**2. Part 1: SGLT2-inhibition and cardiovascular disease**

**2.1 Abstract**

**2.2 Sodium glucose co-transporter 2 as the perfect drug target**

SGLT2-inhibitors were developed as treatment for type 2 diabetes mellitus. The idea behind was simple and obvious: if there is a problem of excess glucose in bloodstream, it can be potentially be solved by increasing the loss of glucose in the kidneys. Sodium glucose co-transporter 2, the main driver for glucose reabsorption in the proximal tubule of the nephron, was the ideal target since the transporter is responsible for 99%(ref) of glucose reuptake following the primary filtration in the glomerulus [Figure 2.1](#fig-1). Early studies of SGLT2 physiology were conducted well before the actual discovery of SGLT2. In 1835, phlorizin - a naturally occurring phenol glucoside and SGLT2-inhibitor - was first isolated from apple tree bark(1), and studies in the 1880s showed that the administration of this isolate caused renal glucosuria(2). It was, however, not until the 1980s that studies of phlorizin as a blocker of renal tubular glucose reabsorption gained traction(3,4), and that the connection to the phlorizin-like phenotype of familial renal glucosuria was found to be caused by genetic variation in the gene encoding SGLT2 (ref).

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| *Figure 2.1: SGLT2 is located on the luminal side of the tubule cells in the proximal tubule of the nephron in the kidney. Glucose enters the cell in Na^+ -coupled transport driven by the Na^+ gradient created by the Na+/K+-ATPase on the interstitial side. Glucose leaves the cell largely through GLUT2 and diffuses passively to the peritubular vessel. SGLT2 is responsible for ~ 90% of glucose reabsorption and the kidney. Image created with BioRender.* |

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| *Figure 2.2: Meta-analysis of cardiovascular risk in SGLT2-inhibitor cardiovascular outcome trials. Adapted from McGuire et al.(5).* |

The first SGLT2-inhibitors or gliflozins went on the market in the early 2010s as novel oral antidiabetic treatment options, shown to reduce plasma glucose and weight (reference). Then, when results from large scale cardiovascular outcome trials came out a few years later, type 2 diabetes mellitus treatment suddenly faced a shift in treatment paradigm: Up until 2015, treatment for type 2 diabetes had mainly been focused on lowering plasma glucose and improving lifestyle, but the results of the EMPA-REG OUTCOME trial revealed the potential of pharmacologically reducing cardiovascular risk along with risk of mortality. The EMPA-REG OUTCOME group found a 35% (95% CI: 15-50) lower risk of hospitalization for heart failure, a 38% (23-51) lower risk of cardiovascular mortality, and a 32% (18-43) lower risk of all-cause mortality for empagliflozin compared to placebo(6). In the following years, the effects were also found in other SGLT2-inhibitor types(7–9) [Figure 2.2](#fig-2) and it became evident that this not only had the potential to change diabetes treatment, but also to prevent cardiovascular outcomes in other at-risk populations(10–12). As a consequence, SGLT2-inhibitors Empagliflozin and Dapagliflozin are now included as class 1 recommended treatments for heart failure with reduced ejection fraction in individuals without diabetes in both European and American guidelines(13,14).

Although several processes that may lead to cardiovascular disease are impacted by the metabolic changes that characterize type 2 diabetes, only hyperglycemia, dyslipidemia, (hypertension, obesity, and sex) will be described in this review to provide a framework for the examinations of the mediators of the relationship between SGLT2-inhibition and cardiovascular disease. Likewise, the mechanistic considerations for the effect of SGLT2-inhibitors on cardiovascular disease and mortality will be the most thorough for potential mechanisms explored in the two original studies included in the thesis.

