GLP-1 receptor agonism to improve cardiometabolic health

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Chapter 1

General introduction and outline of this thesis

GENERAL INTRODUCTION

1. Obesity and type 2 diabetes

Over the past decades, the worldwide prevalence of overweight and obesity has increased dramatically. The World Health Organization defines overweight as a body mass index (BMI), which is the person's weight in kilograms divided by the square of his/her height in meters, greater than or equal to 25 kg/m^2 and obesity as a BMI greater than or equal to 30 kg/m^2. In 2016 it was estimated that since 1975 obesity prevalence had nearly tripled and in 2016 more than 1.9 billion adults were overweight of whom 650 million were obese[1]. The cause of this rise in overweight and obesity is a globally increasing imbalance between energy intake and energy expenditure. This problem is affecting more and more people as a consequence of excessive intake of high-caloric foods and low physical activity due to an increasingly sedentary lifestyle. By disturbing metabolic processes and inducing low grade systemic inflammation, overweight and obesity increase the risk to develop type 2 diabetes, which in turn can lead to cardiovascular diseases. In 2021, it was estimated that 537 million adults aged 20-79 years had diabetes, with type 2 diabetes being the most common type accounting for over 90% of all diabetes. This corresponds to a global prevalence of 10.5% in this age group. However, even though the numbers are already very high, the burden of diabetes is expected to further increase and global prevalence is expected to rise to 783 million people in 2045[2]. In the Netherlands, in 2019, 1.2 million people were living with diabetes, of whom 90.4% with type 2 diabetes[3].

2. Pathogenesis of type 2 diabetes

Type 2 diabetes is a chronic disease involving environmental and genetic factors that is characterized by a disturbed glucose regulation. Under physiological conditions insulin is secreted postprandially by β-cells of the pancreas, via several mechanisms in which rising blood glucose levels and also secretion of gut hormones including glucagon-like peptide-1 (GLP-1) play a role. This causes uptake of glucose in metabolically active tissues, suppression of endogenous glucose production by the liver and inhibition of intracellular lipolysis in adipocytes, thus resulting in storage of glucose and restoring blood glucose levels to the pre-prandial concentration. However, in type 2 diabetes the metabolic tissues do not respond to insulin as they should as a consequence of insulin resistance. Insulin resistance of metabolic organs is already present long before hyperglycemia occurs. However, in the early stage of insulin resistance, insulin secretion by the pancreas is increased to compensate for the increased insulin resistance in order to maintain glucose tolerance. Ultimately, as insulin resistance increases and β-cell function declines, compensatory insulin secretion fails to overcome insulin resistance, resulting in the development of type 2 diabetes with hyperglycemia[4,5].

In addition, insulin resistance plays an important role in the pathogenesis of combined dyslipidemia, which is characterized by high low-density lipoprotein (LDL) cholesterol and plasma triglyceride levels and low high-density lipoprotein (HDL) cholesterol levels via several mechanisms. On the one hand, insulin resistance of white adipose tissue and skeletal muscle results in attenuated lipoprotein lipase (LPL)-mediated catabolism of triglyceride-rich lipoproteins (TRLs), i.e. intestine-derived chylomicrons and liver-derived very-low-density lipoproteins (VLDL). On the other hand, the decreased insulin-mediated inhibition of intracellular lipolysis in white adipocytes subsequently results in increased release of free fatty acids (FFAs) from white adipose tissue that are transported to the liver where FFAs serve as substrate for hepatic production and secretion of triglyceride and cholesterol-containing VLDL. The hepatic insulin resistance further increases VLDL production, which under physiological conditions is suppressed by insulin[6]. The overproduction of TRLs in combination with reduced clearance of TRLs together cause hypertriglyceridemia and high LDL-cholesterol. Attenuated catabolism of TRLs is accompanied by reduced availability of phospholipid-containing surface remnants that are transferred by phospholipid transfer protein (PLTP) to feed the HDL pool. In addition, a high VLDL-TG concentration drives the neutral lipid transfer activity of cholesteryl ester transfer protein (CETP), that is involved in the transfer of triglycerides from VLDL to HDL, coupled to transfer of cholesteryl esters from HDL towards VLDL. This process results in formation of pro-atherogenic VLDL particles and triglyceride-enriched HDL particles that are subsequently hydrolysed and cleared by hepatic triglyceride lipase resulting in decreased HDL-cholesterol levels[7-11].

