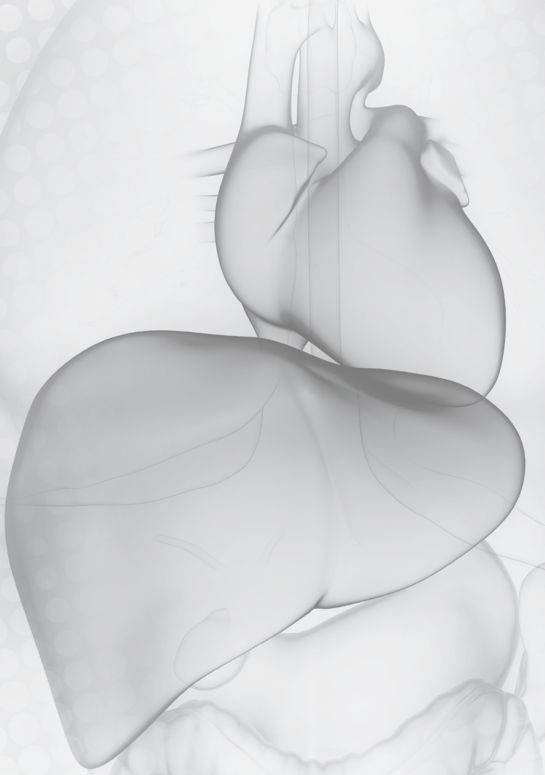
Chapter 1

General introduction and outline of the thesis



GENERAL INTRODUCTION

The number of people with obesity has dramatically increased over the past decades to an estimated number of 396 million adults worldwide with a projected 573 million in 2030, with an even higher calculated prevalence of 1.12 billion in 2030 if corrected for recent trends (1). Simultaneously the worldwide prevalence of type 2 diabetes mellitus (T2DM) is projected to increase from 2.8% in 2000 to 4.4% in 2030 even if the obesity prevalence remains stable (2). Therefore, this will probably be an underestimate of the real future diabetes prevalence, as obesity is a major risk factor for diabetes.

Diabetes is a life-threatening condition. According to the World Health Organization, six deaths every minute are attributable to diabetes. T2DM patients are at increased risk of cardiovascular disease (CVD) and CVD accounts for 52% of deaths in type 2 diabetes (3). The pathogenesis of T2DM and cardiovascular complications involve multiple pathways that are tightly interlinked. Although it is impossible to dissect the individual contributions of the multiple endogenous and exogenous disruptors, and to establish causal relationships between the many pathological phenomena observed in T2DM, the main pathogenetic phenomena identified in T2DM are related to lipid dysfunction, inflammation and glucotoxicity.

In the pathogenesis of T2DM, an important role is attributed to the pathophysiological changes induced by visceral obesity. Traditionally, it has been presumed that the only function of adipose tissue is storage of triglycerides (TG). However, adipose tissue secretes various hormones and cytokines (also referred to as adipokines) that influence glucose and lipid metabolism and energy homeostasis. In obese subjects prone to develop T2DM, increased secretion of adipokines with a pro-inflammatory profile is observed (4). In addition, it has been recognized that excess TG can also be accumulated outside the traditional fat compartments. Ectopic fat is defined as storage of TG in tissues other than adipose tissue, that normally contain only small amounts of fat, such as the liver, skeletal muscle, heart and pancreas.

This thesis is dedicated to the measurement by magnetic resonance imaging (MRI) and spectroscopy (MRS) of ectopic fat accumulation and the intervention with dietary, exercise and pharmacological strategies. The focus is on ectopic TG accumulation in the liver and the heart.
We focused on the liver because this organ is both passively and actively involved in metabolic derangements in both forms of diabetes. Minute shifts in triglyceride distribution between adipose tissue and the plasma compartment, e.g. caused by insulin deficiency (type 1 diabetes) or insulin resistance (type 2 diabetes) results in higher plasma non-esterified fatty acid (NEFA) levels. Depending on extrahepatic factors (e.g. high NEFA and glucose levels,

hyperinsulinemia) the liver will accumulate triglycerides and become steatotic (5). In turn, steatosis is associated with insulin resistance because of the suppressive effects of insulin on glucose and very low-density lipoprotein (VLDL) production, and as a consequence contributes to hyperglycemia and dyslipidemia (6). High plasma concentrations of NEFAs are also associated with fatty acid uptake in the heart, in excess of oxidative requirements, resulting in triglyceride accumulation in the myocardiocytes ("cardiac steatosis"), which is associated with impaired diastolic cardiac function (7,8).

