monitoring to ensure the safety and effectiveness of physical activity interventions. Physicians, exercise physiologists and physical therapists can help patients in assessing their fitness levels and in developing personalized exercise plans. Physical activity not only increases energy expenditure but also promotes fat metabolism. Regular exercise can enhance the body's ability to utilize fat as a main energy source, thus leading to better weight management[35]. It can positively affect lipid profiles by increasing the levels of

HDL-cholesterol and decreasing the levels of LDL-cholesterol, resulting in a reduced risk of cardiovascular diseases[36]. Physical activity also regulates glucose uptake by muscle cells. Regular training can also improve insulin sensitivity. Both aerobic and resistance training are beneficial in ameliorating the metabolic disturbances associated with type 2 diabetes mellitus (T2DM)[37]. Resistance training stimulates muscle protein synthesis, and also triggers the breakdown of muscle proteins, which are rebuilt during the recovery period, thus leading to more resilient muscle tissues[38]. In addition, regular physical activity can lower blood pressure, as exercise promotes vasodilatation, resulting in lower resistance in the blood vessels. These changes lead to improved cardiac output and better efficiency of the cardiovascular system[39].

Identification of patients at high risk of CVDs, and giving appropriate education and treatment is of critical importance in preventing premature mortality. We can differentiate primary, secondary, and primordial prevention[40]. Primordial prevention aims to prevent the development of risk factors leading to CVDs. Optimal body weight can prevent weight excess, high blood pressure, high blood lipid and blood glucose levels as well. Proper diet and physical activity are recommended for achieving this balance. The cessation of smoking can prevent inflammation and endothelial dysfunction. Even though primordial prevention is not discussed frequently enough, it plays a key role not only in the medical but in the socioeconomic aspect of CVDs as well[41].

2.3. Obesity

Overweight and obesity are defined by the World Health Organization (WHO) as abnormal or excessive accumulation of fat, which presents a risk to health[42]. BMI is an index of weight for height that is used to classify the body composition of adults. Its definition is a person's weight in kilograms divided by the square of their height in meters. It provides population-level measurements as it is the same for both sexes and all ages of adults. A body mass index (BMI) over 25 kg/m^2 is considered overweight, while over 30 kg/m^2 is obese[43]. It is worth mentioning, that it has limitations as well, as it may not correspond to the same degree of fatness in different patients, as it is not concise enough to be used for the evaluation of children[44]. For the measurement of children, percentile numbers are more often used. Percentile numbers show how many percent of children of the same age a child exceed the measured variable[45].

More than 4 million people died in 2017 as a consequence of being overweight or obese. Nearly 2 billion adults – 39 % of the world's adult population – were estimated to be obese or overweight according to the latest data. If this trend continues, nearly 20% of the world's adult

population will clinically be declared obese by 2025[46].

The main cause of overweight and obesity is the energy imbalance between calories consumed and expended. Nowadays the intake of energy-dense foods has risen, that are high in sugars and fat, as well as the measures of physical activity have declined. This may be due to the increase of sedentary forms of work, changes in transportation and increasing urbanization. The changes in these patterns are merely the results of different intertwined environmental and societal changes that are not counterbalanced by proper supportive policies[47].

Although obesity is merely one side of the burdens of malnutrition, more people are obese than underweight in almost every region, except sub-Saharan Africa and some regions of Asia[48]. Even though it was once considered as a problem only in first-world countries, both overweight and obesity are rising in both middle- and low-income countries as well. In addition, the majority of obese children live in developing countries, with a higher rate of increase than in developed countries[49]. Undernutrition and obesity are not mutually exclusive terms, they can and often do co-exist in the same patients. Even though high-energy foods contain a vast number of macronutrients, they often lack the necessary micronutrients, such as antioxidants (including vitamin E, vitamin C, beta-carotene and selenium), vitamin D, B-complex vitamins, zinc, iron and calcium[50].

Obesity is associated with numerous diseases, such as T2DM[51], cardiovascular diseases[52], hypertension[53], dyslipidemia[54], specific types of cancer[55] and even pneumological[56], nephrological[57], skeletal muscle[58], rheumatologic[59], dermatologic[60] and neuropsychologic[61] 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 LDL-particles, and low levels of HDL-cholesterol levels[62]. These abnormalities are accompanied with low-grade systemic inflammation as well[63].

2.4. Inflammation

Inflammation is a complex evolutionary biological response to harmful stimuli, such as infection, tissue injury, or other noxious conditions. This immune response allows the neutralization of these harmful stimuli and also the healing of the damaged tissues. One of the primary aims is to arrange cellular homeostasis against injury. This also means, that this adaptive response overwrites standard cellular functions. Thus, the longer this immune response persists,

the more it exhausts normal functions[64]. Although this could be interpreted as a harmful reaction, it is essential to prevent the escalation of the initial damage. While acute inflammation is a normal and necessary part of the body's immune response, chronic inflammation is associated with a wide range of diseases, including obesity, diabetes, cardiovascular disease, and cancer. Chronic inflammation is characterized by the sustained activation of immune cells and the release of pro-inflammatory molecules, such as cytokines and chemokines[65].

The innate immune system is a crucial component of inflammation. Macrophages, mast cells, dendritic cells, neutrophil granulocytes and lymphocytes are necessary for proper defense. Depending on the type of injury, inflammatory pathways differ greatly, as do their target tissues. The immune cells identify bacteria through specific receptors in the case of an infection. This receptor activation leads to the production of inflammatory mediators called cytokines. These molecules escalate the inflammatory progress. These changes include the recruitment of neutrophils, antibodies and complement factors through the increase of vascular endothelial permeability into the site of the infection. These cytokines trigger systemic effects as well, like the production of acute phase proteins (e. g. C-reactive protein (CRP)) by the cells in the liver. These molecules induce the endothelium in the brain and facilitate the secretion of prostaglandins that are responsible for the well-known symptoms associated with inflammation[66].

In recent years, inflammation has emerged as a critical component in the development of insulin resistance and T2DM. Chronic low-grade inflammation is present in the adipose tissue of obese individuals, leading to the production of pro-inflammatory cytokines that impair insulin signaling and promote insulin resistance. Inflammatory molecules can also directly damage pancreatic beta cells, impairing their ability to produce insulin. Furthermore, the link between obesity and inflammation is well established[67]. Adipose tissue is not just a passive energy storage depot but also an active endocrine organ that secretes various hormones and cytokines. Adipose tissue in obese individuals produces a higher level of pro-inflammatory cytokines than in lean individuals. This leads to the recruitment of immune cells into adipose tissue, leading to further inflammation and exacerbating insulin resistance[68]. Inflammation is also linked to the development of cardiovascular diseases, a major complication of diabetes. Chronic inflammation in the arterial wall can lead to the formation of atherosclerotic plaques, increasing the risk of heart attack and stroke[69]. Given the central role of inflammation in the development of obesity, insulin resistance, and diabetes, there is a growing interest in targeting inflammation as a therapeutic strategy for these conditions[70].

2.5. Obesity and inflammation

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[71]. One of these pathways includes the production of cytokines by the adipose tissue, such as TNF-α, IL-6, and interleukin-1 β (IL-1β), among many other molecules. These cytokines trigger an immediate immune response that leads to the recruitment of immune cells (e.g. macrophages) into the adipose tissue that results in an inflammatory response[72]. Obesity can also activate the innate immune system, which results in the production of reactive oxygen species (ROS) and also free radicals. All of these molecules cause oxidative stress and inflammation that damages the tissues[73].

The inflammatory trigger in obesity is metabolic: the cause is the excess consumption of food. Accordingly, adipocytes are sustaining the insult and sustain the inflammatory response, thus damaging metabolic homeostasis. Elevated levels of TNF-α and other inflammatory cytokines (such as IL-6, IL-1β) can be measured not only the in adipose tissue but in the liver, pancreas, brain and possibly in the muscles as well[74]. This inflammatory activation is significant, but it is not on the same scale as in the case of infection or mechanical trauma. The escalation is not restricted to cytokines only, as greater amounts of immune cells can be observed in the adipose tissue as well. This cannot be interpreted as mere macrophage infiltration, because of the vast amount of pro-inflammatory M1 population of macrophages, which have a negative effect on insulin sensitivity. At a molecular level, insulin resistance is promoted by a transition in macrophage polarization from an alternative M2 activation state sustained by signal transducer and activator of transcription 6 and peroxisome proliferator-activated receptors (PPARs) to a more classical M1 activation state promoted by nuclear factor kappa-light-chain-enhancer of activated B cells, activator protein 1, and other signal-dependent transcription factors that play key roles in innate immunity. The infiltration also includes mast cells and natural killer T cells as well, which also contribute to the inflammatory environment[75]. Other studies showed that both the count of cluster of differentiation (CD) 4+ and CD8+ lymphocytes and the CD4+/CD8+ ratio differs among obese patients[76]. These complex mechanisms are still not properly known, although it already shows, that the immune cells are of key importance in the response to obesity.

There are also indirect pathways, for example metabolic dysfunctions like insulin resistance and dyslipidemia that further escalate the inflammation. The accumulation of glucose and lipids in the bloodstream triggers an inflammatory response[77,78]. Obesity is also associated with alterations in the gut microbiome. These changes can lead to an overgrowth of harmful

bacteria that release pro-inflammatory and suppress anti-inflammatory molecules, thus changing the balance of the immune system[79].

2.6. Insulin signaling pathway and insulin resistance

The insulin signaling pathway is a complex set of biochemical reactions that occur in response to insulin. Insulin is a hormone secreted by the pancreatic β-cells, containing two polypeptide chains connected by disulfide bonds, one having 30 amino acid residues while the other has 21. Its secretion is primarily triggered by an elevated blood level of glucose[80]. Insulin plays a key role in regulating glucose metabolism in the body by the promotion of glucose uptake in muscle and fat cells. Notably, it regulates not only metabolism, but gene expression as well: the insulin signal travels from the plasma membrane receptor to insulin-sensitive metabolic enzymes and into the nucleus, where it stimulates the transcription of several genes[81].

Insulin receptor substrate-1 (IRS-1) is one of the target proteins phosphorylated by the insulin receptor. After the phosphorylation has occurred, the phosphorylated tyrosine residue is bound by the Src homology (SH) 2 domain of the growth factor receptor-bound protein 2 (Grb2). Grb2 also contains another protein-binding domain, called SH3, that connects to the proline-rich domain of Son of sevenless molecule, adding it to the receptor complex. The latter catalyzes the replacement of guanosine diphosphate by guanosine triphosphate on Rat sarcoma virus (Ras) protein. Ras is a guanosine nucleotide-binding protein, which activates a protein kinase, Rapidly Accelerated Fibrosarcoma-1 (Raf-1), that triggers a kinase cascade consisting of mitogen-activated protein kinase kinase and extracellular signal-regulated kinase (ERK). ERK is activated by the phosphorylation of both threonine and tyrosine residues, which then mediates specific biological effects of insulin by entering the nucleus and phosphorylating proteins such as erythroblast transformation specific like-1 protein (Elk-1) that modulates the transcription of more than 100 genes[82].

The phosphorylated IRS-1 interacts with other molecules besides IRS-1. Phosphoinositide 3-kinase (PI-3K) is an enzyme that binds to the SH2 domain of IRS-1, and thus activates it. The activated enzyme converts the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3 binds to protein kinase B (PKB), then another protein kinase, phosphoinositide-dependent kinase-1 (PDK1) phosphorylates the latter thus activating it. The activated PKB phosphorylates serine or threonine residues of target proteins. One of the target molecules is glycogen synthase kinase 3 (GSK3). GSK3 is active in its non-phosphorylated form, in which it phosphorylates glycogen synthase, thus inactivating it. This

contributes to the slowing of glycogen synthesis. PKB inactivates GSK3 by phosphorylating it, so that it cannot inactivate glycogen synthase in the liver and muscle cells, resulting in the stimulation of glycogen synthesis. PKB triggers the transport of glucose transporter (GLUT) 4 from the internal vesicles to the plasma membrane, which results in the stimulation of glucose uptake from the blood[83].

A PIP3-specific phosphatase, phosphatase and tensin homolog (PTEN), removes the phosphate from PIP3 to produce PIP2, that no longer binds PBK, thus the signaling chain is broken[84].

Insulin resistance is a condition in which cells become less responsive to the effects of insulin, leading to a decrease in glucose uptake and utilization. This can result in the development of type 2 diabetes and other metabolic disorders[85]. Insulin resistance can develop as a result of a combination of genetic and environmental factors. Some of the most important risk factors for insulin resistance include obesity, physical inactivity, and a diet high in fat and sugar[86].

Sedentary lifestyle and being overweight or obese are two key predictors of insulin resistance and T2DM development. Even though it would seem that the latter's influence is greater than being physically inactive when weighing the magnitude of relative risk of T2DM, lifestyle interventions that include regular physical activity and dietary modification can both delay and even prevent the onset of T2DM. These interventions may act through the increase of fat oxidation, which reduces the accumulation of fatty acid species withing the skeletal muscle, thus resulting in the attenuation of the insulin signaling pathway's inhibition[87].

Increased amount of total fat in the diet may have an adverse effect on insulin sensitivity, although this link is heavily complicated by the relationship between high-fat diet and the development of overweight and obesity. Studies show that the consumption of saturated fats is associated with insulin resistance. The effect of monosaturated, polysaturated and trans fatty acids is controversial regarding the insulin resistance, with the exception of the n-3 polyunsaturated fatty acids, which appears to enhance insulin sensitivity. The available data suggests that a diet less than 30% total fat, especially with the low intake of saturated fats, is the optimal diet for the prevention of insulin resistance and T2DM[88].