3. Visceral adipose tissue, ectopic fat and insulin resistance

Insulin resistance is thus essential in the pathophysiology of type 2 diabetes and the mechanisms resulting in insulin resistance have been studied extensively. Although numerous factors contribute to the development of insulin resistance, overweight and (mainly central) obesity are major risk factors[12-15]. Humans have white adipose tissue compartments in different areas of the body, of which the subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) compartments are most prominent. VAT has been causally linked to insulin resistance and has been shown to be a major contributor to metabolic risk[16-18]. In contrast, SAT does not seem to exert this effect and in line with that, it has been shown that obesity does not increase the risk for development of metabolic disorders in 10-30% of obese individuals[19,20]. This can be explained by the fact that the adipose tissue compartments function as the main lipid storage in our body. In healthy adipose tissue, in a situation of nutrient excess, adipogenesis is increased. However, ongoing exceeding of the buffer capacity of adipose tissue results in adipocyte dysfunction with hypertrophic as well as dying adipocytes and influx of immune cells[21]. Ultimately, this leads to liberation of FFAs from adipocytes and storage of FFAs as triglycerides

around and in other tissues. Storage of triglycerides within non-adipose tissues is called ectopic fat storage and causes disturbance of cellular function and insulin resistance.

VAT and increased ectopic fat play an important role in the development of local and whole-body insulin resistance and type 2 diabetes[21-24]. Ectopic fat accumulation occurs in many different organs that usually contain only a small amount of lipids, such as skeletal muscle, pancreas, heart and liver, resulting in disturbance of the function of these organs[12,13,25]. The detrimental effects of VAT are explained by its high lipolytic activity whereby the released FFAs and pro-inflammatory cytokines are directly delivered to the portal vein, contributing to the development of hepatic steatosis and hepatic insulin resistance[26-28]. In skeletal muscle cells, lipid accumulation results in impaired post-receptor signalling leading to attenuated GLUT-4 trafficking to the cell membrane and diminished glucose uptake into the cell[29]. Since skeletal muscle accounts for around 70% of insulin-mediated glucose uptake, insulin resistance of skeletal tissue is a major factor in the development of type 2 diabetes[30]. Lipid accumulation in the pancreas results in pancreatic steatosis, also called nonalcoholic fatty pancreas disease. It is a frequent clinical condition that might be associated with β-cell dysfunction[31], although other studies report no significant relationship[32,33]. In the heart, lipids can accumulate within cardiomyocytes (i.e. myocardial steatosis) or around the heart within adipocytes of para- and epicardial adipose tissue. Myocardial steatosis is an independent predictor of diastolic dysfunction in patients with type 2 diabetes[34]. Epicardial adipose tissue has been linked to the development and severity of coronary artery disease[35]. Furthermore, the thickness of the epicardial adipose tissue is correlated with whole body insulin resistance[36]. The sequence of events by which nutrient excess leads to cardiometabolic diseases is schematically depicted in **Figure 1**. In the next section, hepatic steatosis will be discussed more extensively.

3.1 Hepatic steatosis

Hepatic steatosis is a condition of ectopic fat accumulation that is characterized by accumulation of lipids within hepatocytes. In the absence of alcohol abuse, the disorder is referred to as nonalcoholic fatty liver disease (NAFLD), a disease strongly linked to obesity[37,38]. Hepatic steatosis is very disruptive for the function of the liver and, it has recently been shown that hepatic steatosis in fact forms a greater risk of incident type 2 diabetes than obesity and waist circumference as a measure of visceral obesity[39]. As described in section 2, as a consequence of hepatic steatosis, dyslipidemia is induced by VLDL overproduction. Furthermore, intracellular lipid accumulation results in reduced stimulation by insulin of glycogen synthase activity and decreased phosphorylation of the forkhead box O (FOXO) transcription factor, resulting in increased transcription of rate-controlling enzymes of gluconeogenesis[29,40].