**2.3 Cardiovascular disease in a metabolic framework**

Cardiovascular mortality has been declining since the 1970s in nearly all regions of the world, especially in high-income countries such as North America, Western Europe, Japan, Australia and New Zealand(15–17), and this can be explained by numerous different trends. In the first half of the 20th century, life expectancy dramatically increased as a result of improved sanitation, vaccines, and antibiotics, and suddenly people lived long enough to experience atherosclerosis, heart disease, and cancer, that together replaced infectious diseases as leading causes of death(18). However, in the 1970’s, epidemiologists from the United States and Australia published studies showing that coronary heart disease mortality surprisingly had begun to decline after peaking in the late 1960’s(19,20), and they were later accompanied by epidemiologists showing similar declines in other regions (DK, Norge?). The declining trend in cardiovascular mortality has continued since and has been explained partly by progress in evidence-based medicine and surgical practices and continuously improved secondary prevention after a cardiovascular event, and partly by changes in risk factors including reductions in low-density lipoprotein cholesterol, systolic blood pressure, and smoking. There is however a problem that is likely to manifest itself in the cardiovascular disease mortality trends in the next few decades and not only be limited to some population groups. Because, while overall cardiovascular mortality is declining, the increasing global epidemic of obesity and type 2 diabetes accounts for an increasing number of cardiovascular deaths(21).  
The prevalence of diabetes continues to rise and it is estimated that by 2045, 693 million people will be suffering from diabetes, presenting a large social, financial, and health system burden across the world(22). Cardiovascular disease is the main cause of morbidity and mortality in type 2 diabetes where cardiovascular events occur 14.6 years earlier (find better number, newer) and are more severe than in individuals without diabetes. It is estimated that more than 50% of individuals with type 2 diabetes will die from a cardiovascular event. Type 2 diabetes is a complex disease and often characterized as a largely lifestyle-associated disease and the increased urbanization and spread of sedentary lifestyle in the recent decades account well for the continuing increase in prevalence. While lifestyle contributes significantly to the development of type 2 diabetes, the Look AHEAD trial demonstrated that improving lifestyle did not improve cardiovascular risk in spite of weight loss and a decrease in HbA1c(23).  
Several clinical trials have investigated the relationship between pharmacologically improved glycemic control in individuals with diabetes and cardiovascular outcomes. The ADVANCE trial (Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation), published in 2008, demonstrated that intensive glucose control using gliclazides targeting an HbA1c level of 6.5% in individuals with type 2 diabetes did not result in a reduction in macrovascular events, nor mortality(24). Additional support for these findings can be found in the 2009 Veterans Affairs Diabetes Trial (VADT)(25) and in the 1998 United Kingdom Prospective Diabetes Study (UKPDS)(26), where intensive glycemic control did not result in significant change in risk of major cardiovascular events or mortality over 7.5 and 10 years of follow-up, respectively. Conversely, the ACCORD trial (Action to Control Cardiovascular Risk in Diabetes), published in 2008, aimed for an HbA1c of 6% in the intensive therapy group, but unexpectedly observed a 22% increased risk of mortality, identifying a previously unrecognized risk associated with intensive glucose lowering in high-risk patients with type 2 diabetes. Due to the elevated mortality, this arm of the trial was prematurely terminated(27). In a meta-analysis of the ADVANCE, VADT, UKPDS, ACCORD, and 3 other trials, no association was found between intensive glycemic control and occurrence of heart failure events(28). In fact, intensive glycemic control with thiazolidinediones even increased the risk of heart failure.  
Collectively, these studies suggest that while therapies for diabetes effectively lower HbA1c levels, they do not necessarily confer cardiovascular benefits, and in some cases, may even increase the risk of cardiovascular events. These findings emphasize that reaching an HbA1c or weight target might not lower cardiovascular risk sufficiently in this high-risk population and ought to be supplemented by other approaches specifically targeting cardiovascular risk in type 2 diabetes management. Notably, none of the above mentioned studies used SGLT2-inhibitor treatment.

Additionally, studies have found that the risk of cardiovascular mortality is higher in women with diabetes than in men with diabetes (29)