Both acute and chronic intake of fructose-sweetened soft drinks suggest that excess fructose intake results in hepatic and whole-body insulin resistance. Some of the studies also showed that hepatic insulin resistance was observed before the whole-body insulin resistance. This could mean that hepatic insulin resistance precedes or even possibly initiates the development of the whole-body insulin resistance. This effect is thought to be secondary to an increase in

hepatic de novo lipogenesis as well as to the decrease of hepatic fatty acid oxidation and to the augmentation of endoplasmic reticulum stress and inflammation[89].

These factors can lead to chronic low-grade inflammation, which impairs insulin signaling and ultimately results in insulin resistance. At the molecular level, insulin resistance is characterized by several key defects in insulin signaling pathways. Insulin signaling begins with the binding of insulin to its receptor on the surface of target cells, such as muscle, liver, and fat cells. This binding triggers a series of molecular events, including the activation of the insulin receptor substrate proteins and the downstream activation of the PI3K/Akt signaling pathway. In insulin-resistant cells, these signaling pathways are impaired, leading to a decrease in glucose uptake and utilization. One of the key mechanisms of insulin resistance is the suppression of IRS protein (The insulin receptor substrate (IRS) proteins are a family of cytoplasmic proteins that integrate and coordinate the transmission of signals from the extracellular to the intracellular environment via transmembrane receptors, thus regulating cell growth, metabolism, survival and proliferation) function, either through serine phosphorylation or other post-translational modifications. This leads to a decrease in downstream signaling and a reduced response to insulin[90]. Chronic low-grade inflammation is thought to be one of the key mediators of insulin resistance. Inflammation can activate several crucial signaling pathways, including the c-Jun NM-terminal kinase and IkappaB kinase-beta pathways (a pivotal regulator of all inducible NF-κB signaling pathways), which can inhibit insulin signaling by phosphorylating IRS proteins and blocking downstream signaling. Inflammation can also induce the production of pro-inflammatory cytokines, such as (tumor necrosis factor- α) TNF-α and (interleukin-6) IL-6, which can directly impair insulin signaling and contribute to insulin resistance. Other factors that have been implicated in the development of insulin resistance include oxidative stress, endoplasmic reticulum stress, and mitochondrial dysfunction. These factors can lead to the accumulation of toxic metabolites and proteins, impairing insulin signaling and ultimately leading to insulin resistance[91].

2.7. Leptin

Leptin is a hormone produced by the adipose tissue that 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, leptin resistance can develop, where 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[92].

Leptin resistance is thought to possibly arise from chronic inflammation, which impairs leptin signaling in the hypothalamus. Leptin resistance has also been implicated in the development of insulin resistance and T2DM. Leptin can directly influence insulin signaling in peripheral tissues, including the liver and skeletal muscle. In obesity and insulin resistance, leptin signaling is impaired, leading to decreased insulin sensitivity. The exact mechanisms by which leptin influences insulin signaling are not fully understood, but several hypotheses have been proposed[93].

One proposed mechanism is that leptin can activate the hypothalamic-pituitary-adrenal axis, leading to the release of cortisol, which impairs insulin sensitivity, however data seem to be contradictory[94]. Another proposed mechanism is that leptin can activate the sympathetic nervous system, leading to increased lipolysis and the release of free fatty acids, which can impair insulin signaling[95]. In addition to its effects on appetite and metabolism, leptin has also been shown to have anti-inflammatory properties. Leptin can suppress the production of pro-inflammatory cytokines and increase the production of anti-inflammatory cytokines. Therefore, leptin may have a protective role in the development of chronic inflammation and related metabolic disorders[96]. Given the critical role of leptin in the regulation of appetite, metabolism, and inflammation, there has been considerable interest in developing leptin-based therapies for the treatment of obesity and related metabolic disorders. However, the development of leptin resistance has posed a challenge for these therapies.

2.8. Leptin resistance

It has been previously observed that obese patients have higher levels of leptin. Despite the elevated amount, leptin does not cause appropriate effects in them. This phenomenon is called leptin resistance. The exact mechanism behind this is not fully understood, nevertheless several theories exist. It is possible, that the chronic inflammation, which is associated with obesity, interferes with the leptin signaling pathways in the brain. It is also possible, that the higher levels of free fatty acids in the blood serum of obese patients can cause leptin resistance, by the impairment of the leptin's transport across the blood-brain barrier. Additionally, the overconsumption of high-fat and high-calorie foods also leads to changes in the reward and pleasure centers of the brain, which leads to a decrease in the sensitivity of leptin's appetites-suppressing effects[97]. These complex interactions between the metabolic, hormonal and neurological factors might all lead to leptin resistance.

Leptin resistance can be inherited, although it is rare. The mutation of the gene, which

produces leptin, leads to ineffective signaling, thus it causes hyperleptinemia and leptin resistance. Similarly, the mutation of leptin's receptor causes the same effect[98]. These mutations are uncommon in the obese population[99]; thus, it is not the most common cause.

Leptin can regulate its own receptor and signaling pathway: in the case of receptor down-regulation leptin resistance might develop[100]. The reduced expression of leptin receptors in the hypothalamus is observed in rodent models both in age-related[101] and diet-induced obesity[102]. The reduction of leptin receptors seems like a result of elevated levels of leptin in the central nervous system. Chronically increased leptin impairs leptin signaling and desensitizes its physiological responses over time. This means, that obesity promotes hyperleptinemia, which causes leptin resistance that leads to further obesity, thus making leptin resistance both a cause and consequence of obesity[103].

2.9. Sodium-glucose cotransporters

Sodium-glucose cotransporters 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[104].

There are six identified SGLT proteins in humans, of which the SGLT1 and SGLT2 receptors have been studied more thoroughly in recent years. Despite the outstanding sequence similarity between SGLT1 and SGLT2, they show different physiological and biochemical properties. While SGLT1 is primarily expressed in the intestines, SGLT2 is most abundant in the renal cortex, where it plays an essential role in renal glucose reabsorption[105].

SGLT1 is primarily found in the small intestine and the renal proximal tubules, while SGLT2 is predominantly expressed in the S1 and S2 segments of the renal proximal tubules. SGLT1 and SGLT2 differ in their affinity for glucose. SGLT2 has a higher affinity for glucose compared to SGLT1, which means that it can transport glucose even when the concentration of glucose in the blood is relatively low. This makes SGLT2 particularly important for maintaining blood glucose levels during periods of fasting or low carbohydrate intake. In addition, other subtypes of SGLT are known as SGLT3, SGLT4 and SGLT5[106].

SGLT1 is primarily found in the small intestine and renal proximal tubules. It plays a critical role in the absorption of glucose and galactose from the lumen of the gut into enterocytes in the small intestines, which are the cells that line the intestine. Once inside the enterocytes, glucose and galactose are transported into the bloodstream through a different transporter called GLUT2. In the renal proximal tubules, SGLT1 is responsible for the reabsorption of glucose from

the filtrate back into the bloodstream, much like SGLT2. However, SGLT1 has a lower affinity for glucose than SGLT2 and is therefore less important for maintaining blood glucose levels during periods of fasting or low carbohydrate intake[107].

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. The SGLT2 gene, also known as SLC5A2, is located on chromosome 16q23.1 and codes for the SGLT2 protein. The gene spans over 35 kilobases and contains 14 exons, which are the portions of the gene that code for the protein. Variations in the SGLT2 gene have been associated with an increased risk of developing type 2 diabetes[108]. One common variant in the SGLT2 gene is a single nucleotide polymorphism (SNP) known as rs61731956, which results in a missense mutation that changes the amino acid sequence of the SGLT2 protein. This SNP has been associated with an increased risk of developing type 2 diabetes, as well as with an increased response to SGLT2 inhibitors[109].

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 has a high affinity for glucose, which means that it can transport glucose even when the concentration of glucose in the blood is relatively low. This allows SGLT2 to play a critical role in maintaining blood glucose levels during periods of fasting or low carbohydrate intake. However, SGLT2 is also responsible for the reabsorption of excess glucose from the urine back into the bloodstream in individuals with diabetes, which can contribute to hyperglycemia (high blood glucose levels)[110].

SGLT3 is a less studied member of the SGLT family. It is expressed in the heart, brain, and skeletal muscle, and it has been suggested to play a role in glucose sensing and regulation of insulin secretion. However, the exact functions of SGLT3 are still unclear and further research is needed to fully understand its role in glucose homeostasis[111].

SGLT4 is expressed in the small intestines and kidneys. Functional studies showed that it is a Na^+-dependent alpha-methyl-D-glucopyranoside transporter. Three rare variants of this gene have also been observed in patients with proliferative diabetic retinopathy, thus it is suggested, that this protein is also expressed in retinal endothelial cells[112].

SGLT5 has been shown to be expressed in kidney tissue. It is a sodium-dependent

mannose and fructose transporter. It is hypothesized, that it is responsible for the reabsorption of these sugars from the filtrate[112].

2.10. SGLT2-inhibitors

2.10.1. The general effects of SGLT2-inhibitors

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[90].

SGLT2 inhibitors have been shown to be effective in lowering hemoglobin A1c (HbA1c) (a measure of long-term blood sugar control), fasting blood glucose levels, and postprandial (after-meal) glucose levels in patients with T2DM. In addition, they have been shown to be effective in improving glycemic control in patients with type 1 diabetes, when used in combination with insulin therapy[113].

Another important benefit of SGLT2 inhibitors is their potential to promote weight loss. By increasing the urinary excretion of glucose, these drugs reduce the number of calories that are absorbed by the body, leading to a reduction in body weight. The weight loss effect of SGLT2 inhibitors is generally modest, averaging around 2-3 kg (4-6 lbs) in clinical trials, but it is sustained over long-term treatment and can be significant for some patients[114].

In addition to their glucose-lowering and weight-loss effects, SGLT2 inhibitors have been shown to have beneficial effects on blood pressure and cardiovascular health. Clinical trials have demonstrated that these drugs can reduce blood pressure by 3-5 mmHg and decrease the risk of cardiovascular events such as heart attack and stroke by up to 14%. These benefits are thought to be due to the diuretic and natriuretic effects of SGLT2 inhibitors, which lead to a reduction in blood volume and vascular resistance[105].

SGLT2 inhibitors, including dapagliflozin, canagliflozin, ipragliflozin, tofogliflozin, luseogliflozin, sotagliflozin, ertugliflozin and empagliflozin, have been studied in several clinical studies for the treatment of type 2 diabetes mellitus. Their selectivity for the SGLT2 receptor shows significant variance. While empagliflozin is 2500×, ertugliflozin is 2000×, dapagliflozin is 1200×, canagliflozin is 250×, sotagliflozin is only 20× more selective for the SGLT2 receptor over SGLT1, which may cause significant differences in the mechanism of action[115]. Several

studies have proved that SGLT2 inhibitors are associated with reduced cardiovascular morbidity and mortality, including heart failure, and vascular diseases[116-119].

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, HFpEF). Numerous potential mechanisms may explain these clinical benefits. First, they have been shown to rapidly lower pulmonary artery pressure that results in decongestion. Second, they may increase myocardial energy production and attenuate systemic microvascular dysfunction that is prevalent not only in the myocardium but in the skeletal muscle as well in these diseases. Furthermore, the improved global endothelial function, the reduced systemic inflammation and oxidative stress, the attenuated insulin resistance and increased fatty acid oxidation all lead to better CV function. Finally, they result in a modest weight reduction as well[117]. According to the 2022 AHA guideline for the management of heart failure, SGLT2 inhibitors have a Class of Recommendation 1A in both HFrEF and HFpEF irrespective of the presence of T2DM[118].

Furthermore, SGLT2 inhibitors have renoprotective effects as well[119]. 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[120], change the activation of the renin-aldosterone-angiotensin system[121] and shift renal fuel consumption towards ketone bodies[122]. Patients treated with SGLT2 inhibitors exhibit improved gluconeogenesis, ketogenesis, and erythrocytosis as well as reduced uricemia, which are the signs of nutrient deprivation signaling and also the main statistical mediators of the ability of SGLT2 inhibitors to reduce the risk of HF hospitalizations ad serious renal events. This promotion of autophagic flux in isolated cells and tissues that do not express SGLT2 and are not exposed to the changes in environmental glucose or ketones may be linked to these drugs' ability to bind directly to sirtuins or mTOR[123]. Until now angiotensin-converting-enzyme inhibitors and angiotensin II receptor blockers have been the only class of agents that were associated with reduced CKD progression risk, although neither was capable to reduce the risk of all-cause mortality. Recent academic results lead to the usage of SGLT2 inhibitors in CKD, as it has showed a significant reduction in the risk of both CKD progression and all-cause mortality[124].

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[125].

Other potential side effects of SGLT2 inhibitors include dehydration, hypotension (low blood pressure), and an increased risk of bone fractures. There have also been rare reports of ketoacidosis, a serious condition in which the body produces high levels of ketones that can lead to coma or death. To minimize the risk of these side effects, patients taking SGLT2 inhibitors should be carefully monitored and educated on the signs and symptoms of these conditions[126].

The main effects of SGLT2 inhibitors beyond their antidiabetic effect are summarized in Figure 1.