Another possible mechanism via which hepatic steatosis can negatively affect metabolic health is via regulation of CETP production. This protein is mainly produced by Kupffer cells and can contribute to a pro-atherogenic lipoprotein profile, as described in section 2[41-43]. It is still largely unknown how CETP production by Kupffer cells is regulated. Interestingly, it has previously been shown that a reduction of hepatic triglyceride content related to pioglitazone treatment[44] or caloric restriction[45] is accompanied by a decrease in circulating CETP, which suggests a role for the hepatic triglyceride content in the regulation of CETP production. However, whether hepatic triglyceride content is indeed related to circulating CETP and whether these factors can also be affected by treatment with GLP-1 receptor agonists, a relatively new class of drugs to treat patients with type 2 diabetes, needs further investigation.

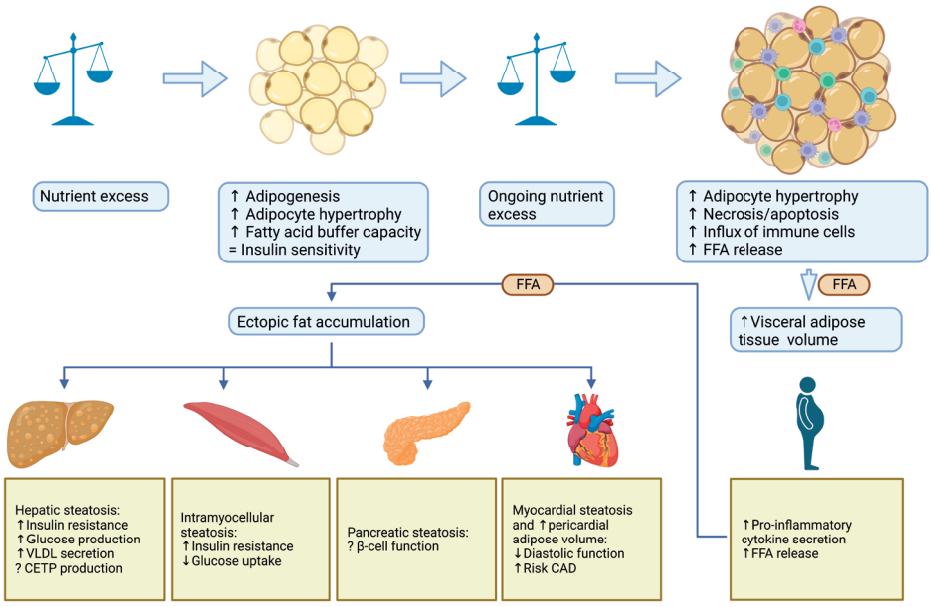

Figure 1. Schematic model of the relation between nutrient excess and cardiometabolic diseases. See section 3 for explanation. CAD, coronary artery disease; CETP, cholesteryl ester transfer protein; FFA, free fatty acid; VLDL, very-low-density lipoprotein. Created with Biorender.com.

4. The disadvantageous phenotype of the South Asian population

Obesity and type 2 diabetes prevalence differ between ethnicities. Of special interest is the population of South Asians, in whom the risk to develop type 2 diabetes as well as cardiovascular diseases is exceptionally high. South Asians originate from the Indian subcontinent, a region that is represented predominantly by India, and is home to approximately one fifth of the world's population. In 2021, in the South-East Asia region

90 million adults aged 20-79 years had diabetes, with an annual mortality of 747,000 diabetes-related deaths[2]. Most South Asians living in the Netherlands originate from Surinam, a former Dutch colony, where many people from India migrated to between the end of the 19th century and the beginning of the 20th century. In 2011 it was estimated that almost 151,000 South Asians were living in the Netherlands, with most of them living in the Hague[46]. Strikingly, a study comparing South Asian Surinamese subjects with ethnic Dutch subjects, showed a prevalence of type 2 diabetes of 16.7% versus 4.2%, respectively, in subjects aged 35-44 years, and of 35.0% versus 8.2%, respectively, in subjects of 45-60 years old[47]. Furthermore, South Asians develop type 2 diabetes at a younger age and at a lower BMI than Western Europeans[48,49].

