Chapter 1
General Introduction
Myocardial triglyceride (TG) content refers to the intracellular TG pool in cardiomyocytes. Myocardial TG stores per se are probably inert, but reflect non-oxidative energy pathways which may negatively influence myocardial function. Myocardial TG stores are tightly regulated by dietary TG intake, plasma TG levels and non-esterified fatty acids (NEFAs), and myocardial fatty acid uptake and oxidation. However, the physiological and pathophysiological relevance of myocardial TGs for cardiac function in humans, especially in metabolic disease, is largely unknown. In this introduction the cardiovascular risk in metabolic disease in general, and the specific potential role for myocardial TGs is discussed, in relation to TG metabolism.

CARDIOVASCULAR RISK IN METABOLIC DISEASE

Excessive caloric intake in combination with decreased physical exercise has led to an increase in the prevalence of obesity and type 2 diabetes mellitus (DM2) in the developed world (1). Obesity and DM2 are major risk factors for cardiovascular disease (2;3). In addition to the effects of insulin resistance and dyslipidemia on cardiovascular disease, a growing amount of evidence suggests a pathophysiological role of increased circulating levels of adipokines released by the adipose tissue (4) and activation of inflammatory pathways (5). Additional to atherosclerosis, obesity and DM2 also induce metabolic changes in the heart (6). The mechanisms by which these metabolic myocardial alterations in obesity and DM2 influence myocardial systolic and diastolic function are not fully elucidated. These metabolic alterations may be reflected in excessive TG accumulation in cardiomyocytes (7;8).

As early as in 1933, it was suggested in autopsy studies that fatty degeneration of the heart is a common finding in obesity, possibly associated with the development of dilated cardiomyopathy (9). However, only in the past decade a syndrome of cardiomyopathy induced by fat accumulation was described in rodents (7).

In human models, the current literature on this issue is limited, mainly due to the challenges faced by the measurement of myocardial lipid accumulation in vivo. There are, however, indications that alterations in metabolic pathways in the heart in obesity and DM2 are also present in humans and may affect myocardial function (10;11). Therefore, the studies presented in this thesis aim to clarify the pathophysiological relevance of myocardial TG accumulation on myocardial function in healthy subjects and in subjects with type 1 diabetes mellitus (DM1) and DM2.

TRIGLYCERIDE AND FATTY ACID METABOLISM

TGs in the circulation are derived from the diet after absorption in the intestines (packed into chylomicrons), and produced by the liver (packed in very low-density lipoproteins, VLDL-TGs).
These particles are hydrolyzed by tissue-specific expression of endothelium-bound lipoprotein lipase (LPL) (12), resulting in TG derived fatty acids. These fatty acids enter the cells of the respective tissues, like myocardium, skeletal muscle and adipose tissue, where they are used for energy requirements, or in case of excessive uptake, the fatty acids are re-esterified and stored as TGs. Fatty acids are not only derived from plasma TGs, because fatty acids also circulate bound to plasma albumin (non-esterified fatty acids = NEFAs) derived from lipolysis of TGs stored in adipose tissue. These NEFAs and the TG derived fatty acids are the sources for fatty acids for the cells to be used for energy requirements, or alternatively to be stored as intracellular TGs.

In healthy conditions, almost all TGs present within the body are stored in adipose tissue, with only a small amount present in non-adipose tissues as the heart (17), the liver (18) and skeletal muscle (19). The amount of TGs stored in these non-adipose tissues is tightly regulated, but when this regulation is only slightly disrupted, TGs can accumulate in these non-adipose tissues. This accumulation is reflected in hepatic steatosis and accumulation of TGs in the pancreas (20), associate with beta cell failure in obesity and DM2 (21).
ENERGY SUBSTRATE METABOLISM IN THE HEART

The heart has a constant need for energy. The healthy heart is mostly dependent of mitochondrial oxidation of plasma fatty acids compared to glucose for energy requirements and adenosine-triphosphate (ATP) synthesis. These fatty acids account for >70% of ATP demand (22). Fatty acids enter the myocardium by passive diffusion or by protein-mediated transport, involving fatty acid transporters (mainly CD36) or fatty acid binding protein, FABP (23). Within the cardiomyocyte, the fatty acids are mainly bound to FABP and are then activated by esterification to fatty acyl-coenzyme A. These long-chain fatty acids can be redirected to TGs in the cardiomyocyte, or can be used for beta oxidation, predominantly in the mitochondria and to a lesser extent in the peroxisomes (24). The end product of beta oxidation (acetyl-coenzyme A) fuels the Krebs cycle, which ultimately generates ATP (22).

Glucose from the plasma is transported through the myocardial cellular membrane. This is regulated both by the gradient of glucose and the availability of glucose transporters (GLUT), mainly GLUT-4 (25). Acetyl-coenzyme A is formed from decarboxylation of pyruvate, which is derived from glycolysis and lactate oxidation (26). This acetyl-coenzyme A, together with acetyl-coenzyme A derived from beta oxidation of fatty acids, enters the Krebs cycle to generate ATP.