The introduction of hepatic and, more recently, cardiac proton magnetic resonance spectroscopy (MRS) enables assessment of hepatic and cardiac triglyceride accumulation in detail *in vivo (9,10)*. Magnetic resonance imaging has also enabled us to study pericardial fat, ectopic fat around the myocardium and cardiac function. Limited data are available on the pathophysiological role of this fat compartment. Due to its close anatomical relation to the myocardium, it has also been proposed to play a role in insulin resistance and cardiac function (11,12). In the current thesis we assessed the effects of lifestyle and pharmacological interventions on these fat compartments.

OUTLINE OF THE THESIS

In **Chapter 2**, the consequences of fat accumulation in the liver, muscle and heart are reviewed and, if known, the underlying pathofysiological pathways. In addition, a review is given on the effect of diets with or without exercise on these ectopic fat localizations and the potential beneficial effects on T2DM.

CVD in T2DM can occur in the absence of traditional risk factors such as hypertension or coronary artery disease (13). Although the origins of CVD in T2DM are complex, it has been hypothesized that hepatic steatosis, which is a common finding in patients with (uncomplicated) T2DM, may play a role. Indeed, several studies in humans showed an association of fatty liver with parameters of cardiovascular dysfunction in T2DM (14-16). These studies have provided hypotheses regarding the liver steatosis as a risk factor for CVD. However, it has not been determined so far whether high liver fat adversely affects metabolic or functional aspects of the heart in asymptomatic patients with uncomplicated T2DM. In **Chapter 3** we assessed the relationship between liver triglyceride content and myocardial metabolism in T2DM patients with verified absence of clinical ischemic heart disease.

Part two of this thesis focuses on nutritional and exercise interventions on ectopic fat accumulation.

In previous studies, it has been shown that short-term caloric restriction leads to increased levels of plasma NEFAs and is associated with a reversible myocardial TG accumulation and a decrease in left ventricular (LV) diastolic heart function in healthy subjects and uncomplicated T2DM (8,17). Although difficult to explain, it cannot be ruled out that these phenomena represent physiological adaptations to low-caloric states (myocardial metabolic flexibility). We hypothesized that in T2DM patients *with* cardiac complications, the flexibility of myocardial TG stores and diastolic heart function may not be present any more. In **Chapter 4**, we assessed the effects of a 3-day very low-caloric diet (VLCD) on myocardial TG content and cardiac function in T2DM patients with cardiac complications. To study effects on tissue-specific ectopic fat distribution, hepatic TG content, visceral fat, subcutaneous fat and pericardial fat volume were also measured before and after the short-term dietary intervention.

Weight reduction and exercise are the cornerstone of lifestyle treatment in T2DM. The effects of weight loss on visceral and subcutaneous fat and insulin sensitivity have been well described. A 16-week VLCD in obese patients with T2DM can already induce major reductions in abdominal fat and hepatic TG content (18). Studies on the long-term effects of a period of VLCD in T2DM have only focused on weight reduction and metabolic effects. The long-term effects of a VLCD on TG accumulation in pericardial fat and other fat compartments have not been studied. In addition, the long-term effects of a VLCD on cardiac function and myocardial TG content have not been studied as well. In **Chapter 5** we describe the effects of a 16-week VLCD and subsequent 14-month follow-up on pericardial fat and other fat compartments in obese insulin-dependent patients with T2DM, using MRI and MRS. In **Chapter 6** we describe the effects of this intervention on cardiac function assessed by cardiac MRI and myocardial triglyceride (TG) content measured by MRS.

It has been well established that a sedentary lifestyle increases the risk of T2DM and that exercise improves metabolic control and cardiovascular risk (19). In most studies on the effects of exercise, dietary interventions are performed as well. Studies on exercise alone show a predominant reduction in visceral abdominal fat in obesity and T2DM and subsequent improvement in insulin sensitivity. As little is known on the effect of exercise alone on organ-specific fat accumulation in T2DM patients, we performed a study, described in **Chapter 7,** in which we assessed the effects of a 6-month exercise intervention on ectopic fat compartments, including hepatic, myocardial and epicardial fat.