The pathophysiological mechanism resulting in the high prevalence of type 2 diabetes in the South Asian population, e.g. as compared to Western Europeans, is not completely understood, but several potential pathophysiological mechanisms have been proposed. An important factor is the body composition of South Asians compared to Western Europeans, with a higher percentage of body fat at comparable BMI[50,51]. Furthermore, adipose tissue is distributed differently, with fat primarily stored in VAT, as well as more other ectopic fat deposition[52,53]. In line, it has been shown that young, lean and healthy South Asian subjects have an increased prevalence of insulin resistance, which is associated with an increased hepatic triglyceride content[54]. In addition, South Asians have an increased size of adipocytes within SAT at smaller SAT volume, suggesting a lower storage capacity (i.e. adipose tissue dysfunction) and possible predisposition to overflow of fat to ectopic depots[52,55]. This probably also contributes to the dyslipidemic, pro-atherogenic lipoprotein profile often present in South Asians, with high levels of LDL-cholesterol and plasma triglycerides and low HDL-cholesterol levels[56,57]. Besides these mechanisms, other factors possibly also contribute to the increased metabolic risk of South Asians. Several studies have reported an unbalanced diet with high saturated fat and carbohydrate intake and low intake of fiber, which could predispose to the development of type 2 diabetes[58,59]. Furthermore, a relatively low resting energy expenditure and brown adipose tissue (BAT) volume, which burns triglycerides and glucose, in the South Asian populations might play a role[60]. Altogether, many pathogenic factors contribute to the disadvantageous metabolic phenotype of South Asians and further studies aimed to decrease the risk to develop disease are warranted.

5. The role of the endocannabinoid system in metabolism

The endocannabinoid system (ECS) is an extensive neuromodulatory system which is composed of endocannabinoids and their receptors cannabinoid receptor type 1 (CB_1) and type 2 (CB_2). Although its functions are still not fully elucidated, the ECS plays an important role in the regulation of appetite, energy balance and metabolism. Accumu-

lating evidence shows that, once obesity has developed, the endocannabinoid system can contribute to further increased obesity and insulin resistance[61]. Binding of endocannabinoids to their receptors results in various effects, including reduced energy expenditure and increased accumulation of VAT. Since activation of the endocannabinoid system can aggravate obesity, reduction of the tone of the endocannabinoid system could be an effective approach to reduce the obesity epidemic. Treatment of obese subjects with rimonabant, an inverse agonist for the CB_1 receptor, induced marked weight loss and improved lipid metabolism, but unfortunately was associated with psychiatric side effects resulting in discontinuation of its clinical use[62,63]. Others have investigated the effect of a small reduction of body weight on circulating endocannabinoid levels, showing variable results: while some studies report a decrease in endocannabinoid levels[64], other studies showed no effect[65,66]. The effects of prolonged caloric restriction and subsequent pronounced weight loss on endocannabinoid levels, however, are still unknown.

6. Treatment of type 2 diabetes

In order to minimize the micro- and macrovascular complications of type 2 diabetes, it is very important to pursue optimal regulation of glucose levels, but also of other cardiovascular risk factors such as cholesterol levels and blood pressure. Since insulin resistance plays a fundamental role in the pathogenesis of type 2 diabetes, and in most patients is caused by obesity, weight reduction is the cornerstone of treatment[67,68]. Indeed, prolonged caloric restriction can substantially improve insulin sensitivity accompanied by reduced hepatic triglyceride content and intramyocellular lipid accumulation[68,69]. Furthermore, insulin sensitivity can be improved by increased physical exercise[70]. However, in addition, pharmacological treatment is often necessary.

In pharmacological management of type 2 diabetes various glucose-lowering agents are currently being used. Usually, the first drug of choice is metformin, which has an excellent safety profile and acts on multiple tissues though various mechanisms, which are still incompletely understood. Important is the suppression of the mitochondrial respiratory chain complex 1 in the liver, resulting in a moderate increase of adenosine monophosphate (AMP) which by activation of AMP-activated protein kinase (AMPK) results in inhibition of gluconeogenesis. This results in reduction of enhanced basal hepatic glucose production[71]. In addition, the UK Prospective Diabetes Study (UKPDS) has shown, albeit in a relatively small number of patients, that the risk of cardiovascular events is decreased by intensive glucose-lowering management with metformin[72]. When glucose control is insufficient with diet and metformin, most commonly, a sulfonylurea is added, which augments insulin secretion by β-cells. Unfortunately, sulfonylureas often cause hypoglycemia and are associated with weight gain[4]. A relatively new therapeutic

modality is the group of sodium-glucose cotransporter 2 (SGLT2) inhibitors, which block reabsorption of glucose in the kidney, resulting in renal glucose disposal via glucosuria. This results in reduction of blood glucose levels and, by urinary loss of calories, induces moderate weight loss[73]. However, further studies are needed to investigate long-term effects on glycemic control and cardiovascular function.