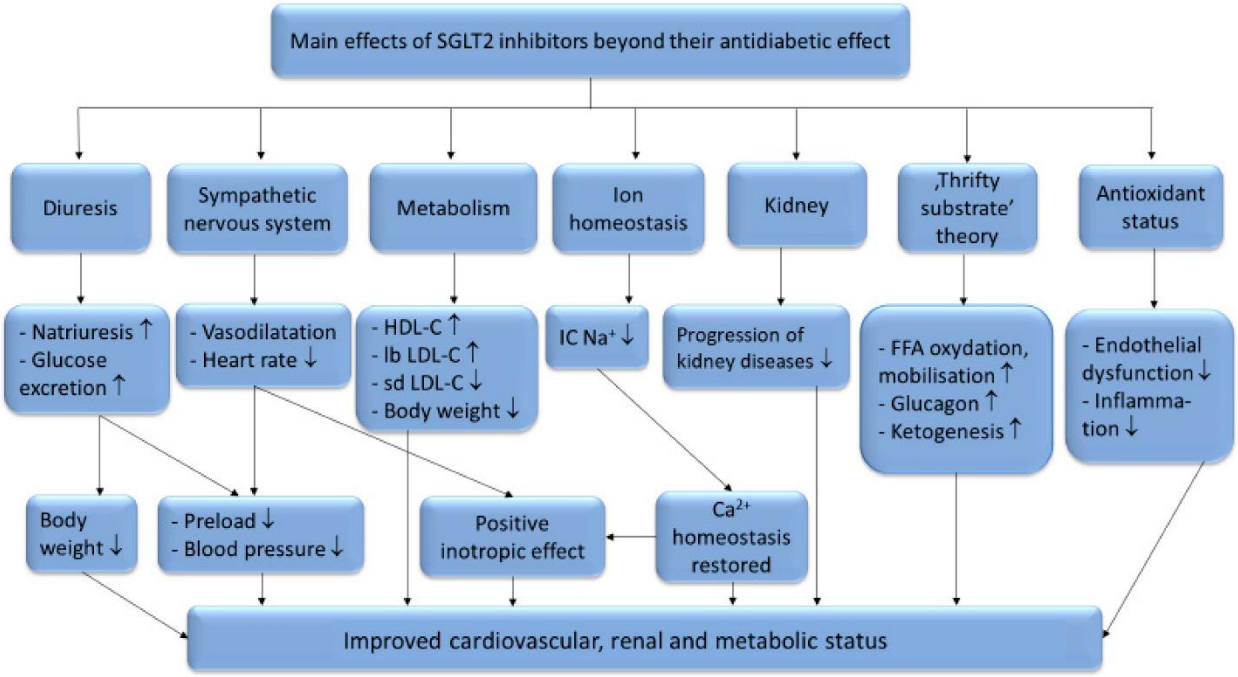

Figure 1. The systematic effects of SGLT2-inhibitors. HDL-C: high-density lipoprotein cholesterol; lb LDL-C: large buoyant low-density lipoprotein cholesterol; sd LDL-C: small dense low-density lipoprotein cholesterol; TG: triglycerides; IC Na+: intracellular sodium-ion; FFA: free fatty acids; ↑: increased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

2.10.2. The SGLT2-inhibitors' Effect on Plasma Lipoprotein Levels

Several studies reported lowered serum levels of total cholesterol (TC) and triglycerides (TG)[127–129] 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. According to Calapkulu et al., LDL-cholesterol level decreased by 13.4 mg/dL after 6 months in diabetic patients with dapagliflozin (10 mg/die)[127]. In contrast, Cha et al. reported an increase of 1.3 mg/dL in LDL level after 24 weeks of dapagliflozin add-on therapy[130], in concordance with the results of Basu et al.

in mice[131]. Furthermore, according to Schernthaner et al., canagliflozin (300 mg/die) caused an 11.7% increase in LDL particles after 52 weeks of therapy in patients with T2DM[132]. According to Basu et al., the cause of this possible increase in the LDL-cholesterol levels could be due to an increased lipoprotein-lipase (LpL) activity and because of a delayed turnover of LDL-cholesterol in the circulation. Canagliflozin reduced the expression of angiopoietin-like protein 4 (ANGPTL4), which is a known inhibitor of LpL in white and brown adipose, skeletal muscle, and heart tissues. With greater LpL-activity both the TG and the VLDL levels decreased. They also observed significantly delayed LDL-cholesterol turnover compared to the control group, which can originate from the lowered hepatic levels of the LDL receptor, which is the major receptor for the clearance of plasma LDL particles[131]. Another important factor is the ratio of the different LDL subclasses. Using gradient gel electrophoresis LDL particles are classified into 4 subclasses, including large (LDL I), intermediate (LDL II), small (LDL III), and very small (LDL IV) LDLs[133]. LDL I and II, are also referred to as large buoyant (lb) LDL, and LDL III and IV as small dense (sd) LDL particles[134,135]. Small dense LDL particles are more prone to induce metabolic disorders[136,137], obesity[138,139], T2DM[140,141] and coronary artery disease (CAD)[142] due to their longer circulation time than that of large LDL particles[143], enhanced ability to penetrate the arterial wall and higher susceptibility to oxidation[144]. It is generally known that modified (oxidized and glycated) LDL particles are highly atherogenic and possess more proinflammatory properties than native LDL molecules[142]. Interestingly SGLT2 inhibitors slightly increase LDL levels yet have beneficial effects on CV morbidity and mortality. The contradiction may be resolved by the results provided by Hayashi et al. showing that dapagliflozin decreased sd LDL, and increased lb LDL levels after 12 weeks of dapagliflozin therapy (5 mg/die) in type 2 diabetic patients[129]. This effect on LDL subclasses ratio may play a significant role in SGLT2 inhibitors' cardioprotective properties[145,146], provided it is a class effect.

Concerning the HDL levels, according to Kamijo et al. after 12 weeks of canagliflozin administration (100 mg/die) the very large high-density lipoprotein and large high-density lipoprotein (LHDL) values showed a significant increase, of 10.9% and 11.5% respectively. These beneficial changes might also contribute to subsequent reduction of cardiovascular outcomes, caused by SGLT2 inhibitors[146].

2.10.3. The SGLT2-inhibitors' Effect on Lipid Regulation

Several signaling molecules have been measured in mice after a 4-week treatment with canagliflozin by Ji et al.[128]. Elevated levels of diacylglycerol O-acyltransferase 2 (DGAT2)

mRNA were reversed by canagliflozin. They also observed an increase in PPAR-α, and a decrease in PPAR-γ levels[128]. DGAT2 is an integral membrane protein which promotes the synthesis and storage of TG in lipid droplets. The PPARs are a group of nuclear receptor proteins that function as transcription factors regulating the expression of several genes. Three types of PPARs have been identified: alpha (α), gamma (γ), and beta/delta (β/δ). While the PPAR-α is expressed mostly in the liver, kidneys, heart, muscle and adipose tissue, it mainly regulates the lipid metabolism in the liver. It is activated under conditions of energy deprivation, and it is necessary for the process of ketogenesis. Activation of PPAR-α promotes the uptake, utilization and catabolism of fatty acids through upregulating the genes that are involved in fatty acid binding and activation, peroxisomal and mitochondrial fatty acid β-oxidation. PPAR-γ regulates fatty acid storage and glucose metabolism. It activates genes stimulating lipid uptake and adipogenesis in fat cells, it also plays a crucial role in adipocyte differentiation. PPAR-γ increases insulin sensitivity through increasing the storage of fatty acids in fat cells, enhancing adiponectin release, inducing fibroblast growth factor 21 (FGF21) and upregulating the CD36 enzyme[147]. This data indicates that canagliflozin suppressed the synthesis of TG and the accumulation of hepatic lipid droplets through the downregulation of DGAT2.

Mice treated with canagliflozin had a significant increase in both hepatic and serum FGF21 levels[148]. FGF21 is a fasting-induced hepatokine that stimulates glucose uptake in adipocytes, but not in other cell types. FGF21 acts through the Ras/MAP kinase pathway. This indicates, that canagliflozin triggered a fasting-like catabolic switch, increasing the adipose lipolysis, hepatic fatty acid oxidation and ketogenesis, potentially via FGF21-dependent mechanisms. In addition, FGF21 can induce sympathetic activation in the central nervous system, leading to energy expenditure and weight loss. According to Osataphan et al., FGF21 was essential for the SGLT2 inhibitor-induced reduction in adipose tissue mass, adipocyte cell size and activation of lipolysis. While canagliflozin reduced adipocyte size, FGF21-null mice had no weight loss, and adipose tissue and cell size even increased in response to canagliflozin, suggesting that FGF21-null mice had impaired sympathetic and lipolytic activity. FGF21 is also responsible for increasing oxidative metabolism, browning and lipolysis in white adipose tissue[148].

According to the findings of Herrera et al., empagliflozin therapy significantly reduced the gene expression as well as the protein levels of CD36 in the atrial tissues of rats after 6 weeks[149]. CD36, also known as fatty acid translocase (FAT), is an integral membrane protein found on the surface of cells that imports fatty acids inside cells. It is also a member of the class B scavenger receptor family. CD36 interacts with several ligands, including collagen types I and IV, oxidized

LDL and long-chain fatty acids as well. It is also involved in the macrophages' phagocytosis. After CD36 binds to a ligand, they are internalized thus long-chained fatty acids and oxidized LDL particles can enter the cells. Since autophagy is decreased in metabolic disorders like diabetes and obesity, this dysregulation is an important factor in the pathophysiology of heart failure[149]. The results show, that empagliflozin may ameliorate the impaired basal cardiac levels of autophagy that would lead to the aggregation of proteins, at least in part through CD36, which contributes to the pathogenesis of cardiometabolic diseases.

Xu et al. showed that empagliflozin elevated AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC-CoA) phosphorylation in skeletal muscle, and increased hepatic and plasma FGF-21 levels. Empagliflozin also increased energy expenditure, heat production, and the expression of uncoupling protein 1 in brown fat and in inguinal and epididymal white adipose tissue. The M1-polarized macrophage accumulation was reduced, while plasma TNFα levels and obesity-related chronic inflammation decreased. In summary, empagliflozin did not just suppress weight gain by inducing fat utilization and browning, but also attenuated obesity-induced inflammation and insulin resistance[150]. Likewise, Osataphan et al. concluded that canagliflozin therapy activated AMPK through the inhibition of mitochondrial complex I, without an increase in ACC-CoA. However, this change in AMPK phosphorylation was not present in lean mice, thus AMPK is not likely to be the major mediator for the increase in fatty acid oxidation and ketogenesis[148]. One of the key molecules in the transport and oxidation of fatty acids is acetyl-CoA carboxylase (ACC), which converts acetyl-CoA to malonyl-CoA. Malonyl-CoA is an inhibitor of carnitine palmitoyltransferase 1, which transports fatty acids into the mitochondria for oxidation. Thus, the inactivation of ACC results in increased fatty acid transport and subsequent oxidation. On the other hand, AMPK may decrease malonyl-CoA levels by regulating malonyl-CoA decarboxylase. Another important role of AMPK is, that it phosphorylates and inactivates 3-hydroxy-3-methylglutaryl-CoA reductase, which is a key enzyme in cholesterol synthesis. AMPK, therefore, regulates fatty acid oxidation and cholesterol synthesis[150]. The mammalian target of rapamycin (mTOR) is a cellular energetic sensor, which is often regulated inversely with AMPK. Osataphan et al. found that after canagliflozin therapy there was a 56% decrease in the hepatic phosphorylation of the mTOR downstream substrate S6 when refeeding in canagliflozin-treated mice. This change was not present in the control groups, thus the decrease in mTOR signaling might be weight-dependent[148].

Empagliflozin therapy reduced the cardiac content of sphingolipids (sphingomyelins and ceramides) and glycerophospholipids, which play an important role in connecting insulin

resistance to cardiac damage, and even in the development of cardiovascular diseases. It is suggested that changes in lipid metabolism within the heart and cardiac lipid accumulation may have an important role in the development of diabetic cardiomyopathy and heart failure. Ceramides, sphingomyelins and glycerophospholipids are associated with lipotoxicity in the heart, thus they have a major impact on the organ's functionality. This suggests, that empagliflozin could regulate the metabolism and the cardiac accumulation of these cardiotoxic lipid molecules, which means, that it could be potentially useful for the prevention and treatment of not only diabetic cardiomyopathy, but also for the management of other cardiovascular diseases that have lipotoxicity in their pathogenesis[149].

2.10.4. The SGLT2-inhibitors' Effect on Metabolism

Chiang et al. investigated a novel SGLT2 inhibitor's, NGI001, effect on non-alcoholic fatty liver disease (NAFLD) and obesity-associated metabolic symptoms in high-fat diet-induced obese mice. According to their results NGI001 prevented adipocyte hypertrophy, inhibited impaired glucose metabolism and regulated the secretion of adipokines associated with insulin resistance. Notably, NGI001 suppressed hepatic lipid accumulation and inflammation. NGI001 ameliorated fat deposition and increased AMPK-phosphorylation, resulting in ACC-CoA phosphorylation. In addition, it blocked the storage of total fat in adipose tissue and alleviated TG accumulation in liver tissue, and the organ's weight decreased likewise. Interestingly, they found that the TG and cholesterol levels in the feces increased. This effect on the lipid excretion through the intestinal system needs further study[151]. Yokono et al. investigated the effects of ipragliflozin on body weight and composition in mice. They found that 4 weeks of therapy suppressed body weight increase despite the small increase in food intake. The reduction of body weight was accompanied by reduced visceral and subcutaneous fat masses. Ipragliflozin lowered the respiratory exchange ratio and decreased the heat production rate from glucose but increased it from fat, thus ipragliflozin mainly promoted the use of fatty acids instead of glucose as an energy source[152]. Other studies showed similar results with different SGLT2 inhibitors, like dapagliflozin[153,154], canagliflozin[155], and empagliflozin[156]. In summary, these results suggest that weight loss during SGLT2 inhibitor therapy may result from the reduction in fat tissue content via enhanced fatty acid utilization.