**2.3.1 Risk of atherosclerotic cardiovascular disease (in individuals with and without type 2 diabetes)**

The Greek word atherosclerosis is composed of the word “athero”, meaning gruel or paste, and “sclerosis”, meaning hardness, and is used to describe plaque build-up and hardening of the arteries. The plaques are formed as cholesterol and lipids accumulate in the arterial wall, triggering inflammation and further accumulation of cellular waste products, calcification and the formation of a fibrous cap. This process can lead to several conditions depending on the localization and severity of the plaque build-up. Frequent manifestations of atherosclerosis include coronary heart disease, ischemic stroke, peripheral artery disease, and heart failure, but also angina pectoris, transient ischemic attack, and aortic aneurysm. Diabetes frequently leads to development of structural heart disease through mechanisms involving myocardial ischemia or infarction(30). The atherosclerotic process is similar in individuals with and without type 2 diabetes but is aggravated by the metabolic changes that characterize type 2 diabetes such as insulin resistance, hyperglycemia and disturbances in the carbohydrate-, fat- and protein metabolism that may cause vascular smooth muscle cell proliferation and induce inflammation. Major risk factors for atherosclerotic cardiovascular disease are smoking, hypertension, hypercholesterolemia, and diabetes. Dyslipidemia in diabetes is characterized by increased levels of low-density lipoprotein cholesterol particles promoting atherosclerosis. Moreover, diabetes contributes to endothelial dysfunction, which facilitates processes such as leukocyte and platelet adhesion, thrombosis, inflammation, and the formation of coronary plaque ulceration.

However, heart failure is not necessarily a result of atherosclerosis and the distinction between different origins of heart failure is important in understanding the different mechanisms and treatment strategies for atherosclerotic and non-atherosclerotic cardiovascular disease.

**2.3.2 Heart failure (Heart failure pathophysiology in individuals without diabetes)**

Heart failure is a common and serious complication of diabetes; it has been found to be the most common presentation of cardiovascular disease after peripheral artery disease in individuals with type 2 diabetes(31). Heart failure often occurs as a result of atherosclerosis that ultimately compromises myocardial blood flow or leads to myocardial infarction, causing ischemic cardiomyopathy, but it can also be the consequence of other disturbances. Both in individuals with and without diabetes, hypertension and chronic kidney disease are major risk factors for heart failure because of the increased cardiac load eventually resulting in left ventricular hypertrophy. In diabetes, metabolic changes can lead to diabetic cardiomyopathy without other obvious causes for cardiomyopathy, such as hypertension, valve disease or ischemic heart disease.  
Heart failure is 2 to 8 times more prevalent in individuals with diabetes compared to individuals without diabetes(32), and it has been shown that a 1 % increase in HbA1c is associated with an increase in risk of heart failure by 8 percent(33). Higher NYHA class has been shown to be associated with lower insulin sensitivity in individuals with heart failure but without a diabetes diagnosis(34), increasing their risk of developing type 2 diabetes. Concomitant diabetes and heart failure may result in further disease progression and a worse prognosis in a manner where each disease independently increases the risk of the other, because of different exacerbating metabolic, pathophysiological, hemodynamic, and neurohormonal factors(rephrase last part).

*2.3.2.1 The pathophysiology of heart failure in diabetes*

The impaired insulin sensitivity and hyperglycemia that define diabetes have consequences for numerous metabolic processes that may lead to diabetic cardiomyopathy, characterized by functional and structural changes of the myocardium due to altered glucose and free fatty acid (FFA) metabolism. Further down the road, as the diabetic disease progresses, comorbidities as hypertension, dyslipidemia, obesity, renal disease, microvascular and neurological complications are likely to further accelerate the heart failure progression. Insulin resistance in the diabetic heart causes impaired glucose uptake into the cardiomyocytes and leads to an increased release of free fatty acid (FFA). This change in substrate availability contributes to abnormalities in energy metabolism and cardiac dysfunction observed in diabetic cardiomyopathy. Excessive free fatty acids can cause lipid accumulation in cardiomyocytes, leading to lipotoxicity, contractile dysfunction, and eventually cardiomyocyte apoptosis(35).  
Hyperglycemia triggers the formation of advanced glycation end products (AGE), which induce collagen cross-linking and contribute to increased fibrosis of the myocardium. This fibrosis leads to heightened myocardial stiffness and impaired cardiac contractility and relaxation(36). In addition, impaired calcium homeostasis regulation, due to altered expression and/or activity of several crucial proteins in the cellular membranes, interferes with cardiomyocyte contractility, and also contributes to cardiomyocyte fibrosis and diastolic dysfunction(37). Furthermore, hyperglycemia systemically and locally activates the renin-angiotensin-aldosterone system (RAAS), resulting in the overproduction of angiotensin II and aldosterone. This causes higher mean arterial pressure, and renal vascular resistance. These hormones promote cardiac hypertrophy and fibrosis(38,39). Additionally, oxidative stress from the elevated production of reactive oxygen species (ROS) and mitochondrial dysfunction may contribute to metabolic substrate dysregulation, cardiac remodeling, impaired calcium handling, and impaired contractility and relaxation of the myocardium(40,41).