Ultimately, if other glucose lowering agents fail, insulin therapy is often required. Unfortunately, treatment with insulin is frequently accompanied by significant weight gain due to its anabolic function, and hypoglycemia is often reported[4,74]. As can be appreciated from the above, most glucose-lowering agents, with the exception of SGLT2 inhibitors, do not reduce body weight or even increase body weight, which eventually results in increasing insulin resistance and requirement of the use of (more) medication such as insulin. Activation of glucagon-like peptide-1 (GLP-1) signalling pathways may be a solution to break this vicious cycle, as will be discussed in the next sections.

7. Treatment of type 2 diabetes with GLP-1 modulators

A relatively new class of drugs to treat patients with type 2 diabetes beneficially modulates GLP-1 signalling. GLP-1 is an incretin hormone that is released after food ingestion by enteroendocrine L-cells in the terminal ileum and colon. GLP-1 binds to receptors present on vagal afferent neurons and in many different tissues such as pancreas and brain. Its main effects are stimulation of glucose-dependent insulin secretion by pancreatic β-cells, inhibition of glucagon release by pancreatic α-cells, delay of gastric emptying and induction of satiety[75]. Native GLP-1 has a very short half-life of less than 2 minutes due to degradation by dipeptidyl peptidase 4 (DPP-4)[76]. Pharmacologically, GLP-1 degradation can be delayed by use of DPP-4 inhibitors. Although no significant side-effects have been reported, the effect of DPP-4 inhibition on insulin secretion and improvement of glucose regulation is only modest. This is probably explained by the fact that DPP-4 inhibitors do not increase plasma GLP-1 levels but merely prolong the effect of physiologically available GLP-1[77,78]. In favourable contrast, long-circulating GLP-1 receptor agonists exert similar effects as native GLP-1 but have much higher therapeutic efficacy because they are modified to be resistant to rapid degradation by DPP-4. Although it has been shown extensively that GLP-1 receptor agonists improve glycemic control and induce weight loss[79-81], the effects on ectopic fat and the cardiovascular system are still unclear.

7.1 Effects of GLP-1 receptor agonism on body weight and ectopic fat

It is well known that GLP-1 receptor agonists induce weight loss and reduce fat mass. Interestingly, this decrease in fat mass does not seem to occur homogeneously in the body. Several studies in animal models have shown that increased GLP-1 receptor

activation reduces hepatic steatosis and VLDL production[82,83] and reduces myocardial steatosis[84], independently of weight loss. However, in humans conflicting results on effects of GLP-1 receptor agonists on the different fat depots have been reported, with studies reporting mainly decrease of VAT[85,86] and epicardial adipose tissue[87], and others reporting no effect on VAT[88]. In addition, the GLP-1 receptor agonists liraglutide[89,90] and exenatide[91] have been shown to reduce hepatic steatosis. Since it is unclear to what extent different adipose tissue compartments are affected by weight loss induced by treatment with GLP-1 receptor agonists, this is an important topic of investigation. Indeed, as explained in section 3, reduction of VAT and ectopic fat would be far more beneficial from a perspective of cardiovascular risk reduction than reduction of SAT.

Finally, although it is well known that the GLP-1 receptor agonists reduce body weight, the exact mechanism behind this effect is still unclear. Although the weight loss can at least be partially explained by a reduction of food intake as a consequence of increased satiety, several studies have indicated that GLP-1 receptor agonists may beneficially impact energy expenditure as well[92-96]. In fact, studies in preclinical models suggest that increased thermogenesis in BAT by GLP-1 receptor agonists contributes to the weight loss[92,93]. However, as reported effects of GLP-1 receptor agonists on energy metabolism are conflicting, further studies to investigate effects including the effects of treatment duration are necessary.

7.2 Effects of GLP-1 receptor agonism on cardiovascular function

Type 2 diabetes predisposes to the development of coronary artery disease. Furthermore, type 2 diabetes is independently associated with a 4 to 5-fold increase in the risk of heart failure, even without presence of prior coronary heart disease[97]. Many patients with type 2 diabetes develop heart failure with preserved ejection fraction (HFpEF). Often, this is preceded by diabetic cardiomyopathy, which is a disorder of the heart muscle characterized by left ventricular diastolic dysfunction as a consequence of impaired relaxation and compliance[98,99]. Unfortunately, pharmacological options to treat or prevent development of diabetic cardiomyopathy remain limited.