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. Also, the SGLT2 receptor was found on the surface of pancreatic α-cells

that can act as a glucose sensor[157]. On the other hand, the increased lipolysis also promotes the production of ketone bodies in the liver[158]. The oxidation of the ketone bodies is energetically more efficient than the oxidation of fatty acids because it results in a higher adenosine triphosphate (ATP)/oxygen ratio than other substrates. According to the 'thrifty substrate' theory under conditions of mild, persistent hyperketonemia, such as during SGLT2 inhibitor therapy, β-hydroxybutyrate is freely taken up by the heart and oxidized instead of fatty acids and glucose[159]. Taking into consideration that ketone bodies serve as an alternative and less expensive myocardial fuel source to the myocardium SGLT2 inhibitors may improve cardiac function and increase mechanical efficiency[160,161]. Furthermore, beta-hydroxybutyrate has antioxidant and antiarrhythmic effects, by inhibiting histone deacetylases, by upregulating mitochondrial biogenesis and, by stabilizing cell membrane potential[159]. Metabolic flexibility means the ability of a muscle to switch between free fatty acids (FFAs) and glucose as the main fuel source based on substrate availability. Patients with diabetes have impaired metabolic flexibility, thus the myocardium becomes more reliant on FFA oxidation. This impairment is the result of the increased FFA delivery to the heart due to peripheral insulin resistance and due to insulin's inability of suppressing lipolysis. This causes increased myocardial FFA oxidation and reduced glucose oxidation. FFAs also impair insulin action by inhibiting insulin signaling pathways, which leads to decreased cellular glucose transport, and even less glucose oxidation. This increase in FFA oxidation decreases cardiac efficiency and causes lipotoxicity. Hyperglycemia causes glucotoxicity, which is associated with ROS overproduction, which has a negative effect on mitochondrial function and other cellular processes. Ketone bodies are an alternative fuel for the cells that played a critical role in human survival. They are almost exclusively synthesized in the liver in the case of high circulating FFA levels, and when the production of ACC-CoA exceeds hepatic cellular energy requirements. The ketone bodies then diffuse into the circulation and extrahepatic tissues, mainly the heart and kidney, providing a major energy source during fasting[122]. It is documented that ketone bodies are mildly elevated when SGLT2 inhibitors are administered to patients[162]. The myocardium is the highest consumer of ketone bodies per unit mass, and it is shown, that despite impairments in skeletal muscle ketone body utilization, myocardial ketone body utilization was preserved in heart failure[163]. However, we must also consider, that euglycemic diabetic ketoacidosis may be a rare, but severe side effect of SGLT2 inhibitor drugs, observed mostly under conditions favorable to excessive ketogenesis, such as increased alcohol consumption, volume loss, infection, stroke, and myocardial infarction[164]. In summary, SGLT2 inhibition in normal conditions through substrate shift offers the myocardium

an alternative fuel that increases cardiac efficiency and decreases lipotoxicity. The main effects of SGLT2 inhibitors on lipid metabolism are summarized in Figure 2.

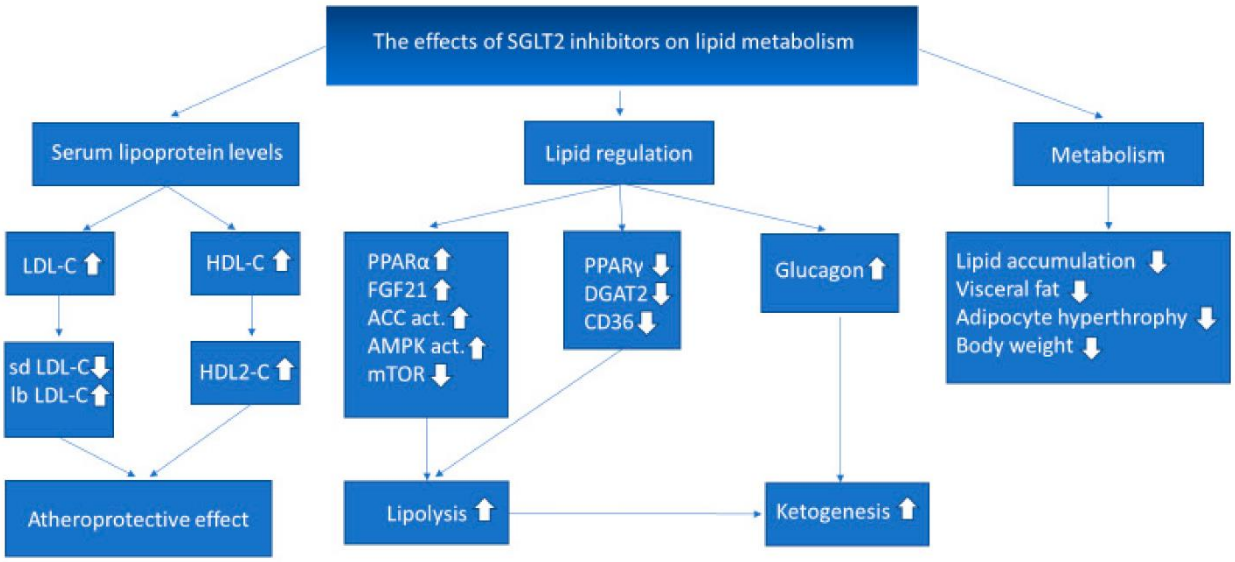

Figure 2. The effects of SGLT2 inhibitor therapy on lipid metabolism. LDL-C: low-density lipoprotein cholesterol; sd LDL-C: small dense low-density lipoprotein cholesterol; lb LDL-C: large buoyant low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; PPARα: peroxisome proliferator-activated 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].

3. Focus and Aims of the present work

3.1. 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.

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

4.1. Materials and methods

4.1.1. 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. Four patients were excluded from the study for different reasons (low compliance, severe epileptic seizure, withdrawal of their consent, and urgent psychiatric ward admission).

4.1.2. Study design

102 patients were recruited into this non-randomized, controlled, cohort clinical study. We assessed their body composition, followed by pre-prandial venous blood collected using a peripheral venous catheter in the cubital vein. The preparation and laboratory procedures were in full accordance with the recommendations of the laboratory kits. Laboratory tests were performed at the Department of Laboratory Medicine, University of Pecs, Pecs, Hungary. The leptin levels were determined using the immunoassay method (Human Leptin ELISA, Biovendor, Czech Republic) at the Department of Biochemistry and Medical Chemistry, University of Pecs, Pecs, Hungary.

4.1.3. 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.

4.1.4. 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.

4.1.5. 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).

4.1.6. 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.

4.2. Results

4.2.1. Study population

Patients' general characteristics were as follows. The mean age for different groups was: 65.95 for group C, 66.40 for group O, 74.58 for group D, 70.90 for group OD, and 65.20 for group ODE. The distribution of sex (male to female percentage) in the groups was as follows: 75–25% for group C, 50–50% for group O, 52.60–47.40% for group D, 68.40–31.60% for group OD, and 75–25% for group ODE. Mean BMI values for different groups were as follows: 26.01 kg/m2 for group C, 34.75 kg/m2 for group O, 26.50 kg/m2 for group D, 35.78 kg/m2 for group OD, and 31.61 kg/m2 for group ODE. All patients had high blood pressure in their medical history. All patients in the diabetic groups (D, OD, ODE) had identified type 2 diabetes mellitus in their medical history, whereas none were reported in the remaining groups (C, O). The percentage of patients with identified cardiovascular disease was 70.40% in group C, 69.40% in group O, 78.60% in group D, 64.30% in group OD, and 73.48% in group ODE. All patients received antihypertensive therapy. All diabetic patients (D, OD, ODE) received antidiabetic therapy, whereas none were administered in the non-diabetic groups (C, O). Empagliflozin was administered only in the ODE group. No other SGLT2 inhibitors were used in our study. The percentage of patients with antihyperlipidemic therapy was as follows: 70% for group C, 75% for group O, 89.47% for group D, 84.20% for group OD, and 80% for group ODE.

4.2.2. 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 (Table 1).

Table 1. Patients' general characteristics, C= control group, n = number of patients, O = obese group, D = diabetic group, OD = obese and diabetic group, ODE = empagliflozin-treated obese and diabetic group, BMI = body mass index, kg = kilogram, m2 = square meter, Body fat = body fat percentage, Visc. fat = visceral fat, HT = high blood pressure, DM = type 2 diabetes mellitus, CVD = cardiovascular disease, ACE = angiotensin-converting enzyme, ARB = angiotensin receptor blocker, SGLT2i = Sodium-glucose co-transporter 2 inhibitor, DPP-4 = Dipeptidyl peptidase-4, SGLT2i = Sodium-glucose cotransporter 2 inhibitors, # = 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.

Group		C (n = 20)	O (n = 20)	D (n = 19)	OD (n = 19)	ODE (n = 20)
Demographics and anthropometrics						
Age, years		65.95±1.98*	66.40±2.23*	74.58±6.38$^{\#,\#\#,***}$	70.90±1.74	65.2±1.86*
Male sex, %		75.00	50.00	52.60	68.40	75.00
BMI, kg/m²		26.01±0.50$^{\#\#,**,***}$	34.75±0.85$^{\#,*,***}$	26.50±0.44$^{\#\#,**,***}$	35.78±0.91$^{\#,*,***}$	31.61±0.77$^{\#,\#\#,**}$
Body fat, %		26.75±6.73$^{\#\#,**}$	38.44±8.38$^{\#,*,***}$	28.12±6.62$^{\#\#,**}$	37.24±6.67$^{\#,*,***}$	30.98±6.06$^{\#\#,**}$
Visc. fat, %)		10.5±0.56$^{\#\#,**,***}$	16.65±0.93$^{\#,*}$	10.89±0.58$^{\#\#,**,***}$	19.01±1.25$^{\#,*,***}$	15.50±0.67$^{\#,*,**}$
Comorbidities						
HT, %		100.00	100.00	100.00	100.0	100.00
DM, %		0.00	0.00	100.00	100.00	100.00
CVD, %		70.00	70.00	78.60	84.70	65.00
Medications						
Antihypertensive drugs, %	**Total**	100.00	100.00	100.00	100.00	100.00
	Beta blocker	85.00	85.00	94.74	84.21	90.00
	ACE inhibitor/ARB	70.00	80.00	94.74	100.00	95.00
	Calcium channel blocker	35.00	30.00	42.11	52.63	55.00
Antihyperlipi-demic drugs, %	**Total**	70.00	65.00	89.47	84.20	80.00
	Statin	70.00	65.00	89.47	84.20	80.00
	Cholesterol absorption inhibitor	00.00	10.00	21.05	15.79	15.00
	Fibrate	5.00	0.00	5.26	10.53	10.00
Antidiabetics, %	**Total**	0.00	0.00	100.00	100.00	100.00
	Biguanide	0.00	0.00	78.95	89.47	100.00
	Insulin	0.00	0.00	21.05	31.58	35.00
	Sulfanylurea	0.00	0.00	10.53	36.84	40.00
	DPP-4 inhibitor	0.00	0.00	5.26	21.05	25.00
	SGLT2i	0.00	0.00	0.00	0.00	100.00

4.2.3. 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 2).

Table 2. 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

4.2.4. 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.

4.2.5. 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 2).

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 ($p = 0.012$) (Table 2).

4.2.6. 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 ($p = 0.048$) (Table 2).

4.2.7. 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%)

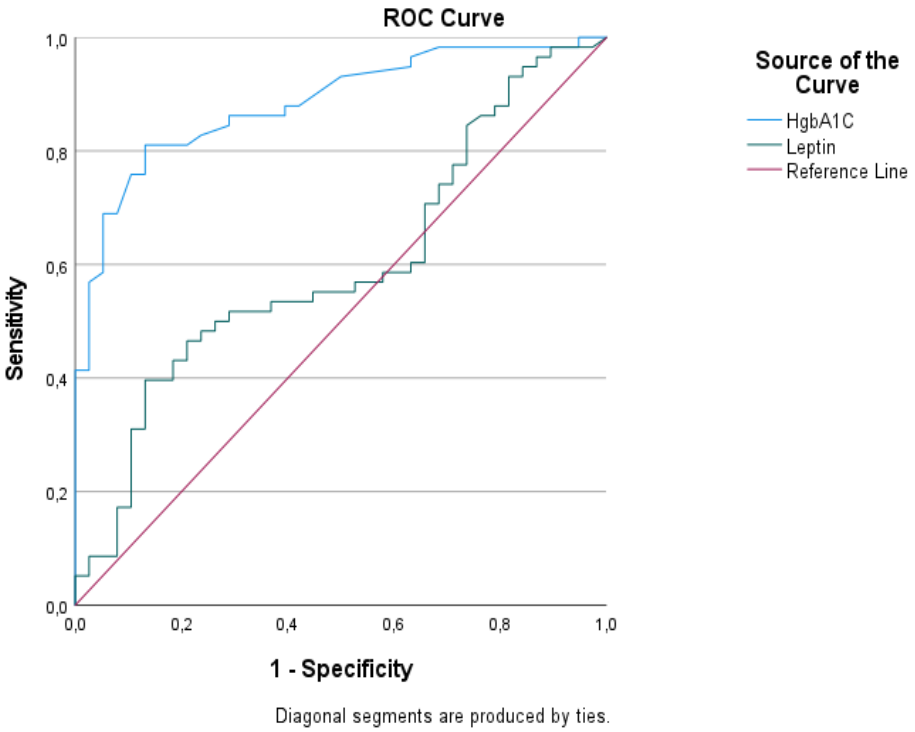

Figure 3. ROC analysis of HgbA1C and leptin to test the diagnostic power for composit endpoint diabetes.

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%).

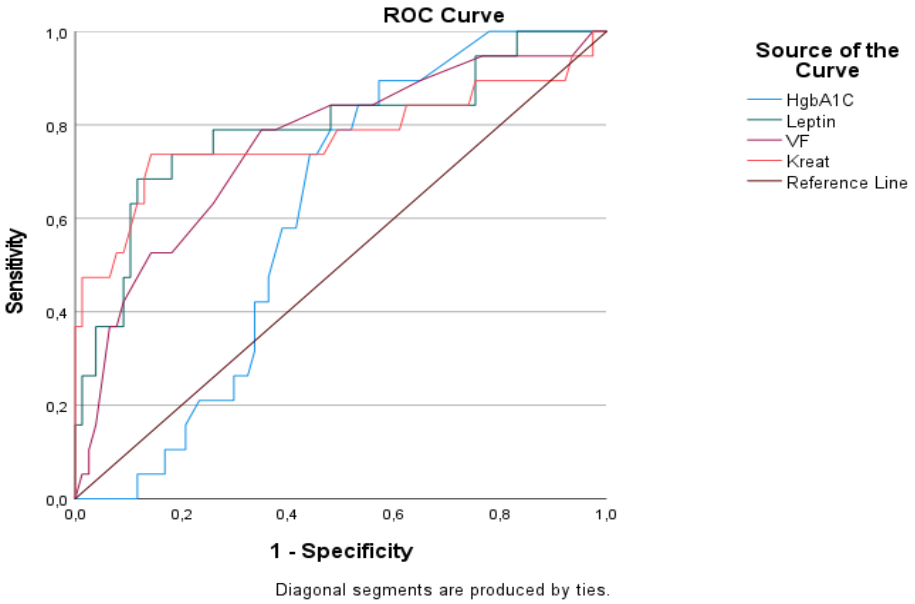

Figure 4. ROC analysis of HgbA1C, leptin, visceral fat (VF) and creatinine (Kreat) to test the diagnostic power for composit endpoint diabetes and obesity.