(While it is well described that type 2 diabetes mellitus and cardiovascular disease share numerous risk factors, such as obesity, dyslipidemia, insulin resistance, low-grade inflammation, and thrombophilia, less is known about causal order of these conditions(42).)

**2.4 Understanding risk factors for cardiovascular disease, type 2 diabetes as an exacerbating factor, and potential mechanisms for SGLT2-inhibitors**

**2.4.1 Glucose**

The relationship between plasma glucose and cardiovascular risk is undeniable. The increased risk of cardiovascular disease in the general population begins even within the normal plasma glucose range [Figure 2.3](#fig-glucose_spline). Mendelian randomization studies have established the causal relationship between higher plasma glucose and risk of myocardial infarction(43,44), free from limitations such as reverse causation which can be an issue in observational analyses.  
Hyperglycemia pathophysiology in type 2 diabetes involves insulin resistance in muscle and liver tissue, impaired beta-cell glucose sensitivity, and an increased insulin secretion, creating a sequence of reciprocal cause and effect which intensifies and aggravates the high glucose level and its negative effects. Hyperglycemia promotes atherosclerosis by acting on several levels of the atherosclerotic process; i.e. endothelial function, metabolic pathways, inflammation, fibrous cap formation, and thrombosis(45), and may explain part of the increased risk of cardiovascular disease in diabetes.

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| *Figure 2.3: Risk of heart failure, myocardial infarction, ischemic heart disease, and all-cause mortality according to plasma glucose in the Copenhagen City Heart Study and Copenhagen General Population Study. Hazard ratios were estimated using Cox regression and restricted cubic splines. The light blue areas indicate the distribution of the potential mediator concentrations in the respective populations. Cox regressions were adjusted for date of birth, sex, body mass index, blood pressure, LDL-cholesterol, HDL-cholesterol, triglycerides, and current smoking status. The median concentration of glucose was used as reference. Only individuals free of event at baseline were included. Adapted from Bechmann et al. (Original article no. 1). CI =confidence interval, HR=hazard ratio, N=number.* |

Furthermore, hyperglycemia drives structural changes through different pathogenic pathways which explain most diabetes complications. These mechanisms include the formation of advanced glycation endproducts (AGEs) to an accelerated degree. AGEs are thought to promote atherogenesis by oxidizing low-density lipoproteins (LDL) and cause changes to the collagen structure in blood vessel intima(36). Furthermore, by the activation of the protein kinase C/diacylglycerol signaling pathway, increased levels of poly(ADPribose) polymerase enzymes that are involved in cellular processes, including DNA repair and programmed cell death; and the oxidative stress burden from both mitochondrial and nonmitochondrial sources (EXPAND AND rephrase). Structural modifications to the myocardium as a result of glycation, that can lead to heart failure, may be explained by alterations to cardiac metabolism found in individuals with diabetes(30).

*2.4.1.1 Renin angiotensin system and glycemic control*

Further illustrating the interlaced mechanisms and complicated cause and effect relationships between hyperglycemia or type 2 diabetes and heart failure, studies have shown that angiotensin II receptor blocker Valsartan, an antihypertensive used in treatment heart failure, improves glycemic control. In the NAVIGATOR trial, 9306 patients with impaired glucose tolerance and established cardiovascular disease or cardiovascular risk factors were randomized to receive valsartan or placebo. In the treatment group, the hazard ratio for diabetes was 0.86 (95% confidence interval: 0.80-0.92, p<0.001), whereas the incidence of cardiovascular outcomes remained unchanged (46). In the DREAM trial, 5269 individuals with impaired fasting glucose levels or glucose tolerance without cardiovascular disease were randomized to ACE-inhibitor ramipril or placebo. The incidence of diabetes or death and median fasting plasma glucose levels did not differ between groups at the end of trial but participants in the treatment group were more likely to have regression to normoglycemia and had a better response to oral glucose tolerance tests(47).  
In a subgroup of participants of the PARADIGM-HF trial, 3778 individuals with diabetes and heart failure with reduced ejection fraction were randomized to receive combined angiotensin II receptor blocker and neprilysin inhibitor sacubitril/valsartan, a drug that has been shown to reduce morbidity and mortality in heart failure(48), or ACE-inhibitor enalapril. Neprilysin is a widely expressed enzyme involved in the breakdown of angiotensin I and II, bradykinin, and glucagon-like peptide 1 (GLP-1). Mean HbA1c levels were reduced more and new use of insulin was 29% lower in the in the sacubitril/valsartan group compared to the enalapril group, suggesting that sacubitril/valsartan might enhance glycemic control in patients with diabetes and HFrEF. In addition to the effects on the renin-angiotensin system, the inhibition of the neprilysin might improve glycemic control through mechanisms related to increases in circulating GLP-1, natriuretic peptides and an altered lipid metabolism(49). (shorten)