Several studies have investigated the effect of GLP-1 receptor agonists on the cardiovascular system in patients with type 2 diabetes. Recently, it was shown in the LEADER trial that liraglutide reduces total cardiovascular mortality in patients with type 2 diabetes and with high cardiovascular risk, and similar effects have been reported for the GLP-1 receptor agonists semaglutide and dulaglutide[100,101]. This risk reduction is at least partially caused by anti-atherosclerotic effects of improved glucose and lipid levels. However, GLP-1 receptor agonists also have direct and indirect effects on the myocardium and multiple preclinical studies have shown therapeutic benefits of GLP-1

receptor agonists on cardiac function[102,103]. Using cardiac magnetic resonance imaging (MRI), effects of GLP-1 receptor agonists on both systolic and diastolic cardiac function and effects on myocardial tissue characteristics can be studied in detail, which has not been done before in humans.

OUTLINE OF THIS THESIS

The aim of thesis was to study the effects of liraglutide on cardiometabolic health, by assessing its effects on the various ectopic fat depots and on cardiovascular function in Dutch South Asian and Dutch Western European subjects. We investigated these effects using MRI and proton magnetic resonance spectroscopy (^{1}H-MRS). In addition, since liraglutide at least partly acts by induction of satiety, we studied effects of dietary restriction on these parameters.

Since South Asians have a high risk to develop type 2 diabetes, which may be related to their high ectopic fat accumulation, in **Chapter 2**, we describe the effects of treatment with liraglutide for 26 weeks on ectopic fat deposition and HbA1c in South Asian patients with type 2 diabetes. In this placebo-controlled, double blind, clinical trial we randomly assigned patients to treatment with liraglutide or placebo added to standard care. Using MRI and ^{1}H-MRS, we assessed effects on SAT, VAT, epicardial and paracardial adipose tissue volume and on myocardial and hepatic triglyceride content. In addition, we assessed the association between the decrease of ectopic fat and the improvement of glucoregulation.

Since liraglutide may be beneficial in the prevention of development of diabetic cardiomyopathy, in the same trial as described in Chapter 2, we investigated effects of liraglutide on cardiac function and myocardial tissue characteristics. The results of this study are described in **Chapter 3**.

In **Chapter 4**, we aimed to assess effects of treatment with liraglutide on energy expenditure and BAT using indirect calorimetry and MRI, respectively. We describe the effects of liraglutide treatment on energy metabolism after 4, 12 and 26 weeks of treatment. Furthermore, we measured the fat fraction in the supraclavicular BAT using chemical-shift water-fat MRI.

CETP is a protein that plays a role in plasma lipid transport and is produced by Kupffer cells. However, the regulation of CETP production by the Kupffer cells is still incompletely understood albeit that the hepatic triglyceride content possibly plays a role. Since it was

recently shown that GLP-1 receptor agonists can reduce hepatic triglyceride content, we assessed whether treatment with liraglutide for 26 weeks decreases CETP concentration in humans. The findings of this study are described in **Chapter 5**. In addition, in this chapter we investigated the relationship between the hepatic triglyceride content and circulating CETP in a large population-based cohort.

Chapter 6 focusses on the effects of prolonged caloric restriction on endocannabinoids in relation to ectopic fat accumulation and cardiac function. Elevated levels of endocannabinoids can contribute to accumulation of VAT and insulin resistance. It has been shown that a very low calorie diet (VLCD; 450 kcal/day) substantially reduces hepatic and myocardial steatosis and improves myocardial function in obese patients with type 2 diabetes *without* coronary artery disease[104]. We now aimed to assess the effects of pronounced weight loss after prolonged caloric restriction on plasma endocannabinoid levels in patients with type 2 diabetes *with* established coronary artery disease, a group with even more increased cardiovascular risk. In addition, we investigated the effects on ectopic fat accumulation and cardiac function. In this chapter we describe the results of a prospective intervention study in which patients with type 2 diabetes and established coronary artery disease followed a 16 week very low calorie diet.

Chapter 7 provides a general discussion and summary of the findings of the studies described in this thesis.

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