4.2.8. 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 2.

5. Discussion

According to the latest data, nearly 2 billion adults (39% of the world's adult population) were estimated to overweight, of whom 671 million (12% of the world's adult population) were considered obese. If current trends continue, it is expected that 1 billion adults, nearly 20% of the world's adult population, will clinically be declared obese by 2025[46]. Obesity, especially the dysfunctional VAT, is the main driver of many metabolic abnormalities including insulin resistance, hyperinsulinemia, glucose intolerance, atherogenic dyslipidemia (high triglyceride and apolipoprotein B levels, increased proportion of small, dense LDL particles, low HDL-cholesterol levels, and small HDL particles), and is associated with a low-grade inflammation[165,166].

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[167]. The serum level of leptin is elevated paradoxically in obesity[168], and this high level of leptin may induce leptin resistance and result in altered glucose metabolism and insulin resistance[169]. Hyperleptinemia has also been associated with increased inflammation, oxidative stress, endothelial dysfunction, atherogenesis, and thrombosis[170]. 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[171].

The link between obesity and T2DM has long been recognized and explains the high prevalence of type 2 diabetes mellitus. T2DM is associated with many vascular complications. Microvascular complications include diabetic kidney disease, retinopathy, and neuropathy, whereas the 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[172]. Novel antidiabetic therapies such as SGLT2 inhibitors provide a new approach to preventing or ameliorating the complications that insulin resistance and hyperglycemia create[173]. SGLT2 inhibitors are potent antihyperglycemic drugs, which inhibit glucose reabsorption in the proximal tubules of the kidney inducing glycosuria and improving blood glucose levels and may reduce body weight through calorie loss. Numerous studies have shown they are associated with reduced cardiovascular morbidity and mortality, including vascular diseases and heart failure[174]. Furthermore, SGLT2 inhibitors have also demonstrated positive reno-metabolic effects[105]. In a cardiovascular outcome trial, the SGLT2 inhibitor empagliflozin proved superior to conventional antidiabetic therapy in reducing the rate major adverse cardiovascular events, mortality, and

hospitalization due to heart failure[175]. SGLT2 inhibitor therapy has been associated with a decrease in serum triglycerides, an increase in HDL cholesterol, and also a small increase in LDL cholesterol level was observed[105]. The presence of metabolic disturbances in obese patients results in oxidative stress[176]. Since obesity and insulin resistance is a major component of metabolic syndrome, it is strongly associated with oxidative stress[177]. The oxidative modification of lipoproteins can result in more atherogenic compounds, which may have a key role in the development of cardiovascular dysfunction in patients with diabetes mellitus[178].

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

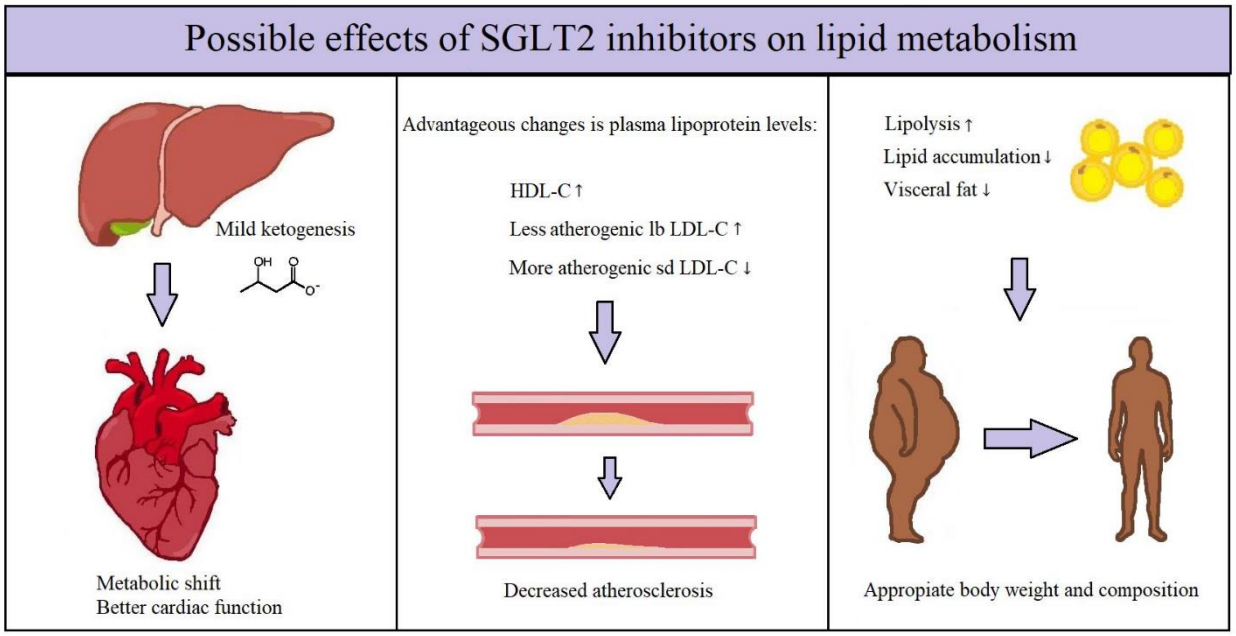

Figure 5. Possible effects of SGLT2 inhibitors on lipid metabolism[105].

Interestingly, these cardiovascular and renoprotective effects occur despite an increase in LDL-cholesterol levels which was observed in several clinical studies[179,180]. Nevertheless, this increase in LDL-C happens in the setting of other beneficial changes in plasma lipoprotein metabolism.

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[181]. 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 (Table 1). In an animal study, empagliflozin suppressed weight gain by shifting energy metabolism towards fat utilization, elevated adenosine monophosphate-activated (AMP) protein kinase, and acetyl coenzyme A (acetyl-CoA) carboxylase phosphorylation in skeletal muscle. Furthermore, empagliflozin increased energy expenditure, heat production and browning, and attenuated obesity-induced inflammation and insulin resistance by polarizing M2 macrophages in white adipose tissue (WAT) and liver[150]. Thus, empagliflozin suppressed weight gain by enhancing fat utilization and browning and attenuated obesity-induced inflammation and insulin resistance.

White adipose tissue is an endocrine organ capable of producing and releasing numerous bioactive substances known as adipokines or adipocytokines. Dysregulated production of adipocytokines is involved in the development of obesity-related diseases. Leptin is one of the most examined adipokines. An increased leptin level is associated with insulin resistance and T2DM development[182]. 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[183]. Furthermore, obesity, hypertension, metabolic syndrome, and endothelial dysfunction are more frequent in T2DM patients with increased leptin levels[183]. In CHD patients, elevated leptin levels were significantly associated with an increased risk of cardiac death, acute coronary syndrome, non-fatal myocardial infarction, stroke, and hospitalization for congestive heart failure[184,185]. Similarly, higher leptin levels were significantly related to the number of stenotic coronary arteries and arterial stiffness in CHD patients[186]. The presence, severity, extent, and lesion complexity of coronary atherosclerosis have been associated with higher leptin levels in CHD patients[187]. Leptin may also affect cardiac remodeling, metabolism, and contractile function[188]. Other effects of leptin include activation of inflammatory responses,

oxidative stress, thrombosis, and atherosclerosis, thereby resulting in endothelial dysfunction and atherosclerotic plaque[171].

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.

A link between increased plasma leptin concentrations and CKD has been reported, which is possibly due to reduced renal clearance[189]. Leptin concentrations gradually increased with the severity of CKD[190]. 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)[191]. Overall, hyperleptinemia has been linked to the presence, severity, and progression of CKD. In our study, creatinine levels were significantly higher with the appearance of diabetes and were the highest among obese patients with T2DM. Among the empagliflozin-treated obese and T3DM patients, the creatinine level was significantly lower eliciting improved renal function (Table 2).

We possess a vast amount of knowledge regarding the cardiovascular and renal effects of SGLT2 inhibitors[105,116,192–194]. In addition to their direct effect on glucose homeostasis, they have many other underlying mechanisms from which not all are fully understood. For instance, SGLT2 inhibitors may also act upon visceral adipose tissue. Dapagliflozin therapy was associated with a decreased circulating leptin level and an increased circulating adiponectin level among patients with type 2 diabetes, which, may contribute to the beneficial effects of SGLT2 inhibitors on metabolic homeostasis, such as improved insulin resistance and reduced cardiovascular risk[195–197]. Furthermore, dapagliflozin displayed significantly lower arterial stiffness in diabetic mice treated with dapagliflozin when compared to untreated diabetic mice[198]. 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[192]. 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 (Table 2). 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.

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[182].

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. It is worthwhile to draw the attention of patients to the need for adequate fluid intake during SGLT2 inhibitor treatment. Unexpectedly, HbA1c levels were the highest in the empagliflozin-treated group. Presumably, this is due to the fact that, in Hungary, SGLT2 inhibitor treatment can only be prescribed to patients with an HbA1c level above 7%. It is important to note, that even though insulin therapy was higher in the empagliflozin treated obese diabetic group, the HbA1c and blood glucose levels were still higher when compared to the obese diabetic group. This also means this group is a more severe patient group in terms of diabetes, thus, the results obtained prove even more crucial.

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[174]. 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[199]. In the EMPA-CARD trial patients with type 2 diabetes and coronary artery disease treated with empagliflozin had lower levels of IL-6, IL-1β and CRP levels compared to a placebo. There were elevations in superoxidase dismutase activity, glutathione, and total antioxidant capacity with empagliflozin[200].

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.

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.

6. Future perspectives

6.1. Body mass index, body fat and visceral fat were significantly lowered in the empagliflozin-treated group. According to several studies, weight loss during SGLT2 inhibitor therapy may result from the reduction in fat tissue content via enhanced fatty acid utilization. Empagliflozin acts against adiposity by the promotion of fat and sugar utilization and by enhancing β-oxidation of free fatty acids. Furthermore, increased energy expenditure was found in empagliflozin-treated mice, as well as increased browning in the white adipose tissue, which suggests that empagliflozin may regulate the proton influx back into the mitochondrial matrix and dissipates oxidative energy as heat instead of the production of adenosine triphosphate. These results may indicate the usage of SGLT2 inhibitor therapy in insulin resistance, fatty liver disease and for weight loss in obese patients without diabetes mellitus. These observations need further investigations on a larger number of patients in clinical settings.

6.2. Leptin levels were significantly higher in obese patients yet were reduced in the empagliflozin-treated group. These results indicate that SGLT2 inhibitor therapy might be useful both in obesity and prediabetes for the prevention of diabetes mellitus. Further investigations are needed with a wider population to clarify the exact effects of SGLT2 inhibitors on leptin.

6.3. Several studies reported lowered serum levels of total cholesterol and triglycerides as a result of SGLT2 inhibitor therapy. Furthermore, it has been observed, that SGLT2 inhibitor therapy decreased the level of small dense LDL particles that are highly atherogenic and possess higher inflammatory properties, while increasing the level of large buoyant LDL particles, which are less likely involved in the development of cardiovascular diseases. This means that even though the level of total cholesterol may slightly increase during SGLT2 inhibition therapy, the dyslipidemia is still attenuated, in terms of atherogenic cholesterol components. SGLT2 inhibitor therapy also increased the levels of very large high-density lipoprotein cholesterol and large high-density lipoprotein cholesterol levels. These complex effects on blood lipid profiles might be especially useful in the treatment of dyslipidemia. These observations need further investigations on a larger number of patients in clinical settings.

6.4. Empagliflozin therapy may regulate the metabolism and the cardiac accumulation of cardiotoxic lipid molecules, such as ceramides, sphingomyelins and glycerophospholipids. According to these results conducted on animal models, cardiomyopathy and other lipotoxic cardiac diseases might be susceptible to SGLT2 inhibitor therapy, however further clinical studies

are needed to fully understand the exact molecular mechanisms. SGLT2 inhibition offers the myocardium an alternative fuel (ketone bodies) via substrate shift that increases cardiac efficiency and decreases lipotoxicity. The usage of SGLT2 inhibition in heart failure is already known, thus it might be important to reconsider its use in other cardiac diseases as well, such as cardiomyopathies These observations need further investigations on a larger number of patients in clinical settings.

7. 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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10. 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: 4.097 (2021)

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 IF: 2,849.
Quartile Ranking: Q2 Impact Factor: 2.849 (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

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

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Abstract: Obesity is a major public health problem worldwide, and it is associated with many diseases and abnormalities, most importantly, type 2 diabetes. The visceral adipose tissue produces an immense variety of adipokines. Leptin is the first identified adipokine which plays a crucial role in the regulation of food intake and metabolism. Sodium glucose co-transport 2 inhibitors are potent antihyperglycemic drugs with various beneficial systemic effects. We aimed to investigate the metabolic state and leptin level among patients with obesity and type 2 diabetes mellitus, and the effect of empagliflozin upon these parameters. We recruited 102 patients into our clinical study, then we performed anthropometric, laboratory, and immunoassay tests. Body mass index, body fat, visceral fat, urea nitrogen, creatinine, and leptin levels were significantly lower in the empagliflozin treated group when compared to obese and diabetic patients receiving conventional antidiabetic treatments. Interestingly, leptin was increased not only among obese patients but in type 2 diabetic patients as well. Body mass index, body fat, and visceral fat percentages were lower, and renal function was preserved in patients receiving empagliflozin treatment. In addition to the known beneficial effects of empagliflozin regarding the cardio-metabolic and renal systems, it may also influence leptin resistance.