*2.4.1.2 SGLT2-inhibitor effects on glycemic control*

SGLT2-inhibitor treatment improves glycemic control almost immediately after starting treatment. HbA1c levels drop with 0.7 - 0.8 percentage points after the first 3 months of treatment but the effect attenuates with longer treatment and the change from baseline after 4 years of follow-up is -0.3 percentage points(50–53). Other glucose-lowering agents have been shown to reduce cardiovascular risk(54). Glucose-lowering drugs with similar glucose-lowering properties, however, do not affect cardiovascular risk equally. (meta-analysis figure). This has been one of the main arguments against improved glycaemic control as the main driver behind lower cardiovascular risk with SGLT2-inhibitors, despite being an established causal risk factor.

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| *Figure 2.4: The effects of hyperglycemia and dyslipidemia on the heart* |

**2.4.2 Lipids and lipoproteins**

Lipids and lipoproteins play a central role in the development and progression of atherosclerotic disease, a discovery that began to take shape in the beginning of the 20th century. While other scientists were beginning to link diabetes pancreas insufficiency (ref), German chemist, Adolf Windaus made a significant discovery that provided the initial indication that the substance present in atherosclerotic plaques was cholesterol. Windaus found that plaques taken from human aortas contained 25 times more cholesterol than healthy aortas. In 1913, Russian pathologist Nikolaj Anitschkow conducted an experiment in which he fed rabbits pure cholesterol, resulting in the development of extensive atherosclerosis. This experiment was the first known attempt to causally link dietary cholesterol and the fatty deposits seen in atherosclerosis(55). Since then, cholesterol has been extensively examined in relation to cardiovascular disease, and it is now well established that LDL cholesterol is causally associated with atherosclerotic cardiovascular disease (56).  
Years of research support the association between cholesterol-rich LDL and other lipoproteins containing apolipoprotein B (apoB), such as very low-density lipoproteins (VLDL) and their remnants, intermediate density lipoproteins (IDL), and lipoprotein(a) [Lp(a)], with the development of atherosclerotic cardiovascular disease.

The effects of hyperglycemia and dyslipidemia are summarized in figure [Figure 2.4](#fig-hypergly-dyslipi).

*2.4.2.1 LDL cholesterol*

Low-density lipoproteins (LDL) are established causal risk factors for atherosclerotic cardiovascular disease and are often pointed to as the primary cause of atherosclerosis.

*2.4.2.2 HDL cholesterol*

discussion

*2.4.2.3 Triglycerides*

*2.4.2.4 Lipoprotein(a)*

*2.4.2.5 SGLT2-inhbitor effects on lipids and lipoproteins*

Triglycerides and LDL cholesterol are causal risk factors for cardiovascular disease and therefore the relationship between SGLT2 inhbitors and lipids and lipoproteins had to be determined as part of the drug development process. The primary investigation of this was part of the early stages of drug development, however, as animal studies did not reveal any effects on lipids and lipoprotein and no clear link between SGLT2 and lipids exist, this relationship has not been in focus. However, as more randomized controlled trials of SGLT2-inhibitors were published a pattern was forming and showing that, unlike in animals, SGLT2 inhibitors affected lipid and lipoprotein levels in humans. In a meta-analysis of 60 randomized placebo-controlled trials, Bechmann et al. (original paper no 2) found that SGLT2-inhibition modestly increased total, LDL, and HDL cholesterol and decreased triglycerides [Figure 2.5](#fig-meta_sglt2_lipids).