Differences in substrate delivery to the heart shift the balance between glucose and fatty acid utilization (26;27). In accordance with this concept, the rate of fatty acid uptake by the heart is primarily determined by the concentration of NEFAs in the blood (28), in addition to glucose concentrations, plasma insulin levels and factors including insulin resistance. Increased myocardial reliability on fatty acids is a hallmark of both DM1 and DM2 (29-31). A mismatch between excessive fatty acid uptake in relation to fatty acid utilization results in re-esterification of fatty acids into TGs. However, in obesity fatty acid oxidation is also increased (32-34). This increased oxidation is paralleled by a decrease in glucose utilization. Accordingly, in obesity and insulin resistance impaired fatty acid oxidation per se is not likely to contribute to the observed ectopic lipid accumulation (35). Excessive fatty acid supply to the myocardium increases fatty acid uptake, and this feature may result in TG accumulation.

Furthermore, the heart uses a small amount of ketone bodies for its energy requirements. Extraction of these ketones by the heart is increased, when the delivery of ketone bodies is increased (36;37), i.e. in poorly regulated diabetes and starvation, when insulin levels are relatively low and plasma NEFAs are increased (38-41). Oxidation of ketone bodies inhibits fatty acid oxidation (36) and can, consequently, contribute to myocardial TG accumulation. A simplified overview of myocardial substrate metabolism is provided in Figure 1.2.
MECHANISMS OF MYOCARDIAL LIPOTOXICITY

An overload of cellular fatty acid uptake in relation to oxidative requirements may result in a process called lipotoxicity. The postulated pathways by which lipotoxicity induces alterations in myocardial function are diverse and are discussed in this paragraph.

The balance between cell division and cell death influences the cellular population of organs and, thereby, the functional capacity of these organs (42). A mismatch between the rate of cell death and the replacement of cells creates a functional deficit. The loss of beta cells in the pancreas in DM2 is an example of this concept, which ultimately results in insulin deficiency and hyperglycemia in DM2. This cell loss is induced by a process called programmed cell death or apoptosis. In addition to apoptotic stimuli like thermal- and chemical stress factors (43), metabolic alterations can contribute to apoptosis as well. In animal studies metabolic factors were involved in this so-called lipoapoptosis, associated with the development of pancreatic beta cell dysfunction and cardiomyopathy (7;21;44-47). Accordingly, obesity-related deposition of TGs in non-adipose tissues is associated with insulin resistance and the development of DM2 (48-53).

When fatty acid overload in cells exceeds the oxidative capacity, surplus fatty acids enter non-oxidative pathways. As mentioned above, this overload will lead to re-esterification of fatty acid derivatives into TGs within the cells, although TGs per se are probably not harmful. However, these TGs are the reflection of increased availability of fatty acid derivatives like diacylglycerol and fatty acyl-coenzyme A. Therefore, intracellular TG might be considered as an inert reflection of the potentially damaging pathways.
Different pathways may lead to cellular dysfunction upon increased availability of fatty acid derivatives. In addition to lipid peroxidation (47) and diacylglycerol, the ceramide pathway seems to be important (7;42). The increase in fatty acyl-coenzyme A levels, resulting from chronic lipid overload, induces de novo synthesis of tumor necrosis factor alfa and ceramide (46), which upregulates the expression of inducible nitric oxide synthase (21). Furthermore, fatty acyl-coenzyme A decreases Akt kinase activity (53), which ultimately decreases the translocation of the GLUT resulting in decreased glucose availability. Moreover, fatty acyl-coenzyme A activates the apoptotic process and serves as a ligand for transcription factors like peroxisomal proliferator-activated receptor alpha, which ultimately alters the structure and function of the heart.

Based on rodent studies, excessive myocardial fatty acid uptake and resulting TG accumulation may be causally involved in the development of disturbed myocardial function in diabetes mellitus (7;10;47;54). The amount of TGs is associated with alterations in myocardial function (7;47;55). Moreover, it may reflect long-chain fatty acid induced activation of calcium channels (56) which may alter cardiac function (57). The mechanisms of lipotoxicity are complex.
as glucose and fatty acid metabolism also interact with each other. For example, fatty acids inhibit Akt 1, resulting in altered insulin signaling and decreased glucose uptake. The involved pathways in lipotoxicity are summarized in Figure 1.3. Taken together, quantification of intracellular TGs may be a representation of these toxic, non-oxidative pathways. Accordingly, when obese rats are treated with troglitazone, myocardial TG accumulation is decreased, associated with a decrease in intracellular content of ceramides, DNA laddering and an improvement in myocardial contractility (7). Furthermore, hyperleptinemia in obese mice prevents the development of lipotoxic cardiomyopathy (54).