Keywords: empagliflozin; type 2 diabetes mellitus; obesity; leptin; lipid metabolism

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1. Introduction

According to the latest data, nearly 2 billion adults (39% of the world's adult population) were estimated to be obese or overweight. If current trends continue, it is expected that 1 billion adults, nearly 20% of the world's population, will clinically be declared obese by 2025 [1]. Obesity is associated with many diseases and abnormalities, such as type 2 diabetes [2], dyslipidemia [3], cardiovascular diseases [4], hypertension [5], certain types of cancer [6,7], pneumological [6], nephrological [8], skeletal muscle [9], rheumatologic [10], dermatologic [11], and neuropsychologic [11] complications, and is it associated with premature mortality. Obesity, especially the dysfunctional visceral adipose tissue (VAT), is the main driver of many metabolic abnormalities including insulin resistance, hyperinsulinemia, glucose intolerance, atherogenic dyslipidemia (high triglyceride and apolipoprotein B levels, increased proportion of small, dense LDL [low-density lipoprotein] particles, low HDL [high-density lipoprotein] cholesterol levels, and small HDL particles), and is associated with a low-grade inflammation [6].

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 [12]. The serum level of leptin is elevated paradoxically in obesity [13], and this high level of leptin may induce leptin resistance and result in altered glucose metabolism and insulin resistance [14]. Hyperleptinemia has also been associated with increased inflammation, oxidative stress, endothelial dysfunction, atherogenesis, and thrombosis [15]. Based on these effects, leptin is attributed to a significant role in the development of cardiovascular diseases. Additionally, patients with type 2 diabetes mellitus scored a higher percentage of hypertension, obesity, metabolic syndrome, and endothelial dysfunction if they had elevated leptin levels [16].

The link between obesity and type 2 diabetes mellitus [T2DM] has long been recognized and explains the high prevalence of type 2 diabetes mellitus. Type 2 diabetes mellitus is associated with many vascular complications. Microvascular complications include diabetic kidney disease, retinopathy, and neuropathy, whereas the 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 [17]. Novel antidiabetic therapies such as sodium glucose co-transporter 2 (SGLT2) inhibitors provide a new approach to preventing or ameliorating the complications that insulin resistance and hyperglycemia create [18]. SGLT2 inhibitors are potent antihyperglycemic drugs, which inhibit glucose reabsorption in the proximal tubules of the kidney inducing glycosuria and improving blood glucose levels, and may reduce body weight through calorie loss. Numerous studies have shown they are associated with reduced cardiovascular morbidity and mortality, including vascular diseases and heart failure [19]. Furthermore, SGLT2 inhibitors have also demonstrated positive reno-metabolic effects [20]. In a cardiovascular outcome trial, the SGLT2 inhibitor empagliflozin proved superior to conventional antidiabetic therapy in reducing the rate of MACE, mortality, and hospitalization due to heart failure [21]. SGLT2 inhibitor therapy has been associated with a decrease in serum triglycerides, an increase in HDL cholesterol, and also a small increase in LDL cholesterol level was observed [20]. The presence of metabolic disturbances in obese patients results in oxidative stress [22]. Since obesity and insulin resistance is a major component of metabolic syndrome, it is strongly associated with oxidative stress [23]. The oxidative modification of lipoproteins can result in more atherogenic compounds, which may have a key role in the development of cardiovascular dysfunction in patients with diabetes mellitus [24,25].

The aim of our study was to investigate certain laboratory parameters such as lipids, inflammatory markers, blood glucose level, glycated hemoglobin [HbA1c] level, kidney function, leptin level, as well as body mass index [BMI], body fat and visceral fat percentage among patients afflicted with obesity and diabetes. We also investigated a subgroup of patients receiving empagliflozin treatment.

2. Results

2.1. Body Mass Index, Body Fat, and Visceral Fat Were Significantly Lower in the Empagliflozin Treated Group

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

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

Visceral fat was significantly lower in the control group (C) when compared to the obese (O) ($p < 0.001$), to the obese and diabetic (OD) ($p < 0.001$), and to the empagliflozin-treated group (ODE) ($p < 0.001$). It was also significantly lower in the diabetic (D) group when compared to the obese (O) ($p < 0.001$), to the obese and diabetic (OD) ($p < 0.001$), and to the empagliflozin-treated group ($p < 0.001$). Visceral fat was significantly lower in the empagliflozin-treated group (ODE) when compared to the obese and diabetic (OD) group ($p < 0.014$). There were no significant differences between the other groups (Table 1).

Table 1. Patients' general characteristics, n = number of patients, BMI = body mass index, kg = kilogram, m^2 = square meter, Body fat = body fat percentage, Visc. fat = visceral fat, HT = high blood pressure, DM = type 2 diabetes mellitus, CVD = cardiovascular disease, SGLT2i = Sodium glucose co-transporter 2 inhibitors.

Group	C (n = 20)	O (n = 20)	D (n = 19)	OD (n = 19)	ODE (n = 20)	Total (n = 98)
Demographics and anthropometrics						
Age, years	65.95 ± 1.98	66.40 ± 2.23	74.58 ± 6.38	70.90 ± 1.74	65.2 ± 1.86	68.52 ± 0.90
Male sex, %	75.00	50.00	52.60	68.40	75.00	64.30
BMI, kg/m²	26.01 ± 0.50	34.75 ± 0.85	26.50 ± 0.44	35.78 ± 0.91	31.61 ± 0.77	31.04 ± 0.52
Body fat, %	26.75 ± 6.73	38.44 ± 8.38	28.12 ± 6.62	37.24 ± 6.67	30.98 ± 6.06	32.93 ± 6.90
Visc. fat, %)	10.5 ± 0.56	16.65 ± 0.93	10.89 ± 0.58	19.01 ± 1.25	15.50 ± 0.67	14.51 ± 0.79
Comorbidities						
HT, %	100.00	100.00	100.00	100.0	100.00	100.00
DM, %	0.00	0.00	100.00	100.00	100.00	59.20
CVD, %	70.40	69.40	78.60	84.70	64.30	73.48
Medications						
Antihypertensive, %	100.00	100.00	100.00	100.00	100.00	100.00
Antidiabetics (other, than SGLT2i), %	0.00	0.00	100.00	100.00	100.00	59.20
Antihyperlipidemic, %	70.00	75.00	89.47	84.20	80.00	79.59

2.2. Hemoglobin Levels Were Significantly Higher among the Empagliflozin Treated Patients

Hemoglobin levels were significantly higher in the empagliflozin-treated group (ODE) when compared to the diabetic (D), and obese and diabetic (OD) groups ($p = 0.004$ and $p < 0.001$, respectively). There was no significant difference between the diabetic (D) and the obese and diabetic (OD) group ($p = 0.850$). The obese group (O) had a significantly higher hemoglobin when compared to the obese and diabetic group (OD) and a significantly lower level when compared to the empagliflozin-treated obese group (ODE) ($p = 0.033$ and $p = 0.007$ respectively) (Table 2).

2.3. Renal Parameters Were Significantly Higher in Diabetic Patients, Yet Were Reduced in the Empagliflozin Treated Group

Urea nitrogen level increases significantly with the appearance of diabetes in obesity (O vs. OD) ($p = 0.002$). In the empagliflozin-treated group (ODE), the urea nitrogen level was significantly lower when compared to the obese and diabetic (OD) group ($p = 0.008$) (Table 2).

Creatinine significantly increases with the appearance of diabetes in the obese groups (O vs. OD) ($p = 0.011$). In the empagliflozin-treated group (ODE), the creatinine level was significantly lower when compared to the obese and diabetic (OD) group ($p = 0.012$) (Table 2).

Table 2. 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.

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
HbA1c, %	5.48 ± 0.08	5.67 ± 0.93	6.72 ± 0.34	6.39 ± 0.15	7.68 ± 0.33
Blood glucose, mmol/L	5.48 ± 0.85	5.62 ± 1.17	6.79 ± 1.95	6.20 ± 1.53	7.01 ± 1.61
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	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

2.4. Blood Glucose and HbA1c Levels Were Significantly Higher in Diabetic Patients, Yet There Was No Significant Difference between the Different Diabetic Groups

Blood glucose and HbA1c levels were significantly lower in the control group (C) when compared with the diabetic (D), the obese and diabetic (OD), 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 (D), the obese and diabetic (OD), 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.

2.5. Leptin Levels Were Significantly Higher in Obese Patients, Yet Were Reduced in the Empagliflozin-Treated Group

Leptin levels were significantly higher with the appearance of obesity (O) ($p = 0.003$) even if obesity was present with diabetes (OD) ($p < 0.001$) when compared to the control (C) group. It was also significantly higher in diabetic patients (D) when compared with the control group (C) ($p = 0.029$). Obese and diabetic patients (OD) had a significantly higher level of leptin when compared to diabetic yet not obese (D) patients ($p = 0.001$). In the empagliflozin-treated group (ODE), the leptin level was significantly lower when compared to the obese and diabetic (OD) group ($p = 0.048$) (Table 2).

2.6. There Were No Significant Differences between the 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.

3. Discussion

In our clinical study, we examined metabolic and inflammatory parameters, kidney function, and leptin levels among patients afflicted with hypertension, obesity, type 2 diabetes, 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 type 2 diabetes even with normal BMI, and were significantly higher in obese non-diabetic patients and were the highest in obese patients with type 2 diabetes. 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 [26]. In our study, BMI, body fat, and visceral fat percentage were the highest among patients with obesity and type 2 diabetes (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 (Table 1). In an animal study, empagliflozin suppressed weight gain by shifting energy metabolism towards fat utilization, elevated adenosine monophosphate-activated [AMP] protein kinase, and acetyl coenzyme A [acetyl-CoA] carboxylase phosphorylation in skeletal muscle. Furthermore, empagliflozin increased energy expenditure, heat production and browning, and attenuated obesity-induced inflammation and insulin resistance by polarizing M2 macrophages in white adipose tissue [WAT] and liver [27]. Thus, empagliflozin suppressed weight gain by enhancing fat utilization and browning and attenuated obesity-induced inflammation and insulin resistance.

White adipose tissue is an endocrine organ capable of producing and releasing numerous bioactive substances known as adipokines or adipocytokines. Dysregulated production of adipocytokines is involved in the development of obesity-related diseases. Leptin is one of the most examined adipokines. An increased leptin level is associated with insulin resistance and T2DM development [28]. In T2DM, a link has also been reported between high leptin concentrations and increased cardiovascular [CV risk], including the presence of microvascular complications and cardiac autonomic dysfunction [29]. Furthermore, obesity, hypertension, metabolic syndrome, and endothelial dysfunction are more frequent in T2DM patients with increased leptin levels [30]. In chronic heart disease (CHD) patients, elevated leptin levels were significantly associated with an increased risk of cardiac death, acute coronary syndrome, non-fatal MI, stroke, and hospitalization for congestive heart failure [31,32]. Similarly, higher leptin levels were significantly related to the number of stenotic coronary arteries and arterial stiffness in CHD patients [33]. The presence, severity, extent, and lesion complexity of coronary atherosclerosis have been associated with higher leptin levels in CHD patients [34]. Leptin may also affect cardiac remodeling, metabolism, and contractile function [35]. Other effects of leptin include activation of inflammatory responses, oxidative stress, thrombosis, and atherosclerosis, thereby resulting in endothelial dysfunction and atherosclerotic plaque [16].

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.

A link between increased plasma leptin concentrations and chronic kidney disease (CKD) has been reported, which is possibly due to reduced renal clearance [36]. Leptin concentrations gradually increased with the severity of CKD [37]. 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) [38]. Overall, hyperleptinemia has been linked to the presence, severity, and progression of CKD. In our study, creatinine levels were significantly higher with the appearance of diabetes and were the highest among obese patients with type 2 diabetes. Among the empagliflozin-treated obese and type 2 diabetic patients, the creatinine level was significantly lower eliciting improved renal function (Table 2).

We possess a vast amount of knowledge regarding the cardiovascular and renal effects of SGLT2 inhibitors [20,39–42]. In addition to their direct effect on glucose homeostasis, they have many other underlying mechanisms from which not all are fully understood. For instance, SGLT2 inhibitors may also act upon visceral adipose tissue. Dapagliflozin therapy was associated with a decreased circulating leptin level and an increased circulating adiponectin level among patients with type 2 diabetes, which, may contribute to the beneficial effects of SGLT2 inhibitors on metabolic homeostasis, such as improved insulin resistance and reduced cardiovascular risk [43–45]. Furthermore, dapagliflozin displayed significantly lower arterial stiffness in diabetic mice treated with dapagliflozin when compared to untreated diabetic mice [46]. 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 [39]. In our study, the leptin level was significantly lower in the empagliflozin-treated obese and type 2 diabetic patients (ODE) when compared to the obese, diabetic patients (OD) treated with other antidiabetics (Table 2). 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.

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 receiver operating characteristic [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 [28].

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. It is worthwhile to draw the attention of patients to the need for adequate fluid intake during SGLT2 inhibitor treatment. Unexpectedly, HbA1c levels were the highest in the empagliflozin-treated group. Presumably, this is due to the fact that, in Hungary, SGLT2 inhibitor treatment can only be prescribed to patients with an HbA1c level above 7%. This also means this group is a more severe patient group in terms of diabetes, thus, the results obtained prove even more crucial.