Increased arterial stiffness is independently associated with coronary artery disease (20). Aortic pulse wave velocity (PWV) is a surrogate marker for central arterial stiffness and aortic PWV is an independent predictor of mortality in T2DM (21). Insulin plays an important role in hemodynamic regulation by modulating vascular tone, probably mediated by nitric oxide. Several animal and human studies suggest that the effect of insulin on vascular tone may

be region-specific for various vascular beds and locations. However, tonometry and Doppler ultrasound do not enable to measure region specific PWV. We developed an MRI protocol enabling regional assessment of the PWV in multiple locations in the aorta. As no studies have been performed on the short-term effect of an oral glucose load on regional aortic stiffness, we assessed the effects of an oral glucose load on local aortic PWV in the ascending and descending aorta using a validated MRI technique for measuring site-specific changes in aortic PWV, which is described in **Chapter 8.**

Angiopoietin-like protein 4 (Angptl4) is involved in a variety of functions including angiogenesis and lipoprotein metabolism and may be associated with dyslipidemia and T2DM. In the context of fatty acid metabolism, Angptl4 suppresses the release of non-esterified fatty acids (NEFA) and their subsequent uptake by underlying tissues such as adipose tissue, skeletal muscle and the heart (22). Paradoxically, Angptl4 increases intracellular lipolysis of TG within adipocytes, thereby raising plasma NEFA levels (23). On its turn, the expression of Angptl4 in a variety of tissues is stimulated in vitro by NEFA and in vivo by fasting, which also increases plasma NEFA. Therefore, a mutual relationship between Angptl4 and plasma NEFA is suggested. Data on the determinants of plasma Angptl4 levels in humans are still scarce as there were no commercial assays for determination of Angptl4 available until recently. To our knowledge, the effect of increased NEFA levels on Angptl4 levels in patients with T2DM is still unknown. We hypothesized that an increase in plasma NEFA levels would increase plasma Angptl4 levels in healthy subjects and T2DM. We therefore performed a study, described in **Chapter 9** to determine the effects of nutritional interventions, resulting in increased plasma NEFA levels, on Angptl4 levels in healthy subjects and T2DM.

Part three of this thesis focuses on pharmacological interventions on ectopic fact accumulation.
Although intensive insulin treatment is the cornerstone of therapy for patients with type 1 diabetes mellitus (T1DM), patients suffer from frequent episodes of hyperglycemia. Several studies point to a major role of metabolic dysregulation in the induction of abnormal intramyocellular lipid accumulation in T1DM, including the heart. Therefore, we hypothesized that episodes of metabolic dysregulation in T1DM due to insufficient insulin provision may also modulate myocardial TG content and possibly myocardial function. In **Chapter 10,** we describe a study in which we evaluate the effects of short-term metabolic dysregulation, caused by protocolized insufficient insulin provision on myocardial TG content and myocardial function in T1DM volunteers who are otherwise well controlled by continuous insulin pumps.

Pericardial fat is a fat compartment surrounding the myocardium and includes epicardial fat and paracardial fat. Controversies exist in the literature on supposed beneficial or adverse

roles of pericardial fat in cardiovascular disorders (24). Fundamental studies suggest a role of pericardial fat in myocardial energy metabolism, whereas epidemiological studies suggest a relationship with coronary artery disease. The class of ppar-gamma agonists plays an important role in differentiation of adipocytes and indeed has documented effects on fat compartments and ectopic fat. However, the effects of ppar-gamma agonists on pericardial fat have not been studied so far. In **Chapter 11**, we describe a prospective study on the effect of pioglitazone versus metformin on pericardial fat volume in patients with T2DM using MRI.

Previous intervention studies with lipid lowering agents in animal models showed that there is an intimate relationship between hepatic fat content and the hepatic expression and plasma levels of cholesteryl ester transfer protein (CETP) (25,26). CETP is a protein that mediates the heteroexchange of cholesteryl esters from HDL to (V)LDL with a simultaneous exchange of TG from (V)LDL to HDL. These studies therefore suggest that plasma CETP could be a marker for hepatic fat content. Since the correlation between hepatic TG content and plasma CETP mass has not been studied in humans, we performed a prospective study, described in **Chapter 12**, in which we evaluated the relationship between hepatic TG content and plasma CETP mass. In this study, we used pioglitazone treatment as a model to study the effects of a change in hepatic TG content on CETP mass in patients with T2DM.

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