In humans, plasma TG levels are an independent predictor of left ventricular relaxation (58). Alterations in left ventricular function (59;60) are associated with altered myocardial (high-energy phosphate) metabolism in patients with DM2 (61) and in patients with hypertension (62). Furthermore, in obesity, plasma levels of NEFAs are associated with myocardial TG content (63) and with left ventricular diastolic function (63;64). These circumstantial lines of evidence indicate that the observations on the effects of fatty derivatives documented in rodent studies may also be applicable in human pathophysiology.

**MYOCARDIAL TRIGLYCERIDES IN HUMANS**

Although experimental studies in rodents suggest a causal relation between myocardial TG content and myocardial function, translational studies on this subject in humans are scarce. One important reason for this lack of human studies is that non-invasive measurement of myocardial TG content is challenging, mainly due to the confounding effects of cardiac and respiratory motion. However, recently, hydrogen 1 magnetic resonance spectroscopy (\(^{1}\)HMRS) became available to assess TG content of the myocardium in humans *in vivo* (17;63;65-67). An example of a \(^{1}\)HMR spectrum of the myocardium is shown in Figure 1.4. This technique has been validated against histological samples for measurement of hepatic TG content (68;69) and skeletal muscle TG content (70).

The \(^{1}\)HMRS measurement of myocardial TG content is technically challenging, and, therefore, not widely available. To obtain accurate measurements *in vivo* it is essential to minimize artifacts induced by cardiac and respiratory motion (66;67;71;72). Recent studies have shown that myocardial TG content correlates with histological verified TG content (17). Therefore, this technique also allows to measure myocardial TG content in the human heart *in vivo*.

**MAGNETIC RESONANCE IMAGING OF THE HEART**

Cardiovascular magnetic resonance (CMR) is perfectly suitable to assess myocardial systolic and diastolic function (73-75). CMR combined with metabolic imaging of TG content by \(^{1}\)HMRS and
phosphorus $^{31}$ ($^{31}$P) spectroscopy provides a potential useful tool to study myocardial substrate metabolism in relation to myocardial function in vivo. The first studies on this subject indicate that myocardial TG content is indeed associated with plasma fatty acid levels and myocardial function (63;76). Moreover, it seems that fatty infiltration in the myocardium precedes the onset of systolic dysfunction and is, therefore, a potential parameter for the evaluation of treatment in the insulin resistant state, even before diabetic cardiomyopathy is present (76). However, the pathophysiological associations between myocardial TG accumulation and myocardial function in humans remain largely uncharacterized.

Therefore, the aim of this thesis is to study myocardial TG content in relation to cardiac function in a variety of metabolic interventions, in healthy subjects and in patients with DM1 and DM2.

**OUTLINE OF THE THESIS**

The studies in this thesis evaluate the relation between myocardial TG content and myocardial function under physiological and pathophysiological circumstances in humans in vivo.
Moreover, we evaluate tissue-specific effects by including the quantification of hepatic TG content, being an extra-cardiac location of ectopic deposition of TGs.

The first part of this thesis documents studies performed in healthy subjects. Chapter 2 evaluates the effects of compensation for the movement artifacts induced by cardiac and respiratory motion on $^1$HMRS measurements. Chapter 3 describes a study on the effects of short-term caloric restriction on myocardial TG content and myocardial function. Furthermore, we evaluate whether extending the model of partial caloric restriction to complete starvation results in more pronounced alterations in Chapter 4. Chapter 5 describes a study on the effects of a hypercaloric, high-fat diet on plasma metabolic parameters, and myocardial TG content in relation to cardiac function.

Part two of this thesis evaluates the flexibility of TG content of the diabetic myocardium in relation to myocardial function in humans in vivo. Therefore, we evaluate the effects of partial caloric restriction on myocardial TG content and myocardial function in patients with DM2 in chapter 6. Moreover, we study the same subjects under the condition of partial caloric restriction during inhibition of adipose tissue lipolysis by administration of acipimox, to specifically assess the contribution of plasma NEFA levels to myocardial TG content and myocardial function. Chapter 7 describes the effects of prolonged partial caloric restriction in severely obese patients with DM2. According to our previous work, we hypothesize that this may result in decreased myocardial TG stores, associated with improved myocardial function as weight loss increases insulin sensitivity (77-79). In patients with DM1 glucoregulation is imperfect and frequent episodes of hyperglycemia and high plasma NEFA levels are frequently present. Therefore, these metabolic alterations may adversely affect myocardial metabolism, with accumulation of myocardial and hepatic TGs as well. Chapter 8 evaluates the effects of controlled partial insulin deprivation for 24 hours with resulting hyperglycemia and increased plasma NEFA levels in otherwise well-controlled patients with DM1 on myocardial TG content and myocardial function.
REFERENCES


37. Forsey RG, Reid K, Brosnan JT. Competition between fatty acids and carbohydrate or ketone bodies as metabolic fuels for the isolated perfused heart. Can J Physiol Pharmacol 1987; 65(3):401-406.


Chapter 1