There was no significant difference in C-reactive protein (CRP) levels among the examined groups; however, some differences were detected. The CRP level was the lowest in the non-obese, non-diabetic group (C). 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 [19]. Among patients receiving empagliflozin treatment (ODE), the CRP level was lower when compared to the obese and diabetic group (OD), 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 [47] In the EMPA-CARD trial patients with type2 diabetes and coronary artery disease treated with empagliflozin had lower levels of interleukin 6, interleukin 1β and CRP levels compared to a placebo. There were elevations in superoxidase dismutase (SOD) activity, glutathione (GSHr), and total antioxidant capacity (TAC) with empagliflozin [48].

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 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. However, 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.

4. Materials and Methods

4.1. Ethics

The study protocol was approved by the Regional Ethics Committee of Pecs (No. 7622—PTE 2019) and was conducted in accordance with the ethical principles stated in the Declaration of Helsinki. Written informed consent was obtained from all patients.

4.2. Patients

102 patients (35 female, 67 male) were enrolled in our study. Patients were recruited from different internal medicine and outpatient departments by various physicians. 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/m^2 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 refusing to sign the consent form. Four patients were excluded from the study for different reasons (low compliance, severe epileptic seizure, withdrawal of their consent, and urgent psychiatric ward admission).

Patients' general characteristics were as follows. The mean age for different groups was: 65.95 for group C, 66.40 for group O, 74.58 for group D, 70.90 for group OD, and 65.20 for group ODE. The distribution of sex (male to female percentage) in the groups was as follows: 75–25% for group C, 50–50% for group O, 52.60–47.40% for group D, 68.40–31.60% for group OD, and 75–25% for group ODE. Mean BMI values for different groups were as follows: 26.01 kg/m^2 for group C, 34.75 kg/m^2 for group O, 26.50 kg/m^2 for group D, 35.78 kg/m^2 for group OD, and 31.61 kg/m^2 for group ODE. All patients had high blood pressure in their medical history. All patients in the diabetic groups (D, OD, ODE) had identified type 2 diabetes mellitus in their medical history, whereas none were reported in the remaining groups (C, O). The percentage of patients with identified cardiovascular disease was 70.40% in group C, 69.40% in group O, 78.60% in group D, 64.30% in group OD, and 73.48% in group ODE. All patients received antihypertensive therapy. All diabetic patients (D, OD, ODE) received antidiabetic therapy, whereas none were administered in the non-diabetic groups (C, O). Empagliflozin was administered only in the ODE group. No other SGLT2 inhibitors were used in our study. The percentage of patients with antihyperlipidemic therapy was as follows: 70% for group C, 75% for group O, 89.47% for group D, 84.20% for group OD, and 80% for group ODE.

4.3. Study Design

102 patients were recruited into this clinical study. We assessed their body composition, followed by pre-prandial venous blood collected using a peripheral venous catheter in the cubital vein. The preparation and laboratory procedures were in full accordance with the recommendations of the laboratory kits. Laboratory tests were performed at the Department of Laboratory Medicine, University of Pecs, Pecs, Hungary. The leptin levels were determined using the immunoassay method (Human Leptin ELISA, Biovendor, Czech Republic) at the Department of Biochemistry and Medical Chemistry, University of Pecs, Pecs, Hungary.

4.4. 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.

4.5. 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), hemoglobin A1C level, and the thyroid stimulating hormone level.

4.6. 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).

4.7. 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.

5. Conclusions

BMI, body fat, and visceral fat values as well as serum creatinine and leptin levels were improved with empagliflozin treatment. High leptin levels and leptin resistance in obesity are associated with insulin resistance, type 2 diabetes, increased risk of CV diseases, low-grade inflammation, and thrombosis. The markedly decreased circulating leptin levels observed in the empagliflozin-treated group may contribute to the known beneficial cardiovascular effects of empagliflozin treatment.

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metabolites

MDPI

Review

The Effects of SGLT2 Inhibitors on Lipid Metabolism

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Abstract: Sodium glucose co-transporter 2 (SGLT2) inhibitors are effective antihyperglycemic agents by inhibiting glucose reabsorption in the proximal tubule of the kidney. Besides improving glycemic control in patients with type 2 diabetes, they also have additional favorable effects, such as lowering body weight and body fat. Several clinical studies have demonstrated their positive effect in reducing cardiovascular morbidity and mortality. Furthermore, the use of SGLT2 inhibitors were associated with fewer adverse renal outcomes comparing to other diabetic agents, substantiating their renoprotective effect in diabetic patients. SGLT2 inhibitors have also remarkable effect on lipid metabolism acting at different cellular levels. By decreasing the lipid accumulation, visceral and subcutaneous fat, they do not only decrease the body weight but also change body composition. They also regulate key molecules in lipid synthesis and transportation, and they affect the oxidation of fatty acids. Notably, they shift substrate utilization from carbohydrates to lipids and ketone bodies. In this review we intended to summarize the role of SGLT2 inhibitors in lipid metabolism especially on lipoprotein levels, lipid regulation, fat storage and substrate utilization.

Keywords: SGLT2 inhibitors; type 2 diabetes mellitus; lipid metabolism

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1. Introduction

There are six identified SGLT (sodium glucose co-transporter) proteins in humans, of which the SGLT1 and SGLT2 receptors have been studied more thoroughly in recent years. Despite the outstanding sequence similarity between SGLT1 and SGLT2, they show different physiological and biochemical properties. While SGLT1 is primarily expressed in the intestines, SGLT2 is most abundant in the renal cortex, where it plays an essential role in renal glucose reabsorption. SGLT2 inhibitors, including dapagliflozin, canagliflozin, ipragliflozin, tofogliflozin, luseogliflozin, sotagliflozin, ertugliflozin and empagliflozin, have been studied in several clinical studies for the treatment of type 2 diabetes mellitus. Their selectivity for the SGLT2 receptor shows significant variance. While empagliflozin is 2500×, ertugliflozin is 2000×, dapagliflozin is 1200×, canagliflozin is 250×, sotagliflozin is only 20× more selective for the SGLT2 receptor over SGLT1, which may cause significant differences in the mechanism of action [1]. Several studies have proved that SGLT2 inhibitors are associated with reduced cardiovascular morbidity and mortality, including heart failure, and vascular diseases [2–6]. The underlying hypothetic mechanisms of SGLT2 inhibitors beyond their antidiabetic effects are summarized in Figure 1.

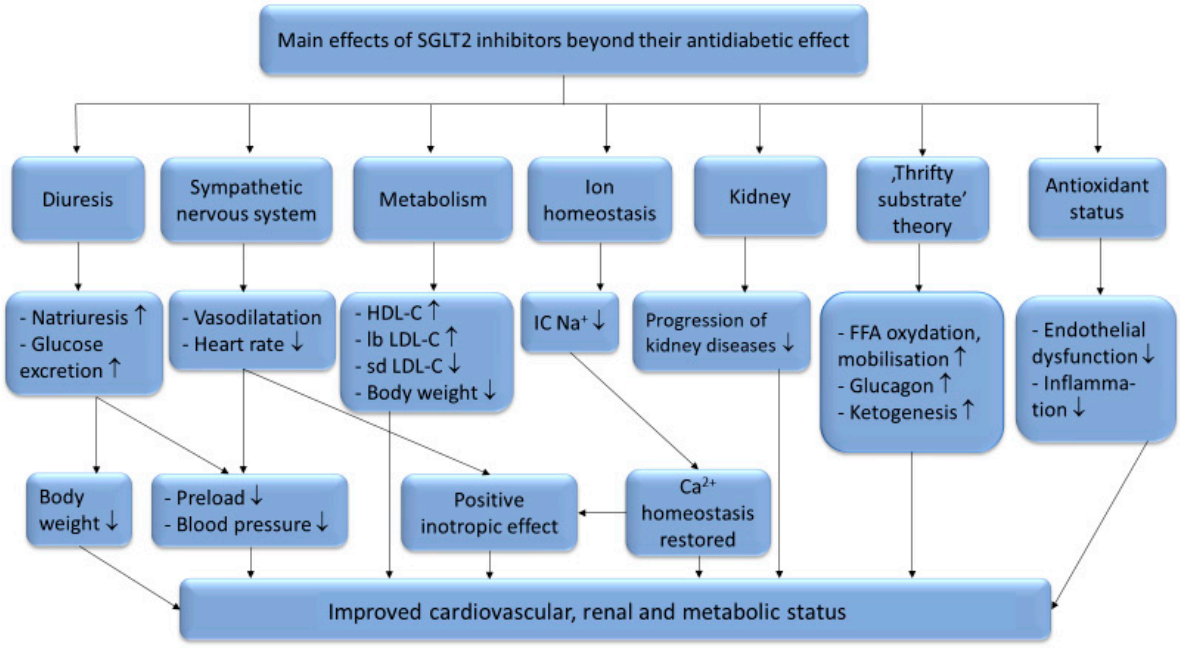

Figure 1. The systematic effects of SGLT2-inhibitors. HDL-C: high-density lipoprotein cholesterol; lb LDL-C: large buoyant low-density lipoprotein cholesterol; sd LDL-C: small dense low-density lipoprotein cholesterol; TG: triglycerides; IC Na^+: intracellular sodium-ion; FFA: free fatty acids; ↑: increased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

Furthermore, SGLT2 inhibitors have renoprotective effects as well [7]. Besides their beneficial effects on the conventional risk factors for kidney disease (such as blood pressure, hyperglycemia, body weight), it has also been hypothesized that they reduce the intraglomerular pressure [8], change the activation of the renin-aldosterone-angiotensin system [9] and shift renal fuel consumption towards ketone bodies [10].

Interestingly, these cardiovascular and renoprotective effects occur despite an increase in low-density lipoprotein cholesterol (LDL-C) levels which was observed in several clinical studies [11,12]. Nevertheless, this increase in LDL-C happens in the setting of other beneficial changes in plasma lipoprotein metabolism. In this review, we aim to analyze the effects of SGLT2 inhibitors on lipid metabolism to better understand their beneficial effects on cardiovascular outcomes despite slightly elevating LDL-C level.

2. Methods

A systematic literature search was conducted to identify all studies that investigated the SGLT2 inhibitor therapy on lipid metabolism in the PubMed (pubmed.ncbi.nlm.nih.gov) database. The searched terms were: SGLT2 inhibitors and metabolic effects, lipoprotein levels, lipid metabolism, lipid regulation and lipid accumulation. Only original research articles, review articles and meta-analyses published in the English language were selected.

3. The SGLT2 Inhibitors' Effect on Plasma Lipoprotein Levels

Several studies reported lowered serum levels of total cholesterol (TC) and triglycerides (TG) [13–15] as a result of SGLT2 inhibitor therapy, however there is a debate regarding the changes observed in the serum levels of high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). According to Calapkulu et al. LDL cholesterol level decreased by 13.4 mg/dL after 6 months in diabetic patients with dapagliflozin (10 mg/die) [13], In contrast, Cha et al. reported an increase of 1.3 mg/dL in LDL level after 24 weeks of dapagliflozin add-on therapy [16], in concordance with the results of Basu et al. in mice [17]. Furthermore, according to Schernthaner et al. canagliflozin (300 mg/die) caused 11.7% increase in LDL after 52 weeks of therapy in patients with type 2 diabetes mellitus [18]. According to Basu et al. the cause of this possible increase in the LDL-C levels could be due to an increased lipoprotein-lipase (LpL) activity and because

of a delayed turnover of LDL in the circulation. Canagliflozin reduced the expression of angiopoetin-like protein 4 (ANGPTL4), which is a known inhibitor of LpL in white and brown adipose, skeletal muscle, and heart tissues. With greater LpL activity both the TG and the VLDL levels decreased. They also observed significantly delayed LDL turnover compared to the control group, which can originate from the lowered hepatic levels of the LDL-receptor, which is the major receptor for the clearance of plasma LDL [17]. Another important factor is the ratio of the different LDL subclasses. Using gradient gel electrophoresis (GGE) LDL particles are classified into 4 subclasses, including large (LDL I), intermediate (LDL II), small (LDL III), and very small (LDL IV) LDLs [19,20]. LDL I and II, also referred to as large buoyant (lb) LDL, and LDL III and IV as small dense (sd) LDL particles [21,22]. Small dense LDL particles are more prone to induce metabolic disorders [23,24], obesity [25,26], type 2 diabetes [20,27] and coronary artery disease (CAD) [28] due to their longer circulation time than that of large LDL particles [29], enhanced ability to penetrate the arterial wall and higher susceptibility to oxidation [30]. It is generally known that modified (oxidized and glycated) LDL particles are highly atherogenic and possess more proinflammatory properties than native LDL molecules [28,31]. Interestingly SGLT2 inhibitors slightly increase LDL level yet have beneficial effects on CV morbidity and mortality. The contradiction may be resolved by the results provided by Hayashi et al. showing that dapagliflozin decreased sd LDL, and increased lb LDL levels after 12 weeks of dapagliflozin therapy (5 mg/die) in type 2 diabetic patients [15]. This effect on LDL subclasses ratio may play a significant role in SGLT2 inhibitors' cardioprotective property [32,33], provided it is a class effect.

Concerning HDL level, according to Kamijo et al. after 12 weeks of canagliflozin administration (100 mg/die) the very large high-density lipoprotein (VLHDL) and large high-density lipoprotein (LHDL) values showed a significant increase, of 10.9% and 11.5% respectively. These beneficial changes might also contribute to subsequent reduction of cardiovascular outcomes, caused by SGLT2 inhibitors [34].