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| *Figure 2.5: Meta-analysis estimates for the effect of SGLT2-inhibitor treatment on lipids and lipoproteins Meta-analysis estimates of the mean difference of the effect of SGLT2-inhibitor treatment versus placebo on plasma total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, and plasma triglycerides. Random effects and fixed effects meta-estimates were calculated using the metafor R package as raw mean differences, and both are shown for each lipid and lipoprotein with 95% confidence intervals in mmol/L and mg/dL. If the mean change from baseline was reported along with a variance statistic, this was preferred for the meta-analysis, otherwise the measurements at follow-up were used. The shown I2s, as measures of between study heterogeneity, are from the random effects models. Adapted from Bechmann et al. (original article no.2). CI = confidence interval, N = number.* |

The mechanism behind these effects are largely unknown and have not been investigated in humans. Basu et al.(57) studied the mechanism behind the increased LDL and decreased triglycerides with SGLT2-inhibition using a mouse model expressing human cholesteryl ester transfer protein (CETP) and apolipoprotein B100 (ApoB100). Mice (n = 40) were assigned to 1 of 4 treatments, vehicle, canagliflozin, an SGLT2 antisense oligonucleotide or insulin. In the canagliflozin group, the effects on LDL cholesterol and triglycerides were similar as in human studies, showing a slight increase in LDL cholesterol and a decrease in triglycerides. To investigate changes in lipoprotein physiology lipoprotein lipase (LpL) activity, LDL receptor and post transcriptional modulator 9 (PCSK9) expression, and angiopoetin-like protein 4( ANGPTL 4) expression were assessed, and the conclusion of the study was that SGLT2 inhibitor and SGLT2 antisense oligonucleotide treatment increased plasma lipolytic activity and LpL acitivity, possibly because of reduced expression of ANGPTL4, especially in the SGLT2 antisense oligonucleotide treated mice. This might explain the reduction in triglyceride levels. Furthermore, the increase in LDL could be due to increased VLDL to LDL turnover and a reduced LDL clearance, possibly because of reduced LDL receptor and PCSK9 expression seen in the SGLT2 antisense oligonucleotide group. In human study, including 80 individuals assigned to either dapagliflozin or sitagliptin, the dapagliflozin group experienced no significant change in LDL cholesterol levels after 12 weeks of treatment. However, there was a notable decrease of 20% in small dense LDL cholesterol levels, accompanied by an increase of 18% in large buoyant LDL cholesterol levels (58). Another study comparing ipragliflozin to continued usual treatment in individuals with type 2 diabetes revealed reductions in total LDL cholesterol and small dense LDL cholesterol levels in the ipragliflozin group when compared to baseline (59). LDL cholesterol comprises various subfractions, giving rise to a heterogeneous group of particles with different sizes and densities, ranging from very small to large. Elevated levels of small and dense LDL particles have been linked to an increased risk of cardiovascular disease, regardless of other LDL subfractions (60,61). Small dense LDL particles tend to circulate in the bloodstream longer than larger LDL particles. Moreover, they are believed to have a higher tendency to penetrate the arterial wall’s intima and are more susceptible to oxidation, potentially increasing their atherogenic potential (62,63). However, further studies are needed to understand the relationship between SGLT2-inhibition, LDL cholesterol subfractions, and cardiovascular risk.

The clinical relevance of the modest increases in total, LDL, and HDL cholesterol and triglycerides is likely to be negligible. Individuals with type 2 diabetes and/or heart failure, who typically receive SGLT2-inhibitor treatment, should be closely monitored for dyslipidemia and treated according to existing guidelines. However, elucidating the effect of SGLT2-inhibitors on lipid metabolism is a step to fully understand the relationship between SGLT2-inhibition and cardiovascular disease.

**2.5 SGLT2-inhibitors reduce risk of cardiovascular disease and mortality**

observational and genetic studies Other not mentioned mechanisms

**2.5.1 Glycemic control**

**2.5.2 Lipid metabolism**

**2.5.3 Blood pressure and RAAS modulation**

**2.5.4 Body mass**

**2.5.5 Natriuresis and osmotic fluid loss**

**2.5.6 Cardiac energy metabolism**

**2.5.7 Na+/H+**

**2.5.8 Reduced inflammation**

**2.5.9 ROS**

**2.5.10 Vascular function**

**2.6 Conclusion and perspectives**

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