4. The SGLT2 Inhibitors' Effect on Lipid Regulation

Several signaling molecules have been measured in mice after a 4-week treatment with canagliflozin by Ji et al. [14]. Elevated levels of diacylglycerol O-acyltransferase 2 (DGAT2) mRNA were reversed by canagliflozin. They also observed an increase in peroxisome proliferator-activated receptor-α (PPAR-α), and a decrease in peroxisome proliferator-activated receptor-γ (PPAR-γ) levels [14]. DGAT2 is an integral membrane protein which promotes the synthesis and storage of TG in lipid droplets. The peroxisome proliferator-activated receptors (PPAR) are a group of nuclear receptor proteins that function as transcription factors regulating the expression of several genes. Three types of PPARs have been identified: alpha (α), gamma (γ), and beta/delta (β/δ). While the PPAR-α is expressed mostly in the liver, kidneys, heart, muscle and adipose tissue, it mainly regulates the lipid metabolism in the liver. It is activated under conditions of energy deprivation, and it is necessary for the process of ketogenesis. Activation of PPAR-α promotes the uptake, utilization and catabolism of fatty acids through upregulating the genes that are involved in fatty acid binding and activation, peroxisomal and mitochondrial fatty acid β-oxidation. PPAR-γ regulates the fatty acid storage and glucose metabolism. It activates genes stimulating lipid uptake and adipogenesis in fat cells, it also plays a crucial role in adipocyte differentiation. PPAR-γ increases insulin sensitivity through increasing storage of fatty acids in fat cells, enhancing adiponectin release, inducing fibroblast growth factor 21 (FGF21) and upregulating the cluster of differentiation 36 (CD36) enzyme [35]. This data indicates that canagliflozin suppressed the synthesis of TG and the accumulation of hepatic lipid droplets through the down regulation of DGAT2.

Mice treated with canagliflozin had significant increase in both hepatic and serum FGF21 levels [36]. FGF21 is a fasting-induced hepatokine that stimulates glucose uptake in adipocytes, but not in other cell types. FGF21 acts through the Ras/MAP kinase pathway. This indicates, that canagliflozin triggered a fasting-like catabolic switch, increasing the

adipose lipolysis, hepatic fatty acid oxidation and ketogenesis, potentially via FGF21-dependent mechanisms. In addition, FGF21 can induce sympathetic activation in the central nervous system, leading to energy expenditure and weight loss. According to Osataphan et al. FGF21 was essential for the SGLT2 inhibitor induced reduction in adipose tissue mass, adipocyte cell size and activation of lipolysis. While canagliflozin reduced adipocyte size, FGF21-null mice had no weight loss, and adipose tissue and cell size even increased in response to canagliflozin, suggesting that FGF21-null mice had impaired sympathetic and lipolytic activity. FGF21 is also responsible for increasing oxidative metabolism, browning and lipolysis in white adipose tissue [36].

According to the findings of Herrera et al., empagliflozin therapy significantly reduced the gene expression as well as the protein levels of CD36 in atrial tissues of rats after 6 weeks [37]. CD36, also known as fatty acid translocase (FAT), is an integral membrane protein found on the surface of cells that import fatty acids inside cells. It is also a member of the class B scavenger receptor family. CD36 interacts with several ligands, including collagen types I and IV, oxidized LDL and long-chain fatty acids as well. It is also involved in the macrophages' phagocytosis. After CD36 binds to a ligand, they are internalized thus long-chained fatty acids and oxidized LDL particles can enter the cells. Since autophagy is decreased in metabolic disorders like diabetes and obesity, this dysregulation is an important factor in the pathophysiology of heart failure. The results show, that empagliflozin may ameliorate the impaired basal cardiac levels of autophagy that would lead to the aggregation of proteins, at least in part through CD36, which contributes to the pathogenesis of cardiometabolic diseases.

Xu et al. showed, that empagliflozin elevated AMPK and ACC-CoA phosphorylation in skeletal muscle, and increased hepatic and plasma FGF-21 levels. Empagliflozin also increased energy expenditure, heat production, and the expression of uncoupling protein 1 in brown fat and in inguinal and epididymal white adipose tissue. The M1-polarized macrophage accumulation was reduced, while plasma TNFα levels and obesity-related chronic inflammation decreased. In summary, empagliflozin did not just suppress weight gain by inducing fat utilization and browning, but also attenuated obesity-induced inflammation and insulin resistance [38]. Likewise, Osataphan et al. concluded that canagliflozin therapy activated AMPK through the inhibition of mitochondrial complex I, without an increase in ACC-CoA. However, this change in AMPK phosphorylation was not present in lean mice, thus AMPK is not likely to be the major mediator for the increase in fatty acid oxidation and ketogenesis [36]. One of the key molecules in the transport and oxidation of fatty acids is acetyl-CoA carboxylase (ACC), which converts acetyl-CoA to malonyl-CoA. Malonyl-CoA is an inhibitor of carnitine palmitoyltransferase 1 (CPT-1), which transports fatty acids into the mitochondria for oxidation. Thus, inactivation of ACC results in increased fatty acid transport and subsequent oxidation. On the other hand, AMP-activated protein kinase (AMPK) may decrease malonyl-CoA levels by regulating malonyl-CoA decarboxylase (MCD). Another important role of AMPK is, that it phosphorylates and inactivates 3-hydroxy-3-methylglutaryl-CoA reductase (MHGCR), which is a key enzyme in cholesterol synthesis. AMPK, therefore, regulates fatty acid oxidation and cholesterol synthesis [38]. Mammalian target of rapamycin (mTOR) is a cellular energetic sensor, which is often regulated inversely with AMPK. Osataphan et al. found that after canagliflozin therapy there was a 56% decrease in the hepatic phosphorylation of the mTOR downstream substrate S6 when refeeding in canagliflozin-treated mice. This change was not present in the control groups, thus the decrease in mTOR signaling might be weight-dependent [36].

Empagliflozin therapy reduced cardiac content of sphingolipids (sphingomyelins and ceramides) and glycerophospholipids, which play an important role in connecting insulin resistance to cardiac damage, and even in the development of cardiovascular diseases. It is suggested that changes in lipid metabolism within the heart and cardiac lipid accumulation may have an important role in the development of diabetic cardiomyopathy and heart failure. Ceramides, sphingomyelins and glycerophospholipids are associated with lipotoxicity in the heart, thus they have a major impact on the organ's functionality. This suggests, that

empagliflozin could regulate the metabolism and the cardiac accumulation of these cardiotoxic lipid molecules, which means, that it could be potentially useful for the prevention and treatment of not only diabetic cardiomyopathy, but also for the management of other cardiovascular diseases that have lipotoxicity in their pathogenesis [37].

5. The SGLT2 Inhibitors' Effect on Metabolism

Chiang et al. investigated a novel SGLT2 inhibitor's, NGI001, effect on non-alcoholic fatty liver disease (NAFLD) and obesity-associated metabolic symptoms in high-fat diet-induced obese mice. According to their results NGI001 prevented adipocyte hypertrophy, inhibited impaired glucose metabolism and regulated the secretion of adipokines associated with insulin resistance. Notably, NGI001 suppressed hepatic lipid accumulation and inflammation. NGI001 ameliorated fat deposition and increased AMPK-phosphorylation, resulting in ACC-CoA phosphorylation. In addition, it blocked the storage of total fat in adipose tissue and alleviated TG accumulation in liver tissue, and the organ's weight decreased likewise. Interestingly, they found that the TG and cholesterol level in the faeces increased. This effect on the lipid excretion through the intestinal system needs further study [39]. Yokono et al. investigated the effects on ipragliflozin on body weight and composition in mice. They found that 4 weeks of therapy suppressed body weight increase despite the small increase in food intake. The reduction of body weight was accompanied by reduced visceral and subcutaneous fat masses. Ipragliflozin lowered the respiratory exchange ratio and decreased the heat production rate from glucose but increased it from fat, thus ipragliflozin mainly promoted the use of fatty acids instead of glucose as an energy source [40]. Other studies showed similar results with different SGLT2 inhibitors, like dapagliflozin [41,42], canagliflozin [43], and empagliflozin [44]. In summary, these results suggest that the weight loss during SGLT2 inhibitor therapy may result from the reduction in fat tissue content via enhanced fatty acid utilization.

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. Also, SGLT2 receptor was found on the surface of pancreatic α-cells that can act as a glucose sensor [45]. On the other hand, the increased lipolysis also promotes the production of ketone bodies in the liver [46]. The oxidation of the ketone bodies is energetically more efficient than the oxidation of fatty acids because it results in a higher ATP/oxygen ratio than other substrates. According to the 'thrifty substrate' theory under conditions of mild, persistent hyperketonemia, such as during SGLT2 inhibitor therapy, β-hydroxybutyrate is freely taken up by the heart and oxidized instead of fatty acids and glucose [47]. Taking into consideration that ketone bodies serve as an alternative and less expensive myocardial fuel source to the myocardium SGLT2 inhibitors may improve cardiac function and increase mechanical efficiency [48,49]. Furthermore, beta-hydroxybutyrate has antioxidant and antiarrhythmic effects, by inhibiting histone deacetylases, by upregulating mitochondrial biogenesis and, by stabilizing cell membrane potential [47]. Metabolic flexibility means the ability of muscle to switch between FFAs and glucose as the main fuel source based on substrate availability. Patients with diabetes have impaired metabolic flexibility, thus the myocardium becomes more reliant on FFA oxidation. This impairment is the result of the increased FFA delivery to the heart due to peripheral insulin resistance and due to insulin's inability of suppressing lipolysis. This causes increased myocardial FFA oxidation and reduced glucose oxidation. FFAs also impair insulin action by inhibiting insulin signaling pathways, what leads to decreased cellular glucose transport, and even less glucose oxidation. This increase in FFA oxidation decreases cardiac efficiency and cause lipotoxicity. The hyperglycemia causes glucotoxicity, which is associated with reactive oxygen species (ROS) overproduction, which has a negative effect on mitochondrial function and other cellular processes. Ketone bodies are alternative fuel for the cells, that played a critical role in human survival. They are almost exclusively synthesized in the liver in the case of high circulating FFA levels, and when the production of ACC-CoA exceeds hepatic cellular energy requirements. The ketone bodies then diffuse into the circulation and into

extrahepatic tissues, mainly the heart and kidney, providing a major energy source during fasting [10]. It is documented that ketone bodies are mildly elevated when SGLT2 inhibitors are administered to patients [50]. The myocardium is the highest consumer of ketone bodies per unit mass, and it is shown, that despite impairments in skeletal muscle ketone body utilization, myocardial ketone body utilization was preserved in heart failure [51]. However, we must also consider, that euglycemic diabetic ketoacidosis (DKA) may be a rare, but severe side effect of SGLT2 inhibitor drugs, observed mostly under conditions favorable to excessive ketogenesis, such as increased alcohol consumption, volume loss, infection, stroke, and myocardial infarction [52]. In summary, SGLT2 inhibition in normal conditions through substrate shift offers the myocardium an alternative fuel that increases cardiac efficiency and decreases lipotoxicity. The main effects of SGLT2 inhibitors on lipid metabolism are summarized in Figure 2.

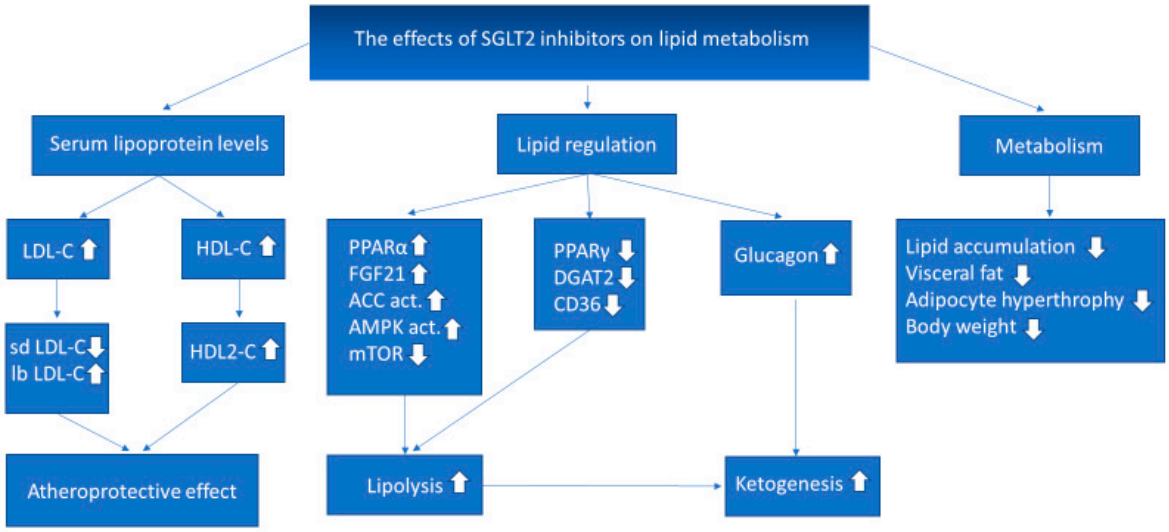

Figure 2. The effects of SGLT2 inhibitor therapy on lipid metabolism. LDL-C: low density lipoprotein cholesterol; sd LDL-C: small dense low-density lipoprotein cholesterol; lb LDL-C: large buoyant low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; PPARα: peroxisome proliferator-activated 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.

6. Conclusions

SGLT2 inhibitors affect the lipid metabolism on several different levels. They decrease lipid accumulation in visceral fat, regulate the serum lipoprotein levels, beneficially change the ratio of LDL particles, reduce lipid oxidation, and shift substrate utilization towards the usage of ketone bodies, which are more efficient in myocardial metabolism, and less reactive oxygen species are created through their oxidation, affect the β-oxidation and the transportation of lipid molecules in the cells. These favorable changes in lipid metabolism may counteract the net increase in LDL level. These findings may show that even though SGLT2 inhibitors are used primarily for the treatment of patients with type 2 diabetes, they may not be restricted merely to these indications in the near future.

7. Strengths and Limitations

Numerous relevant clinical and basic research studies and reviews have been included in this review article to summarize the potential effects of SGLT2 inhibitors on lipid metabolism giving a better understanding about the complex molecular mechanisms offered by these agents.

The limitations of this study are that we have described only the most important molecular mechanisms and searched only PubMed database for English language articles.

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